Low Levels of Heat Shock Proteins-60 and -65 Autoantibodies in Sjögren's Syndrome

Ora Shovman MD[1*], Yaniv Sherer MD[1*], Boris Gilbourd PhD[1], Roberto Gerli MD[2], Elena Bartoloni Bocci MD[2], Francesco delle Monache MD[2], Filippo Luccioli MD[2] and Yehuda Shoenfeld MD[1]

[1] Department of Medicine B and Center of Autoimmune Diseases, Sheba Medical Center, Tel Hashomer, Israel
Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel
[2] Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy

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Abstract

Background: Heat shock proteins are highly conserved immunodominant antigens found in various species. Humoral immune responses to mycobacterial HSP65 and human HSP60 have been established in a number of human autoimmune diseases.

Objective: To assess the prevalence of antibodies to HSP60 kDa and HSP65 kDa in patients with Sjögren's syndrome as compared to normal subjects.

Methods: Thirty-seven patients with SS were compared with normal controls. The antibodies against human HSP60 were measured by the Anti-Human (IgG/IgM) HSP60 ELISA kit. IgGs and IgMs to mycobacterial HSP65 were determined using an enzyme-linked immunosorbent assay with mycobacterial recombinant HSP65 antigens.

Results: The levels of both anti-HSP60 and -HSP65 were lower in patients compared with controls. IgG autoantibodies to HSP60 were significantly different between groups: 162 ± 55.1 ng/ml in controls versus 112.3 ± 30.6 ng/ml in SS patients ($P < 0.001$). The levels among controls of anti-HSP65 IgM isotype were also significantly higher than among the SS patients: 111.6 ± 33.4 U/ml versus 96.1 ± 8.9 U/ml ($P = 0.01$).

Conclusions: The results of the present study show that the levels of different isotypes of anti- HSP60 and HSP65 antibodies were lower in patients with SS than in normal subjects. Additional studies in larger patient populations are required to evaluate the prevalence of these autoantibodies in SS patients.

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Sjogren's syndrome is a chronic organ-specific autoimmune disease characterized by lymphocytic infiltration of the salivary and lacrimal glands, leading to oral and ocular dryness as well as to systemic manifestations such as renal, pulmonary and vascular complications [1]. The etiopathogenesis of SS is largely unknown (although a role has been suggested for a few viruses), but most of the available data suggest that both cellular and humoral immune mechanisms are involved in the pathogenesis of the disease. B cells isolated from SS patients are oligoclonally activated, resulting in hypergammaglobulinemia, elevated levels of circulating immune complexes and non-organ-specific antibodies. The predominance of activated T cells within the exocrine gland infiltrates in SS suggests that T cells play an important role in initiating the disease. However, the putative antigen(s) that trigger activation and generation of these autoreactive T cells are still unknown.

It has been established in recent years that members of the heat shock protein family, especially mycobacterial HSP65 and its human homology HSP60, represent target antigens of the immune response [2]. HSP-specific cellular and humoral immune responses were demonstrated in a number of experimentally induced autoimmune diseases such as adjuvant arthritis in rats [3] and insulin-dependent diabetes mellitus in NOD mice [4]. Moreover, increased T cell response to mycobacterial HSP65 [5] as well as raised levels of circulating antimycobacterial HSP65 and HSP60 antibodies were found in patients with different autoimmune rheumatic diseases [6–14]. In the present study we investigated the different isotypes of antibodies to HSP65 and HSP60 in patients with SS as compared to healthy subjects.

Patients and Methods

The study population comprised 37 consecutive SS patients, all of whom fulfilled the criteria for classification of SS [15]. The sera of 11 age-matched apparently healthy women were obtained from the national blood bank.

Anti-HSP60 antibody determination

The antibodies against human HSP60 were measured by the Anti-Human (IgG/IgM) HSP60 ELISA kit (Stressgen Biotechnologies Corp., Canada) according to the manufacturer's instruction. Concentration of antibodies was determined using the standard anti-HSP60 curve (ng/ml).

Anti-HSP65 antibody determination

For antibody determination, ELISA plates were pre-coated overnight at 4°C with 1 µg/ml (in phosphate-buffered saline) of mycobacterial recombinant HSP65 antigens. Plates were blocked with 1% bovine serum albumin for 2 hours at room temperature. After washing (three times) with PBS 0.05% Tween 20 so-

* Both authors contributed equally to this work.

HSP = heat shock protein
SS = Sjögren's syndrome
Ig = immunoglobulin

PBS = phosphate-buffered saline

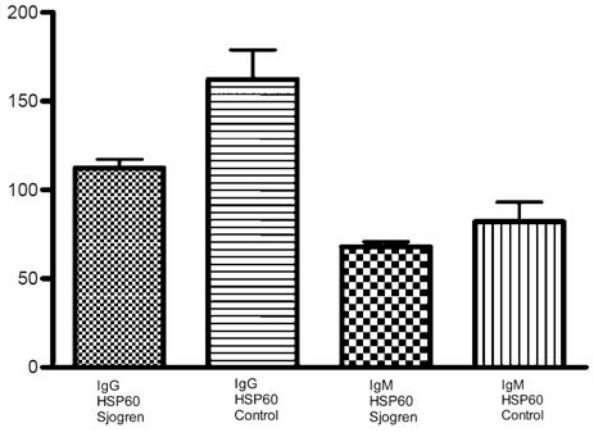

Figure 1. IgG and IgM anti-heat shock protein 60 (HSP60) autoantibodies (ng/ml) in Sjögren's syndrome patients and control subjects. $P < 0.05$ only for IgG.

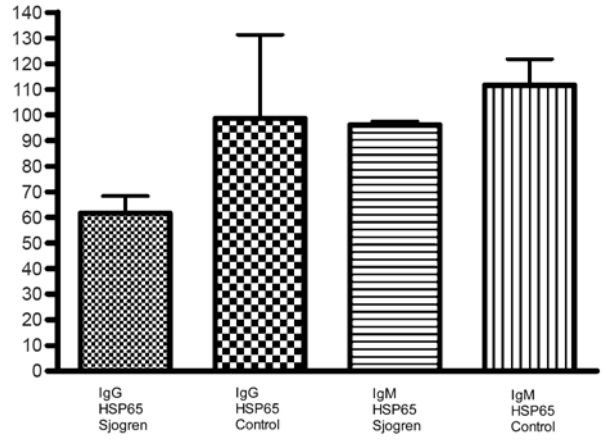

Figure 2. IgG and IgM anti-heat shock protein 65 (HSP65) autoantibodies (U/ml) in Sjögren's syndrome patients and control subjects. $P < 0.05$ only for IgM.

lution, sera of patients diluted 1:200 were added and incubated for 2 hours at room temperature. After additional washings affinity-purified goat anti-human IgG or IgM antibodies conjugated with alkaline phosphatase (Jackson ImmunoResearch, USA) were added for 1 hour at room temperature. The plates were developed using phosphatase substrate (Sigma, USA) and read on an ELISA reader (Anthos HT2, Austria). Results were expressed as arbitrary units per ml (U/ml) using the corresponding standard curve.

Results

The comparison of anti-HSP autoantibodies level between patients with SS and normal subjects showed that levels of IgM autoantibodies to HSP60 were higher in controls than in SS patients (82 ± 37.9 versus 67.9 ± 17.5 ng/ml, respectively; $P > 0.05$). Nonetheless, the levels of IgG autoantibodies to HSP60 were significantly different between groups: 162 ± 55.1 ng/ml in controls versus 112.3 ± 30.6 ng/ml in SS patients ($P < 0.001$) [Figure 1]. A Similar trend was found regarding levels of IgM autoantibodies against HSP65: the levels of the IgM isotype were significantly higher among controls than among patients: 111.6 ± 33.4 versus 96.1 ± 8.9 U/ml ($P = 0.01$). On the other hand, while levels of IgG anti-HSP65 also differed between groups, this difference did not reach statistical significance due to the high standard deviation (98 ± 108.4 versus 61.7 ± 41 U/ml in controls and patients, respectively) [Figure 2].

Discussion

These results emphasize that levels of IgG and IgM autoantibodies against human HSP60 and mycobacterial HSP65 are lower in sera obtained from patients with SS as compared to control subjects. There are only a few studies detailing these autoantibodies in SS patients. Jarjour et al. [16] also found a comparable prevalence of autoantibodies against HSP in sera from SS patients and controls [16]. In contrast, it was demonstrated by Aragona and colleagues [17] that the prevalence of antibodies against H*elicobacter pylori* and its specific HSP60 was higher in 53 SS patients than among controls. Of note is that a similar incidence of anti-HSP60 antibodies was detected in SS patients infected with H. *pylori* and in patients without infection. In another study that included patients with different autoimmune rheumatic diseases, high levels of antibodies against HSP60 were observed in nine SS patients [14].

The relation between HSPs and autoimmune rheumatic diseases has been extensively investigated in recent years. HSP synthesis is increased in order to protect prokaryotic or eukaryotic cells from various insults during periods of stress caused by infection, inflammation, or similar events. Consequently, in several infections and autoimmune diseases, HSPs represent prominent antigens in the humoral and cellular immune response mediated by antibodies and T cells [2]. For instance, a critical role of HSP60 and HSP65 in induction of T cell response has been proved by the involvement of αβ T cells specific for HSP602 in Behcet's disease [5] and CD4 T cell proliferation induced by HSP65 and HSP60 in Takayasu's arteritis [13]. Humoral immune responses to HSP were established in a number of human autoimmune diseases [6–14]. Increased levels of HSP60-specific antibodies in serum were found in atherosclerosis [7], systemic sclerosis [8], psoriasis [9], Kawasaki disease [10], Behcet's disease [11], juvenile chronic arthritis [12], and Takayasu arteritis [13]. Similarly, elevated levels of other HSP-reactive antibodies were detected in some autoimmune diseases. For example, over-expression of HSP90 was found in B and T cells in 20% of patients with systemic lupus erythematosus, and this finding correlated with active central nervous system and cardiorespiratory disorders [18]. In addition, increased antibody levels to HSP90 were found in a group of SLE patients [19], high titers of antibodies against HSP70 were identified in the sera and cerebrospinal fluid of multiple sclerosis patients [20], and a high prevalence of autoantibodies to HSP47 antigen was

SLE = systemic lupus erythematosus

found in patients with mixed connective tissue disease [21]. It should be noted, however, that inconsistent levels of self-HSP-reactive antibodies among patients with rheumatoid arthritis and SLE were reported in other studies [12,22].

Because of controversial reports about the prevalence of HSP antibodies in human autoimmune disease and because these antibodies are occasionally found among healthy subjects [23], their significance with respect to autoimmune disease pathogenesis remains to be determined. It is possible, however, that they play a major role in a given autoimmune disease while playing a minor or no role in another. The results in the present study of decreased IgG HSP60 and IgM HSP65 antibodies in patients with Sjogren's syndrome cast doubt on the real significance of previous partially contrary findings. It is also possible that since anti-HSP antibodies have been reported in association with enhanced atherosclerosis [24,25], their lower levels in SS might imply that they have a protective role in that respect. The implications of immune responses against HSP are still not fully understood, yet they provide additional evidence for the association between infections and autoimmunity.

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Correspondence: Dr. Y. Shoenfeld, Department of Medicine B, Sheba Medical Center, Tel Hashomer 52621, Israel.
Phone: (972-3) 530-2652
Fax: (972-3) 535-2855
email: Shoenfel@post.tau.ac.il