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 (hsps). 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 hsps; hsp 70 is the most conserved and well characterised. It has been suggested recently that hsps 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 (Bioproduct 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 MgCl, 3 mM CaCl, 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>
<thead>
<tr>
<th>Patient No</th>
<th>Sex</th>
<th>Clinical activity*</th>
<th>Clinical manifestation†</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ANFs</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>Very active</td>
<td>Sk, R, N, H, F</td>
<td>2(^{18})</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>Active</td>
<td>Se, R, A, F</td>
<td>2(^{19})</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Active</td>
<td>Sk, O, A, H, F</td>
<td>2(^{20})</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Very active</td>
<td>Sk, O, A, H, F</td>
<td>2(^{21})</td>
</tr>
</tbody>
</table>

*Clinical activity was determined according to the UCH/Middlesex criteria.
†A=arthritis; F=fever high; H=haematological disorder; N=neurological disorder; O=oral ulcer; P=pulmonary disorder; R=renal disorder; Se=serositis; Sk=skin disorder.
‡Titer 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.
with patient

We compared with patients hsp I Figure

filter nitrocellulose and

Results can induce the (cases erythematosus) healthy subject. Hsps are also

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

1 2 3 4

A

B

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 those of a patient with bronchial asthma, and lane 4 those of a normal healthy subject.

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.

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 dithiothreitol, 1 mM ATP, CTP, GTP, and 200 units of RNAsin (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 mM/l respectively, followed by treatment with proteinase 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-hydroxyethylpiperazine-N'2-ethanesulphonic acid)(pH 7.0), one tenth dilution of Denhard'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 x 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 2. Measurement of hsp 70 gene transcription in peripheral blood mononuclear cells of patients with active systemic lupus erythematosus (SLE), patients with bronchial asthma (B), and normal healthy subjects (N). The relative amount (relative counts/10^5 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.
Increased transcription of heat shock protein gene in SLE

We thank Dr Ariga (Hokkaido University) for the generous gift of the human hsp 70 cDNA probe. This study was supported in part by grants from the Ministry of Culture and Education, Science, Japan.

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

Y Deguchi and S Kishimoto

doi: 10.1136/ard.49.11.893

Updated information and services can be found at:
http://ard.bmj.com/content/49/11/893

Email alerting service

These include:
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/