Enhanced expression of the heat shock protein gene in peripheral blood mononuclear cells of patients with active systemic lupus erythematosus

Y Deguchi, S Kishimoto

Abstract
The spontaneous increase in the transcription of the heat shock protein (hsp 70) gene in peripheral blood mononuclear cells of patients with active systemic lupus erythematosus (SLE) is shown by nuclear run on transcription assay. The transcription of hsp 70 gene in the peripheral blood mononuclear cells of five patients with active SLE was more than 10 times greater than that in five normal healthy subjects or three patients with bronchial asthma as controls. This suggests that heat shock proteins may be produced during an active immune response in patients with active SLE and play a part in a change related to lupus of the essential intracellular functions of peripheral blood mononuclear cells.

Systemic autoimmune disorders, such as systemic lupus erythematosus (SLE), are characterised by immunological dysfunction, affecting skin, lung, heart, and muscle. Investigators have directed attention towards immunological abnormalities, and there are few data about the pathophysiology of intracellular molecules of peripheral blood mononuclear cells in autoimmune diseases.

Changes of gene expression in response to heat shock stress (raised temperature) have been described in animal cells. The general effect of heat shock stress is the suppression of protein synthesis, normally produced at a normal temperature, and enhancement and further synthesis of new proteins such as heat shock proteins (hsp). Schlesinger et al showed that various chemical, mechanical and environmental stresses could induce hsp. It has been suggested that these proteins might be important for cell survival under environmental or physiological stresses. The 70 kD hsp (hsp 70) family includes the inducible and cognate 68 to 72 kD hsp; hsp 70 is the most conserved and well characterised. It has been suggested recently that hsp 70s are also developmentally regulated and may have an important and essential role in cell proliferation and differentiation. The half life of RNA transcript for hsp 70 is short.

In this study we examined the transcriptional level of the hsp 70 gene in peripheral blood mononuclear cells of patients with active SLE by nuclear run on transcription assay.

Patients, materials, and methods
PATIENTS AND CONTROLS
We examined five patients (four female, one male) with active SLE. All were receiving prednisolone or azathioprine, or both, and all met the American Rheumatism Association criteria for SLE. The table gives clinical and laboratory data for these patients. We also examined three patients with bronchial asthma who had been receiving 20 mg or more of prednisolone daily for the treatment of asthmatic attacks, and five healthy volunteers who were laboratory or hospital personnel and had no history of use of any drugs known to affect immune functions.

PREPARATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS
Heparinised peripheral blood was diluted two-fold with RPMI 1640 medium (Bioproducts Inc, USA), layered on Ficoll-Paque, and centrifuged at 1800 rpm for 20 minutes. Peripheral blood mononuclear cells were collected from the interface and washed twice with RPMI 1640 medium. The cell population was over 97% viable (trypan blue exclusion).

NUCLEAR RUN ON TRANSCRIPTION ASSAY
To obtain nuclei cells were lysed in a solution containing 10 mM TRIS (pH 7·5), 2 mM MgCl₂, 3 mM CaCl₂, 5 mM dithiothreitol, and 0·02% nonidet P-40, with subsequent centrifugation through 2 M sucrose solution. Thirty million nuclei were suspended in 100 μl of

Clinical and laboratory data for the autoimmune patients in this study

<table>
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<th>Patient No</th>
<th>Sex</th>
<th>Clinical activity</th>
<th>Clinical manifestation</th>
<th>Autoantibodies</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ANF(μg)</td>
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<td>F</td>
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<td>Sk, R, N, H, F</td>
<td>210</td>
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<td>F</td>
<td>Very active</td>
<td>Sk, O, A, H, F</td>
<td>210</td>
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</table>

*Clinical activity was determined according to the UCH/Middlesex criteria.†
†A=arthritis; F=fever; H=haematological disorder; N=neurological disorder; O=oral ulcer; P=pulmonary disorder; R=renal disorder; Se=serositis; Sk=skin disorder.
‡Titre of antinuclear factor (ANF) is less than 2 in normal subjects.
§Anti-dsDNA (anti-double-stranded DNA) is less than 10 U/ml in normal subjects.
50% glycerol solution with 50 mM TRIS (pH 7.5), 5 mM MgCl₂, and 0·1 mM EDTA. The suspension of nuclei was immediately mixed with an equal volume of buffer containing 0·2 M KCl, 5 mM MgCl₂, 5 mM dithioretil, 1 mM ATP, CTP, GTP, and 200 units of RNasain (ribonuclease inhibitor, 500 units; Amersham International plc, England). The preparation was then incubated at 28°C for 20 minutes after addition of 1·85 MBq of ³²P radiolabelled UTP (111 GBq/ml; Amersham Inc). Sodium dodecyl sulphate (SDS) and EDTA solution were added to a final concentration of 1% and 5 mmol/l respectively, followed by treatment with protamine K (1 mg/ml) at 42°C for 30 minutes.

RNA was extracted with phenol and chloroform from the preparation and precipitated with ethanol. The pellet was resuspended in 3 ml of hybridisation buffer, which contained 50% formamide, 0·75 M NaCl, 0·5% SDS, 2 mM EDTA, 50 mM HEPES (N-2-hydroxyethyl-piperazine-N'2-ethanesulphonic acid) (pH 7·0), one tenth dilution of Denhardt’s solution, and denatured salmon sperm DNA (500 μg/ml). Finally, the preparation was applied to the nitrocellulose filter onto which the human hsp 70 cDNA probe (1·2 kb, Bam H1 fragment) or β actin probe (Wako Pure Chemical Industries, Japan) had been dotted. After 24 hours’ incubation the filter was washed three times in 0·1× SSC (0·15 M NaCl+0·015 M sodium citrate) and 0·1% of SDS at 60°C and appropriate solutions, dried, and exposed to x ray film with intensifying screen at -70°C. In some experiments the hybridised dots were excised from the filter and directly counted by betacounter.

**Figure 1**  Representative result of nuclear run on transcription analysis for hsp 70 (A) (48 hour exposure autoradiogram) and β actin gene (B) expression (12 hour exposure autoradiogram). Lanes 1 and 2 denote peripheral blood mononuclear cells of subjects with active systemic lupus erythematosus (cases 2 and 5), lane 3 denotes that of a patient with bronchial asthma, and lane 4 those of a normal healthy subject.

**Figure 2**  Measurement of hsp 70 gene transcription in peripheral blood mononuclear cells of patients with active systemic lupus erythematosus (S), patients with bronchial asthma (B), and normal healthy subjects (N). The relative amount (relative counts/10⁵ cells) of hsp 70 gene transcription was determined with a betacounter for the hybridised dots in the nuclear run on transcription assay. The mean values and standard deviations are shown.

STATISTICAL ANALYSIS
Statistical analysis of the data was by Student’s t test.

Results
We compared the transcriptional level of the hsp 70 gene in peripheral blood mononuclear cells of patients with active SLE with that in patients with bronchial asthma and normal healthy subjects as controls. We used a nuclear run on transcription technique with human hsp 70 cDNA and a β actin probe. We first found a spontaneous increase of the transcriptional level of the hsp 70 gene in peripheral blood mononuclear cells of patients with active SLE (fig 1). The use of β actin probe provided good control as actin synthesis is unaffected by heat stress. We found no significant change in the transcriptional level of the human actin gene in them. We further measured the hybridisation signal by betacounter; fig 2 summarises the transcriptional level of the hsp 70 gene in peripheral blood mononuclear cells of patients with active SLE and control subjects. The transcriptional level of the hsp 70 gene in peripheral blood mononuclear cells of patients with active SLE was found to be more than 10 times greater than that of patients with bronchial asthma or that of normal healthy subjects.

Discussion
Various chemical, mechanical, and environmental stresses, including heat shock response, can induce the transcription of the hsp gene. Heat shock 70 kD and 85 kD (hsp 70 and hsp 85) are major components of human cells. In this study we have shown the spontaneous activation of hsp 70 gene transcription in peripheral blood mononuclear cells of patients with active SLE. In eukaryotes transcription of the heat shock protein gene has been most intensively investigated in drosophila. The activation of hsp gene transcription is mediated by specific sequence elements in the heat shock promoters. Deletion analyses showed that 20 base pairs located about 20 nucleotides upstream from the 5’ portion to the TATA box were essential for induction of the hsp 70 gene in drosophila. The sequences were recognised by multimeric DNA binding proteins. It is not clear how the spontaneous activation of transcription of the hsp 70 gene in peripheral blood mononuclear cells of patients with active SLE is regulated by the similar DNA binding proteins. As hspS are not well known to participate in peripheral blood mononuclear cell functions it is possible that they play an essential part in the intracellular mechanisms of peripheral blood mononuclear cell functions. Hsps are also developmentally regulated and...
Increased transcription of heat shock protein gene in SLE

play a part in cell differentiation and proliferation. Interleukin-2 and mitogens increase hsp 70 mRNA in lymphocytes. It has been shown that hsp70 indeed participate in inflammation—for example, in severe osteoarthritis. We also reported that at protein level the amount of some hsp70 increased in peripheral blood mononuclear cells from patients with SLE. As a result of the present study enhanced transcription of the hsp70 gene is clearly a possible mechanism for the increased amount of hsp products at protein level in peripheral blood mononuclear cells, of patients with active SLE. An understanding of the intracellular events in the peripheral blood mononuclear cells of patients with active SLE involved in hsp70 gene activation, and of the role of hsp products themselves, may provide some new approaches to determining the pathophysiology of chronic inflammation processes such as SLE. The essential significance of the spontaneous activation of the hsp70 gene in the peripheral blood mononuclear cells of patients with active SLE is in progress.

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