The Expression of Molecular Chaperone HSP90 and IL-6 in Patients with Systemic Lupus Erythematosus

HU Shaoxian (胡绍先)1, XU Qing (徐 青)1, XIAO Wenze (萧文泽)1; Huang Melissa2
1Department of Rheumatology and Clinical Immunology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China  
2Johns Hopkins University, Baltimore, Maryland 21210, USA

Summary: To explore the expression and clinical significance of molecular chaperone heat shock protein 90 (HSP90) in peripheral blood mononuclear cells (PBMC) and plasma level of interleukin-6 (IL-6) in patients with systemic lupus erythematosus (SLE), HSP90 was detected in PBMC by Western blot assay and the plasma level of IL-6 was measured by ELISA in 38 SLE patients and 20 normal controls. The correlation analysis was performed between the SLE disease activity index (SLEDAI) and the expression of HSP90 and IL-6. The results showed that there was increased expression of HSP90 in the SLE patients. The active SLE group exhibited higher HSP90 levels (0.82±0.10) than the inactive SLE group (0.54±0.09) (P<0.01). The expression of HSP90 in normal control group (0.37±0.11) showed significant statistical difference as compared to both the inactive and active SLE groups (P<0.01, P<0.01, respectively). The plasma level of IL-6 exhibited a significant increase in both the inactive and active SLE groups (28.99±1.74 pg/mL vs. 44.58±9.15 pg/mL, respectively) compared with normal control group (P<0.01). The expression of HSP90 and IL-6 in SLE patients showed significant positive correlation with SLEDAI scoring (r=0.80, P<0.01; r=0.74, P<0.01, respectively). In addition, there was a positive correlation between the level of IL-6 and HSP90 in SLE patients (r=0.86, P<0.01). The increased expression of molecular chaperone HSP90 and IL-6 may play an important role in the pathogenesis of SLE by regulating autoimmunity.

Key words: systemic lupus erythematosus; heat shock protein 90; interleukin-6

DOI 10.1007/s 11596-006-0609-1

Heat shock proteins (HSPs) participate in protein-folding, subunit-formation, intracellular transportation and protein degradation as molecular chaperones by regulating the activities and functions of targeted proteins. HSPs also play an important role in the duplication and transcription of DNA, intracellular signal transduction and cytoskeleton function. HSPs take part in the regulation of immune function and have close correlation with autoimmune diseases[1]. HSP90 is an important member of HSPs family. Interleukin-6 (IL-6) is a kind of cytokine with multiple biological functions. Therefore, in this study we investigated the clinical implications of HSP90 in peripheral blood mononuclear cells (PBMCs) of the patients with systemic lupus erythematosus (SLE), and the level of IL-6 expression in the corresponding blood plasma by using Western blotting and ELISA.

1 MATERIALS AND METHODS

1.1 Patients and Normal Controls

The SLE group included 38 patients were selected from the patient pool of the Department of Rheumatology of Tongji Hospital of Huazhong University of Science and Technology, Wuha, China. The patients met the diagnostic criteria of SLE by the American Rheumatism Association (ARA, 1982). There were 4 males and 34 females. The patients’ age ranged from 12 to 47 years old and the average age was 30.5±8.6 years old. The patients were divided into two groups according to the SLE Disease Activity Index (SLEDAI)[2]. The inactive phase group contained 15 patients and the active phase group had 23 patients. The normal control group had 20 normal blood donors, including 5 males and 15 females. Their age ranged from 18 to 57 years old and the average age was 33.6±7.8 years old.

1.2 Agents and Antibodies

The materials used included lymphocyte isolation fluid (the Bioengineering Institute of Chinese Academy of Medical Sciences, China), rabbit anti–human HSP90 antibody (Biovision Co., USA), caprine anti-rabbit IgG antibody marked by the horse radish peroxidase (HRP) (Boster Bioengineering Co., China), Homo sapien IL-6 ELISA kit (Jinmei Bioengineering Co., China), total protein detection kit (BIO-Rad Co., USA), ECL lumination agent (Pierce Co., USA).

1.3 Collection of the Samples

Blood (5 mL) was drawn from a peripheral vein and was heparinized. After centrifugation for 3 min at 2000 r/min, 1 mL upper stratum of the blood plasma was collected and stored at −80 °C for the detection of IL-6. The rest of the blood samples was used for harvesting of PBMCs.

1.4 Extraction of PBMCs and Protein

The PBMCs were extracted by the gradient centrifugation according to the literature[3]. The concentration of the cells was adjusted to 1×10^6/mL by phos-
phate-buffered saline (PBS). Thirty μL degenerated buffer solution was added to each 10⁶ PBMC solution and mixed completely and was degenerated by boiling water for 10 min. The sample was cooled at ambient temperature and stored at −80 °C.

1.5 The HSP90 Detection by Western Blot

Equal amount of proteins were extracted from each group. The proteins were separated with the 5 % condensed gel and 8 % separated gel by the SDS-PAGE electrophoresis. The proteins were transferred onto the surface of the nitrocellulose membrane, which was then incubated with PBST buffer prepared with 5 % evaporated skim milk for 1 h at ambient temperature. Initially, the primary antibody, rabbit anti-human HSP90 (1:800), was added into the solution. The nitrocellulose membranes were then incubated overnight at 4 °C, and were washed with PBST buffer three times. Next, the secondary antibody, caprine anti-rabbit IgG (1:2000), marked by HRP was added to the solution. The nitrocellulose membranes were incubated for 1 h at 37 °C, which was followed by three PBST buffer washes. The nitrocellulose membranes were exposed and displayed by the enhanced chemiluminescence (ECL). Then the membranes were washed thoroughly with PBST buffer to terminate the reaction. Gel-Pro Analyzer software was used for semi-quantitative determination of protein bands of Western blot. The result was presented by the ratio of HSP90/β-actin.

1.6 Detection of IL-6

The level of the IL-6 in the blood plasma was detected by using an ELISA kit by strictly following the instructions of the kit.

1.7 Statistical Analysis

The data of each group were expressed as x±s and analyzed by the t-test and the linear correlation by using the SPSS12.0 software.

2 RESULT

2.1 Level of HSP90 in PBMCs

The expressions of HSP90 in the normal control, the inactive phase group and the active phase group of SLE were 0.37±0.11, 0.54±0.09 and 0.82±0.10 respectively. While the level of HSP90 expressed in both the inactive and active phase groups were higher than that of normal control (P<0.01, P<0.01), with the highest expression of HSP90 detected in active phase group. There was a significant difference between the active phase group and the inactive phase group (P<0.01). Fig. 1 shows the electropherograms of HSP90 expression in PBMCs of all three groups.

2.2 Level of IL-6 in Blood Plasma

The levels of IL-6 in the blood plasma of inactive phase group and active phase group of SLE were 28.99±1.74 pg/mL and 44.58±9.15 pg/mL respectively. Both inactive and active phase groups presented significant difference (P<0.01, P<0.01) when compared with the normal control (10.46±4.33 pg/mL). The level of IL-6 in the blood plasma of active phase group was highest and demonstrated the greatest difference when compared with the inactive phase group (P<0.01) (fig. 2).

![Fig. 2](image)

**Fig. 2** The level of IL-6 in blood plasma
1: Control; 2: Inactive phase of SLE; 3: Active phase of SLE

2.3 Correlation Analysis of HSP90, IL-6 and SLEDAI

The levels of both HSP90 and IL-6 of all 38 SLE patients had positive correlation with patients’ SLEDAI (r=0.80, P<0.01; r=0.74, P<0.01) respectively. Similarly, the level of HSP90 also had positive correlation with the level of IL-6 (r=0.86, P<0.01) in SLE patients.

3 DISCUSSION

HSPs is a protein that is abundantly expressed in a wide variety of prokaryotes and eukaryotes. HSPs play important roles in the cellular functions, including self-stabilization and self-protection. HSPs enhance the cellular resistance to damage, accelerate the degradation of paraproteins, maintain the normal cellular function and metabolism, and elevate the survival rate of the cells. As the HSPs from organisms are very similar to human’s, the molecular mimicry of HSPs is one of the important causes of autoimmune diseases. On one hand, human body can recognize the antigens of pathogens with antigen presenting cells (APCs) and T cells are activated. On the other hand, the human body produces sensitized T cells that can recognize self-components or HSPs molecules that form immuno-dominant antigens binding with the pathogens, and then the immune system may attack self-cells by recognizing common antigens between pathogens and host cells. The conservation of the protein structure in HSPs may produce cross-reactive antigen determinants that can lead to autoimmune diseases.

SLE is a common autoimmune disease, the etiology of which is related to with hereditary, infectious, endocrine and environmental factors. SLE affects the T and B lymphocytes of the immune system, inducing abnormal lymphocytic activation and generation of various auton-