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Viral Infection and Serum Antibodies to Heat Shock Protein 70 in the Acute Phase of Ménière's Disease

Federica DiBerardino,[1] Antonio Cesarani,[1] Ales Hahn,[2] and Dario Alpini[3]

[1] *Department of Audiology–Ear, Nose, and Throat, I.R.C.C.S. Fondazione Policlinico, Mangiagalli e Regina Elena, University of Milan, Italy;* [2] *Ear, Nose, and Throat Institute III, Medical Faculty, Charles University, Prague, Czech Republic; and* [3] *Otoneurology Service Sc. Institute, Scientific Institute Maria Nascente–Don Gnocchi Foundation, Milan, Italy*

Abstract: Ménière's disease (MD) is an idiopathic inner-ear disorder characterized by fluctuating hearing loss, episodic vertigo, and tinnitus. Though MD's etiology is unknown, growing evidence suggests that autoimmunity may be involved in its development. The aim of this prospective study was to investigate the presence of anti-heat shock protein 70 (anti-HSP70) antibodies during the acute phase of MD and to relate its presence to the antibody pattern. We examined the sera of 13 patients by Western blot immunoassays for reactivity to bovine inner-ear antigen (anti-HSP70) antibodies. The presence of viral antibodies and autoantibodies (herpes simplex, types 1, 2; herpes zoster; cytomegalovirus; Epstein-Barr; IgM; IgG; cardiolipin; thyroglobulin and thyroperoxidase; and antinuclear, antimitochondrial, and anti-smooth-cell antibodies) were also tested. We found reactivity to HSP70 in only 1 of the 13 MD patients (7.7%), and it occurred during herpes zoster reactivation. We found no relationship between the presence of antibodies to HSP70 and immunological or viral testing.

Key Words: anti–heat shock protein 70 antibodies; autoantibodies; cytomegalovirus; herpesvirus; Ménière's disease

The endolymphatic hydrops associated with Ménière's disease (MD) seems to be due to multifactorial inheritance leading to altered endolymphatic homeostasis. Many etiological factors have been proposed, including functional or anatomical obstructions of endolymphatic flow; abnormalities in endolymph production or absorption [1,2]; genetic anomalies; vasodilation; allergies [3]; viral infections [4,5]; and autoimmunity or inflammatory processes [6–9]. High levels of circulating immune complexes have been found significantly more frequently in MD patients than in control subjects [10], and histological evidence of immune injury to the endolymphatic sac of patients with MD has also been reported [11]. Some have suggested that certain cases of MD may have an altered immunological background, which may be attributable to an autoimmune mechanism that depends on humoral or cellular responses (or both) [12].

Heat shock proteins (HSPs), molecular chaperones of which HSP70 is the prototype, protect cells by dissolving and refolding misfolded or denatured proteins, thus preventing the aggregation and even the resolubilization of injured proteins [13]. HSPs are referred to as *stress proteins*, because they are induced in response to a wide range of physiological and environmental insults, including viral infection, inflammation, fever, malignant transformation, and cellular exposure to oxidizing agents, cytotoxins, and anoxia [14]. HSPs also seem to function as initiators of a host's immune response [15]. The presence of anti-HSP70 antibodies in the sera of patients with progressive sensorineural hearing loss has been evidenced in many reports [16–18], and anti-HSP70 antibodies recently were proposed as a marker of active disease and steroid responsiveness in patients with

Reprint requests: Federica DiBerardino, MD, UO Audiologia–Dip.to di Otorinolaringoiatria, Via Pace 9, 20122 Milan, Italy. Phone, fax: +39 02 5503.3922; E-mail: federica.diberardino@unimi.it

idiopathic, bilateral, progressive sensorineural hearing loss [19]. Previous reports evidenced that anti-HSP70 antibodies occurred in 30–45% of MD patients [7,20,21], whereas a prospective work by Rauch et al. [22] recently demonstrated that anti-HSP70 antibody detection is not clinically useful in MD, as no evidence supports the association between the antibodies' presence and the clinical features or course of MD. Previous reports have shown the presence of anti-HSP70 antibodies but have failed to specify their precise role in MD. For this reason, we tested patients during the disorder's acute phase to investigate the possible relationship between the presence of antibodies to HSP70 and immunological and viral testing.

PATIENTS AND METHODS

The patients presented in this study were part of a larger cohort being prospectively evaluated for progressive inner-ear disorders. Patients were evaluated by two expert neurootologists (CA and AD). Cases to be included were reevaluated by an expert but independent neurootologist (HA). We excluded patients with a history of thermal burns, diabetes mellitus, Alzheimer's disease, cancer, or recent influenza, fever, or ulcerative colitis, as these conditions are associated with increased levels of HSP70. We also excluded those who had recently used medications, such as steroids, that may affect serum HSP70 values.

We enrolled 13 patients (6 men, 7 women; age range, 37–52 years; mean, 43 years) who were referred to us with signs, symptoms, and audiometric criteria consistent with monolateral MD. All the patients had a history of fluctuating and progressive sensorineural hearing loss and two or more episodes of whirling vertigo lasting from 20 minutes to 24 hours, in accordance with the American Academy of Otolaryngology—Head and Neck Surgery diagnostic criteria for MD [23]. All the patients underwent otovestibular diagnosis; the clinical evaluation included a routine medical history, physical examination, and pure-tone audiogram. We performed magnetic resonance imaging to rule out abnormalities of the eighth nerve. The average time that had elapsed from the onset of the hearing loss to the initial examination was 23.8 months, and the median interval between attacks was 15 days. All the patients presented audiometric evidence of progression manifested as a threshold shift greater than 10 dB at low frequencies on serial audiograms recorded less than 3 months apart (active disease). We obtained informed consent from all the patients and took the blood samples during the acute phase of the disorder.

The laboratory test for detecting anti-HSP70 antibodies used the Western blot immunoassay with purified HSP70 antigen from a bovine kidney-cell line. We used enzyme-linked immunosorbent assays to detect antibodies (immunoglobulins M and G [IgM, IgG]) to herpes simplex virus, types 1, 2; cytomegalovirus; varicella-zoster virus (VZV); and Epstein-Barr virus. We also assayed anticardiolipin, antithyroglobulin, antithyroperoxidase, antinuclear, antimitochondrial, and anti-smooth-cell antibodies.

STATISTICAL ANALYSIS

We performed univariate and multiple logistical regression to determine which clinical variables were associated with active hearing and balance symptoms. Two-tailed test values of $p < .05$ were considered statistically significant for all comparisons. We used the SPSS software package for all statistical analyses (version 9.0, SPSS Inc., Chicago, IL).

RESULTS

All patients had active hearing loss and balance symptoms at the time of enrollment, and monolateral disease was present in all cases. We saw no relationship between the pattern of immunoreactivity in the HSP70 Western blot analysis and age, gender, or any of the other tested variables (Table 1). Anti-HSP70 antibodies were detected in only 1 of the 13 patients (7.7%). In that patient, anti-VZV IgM and IgG antibodies were also positive: We took the specimen during the early phase of VZV reactivation at the same time as the acute MD attack but did not detect skin lesions in a subsequent clinical observation. Anti-HSP70 antibodies were absent in another patient with only VZV IgG antibodies. We observed the presence of cytomegalovirus IgG in 5 of the 13 patients (38.46%), whereas Epstein-Barr virus IgG was present in 2 of the 13 (15.38%). In those patients, we observed no relationship between the presence of antibodies and the clinical course of MD.

DISCUSSION

HSPs are selectively expressed in cells after exposure to a range of stress stimuli, including viral infection. HSP species are highly immunogenic and elicit humoral, cytotoxic T-lymphocyte, and natural killer cell responses against viruses, tumors, and infectious diseases. Studies have shown the up-regulated expression of HSP70 during human immunodeficiency virus infections and that HSPs have a role as a vehicle for the delivery of antigens that are targets for natural killer and antibody-dependent cellular cytotoxicity [24]. Acoustic overstimulation and cisplatin injections also induce the synthesis

Table 1. Tested Variables in the Heat Shock Protein 70 Western Blot Analysis

No.	Patient	Sex	HSP70	HSV type 1 and 2 IgM-IgG	HZV IgM/ IgG	CMV IgM/ IgG	EBV IgM/ IgG	Cardiolipin IgM/IgG	Thyro-globulin	Thyro-peroxidase	ANA	AMA	ASMA
1	PO	F	−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−
2	PD	F	−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−
3	CM	M	−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−
4	CL	M	−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−
5	MMT	F	−	−/−	−/−	+/+	−/−	−/−	−/−	−/−	−/−	−/−	−/−
6	BN	F	−	−/−	−/−	−/+	−/+	−/−	−/−	−/−	−/−	−/−	−/−
7	AS	F	+	−/−	+/+	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−
8	CC	F	−	−/−	−/−	+/+	−/−	−/−	−/−	−/−	−/−	−/−	1:20
9	SV	M	−	−/−	−/+	−/−	−/+	−/−	−/−	−/−	−/−	−/−	−/−
10	MS	M	−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−
11	LN	M	−	−/−	−/−	−/+	−/−	−/−	−/−	−/−	−/−	−/−	−/−
12	SA	M	−	−/−	−/−	−/+	−/−	−/−	−/−	−/−	−/−	−/−	−/−
13	CD	F	−	−/−	−/−	−/−	−/−	+/+	−/−	−/−	−/−	1:40	−/−

AMA = antimitochondrial antibodies; ANA = antinuclear antibodies; ASMA = anti-smooth-cell antibodies; EBV = Epstein-Barr virus; CMV = cytomegalovirus; HSP70 = heat shock protein 70; HSV = herpes simplex virus; HZV = herpes zoster virus; IgG = immunoglobulin G; IgM = immunoglobulin M; +/+ = presence of IgM and IgG values higher than normal range; +/− or −/+ = positivity of only IgM or IgG, respectively; −/− = absence of both IgM and IgG.

of HSP72 in the outer hair cells of the cochlea, thus suggesting that an increase in HSP may offer protection or provoke the release of tissue components [25].

We found no relationship between the presence of antibodies to HSP70 and immunological and viral testing. In a previous study, Ruckenstein et al. [26] attempted to establish the value of some immunological and serological tests in MD patients and excluded the role of Lyme disease and otosyphilis in the symptoms. This study detected antiphospholipid antibodies in unilateral MD and higher levels of antinuclear antibodies in bilateral MD, thus suggesting that patients with bilateral MD may be more likely to have a systemic autoimmune process.

Our report evidenced only the simultaneous expression of anti-HSP70 antibodies and VZV IgM. In vitro, the expression and subcellular localization of cellular HSP70 have been examined in VZV-infected human diploid fibroblasts [27], and infection with VZV induces HSP70 expression from 24 hours after infection and its localization to VZV-specific inclusion bodies. This co-occurrence might be caused only by aspecific polyclonal antibody expression during the reactivation of VZV infection and should be confirmed by further studies.

Serum from patients with MD was first tested for the presence of antibodies to HSP70 by Gottschlich et al. [20], who found them in 30% of patients. Subsequent studies by Shin et al. [21] and Rauch et al. [22] showed anti-HSP70 proteins in 33.3% and 47% of patients with MD, respectively. In 1995, Rauch et al. showed that anti-HSP70 antibodies were present in 58.8% of cases of bilateral MD, 37.5% of cases of contralateral delayed endolymphatic hydrops, and 33.3% of cases of unilateral MD with idiopathic, progressive, bilateral sensorineural hearing loss in the second affected ear. The Western blot technique, used to detect antibodies against inner-ear antigens, involved overnight incubation with casein (a cow's milk-derived protein), but no relationship was found between the presence of anti-HSP70 antibodies and allergy to milk in 56 MD patients with milk allergy [28].

Though all these patients were tested during the acute phase of MD, the incidence of HSP70 antibodies in these observed patients was only 7.7%. The lower percentage in our report could be attributed in part to the exiguity of patients but also to the fact that they were affected monolaterally. This underlines the importance of identifying subsets of patients with MD when studying whether immunological factors play a pathogenic role.

Even though the presence of anti-HSP70 antibodies in the sera of patients with progressive sensorineural hearing loss has been reported [16–18], a recent report evidenced that low sensitivity of Western blot immunoassay for patients affected by MD may result from either the long time elapsed from the hearing loss and vertigo to the initial examination or from the increased percentage of cases of systemic autoimmune disease present in patients with idiopathic, progressive sensorineural hearing loss [29]. Conversely, our data confirm the most recent work by Rauch et al. [22], in which anti-HSP70 antibody detection did not offer clinically useful information in MD even in the acute phase; furthermore, there was no evidence of any association between their presence and the antibody pattern. Further studies are needed to find any relation to the viral pattern, but anti-HSP70 antibodies do not seem to be a marker of active MD. Elevated serum HSP70 levels, instead, may predict prognosis in patients with sudden sensorineural hearing loss [30], and a recent report has

hypothesized that an immune response to inner ear-specific HSP70 could be present in a significant number of idiopathic tinnitus cases [31], suggesting different etiological patterns.

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