HSP 60 expression in recurrent oral ulcerations of Behçet's disease

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Objective. The aim was to investigate heat shock protein 60 (HSP60) expression in oral ulcerations of Behçet's disease (BD).
Study design. Biopsy specimens were obtained from patients with BD (n = 11), recurrent aphthous stomatitis (RAS) (n = 11), oral lichen planus (OLP) (n = 11) and healthy individuals (HI) (n = 11). Eight samples in BD and RAS groups were evaluable. All groups were analyzed by biotin streptavidin-aminoethylcarbazole using monoclonal mouse antibodies to HSP60 Ab-1 (clone LK1).
Results. Immunostaining indicative of HSP60 expression in BD and RAS were significantly higher than HI. No significant difference was found between BD and OLP except in the suprabasal layer of epithelium.
Conclusions. Altered expression of HSP60 was found in ulcerative lesions of BD and RAS suggesting that HSP60 has an association with the etiology or chronicity of these inflammatory lesions. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:196-200)**

Behçet's disease (BD) is a systemic disorder of recurrent acute inflammation characterized by major symptoms: genital ulcers, oral ulcers, uveitis, and skin lesions.[1] BD is now recognized as a multisystem vasculitis, which can also affect all types and sizes of blood vessels, joints, lungs, and central nervous and gastrointestinal systems.[2] The disease has a genetic background with an association with HLA-B51 and an increased incidence in countries around the Mediterranean and the Far East, such as Japan and Korea. Besides genetic, environmental, and immunological factors, microbial agents such as streptococci and herpes simplex virus have been implicated in its etiology.[3,4]

Heat shock proteins (HSPs) or stress proteins are highly conserved proteins that are involved in protein synthesis, and folding and translocation of proteins under normal physiological conditions.[5] These proteins are synthesized in large amounts, when cells are exposed to stressful stimuli such as anoxia, infection, toxic agents, and temperature changes.[6]

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Extensive sequence homology between microbial and human HSPs has led to the concept that these proteins might be involved in the etiology and pathogenesis of some immune-mediated disorders.[6] Consistent with this theory, patients with BD have been shown to have antibodies against HSP65 both in the peripheral blood and local fluids such as cerebrospinal fluid.[7-9] Several mycobacterial HSP65 peptides and their human analogues (HSP60) have been shown to stimulate lymphoproliferative response in patients with BD in a specific manner.[10-12] In the oral cavity, anaerobic strains obtained from periodontal sites and mixed saliva of patients with BD produced HSPs.[13] Moreover, in 6 cases of LP, basal keratinocytes stained more intensely for HSP27 and HSP60 in lichen planus than healthy controls.[14]

Currently, there are no data on the local expression of HSP in recurrent oral ulcerations of Behçet's disease. Inasmuch as HSPs expressed in autoimmune inflammatory lesions can contribute to the etiology or persistence of the disease, this study was designed to investigate HSP60 expression in recurrent oral ulcerations of BD and aimed to compare these expressions with its expression in lesions of recurrent aphthous stomatitis (RAS), oral lichen planus (OLP), and normal mucosa of healthy individuals.

MATERIAL AND METHODS

Patients

The study included 11 patients with BD, diagnosed according to International Study Group for Behçet's Disease criteria,[15] with the mean age 36.8 years, 6

females and 5 males with active oral ulcerations for the preceding 1 or 2 days. Patients were those individuals who presented to Istanbul University, Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology between 2002 and 2004. The study also included 33 individuals, with the mean age of 38.7 years, 23 female and 10 male as the control group. The control group consisted of 11 patients with RAS, existing for the preceding 1 or 2 days, diagnosed on the basis of detailed medical history and clinical presentation, 11 patients with OLP who were diagnosed and histopathologically confirmed according to the World Health Organization criteria,[16] and 11 healthy individuals (HI) with no systemic disorders. The study was approved by Istanbul University, Human Ethics Research Committee, and all persons signed informed consents to participate in the study.

Biopsies and histopathologic study

After local anesthesia with articaine HCL 2%, the entire oral ulceration of patients with BD and RAS were taken by 4-mm punch. In addition, 4-mm punch biopsy specimens were obtained from lesional buccal mucosa of patients with OLP and clinically and histologically normal buccal mucosa of HI. The epithelial surface of the biopsy specimens were stained with ink. Samples were fixed in Hollande's solution (copper acetate 75 g, picric acid 120 g, formaldehyde 300 mL, glacial acetic acid 45 mL, distilled water 3000 mL) and embedded in paraffin and processed. Sections from both patients and the controls were stained by hematoxylin-eosin (H-E).

Immunohistochemical study

Immunohistochemical staining procedure was applied to all cases on the same occasion. From each specimen, 3- to 4-μm thick paraffin sections were cut and mounted on positively charged slides (Menzel-glaser-polysine). Monoclonal mouse antibodies to HSP60 Ab-1 (clone LK 1; Neomarkers, Fremont, CA) and Ultravision universal kit (Labvision Corporation, cat. No: TM-015-HA, Neomarkers, Fremont, CA, USA) were used. Sections were deparaffinized in xylene, hydrated through graded alcohol, and washed with phosphate buffered saline (PBS). Endogenous peroxidase activity was prevented by using hydrogen peroxide for 20 minutes. The sections were then washed in PBS. Citrate buffer was used for antigen retrieval. Nonspecific binding was blocked by using a universal blocking reagent (Ultra V block, cat. No: TA-015-UB, Neomarkers). Prediluted primary monoclonal antibodies were applied for 1 hour at room temperature. Slides were incubated for 25 minutes with biotinylated goat anti-mouse and streptavidine peroxidase conjugate was applied for 25 minutes. Aminoethylcarbazole (AEC substrate, cat. No: TA-015-HAS, Neomarkers) was used as chromogen for 15 minutes. Sections were counterstained with Mayer's hematoxylin for 5 minutes and mounted (Ultra-mount, cat.no: TA-060-UM, Neomarkers) and examined under a light microscope. All incubations were done in a humidifying chamber at room temperature. The paraffin block of a breast carcinoma was used as the positive control. Sections in which the primary antibody was replaced with a purified nonimmune mouse immunoglobulin (1:500 dilution, Zymed-Invitrogen, Carlsbad, CA) submitted to the same steps served as negative control.

Immunohistochemical evaluation

The stratified squamous epithelium was divided into 3 layers: basal, suprabasal, and superficial. Vascular endothelial cells and infiltration cells at the lamina propria were also examined under a light microscope. Semiquantitative analysis was assessed by an expert pathologist blinded to the clinical data. Staining intensity of the cells was scored as follows: (–), none; (+), weak; (++), moderate; (+++), dense staining. Each layer was evaluated for the intensity of overall staining. This method was adopted from Bramanti et al.[14]

Statistical analysis

Differences in the HSP60 staining patterns of the study groups were analyzed by Kruskal-Wallis test. Mean differences in each group were analyzed by Dunn's and Tukey tests. Differences were considered significant at P values less than .05.

RESULTS

Three samples of the BD and RAS were determined as having lost epithelium; thus, these samples were not evaluated. Briefly, all groups had positive staining of varying intensity (Table I); however, staining was more intense in patients with BD with respect to the HI group especially at basal epithelium (Fig. 1). HSP60 expression in all layers of the BD group were significantly higher than the scores in the HI group ($P < .05$). HSP60 expression in endothelial cells of biopsy specimens of patients with BD and RAS were similarly determined as weak-moderate staining (Fig. 2). No significant difference in HSP60 expression between the BD group and RAS group was evident ($P > .05$). HSP60 expression in the BD group was significantly higher than the scores in the OLP group only in the suprabasal layer ($P < .05$). HSP60 expression in the OLP group was significantly higher than the scores in the HI group only in the infiltrating cells ($P < .05$). HSP60 expression in the RAS group was significantly

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