Anti-p53 Autoantibody in Systemic Sclerosis: Association with Limited Cutaneous Systemic Sclerosis

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ABSTRACT. ***Objective.*** To determine the prevalence and clinical significance of anti-p53 antibody in patients with systemic sclerosis (SSc).

Methods. Anti-p53 antibody was examined by ELISA and immunoblotting. Findings were correlated with clinical features of disease and other autoantibodies and compared with other connective tissue diseases as well as normal controls. p53 activity to bind target DNA was evaluated by ELISA using a plate coated with oligonucleotide containing the consensus binding site for p53.

Results. IgG anti-p53 antibody levels were elevated in patients with SSc compared to patients with systemic lupus erythematosus ($n = 20$; $p < 0.05$), dermatomyositis ($n = 21$; $p < 0.005$), atopic dermatitis ($n = 17$; $p < 0.0005$), or bullous pemphigoid ($n = 10$; $p < 0.0005$) and normal controls ($n = 21$; $p < 0.0005$). Remarkably, anti-p53 antibody levels were higher in patients with limited cutaneous SSc (lSSc; $n = 30$) than those found in patients with diffuse cutaneous SSc (dSSc; $n = 40$; $p < 0.05$). IgG or IgM anti-p53 antibody levels did not correlate with the presence or levels of other autoantibodies. IgG anti-p53 antibody was associated with longer disease duration ($p < 0.05$) and decreased percentage vital capacity ($p < 0.05$), and correlated negatively with modified Rodnan total skin thickness score ($r = –0.352$, $p < 0.01$). Immunoblotting analysis confirmed the presence of IgG anti-p53 antibody in selected patients with SSc. IgG isolated from sera of selected patients with SSc that contained IgG anti-p53 antibody inhibited the p53 activity relative to normal controls.

Conclusion. IgG anti-p53 antibody was detected in lSSc and dSSc, and was more prominent in lSSc, indicating that IgG anti-p53 antibody is a novel autoantibody associated with lSSc, a milder form of SSc. (First Release Jan 15 2008; J Rheumatol 2008;35:451–7)

Key Indexing Terms:
AUTOANTIBODY
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P53

Systemic sclerosis (SSc) is a multi-organ disorder of connective tissue characterized by excessive fibrosis in the skin and various internal organs. Systemic autoimmunity is one of the central features of SSc, because antinuclear antibodies are positive in over 90% of patients[1]. These autoantibodies react to various intracellular components that are generally critical for cellular function, especially cell mitosis[1]. The autoantibodies associated with SSc include anti-DNA topoisomerase I, anticentromere (ACA), anti-RNA polymerases, anti-U3RNP, and anti-Th/To antibody[1]. Although the pathogenic role of autoantibodies in SSc remains unknown, a relationship between some autoantibodies and ongoing tissue damage has been suggested: anti-topoisomerase I antibody levels correlate closely with disease activity and severity in SSc[2].

The p53 tumor suppressor protein, a nuclear transcription factor, functions by inhibiting cellular proliferation in response to a variety of intrinsic and extrinsic stresses, including DNA damage, hypoxia, and activated oncogenes[3]. p53 protein is activated in a specific manner by posttranslational modifications, which leads to either cell-cycle arrest/DNA repair or cellular apoptosis[3]. Lack of p53 activity results in proliferation and survival of autoreactive lymphocytes as well as tumor cells that would otherwise be eliminated[4]. Mutant p53 protein with a prolonged half-life is associated with accumulation of nonfunctional p53 protein, which leads to the generation of autoantibodies against the mutant p53 protein in certain human malignancies[5]. Such autoantibodies are generated in 30%–50% of patients with mutant p53 in their tumor[6,7].

Anti-p53 antibodies have also been detected in various autoimmune disorders, such as SSc[8,9], systemic lupus erythematosus (SLE)[8-11], type I diabetes[12], autoimmune thyroid dis-

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eases[10,13], and autoimmune hepatitis[14], but are rarely detected in rheumatoid arthritis and Sjögren's syndrome[15]. In SLE, anti-p53 antibody is generated even if there are no p53 mutations in T lymphocytes, suggesting that the development of anti-p53 antibody is not the result of mutations in the T cell-derived p53 gene[9]. A previous study has shown that anti-p53 antibody is also detected in 78% of patients with SSc[8,9]. However, its clinical correlation remained unknown in SSc. We assessed the frequency or levels of anti-p53 antibodies and their clinical correlation in Japanese patients with SSc.

MATERIALS AND METHODS

Serum samples of patients with SSc. Serum samples were obtained from 70 Japanese patients with SSc (61 women, 9 men). All patients fulfilled the criteria proposed by the American College of Rheumatology[16]. These patients were grouped according to the classification system proposed by LeRoy, *et al*[17]: 30 patients (28 women, 2 men) had limited cutaneous SSc (lSSc) and 40 patients (33 women, 7 men) had diffuse cutaneous SSc (dSSc). We classified patients with skin sclerosis limited to hands, face, feet, and forearms as lSSc even if they had anti-topoisomerase I antibodies. Inversely, patients with skin sclerosis on upper arms and trunk were classified as dSSc even if they had ACA. According to the criteria, 6 of 30 patients with lSSc had anti-topoisomerase I antibodies while 2 of 40 patients with dSSc had ACA. The age of patients with SSc [mean ± standard deviation (SD)] was 46 ± 17 years. Patients with dSSc were 46 ± 17 years old while those with lSSc were 45 ± 16 years old. Antinuclear antibody was determined by indirect immunofluorescence using HEp-2 cells (Medical & Biological Laboratories, Nagoya, Japan) as the substrate, and specificities of autoantibody were further assessed by ELISA and immunoprecipitation. Specifically, ACA was determined by the presence of the discrete speckled pattern with indirect immunofluorescence and was confirmed by ELISA using human recombinant CENP-B as antigen (Medical & Biological Laboratories). Anti-topoisomerase I antibody was determined by ELISA using human recombinant topoisomerase I as antigen (Medical & Biological Laboratories) as described[2] and was further confirmed by immunoprecipitation when the titer was low by ELISA. Anti-Th/To antibody was detected by RNA immunoprecipitation as described[18]. The disease duration of patients with lSSc and dSSc was 8.3 ± 9.3 and 3.0 ± 2.9 years, respectively. None of the patients with SSc were treated with oral corticosteroids, D-penicillamine, disease modifying antirheumatic drugs, or other immunosuppressive therapy.

Serum samples of control subjects. In our study, 20 patients with SLE (3 men and 17 women; age 36 ± 12 yrs), 21 patients with dermatomyositis (DM; 3 men, 18 women; age 42 ± 15 yrs), 17 patients with atopic dermatitis (AD; 10 men, 7 women; age 24 ± 5 yrs), and 10 patients with bullous pemphigoid (BP; 3 men, 7 women; age 52 ± 8 yrs) were also examined as disease controls. SLE and DM were diagnosed according to the criteria proposed by the American College of Rheumatology[19] and the criteria proposed by Bohan and Peter[20,21], respectively. Twenty-one age- and sex-matched healthy Japanese persons (15 women, 6 men; age 45 ± 12 yrs) were used as normal controls. Patients with malignancies were excluded from our study. Fresh venous blood samples were centrifuged shortly after clot formation. All samples were stored at –70°C prior to use.

Clinical assessment. Complete medical histories, examinations, and laboratory tests were conducted for all patients. Skin score was measured by the modified Rodnan total skin thickness score (modified Rodnan TSS) as described[22]: the anatomical areas were rated as 0 (normal skin thickness), 1+ (mild but definite skin thickening), 2+ (moderate skin thickening), and 3+ (severe skin thickening), and the modified Rodnan TSS was derived by summation of the scores from all 17 areas (range 0–51). Organ system involvement was defined as described[23]: lung = bibasilar fibrosis on chest radiography and high resolution computed tomography; isolated pulmonary hypertension = clinical evidence of pulmonary hypertension and increased systolic pulmonary arterial pressure (> 35 mm Hg) by Doppler echocardiography, in the absence of severe pulmonary interstitial fibrosis; esophagus = hypomotility shown by barium radiography; joint = inflammatory polyarthralgias or arthritis; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment; kidney = malignant hypertension and rapidly progressive renal failure without any other explanation; and muscle = proximal muscle weakness and elevated serum creatine kinase. Pulmonary function tests, including vital capacity (VC) and diffusion capacity for carbon monoxide (DLCO), were also performed. When the DLCO and VC were < 75% and < 80%, respectively, of the predicted normal values, they were considered to be abnormal. The protocol was approved by Kanazawa University Graduate School of Medical Science and Kanazawa University Hospital, and informed consent was obtained from all patients.

ELISA for anti-p53 antibody. Anti-p53 antibody levels were measured with specific ELISA using recombinant human p53 wild-type protein (Spring Bioscience, Fremont, CA, USA). Briefly, 96-well plates (EIA/RIA plate; Costar, Cambridge, MA, USA) were coated with 1 µg/ml of recombinant human p53 wild-type protein at 4°C overnight. The wells were blocked with 2% bovine serum albumin and 1% gelatin in Tris-buffered saline for 1 h at 37°C and the serum samples (100 µl) diluted to 1:100 were added to triplicate wells for 90 min at 37°C. As a positive control, mouse monoclonal antibody (PAb1801) to p53 (Abcam, Cambridge, MA, USA) diluted to 1:20 was also added to the wells. After washing, the plates were incubated with alkaline phosphatase-conjugated goat anti-human IgG or IgM antibodies (Cappel, Durham, NC, USA) for 1 h at 20°C. Following washing, substrate solution containing 0.91 µg/µl p-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO, USA) in diethanolamine buffer (1 M diethanolamine, 0.5 M $MgCl_2$) was added and the optical density (OD) of the wells at 405 nm was subsequently determined.

Immunoblotting. To confirm the ELISA findings, the presence of anti-p53 antibody was evaluated by immunoblotting analysis. Recombinant human p53 wild-type protein (0.5 µg/lane; Spring Bioscience) was subjected to electrophoresis and electrotransferred to Hybond-P PVDF Membrane (Amersham Biosciences, Piscataway, NJ, USA). The Hybond-P sheets were cut into strips and incubated overnight with serum samples diluted 1:50. One of the strips was also incubated with PAb1801 diluted to 1:1000 as a positive control. The strips were then incubated for 1.5 h with alkaline phosphatase-conjugated goat anti-human IgG antibody (Cappel). Color was developed using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma-Aldrich).

p53 activity inhibition assay. IgG was purified from serum samples using magnetic beads coated with recombinant protein G covalently coupled to the surface (Dynal, Lake Success, NY, USA). Final IgG concentration was measured by spectrophotometer (Gene Quant II, Amersham). The p53 protein activity was quantified by a TransAM p53 transcription factor assay kit (Active Motif, Carlsbad, CA, USA), according to the manufacturer's protocol. Briefly, recombinant human p53 wild-type protein (5 µg) was incubated with purified serum IgG (60 µg) for 30 min at 37°C and was added to triplicate wells of a 96-well plate, to which an oligonucleotide containing the p53 consensus binding site was immobilized. Then the bound p53 was detected with addition of horseradish peroxidase-conjugated anti-p53 antibody and OD was measured by spectrophotometer (Gene Quant II). p53 protein untreated with purified IgG served as positive control.

Statistical analysis. Statistical analysis was performed using the Mann-Whitney U-test for determining the level of significance of differences between sample means, Fisher's exact probability test for comparison of frequencies, and Bonferroni's test for multiple comparisons. Spearman's rank correlation coefficient was used to examine the relationship between 2 continuous variables. A p value < 0.05 was considered statistically significant.

RESULTS

Anti-p53 autoantibody levels in SSc by ELISA. The presence and levels of anti-p53 autoantibodies in serum samples from patients and normal controls were assessed by ELISA (Figure

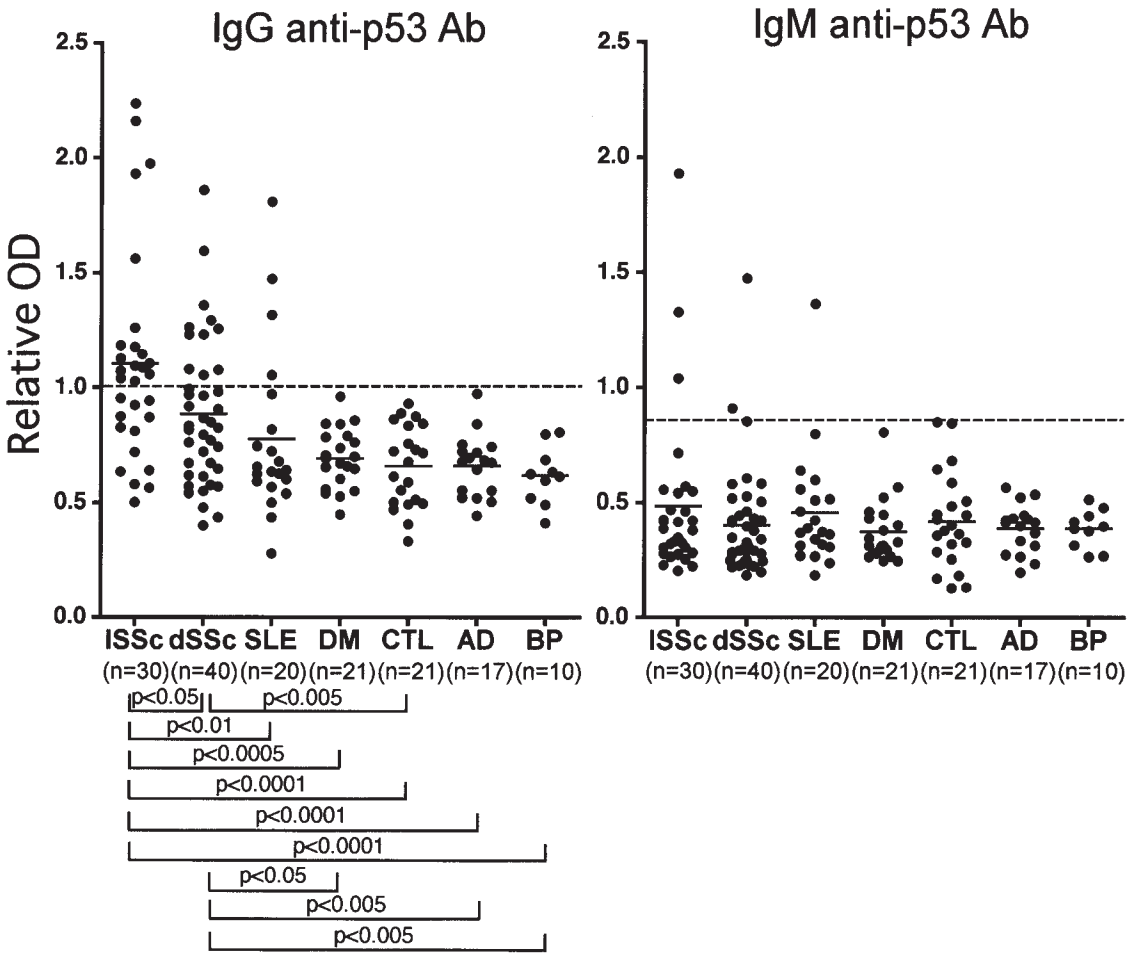

Figure 1. Serum levels of anti-p53 antibody in patients with dSSc, lSSc, SLE, DM, AD, or BP and normal controls (CTL). IgG or IgM anti-53 antibody levels were determined by ELISA using human recombinant wild-type p53 protein. Short bars indicate mean values in each group. Broken lines indicate cutoff values (mean + 2 SD of normal control samples).

1). IgG anti-p53 antibody levels in total patients with SSc were significantly higher than those in patients with SLE ($p < 0.05$), DM ($p < 0.005$), AD ($p < 0.0005$), and BP ($p < 0.0005$), and normal controls ($p < 0.0005$). By contrast, there was no significant difference in IgG anti-p53 antibody levels between patients with SLE, DM, AD, or BP and normal controls. Regarding the SSc subsets, patients with lSSc exhibited significantly elevated IgG anti-p53 antibody levels compared to patients with dSSc ($p < 0.05$) as well as those with SLE ($p < 0.01$), DM ($p < 0.0005$), AD ($p < 0.0001$), or BP ($p < 0.0001$), and normal controls ($p < 0.0001$). IgG anti-p53 antibody levels in patients with dSSc were significantly higher than those found in patients with DM ($p < 0.05$), AD ($p < 0.005$), or BP ($p < 0.005$), and normal controls ($p < 0.005$), but did not differ from those in patients with SLE. By contrast, IgM anti-p53 antibody levels in patients with SSc were similar to those in patients with SLE, DM, AD, or BP, and normal controls. IgG and IgM anti-p53 antibody levels did not correlate with serum levels of other autoantibodies, including antibody against topoisomerase I and centromere, which were determined by autoantibody-specific ELISA (Table 1). Serum samples from patients with SSc that contained high levels of anti-p53 antibody and mouse monoclonal antibody (PAb1801) to p53 showed similar OD values in the same plate (data not shown), indicating that our ELISA system was functioning. Thus, IgG anti-p53 antibody levels were increased in SSc, especially lSSc, but not in other collagen diseases including SLE and DM.

Frequency of anti-p53 antibody positivity and its clinical correlation in SSc. Absorbance values higher than the mean + 2 SD (1.010 for IgG anti-p53 antibody and 0.840 for IgM anti-p53 antibody) of the normal control samples were considered to be positive in our study (Figure 1). IgG anti-p53 antibody was found in 40% (28/70) of total patients with SSc. IgG anti-p53 antibody was detected in 57% (17/30) of patients with lSSc, while it was positive in only 28% (11/40) of patients with dSSc and 20% (4/20) of those with SLE. By contrast, IgG anti-p53 antibody was not detected in patients with DM, AD, or BP, and normal controls. IgM anti-p53 antibody was observed in only 10% (3/30) of patients with lSSc, 5% (2/40)

Table 1. Frequency of autoantibody positivity in patients with SSc.

Antibody	dSSc, n = 40 (%)	lSSc, n = 30 (%)	Total, n = 70 (%)
Anti-topoisomerase I	25 (36)	6 (20)	31 (44)
Anticentromere	4 (10)	22 (73)	26 (37)
Anti-U1RNP	2 (5)	1 (3)	3 (4)
Anti-RNA polymerases I and III	2 (5)	0 (0)	2 (3)
Anti-Th/To	0 (0)	1 (3)	1 (1)
IgG anti-p53	11 (28)	17 (57)	28 (40)
IgM anti-p53	3 (8)	2 (7)	5 (7)
Negative	2 (5)	0 (0)	2 (3)

dSSc: diffuse cutaneous systemic sclerosis; lSSc: limited cutaneous SSc.

of those with dSSc, and 5% (1/20) of those with SLE, whereas it was not detected in patients with DM, AD, or BP, and normal controls.

Regarding the prevalence of other autoantibodies in patients with SSc, ACA and IgG anti-p53 antibody were more frequently detected in patients with lSSc than in those with dSSc (Table 1). However, in a subset of patients with lSSc, IgG anti-p53 antibody positivity did not correlate with the presence of ACA: among patients with lSSc, the prevalence of anti-p53 antibody positivity was higher in patients without ACA (75%) relative to those with this antibody (41%). Thus, IgG anti-p53 antibody in patients with lSSc was present independently of ACA.

Then we assessed clinical correlation of anti-p53 antibody in patients with SSc (Table 2). SSc patients positive for IgG anti-p53 antibody exhibited significantly higher prevalence of lSSc relative to those who were negative (61% vs 31%; $p < 0.01$). Consistent with this finding, IgG anti-p53 antibody levels correlated negatively with modified Rodnan TSS ($r = -0.352$, $p < 0.01$; Figure 2). Further, SSc patients positive for IgG anti-p53 antibody had significantly longer disease duration (7 ± 7 vs 4 ± 5 yrs; $p < 0.05$) and lower frequency of decreased %VC compared to those who were negative (20% vs 43%; $p < 0.05$; Table 2). However, the presence of IgG anti-p53 antibody did not correlate with the presence of any other clinical measures, including ACA and anti-topoisomerase I antibody. Thus, IgG anti-p53 antibody was associated with lSSc and correlated negatively with the severity of skin and lung fibrosis.

Table 2. Clinical laboratory features of SSc patients with elevated serum IgG anti-p53 antibody levels.

	Elevated anti-p53, n = 28	Normal anti-p53, n = 42
Age of onset, yrs, mean ± SD	41 ± 16	49 ± 16
Sex, male:female	1:27	8:34
Duration, yrs, mean ± SD	7 ± 7*	4 ± 5
Disease subset		
dSSc	39**	69
lSSc	61**	31
Clinical features		
Pitting scars	29	45
Contracture of phalanges	39	50
Diffuse pigmentation	46	60
Organ involvement		
Lung	39	45
Decreased % VC	20*	43
Decreased % DLCO	65	75
Pulmonary hypertension	14	14
Esophagus	64	77
Heart	14	17
Kidney	17	23
Joint	18	21
Muscle	11	29
Autoantibodies		
Anti-topoisomerase I	43	45
Anticentromere	46	31

Values are percentages, unless otherwise indicated. * $p < 0.05$; ** $p < 0.01$ vs SSc patients with normal IgG anti-p53 antibody levels. dSSc: diffuse cutaneous systemic sclerosis; lSSc: limited cutaneous SSc.

Immunoblotting analysis for anti-p53 antibody. To confirm the ELISA findings that anti-p53 antibody levels were increased in SSc, immunoblotting analysis using human recombinant p53 protein was performed on selected patients positive for IgG anti-p53 antibody by ELISA. Ten SSc patients positive for IgG anti-p53 antibody by ELISA, 3 SSc patients positive for either anti-topoisomerase I or ACA, but not for IgG anti-p53 antibody by ELISA, 3 patients with SLE and 3 DM patients negative for IgG anti-p53 antibody by ELISA, and 5 healthy individuals were evaluated. PAb1801 reacted with p53 by immunoblotting (lane 2, Figure 3). Similarly, serum samples from the SSc patients positive for IgG anti-p53 antibody by ELISA exhibited reactivity with p53 (lanes 3–6, Figure 3). By contrast, serum samples from the SSc patients positive for anti-topoisomerase I or ACA, but not for IgG anti-p53 antibody by ELISA, did not react with p53 (lane 7 and data not shown). Similarly, serum samples from the SLE or DM patients negative for IgG anti-p53 antibody by

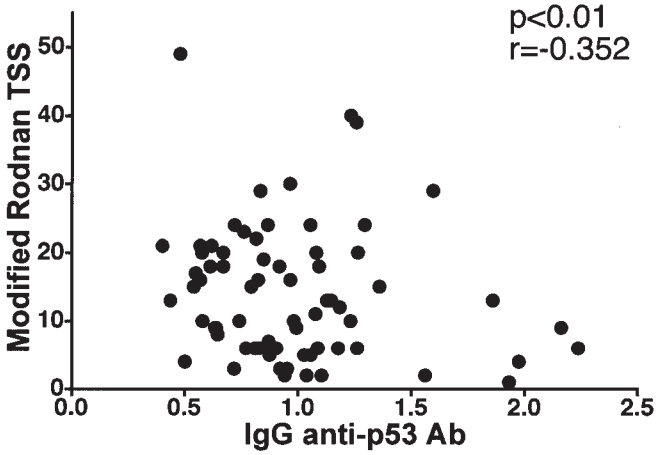

Figure 2. Correlation of IgG anti-p53 antibody levels against modified Rodnan TSS in patients with SSc. Anti-p53 antibody levels were determined by ELISA using human recombinant wild-type p53.

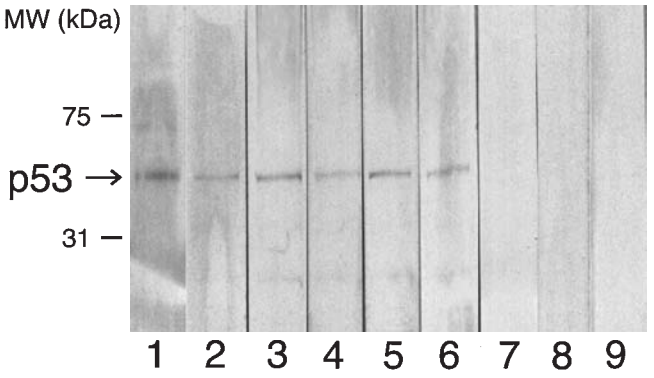

Figure 3. Representative immunoblotting of human recombinant p53 with sera from selected SSc patients positive for IgG anti-p53 antibody. Lane 1, colloidal gold-stained p53; lane 2, mouse monoclonal antibody (PAb1801) to p53; lanes 3–6, serum samples from patients with SSc positive for IgG anti-p53 antibody; lane 7, a serum sample from an SSc patient positive for anti-topoisomerase I antibody, but not for IgG anti-p53 antibody; lane 8, serum sample from SLE patient negative for IgG anti-p53 antibody; lane 9, serum sample from a healthy individual. Markers for molecular weights (kDa) shown to the left.

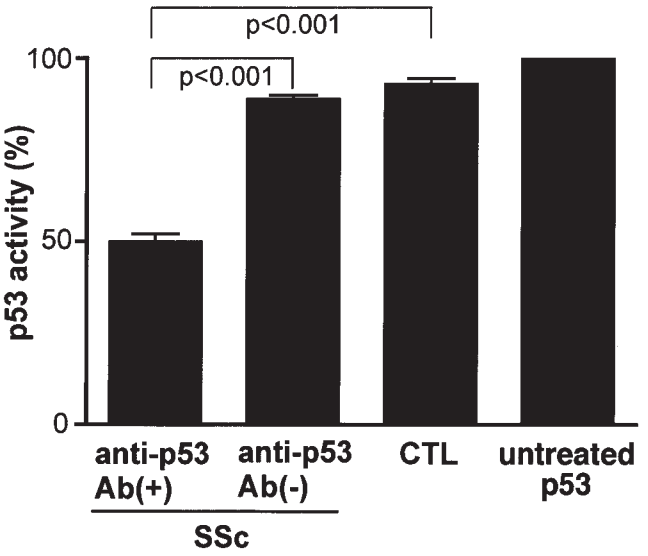

Figure 4. Inhibition of p53 activity by IgG isolated from serum samples that contained anti-p53 antibody. IgG was purified from serum samples of SSc patients positive and negative for IgG anti-p53 antibody and normal controls (CTL). Purified IgG was incubated with p53, and p53 activity was evaluated by binding to wells coated with oligonucleotide containing p53 consensus binding site. p53 activity shown as percentage of untreated p53, defined as 100%. Each histogram shows mean (± SD) results for 10 SSc patients positive for IgG anti-p53 antibody, 10 SSc patients negative for IgG anti-p53 antibody, and 5 healthy individuals.

ELISA did not react with p53 (lane 8 and data not shown). No reactivity with p53 was observed using serum samples from normal individuals (lane 9). Thus, the presence of anti-p53 antibody in patients with SSc was confirmed by immunoblotting analysis.

Inhibition of p53 activity by anti-p53 antibody. To determine the functional relevance of anti-p53 antibody, we assessed whether anti-p53 antibody was able to inhibit the p53 activity. The p53 activity was quantified by an ability of binding to wells coated with an oligonucleotide containing the p53 consensus binding site. Ten selected SSc patients positive for IgG anti-p53 antibody by ELISA, 10 selected SSc patients negative for IgG anti-p53 antibody by ELISA, and 5 healthy individuals were assessed. The p53 activity was not inhibited by purified IgG isolated from healthy individuals (Figure 4). By contrast, purified IgG isolated from serum samples of SSc patients positive for IgG anti-p53 antibody by ELISA significantly inhibited the p53 activity by 50% compared to normal controls ($p < 0.001$). Similarly, PAb1801 inhibited the p53 activity by 70% relative to normal controls. There was no significant correlation between anti-p53 antibody titers and the level of p53 inhibition (data not shown). The p53 activity was not inhibited by IgG isolated from serum samples that contained autoantibodies against topoisomerase I, centromere, or U1RNP, but not IgG anti-p53 antibody. Thus, IgG isolated from serum samples of patients with SSc that contained IgG anti-p53 antibodies was able to inhibit the p53 activity.

DISCUSSION

Our study confirmed that IgG anti-p53 antibody levels were significantly elevated in patients with SSc relative to normal controls. Ours is the first study to reveal that IgG anti-p53 antibody levels were significantly higher in patients with lSSc than in patients with dSSc. Consistent with this finding, IgG anti-p53 antibody levels correlated negatively with the severity of skin and lung fibrosis. The presence of IgG anti-p53 antibody in sera from patients with SSc was also confirmed by immunoblotting analysis. These results indicate that IgG anti-p53 antibody is associated with a milder form of SSc.

Anti-p53 antibody was reported to be detected in 78% of patients with SSc[8]. Comparing the previous study with our current study, the frequency of IgG anti-p53 antibody positivity was 40% in SSc, with higher frequency (57%) in lSSc than that in dSSc (28%). In addition, anti-p53 antibody was observed in 23%–80% of patients with SLE[8-11]. In comparison with this reported prevalence, the frequency of IgG anti-p53 antibody positivity in SLE was relatively lower (20%). This varied frequency may be due to the racial difference, since Chinese patients with SLE did not carry anti-p53 antibody[24]. Additionally, a previous study reported that seropositivity of anti-p53 antibody was more prominent in patients with active SLE such as lupus nephritis[11]. Therefore, the differences in the patient population may contribute to this discrepancy, since none of our patients with SLE exhibited severe complications such as lupus nephritis. Further, the presence of anti-p53 antibody is not a common autoimmune response in connective tissue diseases, as anti-p53 antibody was rarely detected in patients with rheumatoid arthritis, Sjögren's syndrome, or DM[15,25]. In addition, anti-p53 antibody levels in SSc overall were higher than those in SLE, a finding also

observed by Chauhan, *et al*[8]. Thus, although anti-p53 antibody is not specific for SSc, the autoimmune response against p53 in SSc appears to be relatively greater.

A recent study has shown that there is no p53 mutation and no accumulation of mutant p53 in SSc-derived fibroblasts[26]. Consistently, p53 expression in the fibrotic skin from patients with SSc shows no difference from that in normal skin[27]. These findings suggest that anti-p53 antibody is not induced by mutant p53 protein in SSc. SSc is more frequently accompanied by Raynaud's phenomenon relative to other connective tissue diseases. Ischemia and reperfusion injury following Raynaud's phenomenon can generate reactive oxygen species that may result in DNA damage[28,29]. Indeed, many studies have shown that patients with SSc exhibit augmented levels of oxidative stress[30-32]. Wild-type p53 plays a critical role in regulation of this pathogenetic process by inducing cell arrest/DNA repair or induction of apoptosis[3]. In our study, IgG isolated from sera of patients with SSc that contained IgG anti-p53 antibodies could inhibit the p53 activity. However, since p53 is expressed in both the nucleus and cytoplasm[33], it remains unknown whether anti-p53 antibody could react with intracellular p53 and inhibit its activity. In SSc, enhanced apoptosis is detected in the endothelial cells[34]. It has been suggested that the clustering and marked concentration of intracellular autoantigens in the surface blebs of apoptotic cells, and their modification by apoptosis-specific proteolytic cleavage and/or phosphorylation, could induce autoantibody production[35]. Moreover, our study showed that the presence of anti-p53 antibody correlated with longer disease duration. Collectively, it is possible that anti-p53 antibody may be secondarily produced by tissue damage due to the longterm oxidative stress. However, the mechanisms of anti-p53 antibody and its functional relevance *in vivo* require further studies.

In our study, anti-p53 antibody was associated with a milder form of SSc, lSSc. The reasons for this phenomenon remain unknown. However, since the disease duration of lSSc was longer than that of dSSc (8.3 ± 9.3 vs 3.0 ± 2.9 yrs; $p < 0.05$), the correlation of anti-p53 antibody with lSSc may be due to the correlation of this antibody with longer disease duration. A recent study has shown that lpr mice lacking p53 expression exhibit lower autoantibody levels than the B6 lpr mice, suggesting an unanticipated role for p53 in the progression of autoimmunity and the production of autoantibodies[4]. Therefore, it is possible that anti-p53 antibody may play a protective role in the development of SSc. Alternatively, since the autoantibody profile is clearly different between lSSc and dSSc — for example, ACA is closely linked to lSSc, while anti-topoisomerase I antibody correlates with dSSc[1] — the higher prevalence of anti-p53 antibody in lSSc may be reflected by such a distinct autoimmune response in lSSc. Nonetheless, the results of our study suggest that anti-p53 antibody is one of the major autoantibodies in lSSc.

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