Enhancement of c-sis proto-oncogene transcription in bronchoalveolar mononuclear cells from patients with pulmonary sarcoidosis

Y Deguchi, S Kishimoto

Abstract
The expression of c-sis proto-oncogene in bronchoalveolar mononuclear cells was studied in seven patients with pulmonary sarcoidosis. By means of nuclear run on transcription assay, the transcriptional level of c-sis proto-oncogene in bronchoalveolar mononuclear cells was investigated. Expression of c-sis proto-oncogene in bronchoalveolar mononuclear cells was enhanced. Enhancement of c-sis transcription may be involved in the process of activation of bronchoalveolar mononuclear cells in patients with pulmonary sarcoidosis.

Sarcoidosis is a chronic disorder affecting many organs. It is characterised by the accumulation of mononuclear cells and non-caseating epitheliod granulomas. Associated lung disease is common. Investigators have directed attention toward immunological abnormalities.1 There are few data, however, concerning the pathophysiology of pulmonary sarcoidosis. C-sis oncogene, the cellular counterpart of the transforming gene, v-sis, of simian sarcoma virus, is homologous to platelet derived growth factor (PDGF) β chain gene.2 The human c-sis gene was mapped in the long arm of chromosome 22 and is transcribed in several human tumour cell lines3,4 as well as in some normal cell types such as activated macrophages.5

In this study we examined the transcription of c-sis gene in bronchoalveolar mononuclear cells from patients with pulmonary sarcoidosis.

Methods
Seven patients with pulmonary sarcoidosis were investigated. Pulmonary sarcoidosis was diagnosed on clinical grounds and from the biopsy findings of the lesions.1 All of the subjects were examined at our clinic. We also examined five healthy volunteers who were laboratory or hospital staff who had taken drugs known to affect immune function.

Bronchoalveolar lavage (BAL) cells were obtained from patients and controls. Bronchoalveolar mononuclear cells were obtained with Ficoll-Paque method: the cells were suspended in RPMI-1640 medium (Bioproducts Inc., USA), layered on Ficoll-Paque, and centrifuged at 1800 rpm for 20 minutes. Bronchoalveolar mononuclear cells were collected from the interface and washed twice with RPMI-1640 medium. The cell population was over 97%, viable (trypan blue exclusion).

Nuclei were prepared by lysing the cells in the solution containing 10 mM TRIS (pH 7.5), 2 mM MgCl₂, 3 mM CaCl₂, 5 mM DTT and 0.02% of NP40, with subsequent centrifugation through 2 M sucrose solution. Three million nuclei were suspended into 100 μl of 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 (DTT), 1 mM of adenosine triphosphate (ATP), cytosine (CTP), (GTP) and 200 units of RNasin (ribonuclease inhibitor, 500 units, Amersham International plc, Buckinghamshire, England). The preparation was then incubated at 28°C for 20 minutes after 50 μCi of 3²P-labelled UTP (3000 mCi/ml, Amersham Inc.) had been added. Sodium dodecyl sulphate and EDTA solution were added to a final concentration of 1%, and 5 mM, 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 into 3 ml of hybridisation buffer which contained 50% of formamide, 0.75 M NaCl, 0.5% sodium dodecyl sulphate, 2 mM of EDTA, 50 mM of HEPES (pH 7.0), one tenth dilution of Denhardt's solution and denatured salmon sperm DNA (500 μg/ml).6 Finally, the preparation was applied to the nitrocellulose filter on to which the v-sis probe (Oncor Inc, USA) or β actin probe (Wako Pure Chemical Industries, Ltd, Japan) had been dotted. After 24 hours of incubation the filter was washed three times in 0.2 × SSC and 0.1% of sodium dodecyl sulphate at 45°C, dried, and exposed to x-ray film with intensifying screen at −70°C. In all the experiments, the hybridised dot was excised from the filter and directly counted by β counter.7

RNA Preparaton and Northern Blot Assay
Total RNA from the cells was prepared by the guanidinium–thiocyanate and cesium chloride procedure, denatured in glyoxal, and electrophoresed in 1.4% agarose gels (20 μg a lane).8 RNA was transferred to nylon membranes (Biodyne, Pall Inc, Boston, USA). The filters were hybridised in a solution including nick-translated ²P-labelled v-sis (Oncor Inc, Gaithersburg, Maryland, USA) or actin probe

Third Department of Internal Medicine, Osaka University School of Medicine, Fukushima-ku, Osaka 553, Japan
Y Deguchi
S Kishimoto

Correspondence to: Yasuhiro Deguchi, LJH, NIAID, USA, 10687 Weymouth Street #103, Bethesda, Maryland 20814, USA
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patients with pulmonary sarcoïdosis compared with those of healthy control subjects (fig 3).

**Discussion**
In this study we examined the expression of c-sis proto-oncogene in bronchoalveolar mononuclear cells from patients with pulmonary sarcoïdosis. To examine the transcription of c-sis proto-oncogene in bronchoalveolar mononuclear cells we used the nuclear run on transcription assay. As one of the platelet derived growth factor (PDGF) chains (PDGF-β chains, PDGF-2) is encoded by the c-sis proto-oncogene on chromosome 22, bronchoalveolar mononuclear cell activation is associated with c-sis gene expression.́ Recently enhanced expression of c-sis mRNA transcripts was detected in human malignancies.́ Cultured human and bovine endothelial cells express the c-sis transcripts.́ In this study we first showed that the expression of c-sis in bronchoalveolar mononuclear cells from patients with pulmonary sarcoïdosis is enhanced compared with that of healthy controls. Intracellular signal transduction systems to express c-sis transcription might be activated in bronchoalveolar mononuclear cells from patients with pulmonary sarcoïdosis. We also analysed DNA amplification and gross translocation of c-sis proto-oncogene in Southern blot assay. We found neither gene amplification

<table>
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<tr>
<th>c-sis gene</th>
<th>912 (1647)</th>
<th>259 (89)</th>
</tr>
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<tbody>
<tr>
<td>β actin gene</td>
<td>11 490 (2250)</td>
<td>12 301 (1995)</td>
</tr>
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**Table 2 Summary of Northern blot assay for c-sis and β actin expression**

<table>
<thead>
<tr>
<th>c-sis gene</th>
<th>308 (1455)</th>
<th>208 (97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β actin gene</td>
<td>611 (1011)</td>
<td>690 (1226)</td>
</tr>
</tbody>
</table>

**Figure 1** Representative result of nuclear run on transcription assay for c-sis and β actin expression. Lane 1: patient with sarcoïdosis; lane 2: healthy subject.

**Figure 2** Northern blot assay for c-sis (36 hour exposure of autoradiography) and β actin expression (12 hour exposure of autoradiography). Lane 1: healthy subject; lanes 2-8: patients with sarcoïdosis.
c-sis expression in pulmonary sarcoidosis

Figure 3 Western blot assay of bronchoalveolar mononuclear cell lysates for c-sis product (P). Lanes 1, 2, 4-8: patients with sarcoidosis; lanes 3 and 9: healthy subjects.

tion nor gross translocation of c-sis gene in this study (data not shown). It has been recently reported that there are many activation steps for signal transduction systems in mononuclear cells from patients with pulmonary sarcoidosis. Our observation supports the theory that the enhancement of c-sis expression is involved in the activation of bronchoalveolar mononuclear cells from patients with pulmonary sarcoidosis and gives some hints about the understanding of the pathogenesis of chronic inflammatory disorders in pulmonary sarcoidosis.

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