Association of HLA-DR, DQ Genotype With Different β-Cell Functions at IDDM Diagnosis in Japanese Children

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Japanese IDDM patients have been demonstrated to have unique and different HLA associations from white patients. To elucidate the effect of HLA-associated genetic factors on the clinical heterogeneity of IDDM in Japanese people, HLA-DRB1, DQA1, and DQB1 genotypes in 88 childhood-onset Japanese IDDM patients were examined by polymerase chain reaction–sequence-specific oligonucleotide (PCR-SSO) or sequence-specific primers (SSP). Of the 88 IDDM patients, 26 (29.5%) had DRB1*0405-DQA1*0302-DQB1*0401/X (DR4-DQ4/X), 38 (43.2%) had DRB1*0901-DQA1*0302-DQB1*0303/X (DR9-DQ9/X), and 9 (10.2%) were DR4/9-DQ4/9 heterozygous in the present study (X does not contain protective alleles). Clinical heterogeneity such as age distribution at onset, prevalence and serum level of anti-GAD antibodies (GADAb), and residual pancreatic β-cell function after diagnosis were compared between patients with HLA-DR4-DQ4 and DR9-DQ9. The frequency of DR9-DQ9 genotype was significantly higher in the younger (0–10 years) than in the older (11–16 years) age-group of onset, but the frequency of DR4-DQ4 was higher in the older (11–16 years) age-group. Although no association of DR-DQ genotypes with the prevalence and serum level of GADAb was found among newly diagnosed patients, long-standing DR9-DQ9 patients had significantly higher levels of GADAb than those with DR4-DQ4. While no difference in time course of serum C-peptide (CPR) levels was detected between GADAb$^+$ and GADAb$^-$ patients, a remarkable difference was demonstrated between DR9-DQ9 and DR4-DQ4 patients. The residual pancreatic β-cell function was retained more in patients with DR4-DQ4 than in those with DR9-DQ9 at diagnosis through 12–18 months after diagnosis. These results suggest that the DR9-DQ9 genotype may induce stronger autoimmune destructive response (T-helper 1 function) against target β-cells than the DR4-DQ4 genotype does. Our findings may warrant further studies on the association of diabetogenic autoimmune response with HLA class II molecules and contribute to a clarification of interracial differences in HLA-encoded susceptibility to IDDM. *Diabetes* 46:1893–1897, 1997

IDDM is caused by the selective autoimmune destruction of pancreatic β-cells. Both genetic and environmental factors are considered to play a critical role in the pathogenesis of IDDM. Major genetic factors are related to HLA. Recently, studies at the gene level of HLA-DR, DQ molecules have elucidated the HLA associations with the disease more precisely. Japanese IDDM patients have been demonstrated to have unique and different HLA associations from white patients (1–5). Among white patients, HLA-DQB1*0302 and *0201 are positively associated with IDDM, and the amino acid residue 57 (non-Asp) of the DQβ chain is particularly closely associated with susceptibility to IDDM (5–8). In contrast, HLA-DQB1*0303 and *0401, which encode Asp at position 57, are strongly associated with susceptibility to IDDM in Japanese (1–4). In addition, HLA-DRB1*0901 and *0405 have been reported to contribute to the disease only in Japanese subjects, but not in white subjects (4,5).

Some aspects of HLA-associated heterogeneity in the clinical characteristics of IDDM have been reported in white subjects (9–17). However, such studies concerning Japanese patients have been limited (18–20). Kida et al. (18) reported that HLA-DRw9/X (X: not DR4) increased in Japanese IDDM patients who were positive for organ-specific autoantibodies other than islet cell antibodies (ICAs), whereas DR4/X (X: not DRw9) increased in those without autoantibodies. Recently, Kobayashi et al. (20) divided Japanese IDDM patients into groups based on the interval from clinical onset to initiation of insulin therapy, characterizing slowly progressive IDDM (interval, ≥13 months), most of whom were adult-onset (age of onset, >16 years). Kobayashi et al. demonstrated that only HLA-DR4 and DQA1*0301-DQB1*0401 were associated with the slowly progressive group, while three diabetogenic HLA-DQ haplotypes, DQA1*0301-DQB1*0401, DQA1*0301-DQB1*0302, and DQA1*0301-DQB1*0303, were associated with the acute clinical onset group (interval, <3 months).

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CPR, C-peptide; GADAb, anti-GAD antibody; PCR, polymerase chain reaction; RIA, radioimmunoassay; SSO sequence-specific oligonucleotide; SSP, sequence-specific primers; TCR, T-cell receptor; Th, T-helper.

The aim of this study was to determine whether unique HLA-DR, DQ genotypes in Japanese IDDM subjects are associated with any clinical heterogeneity of childhood-onset IDDM. Age distribution at onset, the prevalence and serum level of anti-GAD antibodies (GADAb), and residual pancreatic β-cell function after diagnosis were focused on as the main features of clinical heterogeneity. The present study demonstrated interesting differences in clinical characteristics between patients with HLA-DRB1*0901-DQA1*0302-DQB1*0303 (DR9-DQ9) and DRB1 *0405-DQA1*0302-DQB1*0401 (DR4-DQ4).

RESEARCH DESIGN AND METHODS

Patients. Subjects with IDDM diagnosed according to the criteria of the World Health Organization (WHO) study group (21) were recruited from the Department of Pediatrics, Chiba University School of Medicine and Chiba Children's Hospital. The study group consisted of 88 childhood-onset Japanese patients with IDDM. Their age at onset ranged from 8 months to 16 years (33 male, 55 female; age 2–28 years). Informed consent was obtained from each subject or their parents, and blood samples were taken for DNA isolation and determination of GADAb and C-peptide (CPR) concentrations.

Determination of GADAb. The autoantibodies to GAD were measured by anti-GAD radioimmunoassay (RIA) kit, RIP Anti-GAD Hoechst (Tokyo, Japan). The assay procedure has been reported in detail (22–25). GAD was purified from fresh pig brain and was found to contain two isoforms (65 and 67 kDa). Positive reference serum was defined as containing 256 U at a dilution of 1:60. Sera were considered positive for GADAb if they contained ≥5 U/ml of antibody. The specificity and sensitivity of this assay were recently confirmed to be high at the 1st and 2nd GADAb workshops (26,27).

Determination of HLA genotypes. HLA-DRB1 and DQB1 types were defined by DNA analysis using polymerase chain reaction (PCR) combined with dot-blot hybridization with sequence-specific oligonucleotide (SSO) probes, and HLA-DQA1 types were defined by the PCR–sequence-specific primers (SSP) method. PCR-SSO typing was performed according to the Eleventh International HLA Workshop (11th IHW) protocol (28). DNA panels assayed at the 11th IHW were used in this study. Details of HLA typing procedures used in this study have been reported previously (29–31). The control gene frequencies of HLA-DRB1 and DQB1 in Japanese subjects were referred to in the previous report (32).

Assessment of residual pancreatic β-cell function. The time course of endogenous insulin secretion after insulin therapy was assessed in 34 newly diagnosed IDDM patients by determination of fasting and postprandial serum CPR concentrations. Blood specimens for CPR and blood glucose were drawn after an overnight fast and 2 h after a calorie- and component-controlled breakfast. Total calorie intake was determined by the patient's age, sex, and physical activity. Blood samples were taken first at 1–6 months, and subsequently at 12–18 months and at 24–36 months after the initiation of insulin therapy during visits to the hospital. Serum CPR concentration was determined by RIA with C-Peptide Kit "Daiichi" III (Daiichi Radioisotope, Tokyo, Japan). The lowest CPR concentration (sensitivity) of this kit is 0.03 ng/ml.

Statistical analysis. The χ^2 test or Fisher's exact probability test was used to determine the statistical significance of differences between group frequencies. The unpaired Student's t test was used to compare the levels of GADAb where appropriate, and Mann-Whitney's U test was used to compare the serum levels of CPR among different groups. Results are expressed as mean ± SE.

RESULTS

HLA-DRB1 and HLA-DQB1 allele frequencies of 88 IDDM patients and 608 control subjects are shown in Table 1. Gene frequencies of DRB1*0405, *0901 and DQB1*0303, *0401 were significantly higher in IDDM patients than in control subjects. In contrast, gene frequencies of DRB1*0803, *1501 or *1502 and DQB1*0301, *0601 or *0602 were significantly lower in IDDM patients. Further, 26 (29.5%) of the IDDM patients had DRB1*0405-DQA1*0302-DQB1*0401/X (DR4-DQ4/X), 38 (43.2%) had DRB1*0901-DQA1*0302-DQB1*0303/X (DR9-DQ9/X), and 9 (10.2%) were DR4/9-DQ4/9 heterozygous in the present study (X does not contain the protective alleles, DRB1*0803, *1501 or *1502 and DQB1*0301, *0601 or *0602) (Table 2). These results are compatible with previous studies on HLA genotypes in Japanese IDDM patients (1–5).

TABLE 1
Distribution of HLA-DRB1 and DQB1 alleles among Japanese IDDM children and control subjects

HLA allele	IDDM (n = 176)	Control (n = 1216)	Odds ratio	Pc values
DRB1				
*0405	41 (23.3)	161 (13.3)	1.99	$P < 10^{-3}$
*0901	67 (38.1)	171 (14.1)	3.76	$P < 10^{-5}$
*0803	2 (1.1)	100 (8.3)	0.13	$P < 10^{-2}$
*1501	2 (1.1)	86 (7.1)	0.15	$P < 10^{-2}$
*1502	3 (1.7)	122 (10.1)	0.16	$P < 10^{-3}$
Others	61 (34.7)	576 (47.4)		
DQB1				
*0303	70 (39.8)	181 (14.9)	3.78	$P < 10^{-5}$
*0401	41 (23.3)	158 (13)	2.03	$P < 10^{-3}$
*0301	7 (4.0)	141 (11.6)	0.32	$P < 10^{-2}$
*0601	5 (2.8)	220 (18.1)	0.13	$P < 10^{-5}$
*0602	1 (0.6)	75 (6.2)	0.09	$P < 10^{-2}$
Others	52 (29.5)	441 (36.3)		

Data are n of chromosomes (%).

To clarify the association of HLA-DR, DQ genotypes with age at onset, the 88 patients were subdivided into three groups by onset age; 0–5 years (n = 28), 6–10 years (n = 31), and 11–16 years (n = 29) (Table 2). The frequency of DR9-DQ9 genotype was significantly higher ($P < 0.05$) in the two younger (0–10 years) than in the older (11–16 years) onset group. On the contrary, the frequency of DR4-DQ4 was higher ($P < 0.05$) in the older (11–16 years) group (Table 2).

The prevalence of GADAb in IDDM patients with different HLA genotypes is shown in Table 3. A total of 63.4% of 41 newly diagnosed IDDM and 44.6% of long-standing IDDM patients were positive for GADAb. All of three recent-onset DR4/9-DQ4/9 patients were positive for GADAb. However, no significant association of DR-DQ genotype with the prevalence of GADAb was found among both newly diagnosed and long-standing patients.

GADAb titers in sera were compared among GADAb$^+$ IDDM patients with DR9-DQ9 and DR4-DQ4. Among newly diagnosed patients, there was no significant difference in GADAb titers (DR9-DQ9, mean 50 U/ml, range 5–13,300, n = 10; DR4-DQ4, mean 22 U/ml, range 7–207, n = 8). In contrast, GADAb titers were significantly higher in long-standing (duration of disease, 2.3–14 years) DR9-DQ9 patients (mean 150 U/ml, range 5–27,110, n = 12) than in long-standing (duration of disease, 2.2–14 years) DR4-DQ4 patients (mean 17 U/ml, range 7–106, n = 6, $P < 0.05$).

We analyzed residual pancreatic β-cell function in IDDM patients by measuring serum CPR concentrations before and 2 h after breakfast during 1–6 months, 12–18 months, and 24–36 months after the initiation of insulin therapy in a prospective study. It was notable that serum CPR levels were quite similar between the GADAb$^+$ (n = 20) and GADAb$^-$ (n = 15) IDDM patients; overnight fasting CPR levels, 0.85 ± 0.22 vs. 0.70 ± 0.12 at 1–6 months, 0.58 ± 0.18 vs. 0.55 ± 0.23 at 12–18 months, 0.35 ± 0.11 vs. 0.31 ± 0.16 ng/ml at 24–36 months; postprandial CPR levels, 1.98 ± 0.45 vs. 1.66 ± 0.28 at 1–6 months, 1.21 ± 0.45 vs. 1.35 ± 0.52 at 12–18 months, 0.74 ± 0.26 vs. 0.51 ± 0.19 ng/ml at 24–36 months. In contrast,

TABLE 2
Distribution of HLA class II genotypes by different age-groups at onset

	Total IDDM 0–16 years	Age at onset (years) 0–5	6–10	11–16
n	88	28	31	29
HLA genotypes				
DR4-DQ4/X	26 (29.5)	7 (25.0)	6 (19.4)	13 (44.8)*
DR9-DQ9/X	38 (43.2)	15 (53.6)	15 (48.4)	8 (27.6)*
DR4/9-DQ4/9	9 (10 2)	3 (10 7)	2 (6.5)	4 (13.8)
Others	15 (17.0)	3 (10 7)	8 (25 8)	4 (13 8)

Data are *n* (%). Alleles of DR4-DQ4 are DRB1*0401-DQA1*0302-DQB1*0405. Alleles of DR9-DQ9 are DRB1*0901-DQA1*0302-DQB1*0303. X does not contain protective genotypes described in Table 1. *$P < 0.05$ vs. IDDM children with age at onset, 0–10 years (sum of two age-groups, 0–5 and 6–10).

serum CPR levels were significantly lower in DR9-DQ9 patients ($n = 20$) than in DR4-DQ4 patients ($n = 14$) during these three periods after insulin therapy; overnight fasting CPR levels, 0.31 ± 0.07 vs. 0.98 ± 0.26 at 1–6 months ($P < 0.05$), 0.14 ± 0.04 vs. 0.62 ± 0.20 at 12–18 months ($P < 0.05$), 0.14 ± 0.05 vs. 0.52 ± 0.12 ng/ml at 24–36 months ($P < 0.05$); postprandial CPR levels, 0.97 ± 0.17 vs. 2.71 ± 0.56 at 1–6 months ($P < 0.005$), 0.44 ± 0.09 vs. 1.50 ± 0.53 at 12– 18 months ($P < 0.05$), 0.30 ± 0.07 vs. 0.90 ± 0.31 ng/ml at 24–36 months (NS) (Fig. 1). There was no significant difference in HbA_{1c} level between DR9-DQ9 and DR4-DQ4 patients throughout the periods of follow-up (data not shown).

DISCUSSION

HLA-associated heterogeneity in the clinical characteristics of IDDM has been demonstrated in white subjects (9–17). For example, a lower prevalence of DR3/DR4 heterozygotes has been shown in adult-onset IDDM compared with childhood-onset (9,10). The relation of DR4 to more severe symptoms and ketoacidosis at diagnosis has been demonstrated (11–13), in contrast to the association of DR3 with a more slowly progressing form (13). Younger age at diagnosis and decreased serum CPR levels shortly after diagnosis have been described in Dw3/Dw4 heterozygotes (14,15) and DQB1*0302/*0201 heterozygotes (16). Moreover, Serjeantson et al. (17) reported that among Australians heterozygous for HLA-DR3/DR4, 85% were positive for antibodies to GAD, significantly different from the prevalence of 48% in patients with at least one HLA-DR antigen other than DR3 or DR4.

The polymorphism of HLA molecules has been considered to affect the autoimmune response in several ways, including influencing the binding capacity of antigenic peptides and the repertoire of T-cell receptors (TCR) of responding autoreactive T-cells. HLA class II molecules are expressed on antigen-presenting cells such as macrophages and dendritic cells, B-cells, and activated T-cells, and have critical roles for the activation of helper T-cells (mainly $CD4^+$ T-cells) in the periphery. Antigenic peptides are presented for T-cells in the grooves of HLA class II molecules consisting of α and β chains, and their binding capacity to each class II molecule depends on the amino acid sequence (motif) of the peptide (33). In addition, HLA class II antigens have critical roles for positive and negative selection of immature T-cells in the thymus.

Japanese IDDM patients have been demonstrated to have unique and different HLA association from white patients (1–5). In the present study, childhood-onset IDDM patients were subdivided into two groups based on their HLA genotypes as genetic background, HLA-DRB1*0901-DQA1*0302-DQB1*0303 (DR9-DQ9) and DRB1*0405-DQA1*0302-DQB1*0401 (DR4-DQ4). Both of these HLA haplotypes are unique to Japanese IDDM patients and are the same as those reported previously except for the DQA1 genotype. DQA1*0302 was shown to be associated with IDDM patients in the present study, whereas DQA1*0301 was reported to be associated with Japanese IDDM patients in previous reports (1,3–5). This discrepancy was probably due to a difference in typing methods employed for DQA1 genotype; DQA1*0301 typed in previous reports likely contained both DQA1*0301 and *0302, which are different only at one nucleotide in the third exon. DQA1*0302 codes for a different amino acid (Asp) at position 160 compared with Ala coded by DQA1*0301 (34).

The objective of this study was to clarify the genetic association of HLA with the clinical heterogeneity of Japanese IDDM patients. This study revealed some interesting differences in clinical characteristics between IDDM children with HLA-DR9-DQ9 and those with DR4-DQ4. Although no association of DR-DQ genotypes with the prevalence of GADAb was found among newly diagnosed patients, long-standing DR9-DQ9 patients had significantly higher levels of GADAb than those with DR4-DQ4. Residual pancreatic β-cell function was assessed mainly by a serum CPR level 2 h after breakfast in this study. A postprandial serum CPR level of <2.0 ng/ml was considered to indicate the lack of endogenous insulin secretion, because such a level was detected in 40 (90.9%) of

TABLE 3
The prevalence of GADAb in IDDM patients with different DR-DQ genotype

HLA genotype	Recent-onset (<6 months)	Long-standing (>2 years)
Total	41 (63.4)	56 (44.6)
DR4-DQ4/X	14 (57.1)	14 (50)
DR9-DQ9/X	16 (62.5)	28 (42.9)
DR4/9-DQ4/9	3 (100)	8 (50)
Others	8 (62.5)	6 (33.3)

Data are *n* (GADAb prevalence %). X does not contain protective genotypes described in Table 1.

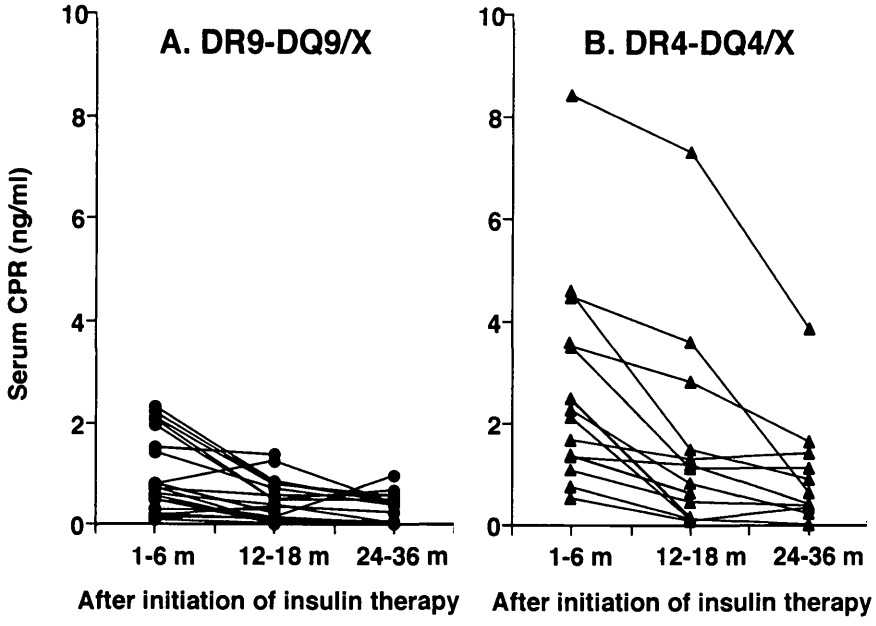

FIG. 1. HLA DR-DQ genotypes and time course of residual pancreatic β-cell function. Blood specimens for CPR were drawn after an overnight fast and 2 h after a calorie- and component-controlled breakfast. Significant differences in postprandial serum CPR were detected between IDDM patients with DRB1*0901-DQA1*0302-DQB1*0303 (DR9-DQ9, *n* = 20; *A*) and DRB1*0405-DQA1*0302-DQB1*0401 (DR4-DQ4, *n* = 14; *B*) at 1–6 m ($P < 0.005$) and 12–18 m ($P < 0.05$).

44 patients, and <1.0 ng/ml was detected in 33 (75.0%) of 44 IDDM patients at 24–36 months after the initiation of insulin therapy; the CPR levels of NIDDM patients were usually >3.0 ng/ml in our hospital (data not shown). IDDM children with HLA-DR9-DQ9 (n = 20) were demonstrated to lose residual pancreatic β-cell function more drastically at diagnosis through 12–18 months after the initiation of insulin therapy than those with DR4-DQ4 (n = 14). Our sample size might be too small to draw any valid conclusion regarding HLA association with pancreatic β-cell destruction. However, the results obtained from the present study suggest that HLA class II genes may induce clinical heterogeneity of IDDM through autoimmune responses, such as the production of autoantibodies and generation of cytotoxic activities against pancreatic β-cells, and may contribute to further studies in other ethnic populations as well as Japanese populations.

Evidence suggesting a pathogenic role for the T-helper 1 (Th1) cells in IDDM has recently been provided by studies of patients (35) and nonobese diabetic (NOD) mice, a spontaneous model of human type 1 diabetes (36,37). $CD4^+$ T-helper (Th) cells, upon antigenic stimulation, differentiate into two distinct subpopulations, each producing its own set of cytokines and mediating separate effector functions (38,39). Th1 cells produce IL-2, tumor necrosis factor β (TNFβ), and IFNγ, thereby activating $CD8^+$ cytotoxic T-cells, macrophages, and inducing delayed-type hypersensitivity (DTH) responses. Th2 cells produce IL-4, IL-5, and IL-10, stimulating production of mast cells, eosinophils, and immunoglobulin G1 (IgG1) and IgE antibodies and possibly suppressing cell-mediated immunity. Therefore, predominance of Th1 cells in IDDM patients may lead to more rapid destruction of autoimmune target cells, pancreatic β-cells. Several factors, including the major histocompatibility complex (MHC) class II haplotype (40), have been demonstrated to influence the differentiation of naive $CD4^+$ T-cells into a specific Th subset (37). Our findings suggest that HLA-DR9-DQ9 might induce a more drastic Th1 response against pancreatic β-cells than DR4-DQ4. Further studies including analysis of IgG subclasses of autoantibodies and cytokine production against β-cells by peripheral T-cells will need to be performed to address these issues.

There is one more interesting point to note. Our findings suggest that the HLA-associated genetic effect by DRB1*0901-DQA1*0302-DQB1*0303 (DR9-DQ9) in Japanese children appeared to be similar to that by DR3/DR4 heterozygotes or by DQB1*0302/*0201 heterozygotes reported in white IDDM patients (14–16). The binding of antigenic peptide to HLA-DR or DQ molecule and the recognition of the antigen-HLA complex by TCR depends on the tertiary structure of the HLA molecule. It may be interesting to determine whether HLA class II molecules in white patients with DR3/DR4 or DQB1*0302/*0201 heterozygotes have physicochemical characters and immunological functions similar to Japanese patients with HLA-DR9-DQ9.

In conclusion, the results in the present study suggest that the DR9-DQ9 genotype may induce stronger autoimmune destructive response (Th1 response) against target β-cells than DR4-DQ4 genotype in Japanese childhood-onset IDDM. Thus, when beginning clinical trials with new treatment to halt the loss of insulin-producing β-cells during the early course of the disease or to prevent the onset of IDDM in Japanese children, we may have to pay particular attention to the HLA genotype when dividing the patients into study groups. In addition, these new findings may help to address the pathogenesis in Japanese IDDM patients at the genetic level, as it appears completely different from white patients.

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