Table 1. The frequencies of myositis-specific, myositis-associated, and anti–CADM-140 antibodies in patients with connective tissue diseases and IPF*

Autoantibodies	PM (n = 61)	DM (n = 42)		RA (n = 50)	SLE (n = 46)	MCTD/OL (n = 27)	Systemic sclerosis (n = 22)	Sjögren's syndrome (n = 7)	IPF (n = 43)
		Classic DM (n = 27)	C-ADM (n = 15)						
Myositis-specific									
Anti-ARS (anti-Jo-1)	10 (16)	6 (22)	0	0	0	0	0	0	0
Anti-ARS (non-anti-Jo-1)	10 (16)	2 (7)	0	0	0	1 (4)	0	0	4 (9)
Anti-SRP	5 (8)	0	0	0	0	0	0	0	0
Anti-Mi-2	0	2 (7)	0	0	0	0	0	0	0
Myositis-associated									
Anti-SSA/Ro	3 (5)	3 (11)	2 (14)	8 (16)	15 (33)	6 (22)	1 (5)	5 (71)	1 (2)
Anti-U1 RNP	2 (3)	2 (7)	0	1 (2)	18 (39)	23 (85)	2 (9)	0	0
Anti–CADM-140	0	0	8 (53)	0	0	0	0	0	0

* Values are the number (%) of patients. Anti-PM/Scl and other myositis-associated autoantibodies were not detected in any of the sera tested. PM = polymyositis; DM = dermatomyositis; C-ADM = clinically amyopathic dermatomyositis; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; MCTD/OL = mixed connective tissue disease/overlap syndrome; IPF = idiopathic pulmonary fibrosis; anti-ARS = anti–aminoacyl-transfer RNA synthetase; anti-SRP = anti–signal recognition particle.

Disease specificity of the anti–CADM-140 antibodies. The frequencies of myositis-specific antibodies, myositis-associated antibodies, and anti–CADM-140 antibodies are summarized in Table 1. Myositis-specific antibodies are found in most patients with myositis, whereas myositis-associated antibodies are frequently found in patients without myositis (12). Among the patients with CTDs or IPF, myositis-specific antibodies (33 with anti-ARS, 5 with anti-SRP, 2 with anti–Mi-2) and myositis-associated antibodies (44 with anti-SSA/Ro, 48 with anti-U1 RNP, none with anti-PM/Scl or other myositis-associated antibodies) were detected. Anti–CADM-140 autoantibodies were found in 19% of sera from patients with DM (especially in 53% with the C-ADM subtype), but were not detected in patients with other CTDs or IPF.

Clinical features of C-ADM patients with anti–CADM-140. Clinical findings were compared between DM patients (including those with C-ADM) with anti–CADM-140 autoantibodies and those without anti–CADM-140 autoantibodies (Table 2). There were no significant differences in the frequencies of skin symptoms. However, the frequency of rapidly progressive ILD was significantly increased in anti–CADM-140–positive patients compared with that in anti–CADM-140–negative patients (50% versus 6%; $P = 0.008$). No myositis-specific antibodies were found in patients with anti–CADM-140; nevertheless, there was no significant

Table 2. Comparison of clinical features in anti–CADM-140–positive versus anti–CADM-140–negative patients with dermatomyositis

Feature	Anti–CADM-140–positive (n = 8)	Anti–CADM-140–negative (n = 34)	*P*
Age at onset, mean ± SD years	44.5 ± 12.7	46.5 ± 15.7	NS
No. male/no. female	2/6	8/26	NS
Gottron's sign or papules	75	88	NS
Heliotrope rash	50	53	NS
Muscle weakness	0	76	0.02
Elevation of CK	25	74	0.03
Fever	25	50	NS
Raynaud's phenomenon	13	24	NS
Arthritis	50	71	NS
Rapidly progressive ILD	50	6	0.008
Malignancy	0	18	NS
MSAs	0	29	NS
MAAs	13	18	NS

* Except where indicated otherwise, values are the percent of patients. NS = not significant; CK = creatine kinase; ILD = interstitial lung disease; MSAs = myositis-specific autoantibodies; MAAs = myositis-associated autoantibodies.

difference in the frequency of these autoantibodies in comparison with the anti–CADM-140–negative group.

None of the 8 patients with anti–CADM-140–positive sera were treated with steroids or other immunosuppressive medications prior to being assessed for C-ADM. All of these patients had Gottron's sign or papules, or periorbital heliotrope erythema and skin biopsy specimens yielding results compatible with DM. None of these patients had muscle weakness. CK levels were in the normal range in 6 patients (75%) and slightly elevated in the remaining 2 patients. Of the 6 patients assessed for the muscle enzyme aldolase, levels were normal in 2 patients. Of the 2 patients who underwent muscle MRI, neither showed findings compatible with a diagnosis of myopathy. Four patients had a muscle biopsy, and 2 of the muscle specimens exhibited mild infiltration of inflammatory cells, but there was no evidence of necrosis of muscle fibers, variation in fiber size, regeneration, or phagocytosis. Of the 7 patients with ILD (88%), 4 developed rapidly progressive disease.

DISCUSSION

We have identified novel autoantibodies (anti–CADM-140 autoantibodies) to an ~140-kd polypeptide in patients with DM. Anti–CADM-140 antibodies were detected specifically in patients with DM, especially those with C-ADM. In addition, anti–CADM-140 antibodies were associated with rapidly progressive ILD.

It has been reported that amyopathic DM may be accompanied by rapidly progressive ILD, especially in Japanese patients and other Asian patients (7). In contrast, rapidly progressive ILD was shown to be rare in patients with amyopathic DM in a North American population (13). In our series, 5 of 15 patients with C-ADM (33%) (4 of whom had anti–CADM-140 antibodies) had rapidly progressive ILD during their clinical course. Rapidly progressive ILD was more frequent in our series compared with that reported previously in North American populations (13). Although the number of patients that we studied was very limited, it remains possible that racial differences are the reason for this discrepancy, because other clinical studies of Japanese patients also demonstrated findings similar to ours (7).

Furthermore, in a recent preliminary report, using immunoprecipitation and immunoblotting of HeLa cell extracts, Targoff et al documented the presence of antibodies to a 155-kd protein and/or Se protein in patients with C-ADM (8). Thirteen of 18 C-ADM sera possessed an anti–155-kd polypeptide antibody, and 6 also immunoprecipitated a 95-kd polypeptide (anti-Se antibody). In contrast, Oddis et al identified the anti-MJ antibody, which was also found to recognize a 140-kd polypeptide, in patients with juvenile DM (14,15). We have been able to conclude that anti–CADM-140 is distinctively different from anti-MJ, because the molecular weights of the immunoprecipitated polypeptides are different. Moreover, the clinical features of anti-MJ are quite different from those associated with anti–CADM-140. Anti-MJ is detected mainly in juvenile DM, has been observed in the US and Argentina, and is clinically characterized by severe DM with a chronic and polycyclic course, sometimes accompanied by vasculitis (14). In order to elucidate the racial differences in the frequency of these antibodies, the examination of a larger number of patients from several different populations is required.

Our results have thus demonstrated the presence of anti–CADM-140 autoantibodies in patients with C-ADM, and these were found to be associated with rapidly progressive ILD. Further studies of this novel autoantibody specificity may provide insight into the pathogenic mechanisms of C-ADM accompanied by rapidly progressive ILD.

ACKNOWLEDGMENTS

We thank Ms N. Fertig for preparing the prototype anti-MJ serum, and Ms Mutsuko Ishida for assisting in RNA immunoprecipitation assays.

REFERENCES

1. Plotz PH, Rider LG, Targoff IN, Raben N, O'Hanlon TP, Miller FW. Myositis: immunologic contributions to understanding cause, pathogenesis, and therapy. Ann Intern Med 1995;122:715–24.
2. Pearson CM. Polymyositis and dermatomyositis. In: Koopman WJ, editor. Arthritis and allied conditions. Baltimore: Williams & Wilkins; 1997. p. 742–61.
3. Hirakata M, Mimori T, Akizuki M, Craft J, Hardin JA, Homma M. Autoantibodies to small nuclear and cytoplasmic ribonucleoproteins in Japanese patients with inflammatory muscle disease. Arthritis Rheum 1992;35:449–56.
4. Targoff IN, Johnson AE, Miller FW. Antibody to signal recognition particle in polymyositis. Arthritis Rheum 1990;33:1361–70.
5. Targoff IN, Reichlin M. The association between Mi-2 antibodies and dermatomyositis. Arthritis Rheum 1985;28:796–803.
6. Sontheimer RD. Would a new name hasten the acceptance of amyopathic dermatomyositis (dermatomyositis sine myositis) as a distinctive subset within the idiopathic inflammatory dermatomyopathies spectrum of clinical illness? J Am Acad Dermatol 2002;46:626–36.
7. Tokiyama K, Tagawa H, Yokota E, Nagasawa K, Kusaba T, TsudaY, et al. Two cases of amyopathic dermatomyositis with fatal rapidly progressive interstitial pneumonitis. Ryumachi 1990;30: 204–11. In Japanese.
8. Targoff IN, Trieu EP, Sontheimer RD. Autoantibodies to 155 kD

and Se antigens in patients with clinically-amyopathic dermatomyositis [abstract]. Arthritis Rheum 2000;43 Suppl 9:S194.
9. Hirakata M, Suwa A, Nagai S, Kron MA, Trieu EP, Mimori T, et al. Anti-KS: identification of autoantibodies to asparaginyl-transfer RNA synthetase associated with interstitial lung disease. J Immunol 1999;162:2315–20.
10. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. Proc Natl Acad Sci U S A 1979;76:4350–4.
11. Lane RJ, Emslie-Smith A, Mosquera IE, Hudgson P. Clinical, biochemical and histological responses to treatment in polymyositis: a prospective study. J R Soc Med 1989;82:333–8.
12. Targoff IN. Laboratory testing in the diagnosis and management of idiopathic inflammatory myopathies. Rheum Dis Clin North Am 2002;28:859–90.
13. Euwer RL, Sontheimer RD. Amyopathic dermatomyositis: a review. J Invest Dermatol 1993;100:124S–7S.
14. Oddis CV, Fertig N, Goel A, Espada G, Confalone Gregorian M, Maldonado Cocco JA, et al. Clinical and serological characterization of the anti-MJ antibody in childhood myositis [abstract]. Arthritis Rheum 1997;40 Suppl 9:S139.
15. Espada G, Confalone Gregorian M, Ortiz Z, Fertig N, Londino AV, Oddis CV, et al. Serum autoantibodies in juvenile idiopathic inflammatory myopathies (IIM) in a cohort of Argentine patients [abstract]. Arthritis Rheum 1997;40 Suppl 9:S140.

Genes and Immunity (2005), 1–5

www.nature.com/gene

BRIEF COMMUNICATION

Interleukin-10 genotypes are associated with systemic sclerosis and influence disease-associated autoimmune responses

LL Hudson[1], KM Rocca[1], M Kuwana[2] and JP Pandey[1]

[1]*Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, USA;* [2]*Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan*

Systemic sclerosis (SSc; scleroderma) is a connective tissue disease, characterized by fibrotic, immunological, and vascular abnormalities. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that modulates collagen production and B-cell survival. To determine if certain IL-10 genotypes are risk factors for the development of SSc and influence disease-associated autoimmune responses, 248 Caucasian and 264 Japanese SSc patients and controls were genotyped for three loci: −3575, −2849, and −2763. Sera from patients were characterized for SSc-associated autoantibodies. In Caucasians, at −3575 and −2763, the frequency of AA homozygotes was higher in patients as compared with controls (P = 0.0005; P = 0.002). In Japanese subjects, the frequency of AC heterozygotes at −2763 was higher, and that of CC homozygotes lower, in patients with diffuse SSc as compared to controls (P = 0.04). Particular IL-10 genotypes were associated with SSc-related autoantibodies. These results suggest that IL-10 genotypes contribute to the etiology of scleroderma.
Genes and Immunity advance online publication, 17 March 2005; doi:10.1038/sj.gene.6364180

Keywords: *systemic sclerosis; interleukin-10; scleroderma; linkage disequilibrium; autoantibodies*

Interleukin-10 (IL-10) is a pleiotropic cytokine produced primarily by monocytes, T cells, and B cells. IL-10 has both activating and inhibitory influences on T cells, is involved in immunoglobulin class switching, and promotes B-cell survival.[1] Additionally, IL-10 modulates the extracellular matrix by inhibiting fibroblast proliferation and collagen production.[2,3] The interindividual differences in IL-10 production levels have a large genetic component.[4–6]

Owing to these properties, there is a growing interest in determining the role of IL-10 genes in autoimmune diseases.[7] Scleroderma is an autoimmune rheumatic disease characterized by extensive fibrosis, thickened skin, vascular alterations, and immunological abnormalities. There are two major subtypes of systemic sclerosis (SSc).[8] Diffuse SSc is the most serious form, characterized by extensive fibrosis of skin and internal organs; development of the disease can be rapid and severe. Limited SSc is a milder disease form, as patients have less involvement of internal organs and slower disease progression. The majority of SSc patients produce antibodies directed at nuclear antigens, which are strongly associated with organ involvement and disease outcome.[9–12]

Correspondence: Dr JP Pandey, Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC 29425-2230, USA. E-mail: pandeyj@musc.edu
Received 29 November 2004; revised 3 February 2005; accepted 3 February 2005

Although the etiology of SSc is unknown, genetic factors are thought to be important. Several polymorphic genes—which are either risk factors for the development of SSc or influence disease-associated autoimmune responsiveness—have been identified. These include human leukocyte antigens (HLA), fibrillin-1, and GM and KM allotypes—genetic markers of γ and κ chains, respectively.[13–15] To determine if allelic variation at IL-10 loci is associated with SSc and SSc-related humoral autoimmune responses, DNA samples from Caucasian and Japanese patients and controls were genotyped at three IL-10 sites (−3575, −2849, and −2763) and patients' sera were characterized for disease-related autoantibodies.

This study was approved by the Institutional Review Board/Ethical Committee for human research of the Medical University of South Carolina and the Keio University School of Medicine. All subjects provided their written informed consent. Caucasian subjects consisted of 105 SSc patients (67 limited, 38 diffuse) and 143 controls presenting at the Rheumatology Clinic of the Medical University of South Carolina. Controls consisted of unrelated patients with osteoarthritis, fibromyalgia, gout, or regional musculoskeletal pain syndromes. Controls with conditions associated with autoimmune or connective tissue diseases were excluded. Japanese subjects consisted of 127 SSc patients (86 limited, 41 diffuse) presenting at Keio University School of Medicine and 137 healthy, ethnically matched controls living in the Tokyo area. All patients fulfilled the

American College of Rheumatology criteria for SSc.[16] Genomic DNA samples were genotyped by PCR-restriction fragment length polymorphism methods. The method for genotyping at −3575 was modified from Moraes *et al*.[17] A 228 base pair (bp) sequence containing the −3575 single-nucleotide polymorphism (SNP) was amplified (F 5′-GGT TTT CCT TCA TTT GCA GC-3′ and R 5′-ACA CTG TGA GCT TCT TGA GG-3′) and digested with the restriction enzyme *Tsp*509I, which cuts this product into 121 and 107 bp pieces in the presence of the T allele. Genotypes at positions −2849 and −2763 were determined using the same primer set (F 5′-ACA TTT CAG AAC AAA TAA AGA AGT CAG-3′ and R 5′-GTG CAG TGG CAT GAT CTC AG-3′) to amplify a 300 bp product including both SNPs. Digestion with *Alw*I results in products of 202, 77, and 21 bp for the G allele, and products of 279 and 21 bp, if the A allele is present at position −2849. Digestion with *Tsp*509I results in products of 174 and 126 bp, if the A allele is present at position −2763; no digestion of the 300 bp amplified product occurs in the presence of the C allele. Indirect immunofluorescence (anticentromere (ACA)), double immunodiffusion and protein immunoprecipitation (anti-topoisomerase I (topo I)), and immunoprecipitation (anti-RNA polymerase I/III (RNAP) and U1 ribonucleoprotein (U1 RNP)) assays were used to identify autoantibodies in patients' sera, as described previously.[12]

The distribution of genotype frequencies was analyzed using Pearson's χ^2 test, except when cell counts were less than or equal to 5; in the latter case, data were analyzed by Fisher's two-tailed exact test. Statistical significance was defined as $P<0.05$. Odds ratio (OR) was calculated to measure the strength of the associations observed. When cell counts were less than or equal to 5, no ORs were determined, as the use of large sample theory to calculate confidence intervals for the ORs can only be justified when all of the expected cell counts are greater than 5.

The distribution of IL-10 genotypes and prevalence of autoantibodies in Caucasian subjects is presented in Table 1. At −3575 and −2763, the frequency of AA homozygotes was significantly higher in patients as compared with controls (29.5 *vs* 11.8%, $P=0.0005$, OR = 3.1, CI 1.6–5.9; 20.6 *vs* 7.2%, $P=0.002$, OR = 3.3, CI 1.5–7.5). At position −2849, the frequency of GG homozygotes was significantly lower in patients as compared to controls (44.2 *vs* 57.4%, $P=0.04$, OR = 0.59, CI 0.35–0.98). Subdivision of the patient population showed that subjects with the limited form of the disease were the primary contributors to the overall variation at −3575 and −2849. Compared to controls, the frequency of AA homozygotes (−3575) was increased (33 *vs* 11.8%, $P=0.0002$, OR = 3.6, CI 1.76–7.43), while that of GG (−2849) was decreased (41.8 *vs* 57.4%, $P=0.03$, OR = 0.53, CI 0.29–0.96) in patients with limited SSc. At −2763, however, subjects in both disease categories contributed to the overall variation. Compared to controls, the frequency of AA homozygotes was higher in both limited and diffuse SSc (21 *vs* 7.2%, $P=0.003$, OR = 3.5, CI 1.48–8.48; 19 *vs* 7.2%, $P=0.03$, OR = 3.0, CI 1.06–8.55). The frequency of the AC genotype at this locus was significantly lower in patients

Table 1 Distribution of IL-10 genotypes and prevalence of autoantibodies in Caucasian subjects

Subjects, N (%)	*−3575 genotype*			*−2849 genotype*			*−2763 genotype*		
	AA	*AT*	*TT*	*AA*	*AG*	*GG*	*AA*	*AC*	*CC*
Total SSc	31 (29.5)[a]	39 (37.1)	35 (33.3)	10 (9.6)	48 (46.2)	46 (44.2)[b]	21 (20.6)[c]	39 (38.2)	42 (41.2)
RNAP+	2 (10.0)	6 (30.0)	12 (60.0)[d]	1 (5.0)	6 (30.0)	13 (65.0)[e]	2 (10.0)	4 (20.0)	14 (70.0)[f]
RNAP−	29 (34.1)	33 (38.8)	23 (27.1)	9 (10.7)	42 (50.0)	33 (39.3)	19 (23.2)	35 (42.7)	28 (34.1)
U1 RNP+	4 (40.0)	4 (40.0)	2 (20.0)	2 (22.2)	5 (55.6)	2 (22.2)	4 (40.0)	5 (50.0)	1 (10.0)[g]
U1 RNP−	27 (28.4)	35 (36.8)	33 (34.7)	8 (8.4)	43 (45.3)	44 (46.3)	17 (18.5)	34 (37)	41 (44.5)
Diffuse SSc	9 (23.7)	15 (39.5)	14 (36.8)	3 (8.0)	16 (43.0)	18 (48.7)	7 (19.0)[h]	10 (27.5)[i]	20 (54.0)
RNAP+	2 (11.8)	5 (29.4)	10 (58.8)[j]	1 (5.9)	5 (29.4)	11 (64.7)	2 (11.8)	3 (17.6)	12 (70.6)
RNAP−	7 (33.3)	10 (47.6)	4 (19.0)	2 (10.0)	11 (55.0)	7 (35.0)	5 (25.0)	7 (35.0)	8 (40.0)
Limited SSc	22 (33.0)[k]	24 (36.0)	21 (31.0)	7 (10.4)	32 (47.8)	28 (41.8)[l]	14 (21.0)[m]	29 (45.0)	22 (34.0)
Controls	17 (11.8)	71 (49.6)	55 (38.6)	7 (5.0)	53 (37.6)	81 (57.4)	10 (7.2)	65 (46.8)	64 (46.0)

[a] $P=0.0005$.
[b] $P=0.04$.
[c] $P=0.002$ (total SSc *vs* controls).
[d] $P=0.005$.
[e] $P=0.03$.
[f] $P=0.003$ (total SSc; RNAP+ *vs* RNAP−).
[g] $P=0.04$ (total SSc; U1 RNP+ *vs* U1 RNP−).
[h] $P=0.03$.
[i] $P=0.03$ (diffuse SSc *vs* controls).
[j] $P=0.02$ (diffuse SSc; RNAP+ *vs* RNAP−).
[k] $P=0.0002$.
[l] $P=0.03$.
[m] 0.003 (limited SSc *vs* controls).

with diffuse disease as compared to controls (27.5 *vs* 46.8%, $P=0.03$, OR = 0.42, CI 0.18–0.93).

As autoantibodies in SSc are associated with disease subgroup and prognostic factors, we analyzed the distribution of IL-10 genotypes among patients with and without disease-associated autoantibodies. The distribution of IL-10 genotypes was significantly different between Caucasian patients positive for antibodies to RNAP and U1 RNP compared to those without these autoantibodies (Table 1). At −3575 in SSc patients as a whole, the frequency of TT homozygotes was higher in subjects with antibodies to RNAP compared to those without these antibodies (60 *vs* 27.1%, $P=0.005$, OR = 4.0, CI 1.46–11.15); this was primarily due to an increased frequency of this genotype in diffuse patients positive for the antibody (58.8 *vs* 19%, $P=0.02$). The frequency of GG homozygotes at −2849 and that of CC at −2763 was higher in patients with anti-RNAP antibodies compared to those without these autoantibodies (65 *vs* 39.3%, $P=0.03$, OR = 2.8, CI 1.03–7.94; 70 *vs* 34.1% $P=0.003$, OR = 4.5, CI 1.5–12.9). In subjects with antibodies to U1 RNP, the frequency of the −2763 CC genotype was lower than in those without these autoantibodies (10 *vs* 44.5%, $P=0.04$). IL-10 genotypes were not associated with anti-topo I or ACA (data not shown).

The distribution of IL-10 genotypes in Japanese SSc patients and controls, and in SSc patients with and without ACA, U1 RNP, and topo I autoantibodies is presented in Table 2. Interestingly, no subjects had the AA genotype at any loci. Examination of the patient population as a whole showed no significant difference in genotype frequencies between patients and controls. Subgroup analysis, however, revealed that in diffuse patients at −2763, the frequency of AC heterozygotes was higher (21 *vs* 8.6%, $P=0.04$, OR = 2.8, CI 1.02–7.79) and that of CC homozygotes lower (79 *vs* 91.4%, $P=0.04$, OR = 0.35, CI 0.13–0.97) than the respective frequencies in the control population.

In the total patient population, the frequency of AT heterozygotes at −3575 was higher (20.8 *vs* 6.8%, $P=0.03$, OR = 3.6, CI 1.04–12.58) and that of TT homozygotes lower (79.2 *vs* 93.2%, $P=0.03$, OR = 0.27, CI 0.08–0.97) in patients with ACA than in those without these autoantibodies. Patients with limited SSc accounted for the increased frequency of the AT genotype in ACA-positive subjects (20.8 *vs* 3.2%, $P=0.02$). The AT heterozygotes at this locus were also more prevalent in the diffuse SSc patients with anti-U1 RNP antibodies than in those lacking these autoantibodies (75 *vs* 5.4%, $P=0.004$). At −2763, the frequency of AC heterozygotes was higher (24.1 *vs* 6.1%) and that of CC homozygotes lower (75.9 *vs* 93.9%) in patients with antibodies to topo I than in those without these autoantibodies ($P=0.005$). Patients with limited SSc were primarily responsible for the increased frequency of AC heterozygotes in anti-topo I-positive subjects (22.6 *vs* 5.5%, $P=0.03$). IL-10 genotypes were not associated with antibodies to RNAP. Other genotype frequencies were similar between total patients or subgroups and controls (data not shown).

In Caucasians, homozygosity for the A allele at −3575 and −2763 was associated with over three-fold higher risk of developing SSc. The protective effects of IL-10 against fibrosis, a hallmark feature of scleroderma, could

Table 2 Distribution of IL-10 genotypes and prevalence of autoantibodies in Japanese subjects[a]

Subjects, N (%)	*−3575 genotype*		*−2849 genotype*		*−2763 genotype*	
	AT	*TT*	*AG*	*GG*	*AC*	*CC*
Total SSc	12 (9.5)	115 (90.5)	2 (1.6)	122 (98.4)	18 (14.5)	106 (85.5)
ACA+	5 (20.8)[b]	19 (79.2)	2 (8.7)	21 (91.3)	1 (4.0)	24 (96)
ACA−	7 (6.8)	96 (93.2)	0	101 (100)	17 (17.2)	82 (82.8)
Topo I+	7 (11.9)	52 (88.1)	0	59 (100)	14 (24.1)[c]	44 (75.9)
Topo I−	5 (7.4)	63 (92.6)	2 (3.1)	63 (96.9)	4 (6.1)	62 (93.9)
Diffuse SSc	5 (12.2)	36 (87.8)	0	40 (100)	8 (21)[d]	30 (79.0)
U1 RNP+	3 (75)[e]	1 (25)	0	4 (100)	2 (66.7)	1 (33.3)
U1 RNP−	2 (5.4)	35 (94.6)	0	36 (100)	6 (17.1)	29 (82.9)
Limited SSc	7 (8.1)	79 (91.2)	2 (2.3)	82 (97.6)	10 (11.6)	76 (88.4)
ACA+	5 (20.8)[f]	19 (79.2)	2 (8.7)	21 (91.3)	1 (4.0)	24 (96)
ACA−	2 (3.2)	60 (96.8)	0	61 (100)	9 (14.8)	52 (85.2)
Topo I+	2 (6.7)	28 (93.3)	0	29 (100)	7 (22.6)[g]	24 (77.4)
Topo I−	5 (8.1)	51 (91.1)	2 (3.6)	53 (96.4)	3 (5.5)	52 (94.5)
Controls	6 (4.4)	131 (95.6)	3 (2.4)	124 (97.6)	10 (8.6)	106 (91.4)

[a]No subjects had the AA genotype.
[b]$P=0.03$ (total SSc; ACA+ *vs* ACA−).
[c]$P=0.005$ (total SSc; topo I+ *vs* topo I−).
[d]$P=0.04$ (diffuse SSc *vs* controls).
[e]$P=0.004$ (diffuse SSc; U1 RNP+ *vs* U1 RNP−).
[f]$P=0.02$ (limited SSc; ACA+ *vs* ACA−).
[g]$P=0.03$ (limited SSc topo I+ *vs* topo I−).

contribute to the immunological mechanisms underlying these associations. IL-10 reduces tumor necrosis factor-α (TNF-α)-induced proliferation of fibroblasts and decreases production of type 1 collagen and fibronectin by fibroblasts.[2,3] As mentioned before, the quantitative expression of IL-10 is highly heritable, and IL-10 genotypes contribute to this phenomenon. Indeed, the risk-conferring genotypes in Caucasians in this study—AA (−3575) and AA (−2763)—are strongly associated with low production of this cytokine.[6]

In the Japanese subjects, there were no AA homozygotes and the only genotype at −2763 carrying the A allele, the AC heterozygote, was associated with over two-fold increased risk of diffuse SSc. Thus, in these A allele carrying subjects, the IL-10 levels may be too low to afford protection from fibrosis, resulting in a higher risk of developing scleroderma. Homozygosity for the G allele at −2849 was marginally associated with protection from the disease in Caucasians, which is in line with the reported over-representation (although not statistically significant) of this allele in IL-10 high producers.[6]

Since fibrosis is more extensive in patients with diffuse SSc than limited SSc, in our subgroup analyses we had expected the diffuse patients to account for most of the variation observed in the total patient population. This appears to be the case for the Japanese subjects, where the frequency of AC heterozygotes was higher in diffuse, but not in limited, patients than that in the controls. In Caucasians, however, patients from both categories contributed to the overall variation at −2763, while those with the limited form of the disease were the primary contributors to the differences at −3575 and −2849 loci. Clearly, other factors, in addition to IL-10 genotypes, contribute to the clinical phenotype of SSc patients. The results presented here are at variance with those reported by Crilly *et al*,[18] who found no association between IL-10 genotypes and SSc as a whole, but reported a decreased frequency of a genotype associated with high IL-10 production in patients with the diffuse form of the disease. The two studies, however, are not comparable: Crilly *et al* studied the IL-10 sites in the proximal region of the promoter, whereas we examined distal SNPs. Other differences include the study population—homogeneous Scottish Caucasians in the study by Crilly *et al vs* heterogeneous North American Caucasians and homogeneous Japanese in the present study.

In addition to being risk factors for the development of the disease, particular genotypes in both Caucasian and Japanese populations contributed to disease-related autoantibodies associated with different clinical phenotypes of scleroderma. In Caucasian subjects, homozygosity for the T, G, and C alleles at −3575, −2849, −2763, respectively, was associated with the presence of anti-RNAP antibodies. At −3575, patients with the diffuse form of the disease contributed to this association; at the other two loci, no disease subtype-associated differences were found. In the Japanese patients, heterozygosity at −3575 was associated with the prevalence of ACA, while that at −2763 was associated with the presence of anti-topo I antibodies. Patients with the limited form of the disease contributed to the variation at both loci.

Since this is the first study of this type for scleroderma, these results cannot be compared directly with other studies. Involvement of IL-10 genotypes in humoral immunity to autoantigens is in accord with the enhancing effect of this cytokine on B-cell survival. However, no consistent pattern of autoreactivity, explainable by the known associations between particular genotypes and the cytokine levels, emerges. It might be relevant to point out that the autoantibodies examined here are strongly associated with particular HLA alleles, and for ACA, a stronger association has been found with certain promoter region determinants of the TNF-α gene.[14,19] Perhaps simultaneous examination of HLA, TNF-α and IL-10 loci in a large study population would shed further light on humoral autoreactivity in scleroderma.

The reason for the observed ethnic differences in genetic association is not clear. Genetic heterogeneity might in part explain the ethnic disparities observed here.[20] Differences in allele frequencies and linkage disequilibrium among populations originating from different continents may also contribute to ethnically restricted associations.[21] Additionally, the low frequency of A alleles in the Japanese subjects may have reduced the statistical power to detect an association between IL-10 genotypes and disease risk in this ethnic group. In addition to differences in allele frequencies between the groups, it is likely that multiple genes interact in an epistatic manner to cause SSc, and differences in gene frequencies at these loci result in differences in relative risk to develop the disease in different ethnic groups. Evidence for such epistatic interactions in SSc has been presented elsewhere.[15]

In addition to the possible influence of IL-10 genes (via their gene products) on SSc pathogenesis discussed above, the observed associations could also be explained by linkage disequilibrium between the IL-10 alleles and those of another, as yet unidentified, locus for SSc. Such case–control studies cannot distinguish between the two possibilities. To our knowledge, this is the first report of an association between IL-10 genotypes and SSc-related autoimmune responses. Although some of the IL-10 genotype associations with SSc and SSc-related autoimmune responses reported here are highly significant and can, at least partially, be explained by the known immunological properties of IL-10, they must be followed by confirmation in an independent study population to be of wider significance.

Acknowledgements

This work was supported in part by the US Department of Energy cooperative agreement DE-FC09-02CH11109. We are grateful to the study subjects for their blood donation and to the physicians for facilitating the patient participation.

References

1 Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; **19**: 683–765.
2 Moroguchi A, Ishimura K, Okano K, Wakabayashi H, Maeba T, Maeta H. Interleukin-10 suppresses proliferation and remodeling of extracellular matrix of cultured human skin fibroblasts. *Eur Surg Res* 2004; **36**: 39–44.
3 Yamamoto T, Eckes B, Krieg T. Effect of interleukin-10 on the gene expression of type I collagen, fibronectin, and decorin in

human skin fibroblasts: differential regulation by transforming growth factor-beta and monocyte chemoattractant protein-1. *Biochem Biophys Res Commun* 2001; **281**: 200–205.
4 Westendorp RG, Langermans JA, Huizinga TW, Verweij CL, Sturk A. Genetic influence on cytokine production in meningococcal disease. *Lancet* 1997; **349**: 1912–1913.
5 Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. *Proc Natl Acad Sci USA* 1998; **95**: 9465–9470.
6 Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. *J Immunol* 2001; **166**: 3915–3922.
7 Roncarolo MG, Battaglia M, Gregori S. The role of interleukin 10 in the control of autoimmunity. *J Autoimmun* 2003; **20**: 269–272.
8 LeRoy EC, Black C, Fleischmajer R *et al*. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; **15**: 202–205.
9 Weiner ES, Earnshaw WC, Senecal JL, Bordwell B, Johnson P, Rothfield NF. Clinical associations of anticentromere antibodies and antibodies to topoisomerase I. A study of 355 patients. *Arthritis Rheum* 1988; **31**: 378–385.
10 Gilchrist FC, Bunn C, Foley PJ *et al*. Class II HLA associations with autoantibodies in scleroderma: a highly significant role for HLA-DP. *Genes Immun* 2001; **2**: 76–81.
11 Tan EM, Chan EK, Sullivan KF, Rubin RL. Antinuclear antibodies (ANAs): diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. *Clin Immunol Immunopathol* 1988; **47**: 121–141.
12 Kuwana M, Kaburaki J, Okano Y, Tojo T, Homma M. Clinical and prognostic associations based on serum antinuclear antibodies in Japanese patients with systemic sclerosis. *Arthritis Rheum* 1994; **37**: 75–83.
13 Tan FK, Wang N, Kuwana M *et al*. Association of fibrillin 1 single-nucleotide polymorphism haplotypes with systemic sclerosis in Choctaw and Japanese populations. *Arthritis Rheum* 2001; **44**: 893–901.
14 Arnett FC. HLA and autoimmunity in scleroderma (systemic sclerosis). *Int Rev Immunol* 1995; **12**: 107–128.
15 Kameda H, Pandey JP, Kaburaki J, Inoko H, Kuwana M. Immunoglobulin allotype gene polymorphisms in systemic sclerosis: interactive effect of MHC class II and KM genes on anticentromere antibody production. *Ann Rheum Dis* 1998; **57**: 366–370.
16 Masi AT. Classification of systemic sclerosis (scleroderma): relationship of cutaneous subgroups in early disease to outcome and serologic reactivity. *J Rheumatol* 1988; **15**: 894–898.
17 Moraes MO, Santos AR, Schonkeren JJ *et al*. Interleukin-10 promoter haplotypes are differently distributed in the Brazilian versus the Dutch population. *Immunogenetics* 2003; **54**: 896–899.
18 Crilly A, Hamilton J, Clark CJ, Jardine A, Madhok R. Analysis of the 5′ flanking region of the interleukin 10 gene in patients with systemic sclerosis. *Rheumatology (Oxford)* 2003; **42**: 1295–1298.
19 Sato H, Lagan AL, Alexopoulou C *et al*. The TNF-863A allele strongly associates with anticentromere antibody positivity in scleroderma. *Arthritis Rheum* 2004; **50**: 558–564.
20 Wandstrat A, Wakeland E. The genetics of complex autoimmune diseases: non-MHC susceptibility genes. *Nat Immunol* 2001; **2**: 802–809.
21 Goddard KA, Hopkins PJ, Hall JM, Witte JS. Linkage disequilibrium and allele-frequency distributions for 114 single-nucleotide polymorphisms in five populations. *Am J Hum Genet* 2000; **66**: 216–234.