Immunogenetic Features in 120 Japanese Patients with Idiopathic Inflammatory Myopathy

TAKEFUMI FURUYA, MASAYUKI HAKODA, NAOYUKI TSUCHIYA, SHIGERU KOTAKE, NAOMI ICHIKAWA, YUKI NANKE, AYAKO NAKAJIMA, MEGUMI TAKEUCHI, MAKOTO NISHINARITA, HIROBUMI KONDO, AYA KAWASAKI, SHIO KOBAYASHI, TSUNEYO MIMORI, KATSUSHI TOKUNAGA, and NAOYUKI KAMATANI

ABSTRACT. ***Objective.*** To examine the role of HLA-DRB1 and tumor necrosis factor (TNF) promoter genotypes in the development and the autoantibody profiles of idiopathic inflammatory myopathy (IIM) in Japanese patients.

Methods. HLA-DRB1 and TNF promoter genotypes were determined, and serum antinuclear autoantibodies were identified in 120 adult Japanese patients with IIM [72 with dermatomyositis (DM), 30 with polymyositis (PM), 18 with myositis overlapping with other collagen vascular diseases], as well as in 265 controls.

Results. Forty-two patients (35%) were positive for myositis-specific autoantibodies (MSA), including 37 (31%) for anti-aminoacyl-tRNA synthetase (ARS) autoantibodies. Allele carrier frequency of HLA-DRB1*0803 was increased in the patients with IIM [p = 0.02, corrected p (pc) NS, 23% vs 14%, odds ratio (OR) = 1.9 (95% confidence interval, CI = 1.1–3.2)], with PM [p = 0.006, pc NS, 33%, OR 3.1 (95% CI 1.3–7.1)], and with anti-ARS autoantibodies [27%, p = 0.04, OR 2.3 (95% CI 1.0–5.1)] compared with controls. DRB1*0405 was increased in patients with anti-ARS autoantibodies compared with controls [41% vs 25%, p = 0.04, pc NS, OR 2.1 (95% CI 1.0–4.3)]. TNF promoter genotype was associated with the presence of interstitial lung disease (ILD). The carriage of a TNF-α haplotype formed by –1031C, –863A, and –857C was increased in the patients with ILD versus those without ILD [33% vs 18%, p = 0.05, pc NS, OR 2.3 (95% CI 0.94–5.5)].

Conclusion. HLA-DRB1 alleles were associated with development of IIM and MSA in a Japanese population. (J Rheumatol 2004;31:1768–74)

Key Indexing Terms:
IDIOPATHIC INFLAMMATORY MYOPATHY HLA-DRB1 AUTOANTIBODIES
JAPANESE TUMOR NECROSIS FACTOR-α PROMOTER

From the Institute of Rheumatology and Department of Neurology, Tokyo Women's Medical University, Tokyo; Department of Clinical Studies, Radiation Effects Research Foundation, Hiroshima; Department of Rheumatology, Taga General Hospital, Ibaraki; Department of Internal Medicine, Kitasato University School of Medicine, Kanagawa; Department of Human Genetics, Graduate School of Medicine, The University of Tokyo; and Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Supported by a grant-in-aid from the Ministry of Education, Culture, Science and Technology of Japan.

T. Furuya, MD, PhD, Instructor; S. Kotake MD, PhD, Assistant Professor; N. Ichikawa, MD, PhD, Instructor; Y. Nanke, MD, PhD, Assistant Professor; A. Nakajima, MD, PhD, Assistant Professor; N. Kamatani, MD, PhD, Director and Professor, Institute of Rheumatology, Tokyo Women's Medical University; M. Hakoda, MD, PhD, Chief, Division of Clinical Laboratories, Department of Clinical Studies, Radiation Effects Research Foundation; M. Takeuchi, MD, PhD, Assistant Professor, Department of Neurology, Tokyo Women's Medical University; M. Nishinarita, MD, PhD, Department of Rheumatology, Taga General Hospital; H. Kondo, MD, PhD, Professor, Department of Internal Medicine, Kitasato University School of Medicine; A. Kawasaki, BSc, Technician; N. Tsuchiya, MD, PhD, Associate Professor; K. Tokunaga, PhD, Professor, Department of Human Genetics, Graduate School of Medicine, The University of Tokyo; S. Kobayashi, AS, Research Assistant; T. Mimori, MD, PhD, Professor, Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine.

Address reprint requests to Dr. T. Furuya, Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjuku-ku, Tokyo 162-0054, Japan. E-mail: furuyat@ior.twmu.ac.jp

Submitted August 12, 2003; revision accepted March 25, 2004.

We previously reported the association of HLA class I and class II alleles with idiopathic inflammatory myopathy (IIM) by analyzing 84 Japanese patients[1]. Our previous study did not examine myositis-specific autoantibodies [MSA; anti-aminoacyl-tRNA synthetases (ARS), anti-signal recognition particle (SRP)] or myositis associated autoantibodies (MAA; anti-RNP, Ro, La, Ku) except for anti-Jo-1 autoantibody.

MSA appear to define clinical features, prognosis, and response to treatment[2]. In Caucasian patients, IIM in the presence of MSA is reportedly associated with HLA-DRB1*0301[3]. Although HLA-DRB1*0301 is common (around 20%) in the Caucasian population, it is very rare (0.1–0.2%) in Japanese[4]. There have been only a few small studies analyzing HLA alleles of patients with IIM among Japanese[1,5,6]. There have been no reports of an association of HLA alleles with MSA in Japanese.

In our previous study, HLA-DRB1*08 alleles were significantly increased in the Japanese patients with IIM compared with controls[1]. However, the actual mechanism by which HLA regulates susceptibility to myositis remains to be defined. Tumor necrosis factor-α (TNF-α), encoded within the MHC, is a potent proinflammatory cytokine with

a wide range of activities. It plays a critical role in the pathogenesis of inflammatory or autoimmune diseases[7]. A recent report showed blockade of TNF may be beneficial for dermatomyositis (DM) and polymyositis (PM)[8].

Among the single nucleotide polymorphisms (SNP) of the 5'-flanking region of the TNF gene, the –308A allele has been shown to be associated with high promoter activity[9] as well as susceptibility/severity to juvenile[10] and adult[11] IIM. However, the frequency of –308A allele is very low in the Japanese population (less than 3%)[12].

New SNP, which may affect transcriptional activity, were recently reported at position –1031(T/C), –863(C/A), and –857(C/A) in the 5'-flanking region of TNF, and each substitution was found in a substantial proportion of the Japanese population[12]. Four major haplotypes were identified, namely, TCC, TCT, CAC, and CCC, which we tentatively designated TNF-U01, U02, U03, and U04, respectively[13]. The associations of TNF-U03 with Crohn's disease[14] and TNF-U01 with asthma[15] have been reported in the Japanese.

In the present study, we aimed to confirm our previous findings of an association of HLA-DRB1*08 alleles with Japanese IIM patients by analyzing a larger number of patients, and to examine whether the polymorphisms of HLA-DRB1 and TNF promoter contribute to susceptibility and autoantibody production among Japanese with IIM.

MATERIALS AND METHODS

Patients. One hundred twenty Japanese patients with IIM seen from September 1994 to December 2001 were studied: 86 at Institute of Rhematology; 7 at Department of Neurology, Tokyo Women's Medical University (Tokyo); 19 at Kitasato University School of Medicine (Sagamihara, Kanagawa); 6 at Taga General Hospital (Hitachi, Ibaraki); and 2 at Tokyo Metropolitan Otsuka Hospital (Tokyo). The mean ± standard deviation (SD) age was 47.9 ± 14.8 years. Thirty of the patients were men and 90 were women. Among these patients, 72 and 30 were classified as having dermatomyositis (DM) and polymyositis (PM), respectively, using criteria proposed by Bohan and Peter[16,17]. Among them, 52, 36, and 14 patients were diagnosed as definite, probable, and possible DM/PM, respectively. The mean ± SD age of DM and PM patients was 47.1 ± 14.4 years and 51.8 ± 13.7 years, respectively. Among 72 DM and 30 PM patients, 19 and 9 were men, respectively. Heliotrope and Gottron rash were used to classify the patients as DM. Three patients with DM also had malignancy at the time of diagnosis (uterine carcinoma, malignant lymphoma, and lung cancer).

Eighteen patients had myositis overlapping with other collagen vascular diseases. They fulfilled both sets of Bohan and Peter criteria for myositis[16,17], as well as criteria for primary collagen diseases. Among them, 6, 8, and 4 patients were diagnosed as definite, probable, and possible DM/PM, respectively. Twelve of the patients met the American College of Rheumatology (ACR) preliminary classification criteria for systemic sclerosis[18], 4 met the ACR criteria for systemic lupus erythematosus (SLE)[19], and 2 met the ACR criteria for rheumatoid arthritis[20]. The mean ± SD age of overlap disease patients was 44.4 ± 17.1 years. Among 18 overlap disease patients, 2 were men and 16 were women.

Among the patients, 64 (56%) were diagnosed as having interstitial lung disease (ILD). ILD was defined by the presence of pulmonary fibrosis seen by chest radiography and/or computed tomography. Information regarding ILD was not available for 5 patients including one patient with anti-ARS autoantibody.

After patients gave informed consent, blood samples were obtained.

Controls. The control group consisted of 265 healthy laboratory personnel and students, 148 of the controls were men, 116 were women, and one was unknown. All controls were unrelated Japanese living in the Tokyo area. HLA-DRB1 and TNF promoter genotypes of the control group have been reported[21]. The central part of Japan has been shown to be relatively homogeneous with respect to genetic background[22], permitting the case-control approach to be employed in this study.

Autoantibody studies. We analyzed the plasma samples obtained from September 1994 to December 2001, which had been stored at –20°C until analysis. Autoantibodies were identified by RNA immunoprecipitation technique using HeLa cell extract as an antigen source[23].

Genomic DNA. Genomic DNA from patients and healthy individuals was purified from peripheral blood leukocytes using a standard phenol-chloroform extraction procedure or the QIAamp blood kit (Qiagen, Hilden, Germany).

HLA typing. The HLA-DRB1 genotype was determined using a polymerase chain reaction (PCR) restriction fragment length polymorphism method[24].

TNF promoter genotyping. TNF promoter allele formed by SNP at –1031, –863, and –857 was determined using the sequence-specific oligonucleotide probing followed by melting-curve analysis in a real-time PCR machine (LightCyclerTM, Roche Diagnostics, Mannheim, Germany) based on fluorescence resonance energy transfer technology. The detailed genotyping method is described in a previous report[25].

Statistical analysis. For the comparison of genotypes, allele carrier frequencies (homozygotes and heterozygotes combined) were compared. Statistical significance of the differences between groups was determined by chi-square analysis or Fisher's exact probability test. Corrected p (pc) values were obtained by multiplying the observed p values by the number of alleles examined; namely, 23 for HLA-DRB1, 12 for HLA-DR (serologic specificity), and 4 for TNF. The odds ratio (OR) with 95% confidence interval (95% CI) was calculated. To determine the sex differences in genetics, each allele carrier frequency was compared among patients and controls in each sex.

RESULTS

Autoantibody frequencies in different myositis syndromes. Frequencies of MSA and MAA in different forms of myositis are shown in Table 1. Among 120 patients with IIM, 42 (35%) were positive for MSA, including 37 (31%) for anti-ARS and 5 (4%) for anti-SRP autoantibody. Of the anti-ARS autoantibodies, anti-Jo-1 autoantibody was the most common (15%), and anti-EJ (9%) autoantibody was the second. Anti-SRP autoantibody was found exclusively in patients with PM.

MSA occurred more commonly in patients with PM (63%) than in those with DM [26%, p = 0.0004, OR 4.8 (95% CI 1.9–12.0)] or in patients with overlap disease [22%, p = 0.006, OR 6.0 (95% CI 1.6–23.0)]. Anti-ARS autoantibodies were more common in PM (47%) versus DM [26%, p = 0.046, OR 2.4 (95% CI 1.0–5.9)]. Anti-Jo-1 was present in 27% of patients with PM versus 11% with DM [p = 0.049, OR 2.9 (95% CI 0.98–8.7)]. Six DM patients, 4 PM, and one with overlap disease were positive for anti-EJ autoantibody.

Among 36 patients with anti-ARS autoantibodies, 28 (78%) had complicating ILD, while 36 (46%) of the remaining 79 antibody-negative patients had this pulmonary complication [p = 0.001, OR 4.2 (95% CI 1.7–10.3)].

Table 1. Autoantibody frequencies in Japanese patients with IIM. Values are the number (%) of patients with autoantibodies.

Autoantibody	All IIM, n = 120	DM, n = 72	PM, n = 30	Overlap, n = 18
Myositis-specific				
Anti-aminoacyl-tRNA synthetases	37 (31)	19 (26)	14 (47)*	4 (22)
Anti-Jo-1	18 (15)	8 (11)	8 (27)**	2 (11)
Anti-EJ	11 (9)	6 (8)	4 (13)	1 (6)
Anti-PL-7	4 (3)	2 (3)	2 (7)	0
Anti-PL-12	3 (3)	3 (4)	0	0
Anti-OJ	1 (1)	0	0	1 (6)
Anti-SRP	5 (4)	0	5 (17)	0
Any of above	42 (35)	19 (26)	19 (63)†	4 (22)
Myositis-associated				
Anti-tRNA	1 (1)	0	0	1 (6)
Ro (SSA)	21 (18)	11 (15)	8 (27)	2 (11)
U1-RNP	11 (9)	1 (1)	1 (3)	9 (50)††
U2-RNP	1 (1)	0	0	1 (6)
La (SSB)	1 (1)	1 (1)	0	0
Ku	1 (1)	0	0	1 (6)

IIM: idiopathic inflammatory myopathy; DM: dermatomyositis; PM: polymyositis; Overlap: overlap disease patients. * p = 0.046, OR 2.4 (95% CI 1.0–5.9) vs DM; ** p = 0.049, OR 2.9 (95% CI 0.98–8.7) vs DM; † p = 0.0004, OR 4.8 (95% CI 1.9–12.0) vs DM, p = 0.006, OR 6.0 (95% CI 1.6–23.0) vs Overlap; †† $p = 4 \times 10^{-9}$, OR 71 (95% CI 8.0–627.6) vs DM, p = 0.0001, OR 29 (95% CI 3.2–261.0) vs PM.

Among myositis-associated autoantibodies, anti-Ro (SSA) was most commonly found in myositis patients (18%), while its frequency did not differ significantly among the 3 groups. In contrast, anti-U1 RNP autoantibody frequency was significantly increased in overlap disease patients (50%) compared with DM [$p = 4.4 \times 10^{-9}$, OR 71 (95% CI 8.0–627.6)] or PM [p = 0.0001, OR 29 (95% CI 3.2–261.0)]. All patients with anti-U1 RNP autoantibody were women.

HLA-DRB1 alleles in patients with myositis. Among all IIM patients, allele carrier frequency of HLA-DRB1*0803 was significantly increased [p = 0.02, pc NS, OR 1.9 (95% CI 1.1–3.2)] as compared with controls (Table 2). When DRB1*0802 and 0803 were combined, the association with all IIM remained significant [p = 0.02, pc NS, OR 1.8 (95% CI 1.1–3.0)]. DRB1*1302 was significantly decreased in all IIM patients [p = 0.01, pc NS, OR 0.4 (95% CI 0.21–0.85)] compared with control patients (Table 2).

Carrier frequency of DRB1*0101 was significantly increased in patients with overlap disease [p = 0.002, pc NS, OR 4.6 (95% CI 1.6–13.3)] compared with control patients (Table 2). Although DRB1*0803 appeared increased in both DM and PM groups compared with controls (Table 2), the difference was significant only in the PM group [p = 0.006, pc NS, OR 3.1 (95% CI 1.3–7.1)]. When DRB1*0802 and *0803 alleles were combined, a significant increase was observed in PM compared with controls [p = 0.02, pc NS, OR 2.5 (95% CI 1.2–5.6)]. The associations of HLA-DRB1*08 or *0803 with IIM or PM, which we previously reported[1], were confirmed in this study with a larger number of patients.

Carrier frequency of the HLA-DRB1 first hypervariable region sequence (9EYSTS13), shared by DR3, DR5, DR6, and DR8 alleles, reported as candidate epitope for IIM[26], was not different between all IIM patients and controls.

HLA-DRB1 alleles in autoantibody subsets of myositis. Carrier frequency of DRB1*0405 was increased in patients with anti-ARS autoantibodies compared with controls [p = 0.04, pc NS, OR 2.1 (95% CI 1.0–4.3)] (Table 2). HLA-DRB1*0802 [p = 0.047, pc NS, OR 2.6 (95% CI 0.98–7.2)] and *0803 [p = 0.04, pc NS, OR 2.3 (95% CI 1.0–5.1)] were increased in the patients with anti-ARS autoantibody compared with controls (Table 2). The frequency of HLA-DRB1*1502 was significantly decreased in patients with anti-ARS autoantibody compared with controls [p = 0.03, pc NS, OR 0.22 (95% CI 0.05–0.96)] and MSA negative patients [p = 0.007, pc NS, OR 0.16 (95% CI 0.034–0.70)] (Table 2). The frequency of 9EYSTS13 carriers was not significantly different among MSA negative patients, the patients with anti-ARS autoantibodies, and controls (Table 2).

TNF promoter haplotypes in myositis patients and autoantibody subsets of myositis. Four TNF haplotypes (U01, U02, U03, and U04) were present (Table 3). No significant differences in TNF haplotype distribution were observed between IIM patients and controls, or among patients with PM, DM, or overlap disease.

HLA-DRB1 and TNF promoter genotypes with and without

Table 2. Carrier frequencies of *HLA-DRB1* alleles in Japanese IIM patients and controls. Values are the number (%) of subjects carrying each *HLA-DRB1* allele (homozygotes and heterozygotes combined).

HLA–DRB1	Clinical Subsets				Antibody Subsets		
	All IIM, n = 120	DM, n = 72	PM, n = 30	Overlap, n = 18	MSA (–), n = 78	ARS (+), n = 37	Controls, n = 265
*0101	21 (18)	12 (17)	3 (10)	6 (33)***	15 (19)	6 (16)	26 (10)
*0401	1 (1)	1 (1)	0	0	1 (1)	0	6 (2)
*0403	6 (5)	3 (4)	2 (7)	1 (6)	3 (4)	3 (8)	13 (5)
*0405	35 (29)	24 (33)	6 (20)	5 (28)	19 (24)	15 (41)[#]	65 (25)
*0406	7 (6)	5 (7)	1 (3)	1 (6)	5 (6)	2 (5)	19 (7)
*0407	0	0	0	0	0	0	6 (2)
*0410	4 (3)	2 (3)	2 (7)	0	3 (4)	1 (3)	5 (2)
*0802	11 (9)	8 (11)	2 (7)	1 (6)	5 (6)	6 (16)[§]	18 (7)
*0803	28 (23)*	14 (19)	10 (33)[†]	4 (22)	16 (21)	10 (27)[§§]	37 (14)
*0901	29 (24)	15 (21)	6 (20)	8 (44)	19 (24)	10 (27)	77 (29)
*1101	1 (1)	0	1 (3)	0	0	1 (3)	5 (2)
*1201	5 (4)	5 (7)	0	0	2 (3)	3 (8)	19 (7)
*1202	5 (4)	3 (4)	2 (7)	0	3 (4)	2 (5)	11 (4)
*1302	11 (9)**	8 (11)	3 (10)	0	8 (10)	2 (5)	51 (19)
*1401	8 (7)	7 (10)	1 (3)	0	7 (9)	1 (3)	13 (5)
*1403	5 (4)	4 (6)	1 (3)	0	4 (5)	1 (3)	10 (4)
*1405	4 (3)	2 (3)	1 (3)	0	2 (3)	1 (3)	17 (6)
*1406	2 (2)	2 (3)	0	0	2 (3)	0	7 (3)
*1501	18 (15)	8 (11)	6 (20)	4 (22)	12 (15)	4 (11)	29 (11)
*1502	26 (22)	17 (24)	5 (17)	4 (22)	21 (27)	2 (5)[##]	54 (20)
*1602	4 (3)	1 (1)	2 (7)	1 (6)	3 (4)	1 (3)	8 (3)
Others	2 (2)	0	0	0	2 (3)	0	13 (5)
9EYSTS13	70 (58)	46 (64)	20 (67)	5 (28)[††]	43 (55)	24 (65)	161 (61)

MSA: myositis-specific autoantibody; ARS: anti-aminoacyl-tRNA synthetase autoantibody. * p = 0.02, p_c NS, OR 1.9 (95% CI 1.1–3.2) vs controls; ** p = 0.01, p_c NS, OR 0.4 (95% CI 0.21–0.85); *** p = 0.002, p_c NS, OR 4.6 (95% CI 1.6–13.3) vs controls; [†] p = 0.006, p_c NS, OR 3.1 (95% CI 1.3–7.1) vs controls; [††] p = 0.006, OR 0.25 (95% CI 0.086–0.72) vs controls; p = 0.0006, OR 0.22 (95% CI 0.07–0.68) vs DM; p = 0.009, OR 0.19 (95% CI 0.053–0.69) vs PM; [#] p = 0.04, p_c NS, OR 2.1 (95% CI 1.0–4.3) vs controls; [§] p = 0.047, p_c NS, OR 2.6 (95% CI 0.98–7.2) vs controls; [§§] p = 0.04, p_c NS, OR 2.3 (95% CI 1.0–5.1) vs controls; [##] p = 0.03, p_c NS, OR 0.22 (95% CI 0.05–0.96) vs controls; p = 0.007, p_c NS, OR 0.16 (95% CI 0.034–0.70) vs MSA negative patients.

Table 3. Carrier frequency of *TNF* promoter genotypes in Japanese patients with IIM and controls. Values are number (%) of patients carrying each *TNF* allele.

TNF	Clinical Subsets				Antibody Subsets		
	All IIM, n = 120	DM, n = 72	PM, n = 30	Overlap, n = 18	MSA (–), n = 77	ARS (+), n = 37	Controls, n = 265
U01	103 (86)	60 (83)	26 (87)	17 (94)	68 (88)	29 (78)	232 (88)
U02	40 (33)	26 (36)	9 (30)	5 (28)	23 (30)	14 (38)	83 (31)
U03	30 (25)	20 (28)	6 (20)	4 (22)	19 (25)	11 (30)	69 (26)
U04	7 (6)	3 (4)	2 (7)	2 (11)	3 (4)	2 (5)	14 (5)

MSA: myositis-specific autoantibody; ARS: anti-aminoacyl-tRNA synthetase autoantibody.

ILD. No significant association was observed between HLA-DRB1 and the presence or absence of ILD (Table 4). When the TNF promoter was examined, carrier frequency of TNF-U03 was found to be increased in patients with ILD as compared with those without ILD [33% vs 18%, p = 0.05, pc NS, OR 2.3 (95% CI 0.94–5.5)].

Analysis of sex effect. The sex distributions were not significantly different among DM, PM, and overlap disease patients. To examine the sex-specific effects, the frequencies of HLA-DRB1 and TNF alleles were compared between patients and controls in each sex separately (Table 5). When the HLA-DRB1 carrier frequencies were compared, DRB1*0101 was increased in female patients with IIM compared with female controls [p = 0.05, pc NS, OR 2.1 (95% CI 0.99–4.4)]. DRB1*0405 was increased in male IIM patients [p = 0.02, pc NS, OR 2.6 (95% CI 1.1–5.8)] and

Table 4. Carrier frequencies of *HLA-DRB1* and *TNF* promoter genotypes in Japanese IIM patients with and without interstitial lung disease (ILD). Values are the number (%) of patients.

Genotype	ILD (+), n = 64	ILD (–), n = 51	p	OR (95% CI)
*HLA-DRB1*0405*	20 (31)	11 (22)	NS	—
*HLA-DRB1*0803*	14 (22)	13 (25)	NS	—
TNF-U01	55 (86)	44 (86)	NS	—
TNF-U02	19 (30)	17 (33)	NS	—
TNF-U03	21 (33)	9 (18)	0.05	2.3 (0.94–5.5)
TNF-U04	2 (3)	4 (8)	NS	—

NS: not significant.

male IIM patients without MSA [p = 0.05, pc NS, OR 2.5 (95% CI 0.98–6.5)] compared with male controls. DRB1*0803 was increased in both male and female IIM patients compared with individual control subjects. The carrier frequency was significantly increased in male patients with IIM without MSA [p = 0.045, pc NS, OR 2.7 (95% CI 0.99–7.5)] and female IIM patients with anti-ARS autoantibody [p = 0.04, pc NS, OR 2.8 (95% CI 1.0–7.5)] compared with individual controls. The distributions of TNF haplotypes were not significantly different between patients and controls even when male and female patients were analyzed separately (data not shown).

DISCUSSION

The number of patients analyzed in our study is the largest among the Japanese IIM studies examining autoantibodies and HLA-DRB1. The distribution of autoantibody subsets in our series was similar to that in a previous Japanese study examining 91 IIM patients[27]. Anti-EJ was the second most common MSA and seemed to be more common in our series than in those reported in Caucasians[5]. Anti-EJ was associated with PM as well as DM in our series, whereas others found that this autoantibody was associated with DM[28,29]. Ohsone, et al also reported that 4 out of 13 Japanese patients with anti-ARS autoantibodies had anti-EJ, and anti-EJ was the second most common anti-ARS autoantibody. We did not examine anti-Mi-2 autoantibody in this study because this autoantibody has been reported to be very rare (0%) in Japanese IIM patients[5].

In this study, our previous findings of an association of HLA-DRB1*08 and *0803 with Japanese IIM (n = 84)[1] were confirmed by analyzing a larger number of patients (n = 120). We also found significant associations of HLA-DRB1*08 and *0803 with anti-ARS autoantibodies. Since the most common MSA are directed against ARS, these 2 results are consistent. The genetic risk factors for IIM and MSA appear to be different between Japanese (DR8,DRB1*0803) and Caucasians (DR3,DRB1*0301)[3].

DR8 alleles are reportedly increased in African-American IIM patients[5], Hispanic SLE patients[30], and Korean autoimmune thyroiditis patients[31]. Both SLE[30] and autoimmune thyroiditis[32] have been reported to be associated with DR3 in Caucasians. Thus, DR8 alleles might be related to the susceptibility to IIM, SLE, and autoimmune thyroiditis in the ethnic groups in which DR3 alleles are rare. It was suggested that DR8 gene was generated by a gene contraction event in a primordial DR52 haplotype (DR3, DR11, to DR14)[33]. DR3 and DR8 nucleotide sequences have been reported to be similar at introns 4 and 5[34] as well as the 5' end[33]. These shared sequences between DR3 and DR8 may be related to the pathogenesis of IIM.

Arnett, *et al*[5] reported that DR2 alleles (DRB1*15 alleles) were decreased in Caucasian and African-American patients with PM. Also, we found that DRB1*1502 was significantly decreased in Japanese patients with anti-ARS autoantibodies compared with controls. Thus, Japanese patients with IIM may share some common features of DRB1 alleles with other ethnic groups.

We found an association between DRB1*0405 and anti-ARS autoantibodies in Japanese IIM patients. And the frequency of HLA-DRB1*0405 seemed to be increased in DM patients compared with controls; however, the difference was not statistically significant. Horiki, et al reported a significant association between Japanese PM patients with ILD and DRB1*0405[6]; however, our current study was not able to confirm this. HLA-DRB1*0405 was associated with anti-ARS autoantibodies in our present study. Since 28 (78%) patients with anti-ARS autoantibodies had complicating ILD, it may be possible that the association reported

Table 5. Carrier frequencies of *HLA-DRB1* alleles in Japanese IIM patients and controls by sex. Values are the number (%) of patients.

HLA-DRB1	Male				Female			
	All IIM, n = 30	MSA (–), n = 21	ARS (+), n = 8	Controls, n = 148	All IIM, n = 90	MSA (–), n = 57	ARS (+), n = 29	Controls, n = 116
*0101	1 (3)	0	1 (13)	12 (8)	20 (33)*	15 (26)	5 (17)	14 (12)
*0405	13 (43)**	9 (43)***	4 (50)	34 (23)	22 (24)	9 (16)	11 (38)	31 (27)
*0803	9 (30)	7 (33)†	2 (25)	23 (16)	19 (21)	9 (16)	8 (28)††	14 (12)

MSA: myositis-specific autoantibody; ARS: anti-aminoacyl-tRNA synthetase autoantibody. * p = 0.05, p_c NS, OR 2.1 (95% CI 0.99–4.4); ** p = 0.02, p_c NS, OR 2.6 (95% CI 1.1–5.8); *** p = 0.05, p_c NS, OR 2.5 (95% CI 0.98–6.5); † p = 0.045, p_c NS, OR 2.7 (95% CI 0.99–7.5); †† p = 0.04, p_c NS, OR 2.8 (95% CI 1.0–7.5).

by Horiki, *et al* was primarily due to the association with anti-ARS autoantibodies. In Japanese, DRB1*0405 allele is associated with rheumatoid arthritis[35], Crohn's disease[14], arthritis, and rheumatoid factor in systemic sclerosis[36]. These results suggest a similar genetic background among these autoimmune diseases in Japanese.

Since the HLA-DQA1*0501 or *0401 allele was reported to be commonly increased in Caucasian and African-American patients with IIM, while different DRB1 alleles were associated with the disease in each ethnic population, Arnett, *et al* suggested that susceptibility to IIM may be localized in the DQA1 locus[5]. However, we[1] and others[5,6] did not observe that the susceptibility to IIM was primarily associated with the DQA1 locus in the Japanese. We also examined distribution of MSA in 83 IIM patients for whom DQA1 alleles were analyzed in our previous study and found no significant association of MSA with DQA1 alleles (data not shown).

We found an association of TNF-U03 with ILD in this study. Previous studies concerning the promoter activity of TNF-U03 are conflicting, i.e., increased[12], unchanged[37], or decreased[38]. TNF is considered to have a disease-promoting effect for ILD[39,40] and some reports suggest that blockade of TNF may be beneficial for ILD[41]. TNF-U03 was recently reported to show a higher binding to the transcriptional factor OCT-1, compared with the common TNF-U01 allele[42].

Our results suggest that the association of HLA-DRB1*0101 and HLA-DRB1*0405 with IIM may be influenced by sex. Although previous IIM studies have not showed any sex differences in genetics, we[43] and others[44,45] have reported significant sex influences on modifier loci in other autoimmune diseases. Our data suggest that the association of some loci with IIM may be influenced by sex.

We confirmed in this study our previous findings of immunogenetic features of Japanese patients with IIM. Further studies of non-HLA as well as HLA genes are needed for determining the genetic contribution of the susceptibility and the pathogenesis of IIM.

ACNOWLEDGMENT

We thank Dr. R.L. Wilder for critical review of the manuscript.

REFERENCES

1. Furuya T, Hakoda M, Higami K, et al. Association of HLA class I and class II alleles with myositis in Japanese patients. J Rheumatol 1998;25:1109-14.
2. Love LA, Leff RL, Fraser DD, et al. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine Baltimore 1991;70:360-74.
3. Shamim EA, Rider LG, Miller FW. Update on the genetics of the idiopathic inflammatory myopathies. Curr Opin Rheumatol 2000;12:482-91.
4. Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T. Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In: Sasazuki T, editor. HLA 1991: Proceedings of the Eleventh International Histocompatibility Workshop and Conference. Oxford: Oxford University Press; 1992.
5. Arnett FC, Targoff IN, Mimori T, Goldstein R, Warner NB, Reveille JD. Interrelationship of major histocompatibility complex class II alleles and autoantibodies in four ethnic groups with various forms of myositis. Arthritis Rheum 1996;39:1507-18.
6. Horiki T, Ichikawa Y, Moriuchi J, et al. HLA class II haplotypes associated with pulmonary interstitial lesions of polymyositis/dermatomyositis in Japanese patients. Tissue Antigens 2002;59:25-30.
7. Eigler A, Sinha B, Hartmann G, Endres S. Taming TNF: strategies to restrain this proinflammatory cytokine. Immunol Today 1997;18:487-92.
8. Hengstman GJ, van den Hoogen FH, Barrera P, et al. Successful treatment of dermatomyositis and polymyositis with anti-tumor-necrosis-factor-alpha: preliminary observations. Eur Neurol 2003;50:10-5.
9. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 1997;94:3195-9.
10. Pachman LM, Fedczyna TO, Lechman TS, Lutz J. Juvenile dermatomyositis: the association of the TNF alpha-308A allele and disease chronicity. Curr Rheumatol Rep 2001;3:379-86.
11. Werth VP, Callen JP, Ang G, Sullivan KE. Associations of tumor necrosis factor alpha and HLA polymorphisms with adult dermatomyositis: implications for a unique pathogenesis. J Invest Dermatol 2002;119:617-20.
12. Higuchi T, Seki N, Kamizono S, et al. Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. Tissue Antigens 1998;51:605-12.
13. Matsushita M, Tsuchiya N, Nakayama T, et al. Allele typing of human TNFA 5'-flanking region using polymerase chain reaction-preferential homoduplex formation assay (PCR-PHFA): linkage disequilibrium with HLA class I and class II genes in Japanese. Tissue Antigens 1999;54:478-84.
14. Kawasaki A, Tsuchiya N, Hagiwara K, Takazoe M, Tokunaga K. Independent contribution of HLA-DRB1 and TNF alpha promoter polymorphisms to the susceptibility to Crohn's disease. Genes Immun 2000;1:351-7.
15. Noguchi E, Yokouchi Y, Shibasaki M, et al. Association between TNFA polymorphism and the development of asthma in the Japanese population. Am J Respir Crit Care Med 2002;166:43-6.
16. Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975;292:403-7.
17. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344-7.
18. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 1980;23:581-90.
19. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
20. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
21. Shibue T, Tsuchiya N, Komata T, et al. Tumor necrosis factor alpha 5'-flanking region, tumor necrosis factor receptor II, and HLA-DRB1 polymorphisms in Japanese patients with rheumatoid arthritis. Arthritis Rheum 2000;43:753-7.
22. Tokunaga K, Imanishi T, Takahashi K, Juji T. On the origin and dispersal of East Asian populations as viewed from HLA haplotypes. In: Szathmary EJ, editor. Prehistoric Mongoloid

dispersals. Oxford: Oxford University Press; 1996:187-97.
23. Hirakata M, Suwa A, Nagai S, et al. Anti-KS: identification of autoantibodies to asparaginyl-transfer RNA synthetase associated with interstitial lung disease. J Immunol 1999;162:2315-20.
24. Uryu N, Maeda M, Ota M, Tsuji K, Inoko H. A simple and rapid method for HLA-DRB and -DQB typing by digestion of PCR-amplified DNA with allele specific restriction endonucleases. Tissue Antigens 1990;35:20-31.
25. Tsuchiya N, Kawasaki A, Tsao BP, Komata T, Grossman JM, Tokunaga K. Analysis of the association of HLA-DRB1, TNF alpha promoter and TNFR2 (TNFRSF1B) polymorphisms with SLE using transmission disequilibrium test. Genes Immun 2001;2:317-22.
26. Goldstein R, Duvic M, Targoff IN, et al. HLA-D region genes associated with autoantibody responses to histidyl-transfer RNA synthetase (Jo-1) and other translation-related factors in myositis. Arthritis Rheum 1990;33:1240-8.
27. 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.
28. Hirakata M, Suwa A, Takeda Y, et al. Autoantibodies to glycyl-transfer RNA synthetase in myositis. Association with dermatomyositis and immunologic heterogeneity. Arthritis Rheum 1996;39:146-51.
29. Targoff IN. Autoantibodies to aminoacyl-transfer RNA synthetases for isoleucine and glycine. Two additional synthetases are antigenic in myositis. J Immunol 1990;144:1737-43.
30. Reveille JD, Moulds JM, Ahn C, et al. Systemic lupus erythematosus in three ethnic groups: I. The effects of HLA class II, C4, and CR1 alleles, socioeconomic factors, and ethnicity at disease onset. LUMINA Study Group. Lupus in minority populations, nature versus nurture. Arthritis Rheum 1998;41:1161-72.
31. Cho BY, Chung JH, Shong YK, et al. A strong association between thyrotropin receptor-blocking antibody-positive atrophic autoimmune thyroiditis and HLA-DR8 and HLA-DQB1*0302 in Koreans. J Clin Endocrinol Metab 1993;77:611-5.
32. Tandon N, Zhang L, Weetman AP. HLA associations with Hashimoto's thyroiditis. Clin Endocrinol Oxford 1991;34:383-6.
33. Andersson G, Lindblom B, Andersson L, Gorski J, Mach B, Rask L. The single DR beta gene of the DRw8 haplotype is closely related to the DR beta 3III gene encoding DRw52. Immunogenetics 1988;28:1-5.
34. Svensson AC, Setterblad N, Sigurdardottir S, Rask L, Andersson G. Primate DRB genes from the DR3 and DR8 haplotypes contain ERV9 LTR elements at identical positions. Immunogenetics 1995;41:74-82.
35. Higami K, Hakoda M, Matsuda Y, Ueda H, Kashiwazaki S. Lack of association of HLA-DRB1 genotype with radiologic progression in Japanese patients with early rheumatoid arthritis. Arthritis Rheum 1997;40:2241-7.
36. Kuwana M, Inoko H, Kameda H, et al. Association of human leukocyte antigen class II genes with autoantibody profiles, but not with disease susceptibility in Japanese patients with systemic sclerosis. Intern Med 1999;38:336-44.
37. Uglialoro AM, Turbay D, Pesavento PA, et al. Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-alpha gene promoter. Tissue Antigens 1998; 52:359-67.
38. Skoog T, van't Hooft FM, Kallin B, et al. A common functional polymorphism (C→A substitution at position -863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. Hum Mol Genet 1999;8:1443-9.
39. Miyazaki Y, Araki K, Vesin C, et al. Expression of a tumor necrosis factor-alpha transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. J Clin Invest 1995;96:250-9.
40. Piguet PF, Collart MA, Grau GE, Sappino AP, Vassalli P. Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. Nature 1990;344:245-7.
41. Vassallo R, Matteson E, Thomas CF Jr. Clinical response of rheumatoid arthritis-associated pulmonary fibrosis to tumor necrosis factor-alpha inhibition. Chest 2002;122:1093-6.
42. Hohjoh H, Tokunaga K. Allele-specific binding of the ubiquitous transcription factor OCT-1 to the functional single nucleotide polymorphism (SNP) sites in the tumor necrosis factor-alpha gene (TNFA) promoter. Genes Immun 2001;2:105-9.
43. Furuya T, Salstrom JL, McCall-Vining S, et al. Genetic dissection of a rat model for rheumatoid arthritis: significant gender influences on autosomal modifier loci. Hum Mol Genet 2000;9:2241-50.
44. del Rincon I, Battafarano DF, Arroyo RA, Murphy FT, Escalante A. Heterogeneity between men and women in the influence of the HLA-DRB1 shared epitope on the clinical expression of rheumatoid arthritis. Arthritis Rheum 2002;46:1480-8.
45. Meyer JM, Han J, Moxley G. Tumor necrosis factor markers show sex-influenced association with rheumatoid arthritis. Arthritis Rheum 2001;44:286-95.