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Original Article

Detection of Anti-Topoisomerase I Autoantibody in Patients with Silicosis

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Abstract

Objectives: The aim of this study was to detect anti-topoisomerase l (anti-topo I) autoantibodies, which are known to be limited in systemic sclerosis patients, in silicosis patients with no clinical symptoms of autoimmune disease.

Methods: Serum anti-topo I autoantibodies were detected using ELISA. Differences in clinical parameters between patients with and without anti-topo I autoantibodies were analyzed.

Results: Seven of 69 patients had anti-topo I autoantibodies. These 7 patients showed elevated $PaCO_2$ values (P=0.0212), and inverse correlations between serum soluble Fas levels and $PaCO_2$ values were found.

Conclusion: Anti-topo I autoantibodies were detected in 10.1% of silicosis patients without any clinical symptoms of autoimmune disease. The findings here suggest that the genesis of anti-topo l autoantibodies might be related to pulmonary involvement or lung fibrosis associated with progression of silicosis.

Key words: silicosis, topoisomerase I, autoantibody, autoimmunity, apoptosis

Introduction

Patients with silicosis are characterized not only by respiratory disorders, but also by various immunological abnormalities such as hypergammaglobulinemia, the appearance of antinuclear antibodies (ANA) and complications of autoimmune diseases including systemic sclerosis (SSc) and systemic lupus erythematosus (SLE)[1–3]. We investigated the mechanisms involved in the immunological disturbances found in silicosis, focusing on the Fas-mediated apoptotic pathway, because abnormalities in Fas and related molecules have been reported in human idiopathic autoimmune diseases such as SLE and rheumatoid arthritis[4–6]. We found that serum soluble Fas (sFas) levels were elevated in silicosis patients with no clinical symptoms of autoimmune disease[7], and that the sFas message was dominantly expressed in peripheral blood mononuclear cells (PBMC) derived from these patients[8]. Based on these investigations, we concluded that dysregulation of the Fas-mediated apoptotic pathway may play an important role in the pathogenesis of the immunological abnormalities found in patients with silicosis. Furthermore, we found that silica compounds act as superantigens to activate human T cells polyclonally *in vitro*[9] and cause activation-induced cell death in these cells[10].

Autoantibodies against DNA topoisomerase I (topo I) have been reported to be specific to SSc, and to identify a subset of patients with diffuse cutaneous involvement, pulmonary interstitial fibrosis, and peripheral vascular disease[11–14]. However, anti-topo I autoantibodies have been detected in patients with silica-associated SSc[15] or silicone breast implants[16]. In this study, we analyzed sera for anti-topo I autoantibodies in silicosis patients with no clinical symptoms of autoimmune disease.

Materials and Methods

Patients and sera

Serum samples were obtained from 69 Japanese silicosis patients (62 males and 7 females, average age 68.3±6.9 years) with no clinical symptoms of autoimmune disease including sclerotic skin, Raynaud's phenomenon, facial erythema, arthralgia or malignant tumors. Specimens were taken only from patients who gave informed consent.

Detection of anti-topo I autoantibodies

Serum anti-topo I autoantibodies were detected using a commercially available ELISA kit (MBL, Nagoya, Japan) according to the manufacturer's instructions. Briefly, 1:201 diluted serum samples were incubated in microtiter plates coated with recombinant topo I at room temperature for 1 hr. After washing, peroxidase conjugated anti-human IgG and IgA mixture was added, and samples were incubated for 1 hr. Then the wells were washed and treated with a peroxidase substrate mixture (TMB and H_2O_2) for

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30 min. The optical density at 450 nm was read using a microplate reader (Model 450, BIO-RAD, USA). Index values ≥10 were considered positive according to the criterion defined by the manufacturer.

Clinical parameters of silicosis

Statistical differences between silicosis patients with and without anti-topo I autoantibodies were analyzed in terms of multiple clinical parameters for respiratory disorders and immunological abnormalities. The parameters used were as follows:

A. Pulmonary parameters: (i) duration of silica exposure (years); (ii) radiological grades (PR, profusion rate according to the 1980 ILO international classifications of radiographs of pneumoconioses); (iii) subjective dyspnoea; (iv) PaO_2 (torr); (v) $PaCO_2$ (torr); (vi) A-aDO_2 (torr); (vii) vital capacity (VC) (l); (viii) percent VC (%); (ix) forced expiratory volume in one second ($FEV_{1.0}$) (l); (x) $FEV_{1.0}$% (%); (xi) 25% minute volume/height ($\dot{V}_{25}$/H) (l/s); (xii) peak flow rate (l/s)

B. Immunological parameters: (i) titer of ANA; (ii) serum IgG levels (mg/dl); (iii) membrane Fas (mFas) expression on the surface of peripheral blood lymphocytes (%); (iv) serum soluble Fas (sFas) levels (ng/ml); (v) serum sFas ligand (sFasL) levels (ng/ml); (vi) soluble/membrane Fas mRNA expression ratios.

Details of materials and methods for B (i), (iii), (iv) and (v) and B (vi) were previously reported by Tomokuni et al.[7,17] and Otsuki et al.[8], respectively.

Statistical analysis

Values are expressed as the mean±S.D. Statistical differences between silicosis patients with and without anti-topo I autoantibodies (anti-topo I (+) and anti-topo I (–) groups) were calculated using the chi-square test for radiological classification, subjective dyspnoea and titer of ANA. Significance of differences between mean values was determined by Student's *t* test. In addition, Pearson's correlation coefficient was also used to examine correlations between serum sFas levels and $PaCO_2$ values, or $FEV_{1.0}$% in the anti-topo I (+) group or (–) group, and deviations were examined by Fisher's Z-transformation. $P<0.05$ was considered significant.

Results

Anti-topo I autoantibodies in silicosis patients

Seven of 69 patients with silicosis (10.1%) had anti-topo I autoantibodies detected by ELISA. Patients' characteristics in the anti-topo I (+) group and (–) group are shown in Table 1.

Table 1 Characteristics of silicosis patients

	Total	Group	
		Anti-topo I Ab* (+)	Anti-topo I Ab (–)
Number	69	7	62
Age (years)	68.3±6.9	67.7±7.0	68.5±7.0
Gender (male : female)	62 : 7	7 : 0	55 : 7
Duration of silica exposure (years)	27.7±8.7	30.1±6.8	27.4±8.9

Values are the mean±S.D.

* Anti-Topo I Ab=Anti-topoisomerase I autoantibody

Statistical differences in multiple clinical parameters between the anti-topo I (+) and (–) groups in silicosis patients

As shown in Table 2, there were no significant differences in radiological classification or subjective dyspnoea between anti-topo I (+) and (–) groups of silicosis patients. Among the parameters of respiratory function, only the $PaCO_2$ values in the anti-topo I (+) group were significantly higher (42.9±12.8 torr, P=0.0212) than those in the anti-topo I (–) group (37.1±4.9 torr). In addition, the anti-topo I (+) group tended to show a lower $FEV_{1.0}$% (58.2±14.3%) than the anti-topo I (–) group (66.6±14.2%), although it was not significant (P=0.2107). These observations suggest that the anti-topo I (+) group had a tendency towards increased $PaCO_2$ values and reduced $FEV_{1.0}$%, which suggests that constrictive airway diseases or pulmonary fibrosis had occured in this group. However, in terms of immunological parameters of the silicosis patients, particularly in the Fas/Fas ligand pathway, there were no significant differences between the anti-topo I (+) and (–) groups. Thus, we examined the correlations between serum sFas levels and $PaCO_2$ values, or $FEV_{1.0}$% in each group.

Correlations between serum sFas levels and $PaCO_2$ values or $FEV_{1.0}$% in the anti-topo I (+) group or (–) group

As shown in Fig. 1a, silicosis patients in the (+) group showed a significant inverse correlation between serum sFas levels and $PaCO_2$ values (r=–0.763, P=0.0447), although patients in the (–) group did not (Fig. 1b). Furthermore, serum sFas levels indicated a tendency to correlate with $FEV_{1.0}$% in silicosis patients in the (+) group (r=0.828) even though the P value was 0.0949 (Fig. 1c), while there was no correlation in the (–) group (Fig. 1d).

Discussion

In this study, anti-topo I autoantibodies were detected in 10.1% of all silicosis patients with no clinical symptoms of autoimmune disease. Although anti-topo I autoantibodies have been specifically reported in patients with idiopathic SSc[11] and silica-associated SSc[15], anti-topo I autoantibodies were also detected in silicosis patients with no clinical manifestations of SSc. To clarify the association between clinical symptoms and anti-topo I autoantibodies in silicosis, we investigated the statistical differences in multiple clinical parameters between the anti-topo I (+) and (–) groups of silicosis patients.

We found that dysregulation of the Fas-mediated apoptotic pathway might play an important role in the pathogenesis of the immunological abnormalities found in silicosis patients[7,8,17–19]. Therefore, it is suggested that the genesis of anti-topo I autoantibodies is associated with the abnormalities of apoptosis-related molecules in silicosis patients. However, there were no significant differences in the immunological parameters such as serum sFas and sFasL between the anti-topo I (+) and (–) groups. However, the anti-topo I (+) group had a tendency towards increased $PaCO_2$ values and reduced $FEV_{1.0}$% compared with the anti-topo I (–) group. In addition, silicosis patients in the anti-topo I (+) group showed inverse correlations between the serum sFas levels and $PaCO_2$ values, although patients in the anti-topo I (–) group did not. Moreover, our previous studies[7,19] on dysregulation of the Fas/FasL pathway showed that the same series of patients with silicosis or SLE showed higher serum sFas levels and over-expres-

Table 2 Clinical features of silicosis patients

Clinical parameters	Group			
	Anti-topo I Ab (+)	Number	Anti-topo I Ab (–)	Number
Pulmonary parameters				
Radiological classification				
PR0		0		3
PR1		1		10
PR2		1		7
PR3		0		3
PR4		5		39
Subjective dyspnoea				
Slight		4		35
Moderate		0		18
Severe		3		9
Respiratory function				
PaO_2 (torr)	84.0±15.2	7	84.6±11.9	59
$PaCO_2$ (torr)	42.9±12.8*	7	37.1±4.9	59
A-aDO_2 (torr)	15.2±13.3	7	21.1±10.4	59
VC (l)	2.34±1.13	5	2.08±0.67	52
%VC (%)	67.3±25.2	5	68.1±19.3	53
$FEV_{1.0}$ (l)	1.54±0.96	5	1.42±0.58	52
$FEV_{1.0}$% (%)	58.2±14.3	5	66.6±14.2	53
$\dot{V}_{25}$/H (l/s)	0.25±0.13	5	0.31±0.16	53
Peak flow late (l/s)	3.19±2.78	5	2.99±1.70	47
Immunological parameters				
Titer of ANA				
<1:40		0		12
1:40 or 1:80		3		21
≥1:160		4		29
IgG (mg/dl)	1,653.3±492.4	6	1,459.3±500.9	59
mFas (%)	62.8±13.5	5	55.0±14.2	59
sFas (ng/ml)	2.46±0.64	7	2.54±0.72	62
sFasL (ng/ml)	0.15±0.09	7	0.16±0.07	62
s/m Fas ExR	1.52±0.99	6	1.58±0.59	62

Values are the mean±S.D. * P=0.0212.

Anti-Topo I Ab, anti-topoisomerase I autoantibody; PR, profusion rate; VC, vital capacity; $FEV_{1.0}$, forced expiratory volume in one second; $\dot{V}_{25}$/H, 25% minute volume/height; ANA, antinuclear antibody; mFas, membrane Fas; sFas, soluble Fas; sFasL, soluble Fas ligand; s/m Fas ExR, soluble/membrane Fas expression ratio.

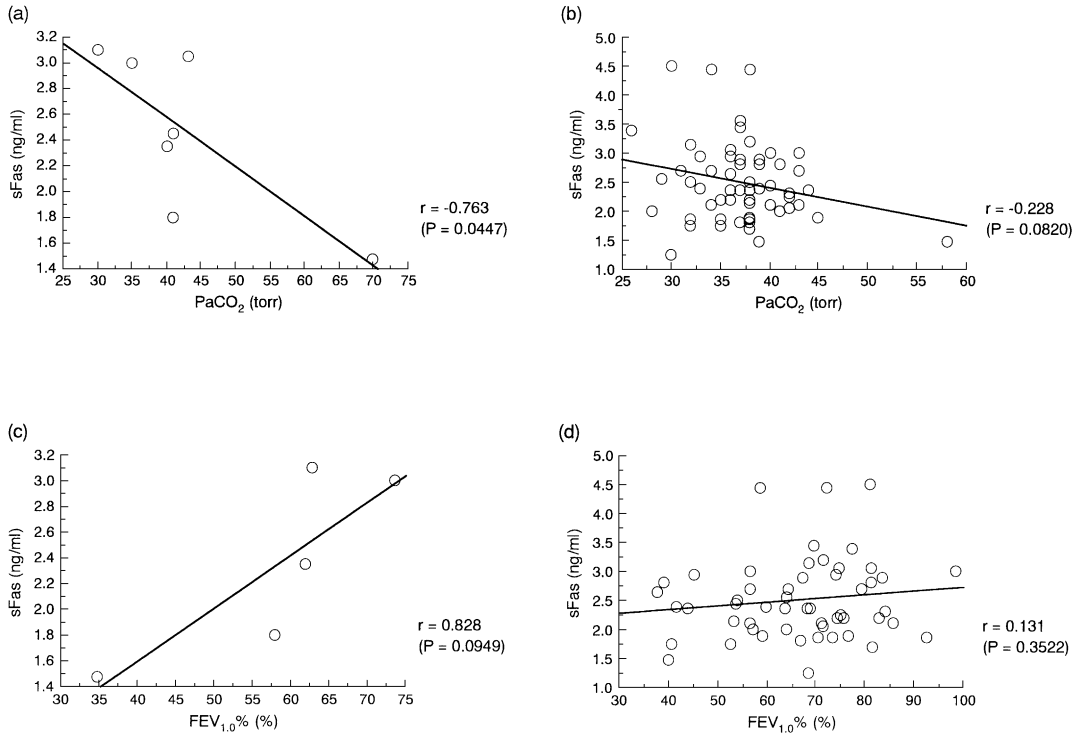

Fig. 1 Correlations between serum soluble Fas (sFas) levels and $PaCO_2$ values (a, b), or $FEV_{1.0}$% (c, d) in silicosis patients with anti-topo I autoantibodies (a, c) or without (b, d). r: Pearson's correlation coefficient.

sion of the decoy receptor 3 gene in peripheral blood mononuclear cells, while SSc patients did not. These findings suggest that the genesis of anti-topo I autoantibodies is related to pulmonary involvement or lung fibrosis associated with progression of silicosis, and that it may have little association with dysregulation of the Fas-mediated apoptotic pathway.

Based on the findings of the present study and those of the recent study of the predominant genesis of anti-topo I autoantibodies in silica-associated SSc[15], silicosis patients with anti-topo I autoantibodies may have both lung fibrosis in silicosis and pulmonary fibrosis caused by SSc-like immunological abnormalities, even though they have no clinical symptoms of SSc. It remains to be clarified whether silicosis patients with anti-topo I autoantibodies show a tendency towards developing SSc, or whether other distinct factors are necessary to cause a progression of SSc. Future analyses of anti-topo I autoantibodies and molecules related to Fas-mediated apoptosis will be necessary when examining the parameters of workers exposed to silica dust.

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