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

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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 epithelioid 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 similar 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 lines^{3,4} 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 $MgCl_2$, 3 mM $CaCl_2$, 5 mM DTT and 0.02% of NP40, with subsequent centrifugation through 2 M sucrose solution. Three million nuclei were suspended into 100 μ l of 50% glycerol solution with 50 mM TRIS (pH 7.5), 5 mM $MgCl_2$, 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 $MgCl_2$, 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 uCi of ³²P-radiolabelled 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 μ g/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 $\times$ 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 PREPARATION 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 μ g a lane).⁸ RNA was transferred to nylon membranes (Biodyne, Pall Inc, Boston, USA). The filters were hybridised⁹ in a solution including nick-translated ³²P-labelled v-sis (Oncor Inc, Gaithersburg, Maryland, USA) or actin probe

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Accepted for publication 8 November 1989

Table 1 Summary of nuclear run on transcription analysis for *c-sis* and β actin gene expression

	Mean (SD) hybridisation counts (cpm)	
	Patients with sarcoidosis	Healthy subjects
<i>c-sis</i> gene	9122 (1647)	259 (89)
β actin gene	11 490 (2250)	12 301 (1995)

(Wako Pure Chemical Industries, Ltd, Japan) (specific activity, 1.5×10^8 cpm/mg). The final washes were done under conditions with $0.2 \times \text{SSC}$ at 45°C for one hour before autoradiography. In all experiments the hybridised parts of the filters were excised and directly counted by β counter.¹⁰

WESTERN BLOT ASSAY

Bronchoalveolar cell lysates were prepared and loaded on to sodium dodecyl sulphate-polyacrylamide gels under non-reducing conditions. Samples were loaded on to each lane at an equivalent 100 μg of protein from each sample. Electrophoresis to nitrocellulose was performed by the method of Burnette.¹¹ Enzyme linked immunoassays were performed by a method of Bio-Rad with rabbit anti-human platelet derived growth factor B chains (Genzyme, Boston, USA) and goat anti-rabbit IgG-alkaline phosphatase conjugate (Bio-Rad, San Francisco, California, USA). Enzymatic development was done by standard procedures for each reaction.¹¹

Results

Table 1 and fig 1 show that the transcription of *c-sis* proto-oncogene in bronchoalveolar mononuclear cells from patients with pulmonary sarcoidosis is enhanced by nuclear run on transcription assay. In contrast, the use of a probe for the β actin gene showed that both bronchoalveolar mononuclear cells from patients with pulmonary sarcoidosis and healthy subjects expressed similar levels of the actin gene transcription. The normal size *c-sis* mRNA (4.2 kilobase pairs) increased in bronchoalveolar mononuclear cells from the patients with pulmonary sarcoidosis by Northern blot assay (table 2, fig 2). We further tried to assess the *c-sis* (platelet derived growth factor β chains) expression at the protein level. Western blot assay with anti-human platelet derived growth factor β chain antibody show that the amount of this protein increases in the bronchoalveolar mononuclear cells from

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

Table 2 Summary of Northern blot assay for *c-sis* and β actin expression

	Mean (SD) hybridisation counts (cpm)	
	Patients with sarcoidosis	Healthy subjects
<i>c-sis</i> gene	3088 (1455)	208 (97)
β actin gene	6114 (1011)	6905 (1226)

patients with pulmonary sarcoidosis 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 sarcoidosis. 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.² Recently enhanced expression of *c-sis* mRNA transcripts was detected in human malignancies.⁴ Cultured human and bovine endothelial cells express the *c-sis* transcripts.⁵ In this study we first showed that the expression of *c-sis* in bronchoalveolar mononuclear cells from patients with pulmonary sarcoidosis 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 sarcoidosis. We also analysed DNA amplification and gross translocation of *c-sis* proto-oncogene in Southern blot assay. We found neither gene amplifica-

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 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.¹ 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.

We thank Drs T Tachibana, T Ohkubo, and P Cullen for their valuable advice and comments. This study was supported in part by

grants from the Ministry of Culture and Education and Science in Japan.

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