Original Article

Combination of HLA-A24, -DQA1*03, and -DR9 Contributes to Acute-Onset and Early Complete β-Cell Destruction in Type 1 Diabetes

Longitudinal Study of Residual β-Cell Function

Koji Nakanishi[1,2] and Hidetoshi Inoko[3]

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/6/1862/660880/zdb00606001862.pdf by guest on 21 May 2024

To elucidate the genetic factors contributing to heterogeneity of the rate of β-cell destruction in type 1 diabetes, we investigated the relationship between the time course of complete β-cell loss and HLA class I and II alleles. HLA allele frequencies were also examined among subgroups classified by the mode of onset. The subjects were 266 type 1 diabetic patients (among whom 196 patients were studied longitudinally) and 136 normal control subjects. Earlier complete loss of β-cell function was observed in patients who possessed both HLA-A24 and HLA-DQA1*03 and in patients who had HLA-DR9, compared with those without these HLA alleles (P = 0.0057 and 0.0093, respectively). Much earlier complete β-cell loss was observed in the patients who possessed all of HLA-A24, -DQA1*03, and -DR9 compared with the remaining patients (P = 0.0011). The combination of HLA-A24, -DQA1*03, and -DR9 showed a higher frequency in acute-onset than slow-onset type 1 diabetes (P = 0.0002). In contrast, HLA-DR2 was associated with a slower rate of progression to complete β-cell loss. These results indicate that the combination of HLA-A24, -DQA1*03, and -DR9 contributes to the acute-onset and early complete β-cell destruction, whereas HLA-DR2 has a protective effect against complete β-cell loss in type 1 diabetes. *Diabetes* 55:1862–1868, 2006

The temporal profile of β-cell destruction in type 1 diabetes is heterogeneous, which leads to heterogeneity in the mode of onset (1) and to variations of a substantial residual β-cell mass in type 1 diabetes (2,3). According to the recent classification by the American Diabetes Association (ADA) and the World Health Organization, type 1 diabetes is divided into type 1A diabetes (immune mediated) and type 1B diabetes (idiopathic) (1,4). Type 1A diabetes includes the classical case subjects with the acute onset of hyperglycemia and ketosis or ketoacidosis and patients who progress slowly through a non–insulin-dependent stage to an insulin-dependent state with persistent islet autoantibodies (latent autoimmune diabetes of adults [LADA]) (1,5,6). Other patients who show the sudden onset of hyperglycemia with ketosis or ketoacidosis plus a rise of pancreatic exocrine enzymes have been reported as one form of potential type 1B diabetes (fulminant type 1 diabetes) (7). Such heterogeneity in the mode of onset is thought to result from the variations in the rate of β-cell destruction up to the time of insulin dependency (1). Even after insulin becomes necessary, the level of residual β-cell function is also heterogeneous (2,3,8,9). Some patients lose β-cells completely soon after the onset, whereas others retain minute residual β-cells over a long period (2,3). These findings all suggest that the natural course of β-cell destruction is heterogeneous in type 1 diabetes.

Susceptibility to type 1 diabetes is conferred by multiple genes (10), among which the HLA class II region has the strongest influence on the susceptibility to this disease (10). In the Japanese population, the DRB1*0405-DQA1*03-DQB1*0401 or DQB1*0302, DRB1*0901-DQA1*03-DQB1*0303, and DRB1*0802-DQA1*03-DQB1*0302 haplotypes are positively associated with type 1 diabetes, whereas the DRB1*1501-DQA1*0102-DQB1*0602 and DRB1*1502-DQA1*0103-DQB1*0601 haplotypes have a negative association (11,12). There is also evidence suggesting that the HLA class I region is another susceptibility locus for type 1 diabetes in humans (13–17) and mice (18,19). Regarding genetic influences on the extent of β-cell destruction, our previous cross-sectional study demonstrated a strong association between HLA-A24 (A*2402) and complete β-cell destruction in patients with type 1 diabetes (2,13). To better elucidate the genetic factors that regulate the natural course of β-cell destruction in type 1 diabetes, however, longitudinal observation of β-cell function is necessary.

Because the mode of onset of type 1 diabetes (acute, slow, or fulminant) is dependent on the rate of β-cell loss until the occurrence of insulin dependency, it could be hypothesized that the mode of onset of diabetes is also closely related to the rate of β-cell destruction during the entire clinical course of type 1 diabetes. Based on this

From the [1]Department of Endocrinology and Metabolism, Toranomon Hospital, Tokyo, Japan; the [2]Okinaka Memorial Institute for Medical Research, Tokyo, Japan; and the [3]Department of Molecular Life Science, Division of Molecular Medical Science and Molecular Medicine, Tokai University Graduate School of Medicine, Kanagawa, Japan.

Address correspondence and reprint requests to Koji Nakanishi, MD, PhD, Department of Endocrinology and Metabolism, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. E-mail: koji01@toranomon.gr.jp.

Received for publication 16 August 2005 and accepted in revised form 27 February 2006.

ADA, American Diabetes Association; CPR, C-peptide response; LADA, latent autoimmune diabetes of adults; RFLP, restriction fragment–length polymorphism.

DOI: 10.2337/db05-1049

© 2006 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

assumption, we first tried to identify the HLA class I or class II alleles that contribute to early complete β-cell destruction by longitudinal observation of residual β-cell function. Then we evaluated the relationships between such HLA class I or class II alleles and the mode of onset of type 1 diabetes. As a result, we identified specific combination of HLA class I and class II alleles that contributed to acute and early β-cell destruction in type 1 diabetes.

RESEARCH DESIGN AND METHODS

The subjects consisted of 266 patients with type 1 diabetes, including 151 men and 115 women aged 34 ± 14 years (means ± SD) at the onset of diabetes, and 136 normal control subjects. The relationship between the mode of onset of diabetes and each HLA allele was analyzed in these subjects. Our longitudinal investigation of residual β-cell function was designed as a historical cohort study (20), and the onset of diabetes was defined as the starting point. To determine the temporal profile of β-cell destruction as precisely as possible, 70 patients in whom the first assessment of residual β-cell function revealed complete β-cell loss at >5 years after the onset of diabetes were excluded from the longitudinal study, because the time of complete β-cell loss was unknown over the 5-year period in these patients. By setting this 5-year limit, the error in determining the time of complete β-cell destruction was reduced to less than about one-tenth (<5 years) of the longest observation period (0.08–48.0 years; median 10 years). As a result, 196 patients with type 1 diabetes, including 115 men and 81 women aged 36 ± 14 years (means ± SD) at the onset of diabetes, were included in the longitudinal study. Some of these patients have been reported previously (2,13,21). All of the patients were Japanese residents of the Tokyo and Yokohama areas. A diagnosis of type 1 diabetes was made according to the ADA guidelines (1). In addition, daily urinary C-peptide excretion of <6.6 nmol/day or an integrated serum C-peptide value during the 100-g oral glucose tolerance test of <3.3 nmol/l was used to define type 1 diabetes, as described previously (2,6,13,21). Autoantibodies to GAD were positive at the onset of diabetes in 82.6% (123 of 149) of our patients. This study was approved by the Committee for Investigations Involving Human Subjects of Toranomon Hospital. All patients gave informed consent before enrollment in the study.

Evaluation of residual β-cell function. A sensitive C-peptide radioimmunoassay was used to assess residual β-cell function (8). We have previously defined a serum C-peptide response (CPR) of <0.033 nmol/l after a 100-g oral glucose load (ΔCPR) as indicating complete β-cell destruction (2,21). To allow more convenient assessment of residual β-cell function over time, a fasting serum C-peptide level below the detection limit (0.017 nmol/l) or a level <0.033 nmol/l at 2–3 h postprandially was also used as a criterion indicating complete β-cell destruction in this study. The time of 2–3 h postprandially was selected because the maximal serum C-peptide level was measured at this point in type 1 diabetic patients undergoing a 100-g oral glucose tolerance test (8). The relationships between these three serum C-peptide criteria for complete β-cell loss were analyzed in 57 of the 266 type 1 diabetic patients.

Assessment of residual β-cell function was done as follows. In 169 patients, the first measurement of serum C-peptide was performed at 5.9 ± 7.9 years (means ± SD) (median 2 years; range 0.08–37 years) after the onset of diabetes, and serum C-peptide was measured a total of 5.1 ± 2.9 times (median 10 times; range 2–14 times) during a disease duration of 13.3 ± 10.5 years (median 10 years; range 0.1–48 years). Complete β-cell loss that occurred among these 169 patients was confirmed by the next measurement of serum C-peptide in all (n = 54) but two patients. In 16 patients, complete β-cell loss was detected by the first assessment of β-cell function performed at 1.1 ± 0.33 years (median 0.45 years; range 0.08–4 years) after the onset of diabetes. In 13 of these 16 patients, complete β-cell loss was confirmed by the next measurement of serum C-peptide. In the four patients who underwent serum C-peptide measurement a total of three times, complete β-cell loss was observed >5 years (median 8 years; range 6–14 years) after the detection of residual β-cell function by the second assessment. Based on the above-mentioned 5-year limit, these patients were censored at the last time when residual β-cell function was detected because time of complete β-cell loss was unknown over the 5-year period. In seven patients, the presence of residual β-cell function was only investigated by one test at 4–24 years (median 14 years) after the onset of diabetes.

HLA typing. HLA-DR and -DQ alleles were typed by the previously described PCR–restriction fragment–length polymorphism (RFLP) methods (22–24). HLA-A, -C, -B, and -DR antigens were typed by the microcytotoxicity test (25), except in 38 patients for whom only HLA-A alleles were determined by PCR-RFLP method (26). In 91 patients, serological data of HLA-A alleles were confirmed by PCR-RFLP method. The DRB1-DQA1-DQB1 haplotype was established based on known linkage disequilibrium data (27).

Definition of the subtypes of type 1 diabetes. According to the time from the diagnosis of diabetes to the start of insulin therapy, we divided the patients into those with acute-onset (≤12 months) type 1 diabetes (n = 138) and slow-onset (>12 months) type 1 diabetes (n = 99), as described previously (28,29). By reviewing the medical records, we detected patients who developed ketosis or ketoacidosis within 1 week of the onset of hyperglycemic symptoms and had a concomitant rise of pancreatic exocrine enzymes at the onset, and we classified such patients as having fulminant type 1 diabetes (n = 11) (7). Eighteen patients could not be allocated to a subgroup because their medical records were incomplete.

Statistical analysis. The Kaplan-Meier method was used to estimate the cumulative incidence of complete β-cell destruction, and differences between the cumulative incidence curves were assessed by the log-rank test (30). Person-time incidence rates were expressed as the number of events per 100 patient-years based on the ratio of the observed number of events to the total number of patient-years of exposure. Cox's proportional hazards model (30) was used to examine the combined influence of the variables on the risk of complete β-cell loss in type 1 diabetes. The Mann-Whitney U test was used for unpaired data. Differences of frequency in two or three groups were assessed by Fisher's exact probability test or the contingency χ^2 test, respectively. To calculate corrected P values, the raw values were multiplied by the number of alleles examined at each locus. Results are expressed as means ± SD. Statistical significance was defined as $P < 0.05$. All analyses were performed with the Stat View 4.02J statistical package and Survival Tools for StatView (Abacus Concepts, Berkeley, CA).

RESULTS

Validity of serum C-peptide criteria for assessing complete β-cell loss. The correspondence between judgment of complete β-cell destruction based on ΔCPR versus the fasting serum C-peptide level or based on ΔCPR versus serum C-peptide level at 2–3 h postprandially was 84.2% (48 of 57) and 93.0% (54 of 57), respectively.

Relationship between the temporal profile of complete β-cell loss and HLA class I or II alleles

HLA-A24 and HLA-DQA1*03. Because the combination of HLA-A24 and HLA-DQA1*03 was associated with accelerated β-cell destruction in a previous cross-sectional study (13), we first examined the effect of this allele combination on the temporal profile of complete β-cell loss. Among 177 patients who could be typed for the HLA-A and -DQA1 loci, all but 1 (99.4%) had HLA-A24 and/or HLA-DQA1*03, which showed a higher prevalence than in the normal control subjects (86.8%, 118 of 136, $P <$ 0.0001). The cumulative incidence of complete β-cell loss differed among the patients with both HLA-A24 and HLA-DQA1*03 (n = 98), those with HLA-DQA1*03 only (n = 59), and those with HLA-A24 only (n = 19) (4.22, 2.37, and 0.90 per 100 patient-years, respectively, P = 0.012) (Fig. 1*A*). Patients who possessed both HLA-A24 and HLA-DQA1*03 (n = 98) showed earlier complete loss of β-cell function than the remaining patients (n = 79) (4.22 vs. 1.89 per 100 patient-years, P = 0.0057). Among 146 type 1 diabetic patients with HLA-DQA1*03, there were 225 DQ haplotypes involving DQA1*03, which had the following breakdown: 82 (36.4%) were DQA1*03-DQB1*0303, 88 (39.1%) were DQA1*03-DQB1*0401, 44 (20.0%) were DQA1*03-DQB1*0302, 6 (2.7%) were DQA1*03-DQB1*0402, and 5 (2.2%) were DQA1*03-DQB1*0301. All of the HLA-A24 alleles typed at the DNA level (n = 75) were A*2402.

HLA-DR9. Patients with HLA-DR9 (DRB1*0901) (n = 69) showed earlier complete loss of β-cell function than those without HLA-DR9 (n = 116) (4.53 vs. 2.27 per 100 patient-years, P = 0.0093) (Fig. 1*B*). Homozygotes for HLA-DR9 (n = 26) showed earlier complete β-cell loss than the remaining patients (n = 170) (P = 0.0004) and earlier loss

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/6/1862/660880/zdb00606001862.pdf by guest on 21 May 2024

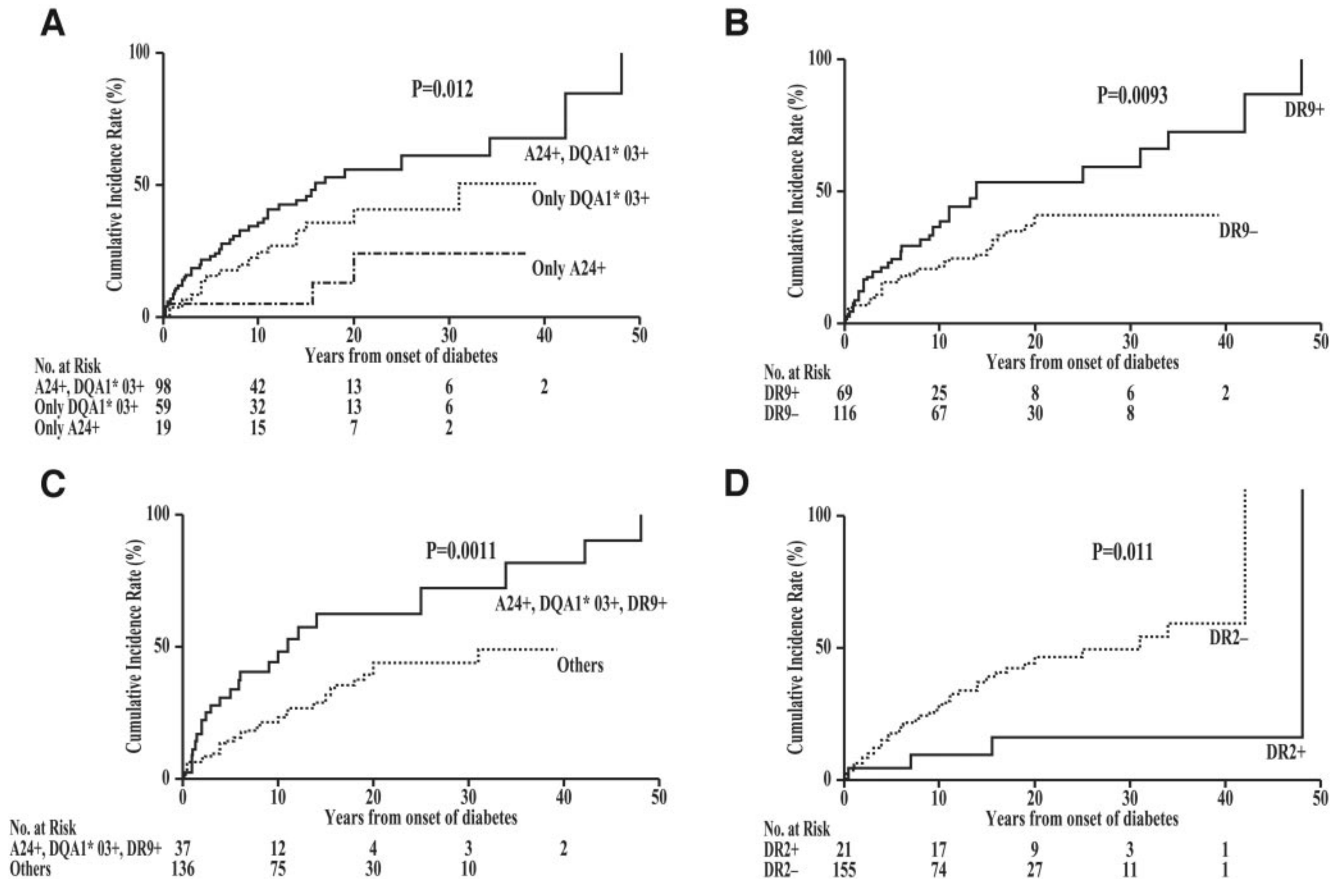

FIG. 1. Cumulative incidence rates of complete β-cell destruction in type 1 diabetic patients with both HLA-A24 and HLA-DQA1*03, those with HLA-DQA1*03 only, and those with HLA-A24 only (*A*); those with or without HLA-DR9 (*B*); those with the three-allele combination of HLA-A24, HLA-DQA1*03, and HLA-DR9 and those without this allele combination (*C*); and those with or without HLA-DR2 (*D*). For analysis of the influence of HLA-DR2 on β-cell destruction (*D*), patients with fulminant type 1 diabetes were excluded.

than in heterozygotes for HLA-DR9 (n = 42) (P = 0.023) (7.46, 2.51, and 3.28 per 100 patient-years, respectively). A higher cumulative incidence of complete β-cell loss was also observed in patients with the DQA1*03-DQB1*0303 haplotype (n = 60), which is in linkage disequilibrium with HLA-DR9 (27), compared with those without this haplotype (n = 103) (4.33 vs. 2.55 per 100 patient-years, P = 0.0497). The frequency of HLA-A24 plus HLA-DQA1*03 did not differ between patients with HLA-DR9 (57.4%, 39 of 68) and patients without HLA-DR9 (55.1%, 59 of 107) (P = 0.88).

HLA-A24, HLA-DQA1*03, and HLA-DR9. There were no other HLA alleles or haplotypes that showed an association with earlier complete β-cell loss, including HLA-A24 (P = 0.17), HLA-DQA1*03 (P = 0.11), HLA-A2 (P = 0.33), HLA-B54 (P = 0.49), HLA-DR4 (P = 0.80), DQA1*03-DQB1*0302 (P = 0.31), DQA1*03-DQB1*0401 (P = 0.78), and other alleles or haplotypes (HLA-A11, -A26, -A31, -A33, -B35, -B39, -B44, -B52, -B60, -B61, -B62, -Cw1, -Cw3, -Cw4, -Cw7, -DR1, -DR6, -DR8, and DQA1*0102-DQB1*0604 [data not shown]). Accordingly, the combined effect of HLA-A24, HLA-DQA1*03, and HLA-DR9 was examined. The patients who possessed all of HLA-A24, HLA-DQA1*03, and HLA-DR9 (n = 37) showed complete loss of β-cell function much earlier than those who did not have this three-allele combination (n = 136) (6.19 vs. 2.45 per 100 patient-years, P = 0.0011) (Fig. 1*C*).

HLA-DR2. In contrast, the incidence of complete β-cell destruction tended to be lower in patients with HLA-DR2 (n = 26) than in those without HLA-DR2 (n = 159), but the difference was not significant (1.75 vs. 3.31 per 100 patient-years, P = 0.077). When we excluded patients with fulminant type 1 diabetes (n = 9) from the analysis, possession of HLA-DR2 (n = 21) was associated with a lower rate of progression to complete β-cell loss than in patients without HLA-DR2 (n = 155) (0.95 vs. 3.08 per 100 patient-years, P = 0.011) (Fig. *1D*). Among the 21 patients with HLA-DR2, 10 patients had the DRB1*1501-DQA1*0102-DQB1*0602 haplotype, 10 patients had the DRB1*1502-DQA1*0103-DQB1*0601 haplotype, and 1 patient had the DRB1*1501-QA1*05-DQB1*0301 haplotype. The frequency of HLA-A24 plus HLA-DQA1*03, HLA-DR9 alone, and the combination of HLA-A24, HLA-DQA1*03, and HLA-DR9 did not differ between the patients with or without HLA-DR2 (data not shown).

Clinical characteristics and HLA alleles. Clinical characteristics, including sex distribution, age at onset of diabetes, BMI at onset, and time until insulin therapy, were compared between patients with or without the above-mentioned HLA alleles or allele combinations. Patients with both HLA-A24 and HLA-DQA1*03 had an older age at onset (38.5 ± 14.2 years) than the remaining patients (32.7 ± 12.1 year, P = 0.0084). The time until insulin therapy was shorter in patients with the combination of HLA-A24, HLA-DQA1*03, and HLA-DR9 (10.5 ± 16.2 months) than in the remaining patients (27.5 ± 47.2 months, P = 0.014). There were no differences in the clinical characteristics in other comparisons.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/6/1862/660880/zdb00606001862.pdf by guest on 21 May 2024

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/6/1862/660880/zdb00606001862.pdf by guest on 21 May 2024

Mode of onset and HLA alleles. Complete β-cell destruction occurred in the order of fulminant (n = 10), acute-onset (n = 97), and slow-onset type 1 diabetes (n = 85) (15.88, 3.93, and 1.71 per 100 patient-years, respectively, $P < 0.0001$). Therefore, the frequencies of HLA alleles, allele combinations, and haplotypes that influenced complete β-cell destruction were investigated to search for genetic factors influencing the mode of onset by including the patients without longitudinal data on residual β-cell function (Table 1).

Although differences in the frequency of HLA-A24 did not reach significance among the three subgroups of patients (P = 0.051), its frequency was higher in acute-onset type 1 diabetes than in slow-onset type 1 diabetes (P = 0.0023). For the combination of HLA-A24 plus HLA-DQA1*03, the frequency differed among the three groups (P = 0.016) and was higher in acute-onset than slow-onset type 1 diabetes (P = 0.0068). Furthermore, when the combination of HLA-A24, HLA-DQA1*03, and HLA-DR9 was analyzed, the difference in the frequency of this three-allele combination among the three groups became striking (P = 0.0005). Frequency of this three-allele combination was also strikingly higher in acute-onset than slow-onset type 1 diabetes (P = 0.0002). Neither the frequency of patients with HLA-DR9 nor that of homozygote for HLA-DR9 showed a significant difference among or between the subgroups of patients. In comparison with normal control subjects, the alleles or allele combinations that showed an increase in patients with acute-onset type 1 diabetes were HLA-A24 plus HLA-DQA1*03 (68.5% [85 of 124] vs. 41.2% [56 of 136], $P <$ 0.0001), HLA-DR9 alone (44.8% [60 of 134] vs. 26.5% [36 of 136], P = 0.0022, Pc = 0.020), and the combination of HLA-A24, HLA-DQA1*03, and HLA-DR9 (35.8% [44 of 123] vs. 16.9% [23/136], P = 0.0006).

Although the frequency of HLA-DR2 also differed among the three subgroups of patients (P = 0.0027), it was higher in patients with fulminant type 1 diabetes than in those with acute-onset type 1 diabetes (P = 0.0005) or slow-onset type 1 diabetes (P = 0.0058) (Table 1), whereas there was no significant difference from the frequency in normal control subjects (45.5% [5 of 11] vs. 30.9% [42 of 136], P = 0.33). The haplotype of DRB1*1501-DQA1*0102-DQB1*0602 also showed the same trends (Table 1).

Multivariate analysis. To identify variables making an independent contribution to complete β-cell loss in type 1 diabetes, Cox's proportional hazards model was applied to data from the longitudinal study of residual β-cell function in 196 patients. In this model, the response variable was the time until the occurrence of complete β-cell loss or the time until the final measurement of residual β-cell function. The covariates examined included the sex and possession of the combination of HLA-A24, HLA-DQA1*03, and HLA-DR9 as categorical variables and age at onset (range 8–73 years), BMI at onset (range 13.2–31.3 kg/m^2), and time from the onset of diabetes to initiation of insulin therapy (range 0–300 months) as continuous variables. In this model, significant independent risk factors were male sex (hazard ratio 1.87 [95% CI 1.08–3.24]; P = 0.026), age at onset (1.03 per 1-year increase [1.01–1.05]; P = 0.0009), the time from the onset of diabetes to initiation of insulin therapy (0.97 per 1-month increase [0.96–0.99]; P = 0.0006), and possession of the HLA-A24, HLA-DQA1*03, and HLA-DR9 combination (1.79 [1.04–3.08]; P = 0.036). When HLA-A24 plus HLA-DQA1*03, HLA-A24 alone,

TABLE 1
Frequencies of HLA alleles, allele combinations, and haplotypes in each subgroup of type 1 diabetic patients

HLA allele, allele combination, and haplotype	Fulminant type 1 diabetes (n = 11)	Acute-onset type 1 diabetes (n = 138)	Slow-onset type 1 diabetes (n = 99)	P value (Pc)			
				Among 3 groups	Fulminant vs. acute	Fulminant vs. slow	Acute vs. slow
A24	80.0 (8/10)	72.9 (97/133)	58.5 (55/94)	0.051	0.63	0.19	0.0023 (0.018)
A24 and DQA1*03	50.0 (5/10)	68.5 (85/124)	49.4 (44/89)	0.016	0.30	>0.99	0.0068
DR9	36.4 (4/11)	44.8 (60/134)	32.3 (30/93)	0.16	0.59	0.78	0.058
DR9/9	9.1 (1/11)	16.4 (22/134)	8.6 (8/93)	0.20	>0.99	>0.99	0.11
A24, DQA1*03, and DR9	20.0 (2/10)	35.8 (44/123)	12.6 (11/87)	0.0005	0.49	0.62	0.0002
DR2	45.5 (5/11)	9.7 (13/134)	12.9 (12/93)	0.0027 (0.024)	0.0005 (0.0045)	0.0058 (0.052)	0.45
DRB1*1501-DQA1*0102-DQB1*0602	27.3 (3/11)	4.3 (5/115)	4.8 (4/83)	0.0069	0.022	0.033	>0.99
DRB1*1502-DQA1*0103-DQB1*0601	18.2 (2/11)	5.2 (6/115)	7.2 (6/83)	0.25	0.15	0.24	0.56
DQA1*03	63.6 (7/11)	94.5 (120/127)	87.6 (78/89)	0.0022 (0.015)	0.0056 (0.039)	0.058	0.084

Data are n (%). The frequencies of HLA alleles, allele combinations, and haplotypes that affected β-cell destruction in the longitudinal study are shown. No other HLA alleles and haplotypes showed differences of distribution among or between the subgroups of type 1 diabetic patients. For calculation of percentages, the number of subjects who could be typed for the corresponding allele, allele combination, or haplotypes was used as the denominator. A corrected P value (Pc) is shown when the P value in multiple comparison was <0.05.

HLA-DR9 alone, or HLA-DR2 alone was incorporated into this model instead of the three-allele combination (HLA-A24, HLA-DQA1*03, and HLA-DR9), neither the two-allele combination nor the individual HLA alleles were a significant risk factor ($P = 0.061$, 0.33, 0.059, and 0.11, respectively).

DISCUSSION

In the present study, the HLA alleles that contributed to both early complete β-cell destruction and acute onset of diabetes were the two-allele combination of HLA-A24 and HLA-DQA1*03 and the three-allele combination of HLA-A24, HLA-DQA1*03, and HLA-DR9. The three-allele combination had a stronger influence than the two-allele combination. Multivariate analysis showed that only the three-allele combination of HLA-A24, HLA-DQA1*03, and HLA-DR9 was a significant independent risk factor for complete β-cell loss among the HLA alleles tested as covariates. Each HLA allele in this three-allele combination is already known as a risk allele for type 1 diabetes or for β-cell destruction. HLA-A24 (A*2402) is associated with complete β-cell destruction in type 1 diabetic patients who have other susceptible HLA alleles (2), whereas HLA-A24 and HLA-DQ haplotypes involving HLA-DQA1*03 accelerate β-cell destruction in an additive manner (13). In addition, not only HLA-DQ haplotypes involving HLA-DQA1*03 but also HLA-A24 itself contribute to susceptibility to type 1 diabetes (13–16). Besides the contribution of HLA-DR9 to susceptibility to type 1 diabetes (11,12), HLA-DR9-DQ9 was associated with lower serum C-peptide levels in type 1 diabetic patients compared with HLA-DR4-DQ4 over a relatively short disease duration (~3 years) (31). Although HLA-DR9, especially in homozygotes, contributed to early complete β-cell destruction in our longitudinal study, it did not discriminate acute-onset from slow-onset type 1 diabetes despite an increased frequency in acute-onset patients compared with normal control subjects, as reported previously (32). Taking these findings into consideration, our results indicate that a combination of HLA class I and class II alleles, each of which contributes to susceptibility to type 1 diabetes or promotes β-cell destruction, results in the further potentiation of β-cell destruction in type 1 diabetes.

The mechanism by which accumulation of specific HLA class I and class II (DR and DQ) alleles causes faster and more severe destruction of β-cells in type 1 diabetes remains unclear. MHC class II molecules on antigen-presenting cells present antigenic peptides to $CD4^+$ T-cells, whereas MHC class I molecules on target cells present antigenic peptides to $CD8^+$ T-cells (33). In addition, HLA-DR and -DQ molecules show functional complementarity in antigen presentation (34). In fact, the profiles of peptide eluted from HLA-DR and -DQ molecules after pulsing with islet antigens are different (35). Thus, the combination of specific HLA-A, -DR, and -DQ alleles may have synergic and complementary effects on various steps of the immune response. However, the frequency of this three-allele combination was only 36% in acute-onset type 1 diabetes, so there remains the possibility that non-HLA genes (10) or environmental factors such as viral infection (36) also promote β-cell destruction in type 1 diabetes.

Another finding of this study was the contrasting distribution of HLA-DR2 between type 1B (fulminant type 1) diabetes and type 1A (acute-onset and slow-onset type 1) diabetes. In fulminant type 1 diabetes, the frequency of HLA-DR2 was not lower than in normal control subjects and was thus higher than in acute-onset or slow-onset type 1 diabetes. Although our study population only included a small number of patients with fulminant type 1 diabetes, an increased frequency of HLA-DR2 in fulminant type 1 diabetes compared with acute-onset type 1 diabetes was also detected by a nationwide survey performed in Japan (32). This difference in the frequency of HLA-DR2 between fulminant type 1 diabetes and type 1A diabetes may be due to a different pathogenesis of the former (37). In contrast, HLA-DR2 was protective against early complete β-cell destruction in type 1A diabetes, a result that was consistent with the findings of our previous cross-sectional study (21).

As clinical parameters, male sex, an older age at onset, and shorter time until insulin therapy were independent risk factors for early complete β-cell destruction in type 1 diabetes. Male sex was previously shown to be a risk factor for insulin dependence in LADA (38), but the association of an older age at onset with early complete β-cell destruction contradicts the results of a previous large-scale study (3). This may have been due to a wide-ranging age of onset in our study population as a result of including patients with fulminant type 1 diabetes, which develops later than acute-onset type 1 diabetes (39). The higher age of onset in our patients compared with Caucasians may also be due to the inclusion of patients with LADA. A close relationship between the mode of onset of diabetes and the rate of β-cell destruction is consistent with our cross-sectional study that showed preservation of residual β-cell function in LADA compared with acute-onset type 1 diabetes (29). Because our patient population was limited to Japanese subjects, studies performed in other racial groups (including patients with fulminant type 1 diabetes or LADA) will be necessary to confirm the cumulative effect of HLA alleles presented in this study.

Although our study demonstrated the temporal profile of β-cell destruction in type 1 diabetes and detected an HLA allele combination that contributed to early and acute complete β-cell destruction, its retrospective design and one-point determination of serum C-peptide are limitations with respect to delineating longitudinal changes of residual β-cell function. Complete β-cell destruction could have occurred at an earlier time than when it was detected. The potential bias may also be introduced by the exclusion of the patients whose first C-peptide test revealed complete β-cell loss at >5 years after the onset of diabetes from the longitudinal study. Long-term prospective studies on residual β-cell function using the stimulated CPR would be preferable, as suggested by the ADA workshop report (40).

In summary, we demonstrated that a combination of specific HLA class I and class II alleles contributed to acute and early complete β-cell destruction in Japanese patients with type 1 diabetes.

ACKNOWLEDGMENTS

This work was partly supported by the Japanese Ministry of Education, Science, and Culture (Grant 14571117).

We thank Fumie Takano for her secretarial work.

Parts of this work were presented at the 64th scientific session of the American Diabetes Association, Orlando,

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/6/1862/660880/zdb00606001862.pdf by guest on 21 May 2024

Florida, 4–8 June 2004 and at the 41st annual meeting of the European Association for the Study of Diabetes, Athens, Greece, 12–15 September 2005.

REFERENCES

1. American Diabetes Association: Diagnosis and classification of diabetes mellitus. *Diabetes Care* 28 (Suppl. 1): S37–S42, 2005
2. Nakanishi K, Kobayashi T, Murase T, Nakatsuji T, Inoko H, Tsuji K, Kosaka K: Association of HLA-A24 with complete β-cell destruction in IDDM. *Diabetes* 42:1086–1093, 1993
3. The DCCT Research Group: Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual β-cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCCT). *J Clin Endcrinol Metab* 65:30–36, 1987
4. Alberti KGMM, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
5. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR: Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non–insulin-dependent onset of disease. *Diabetes* 42:359–362, 1993
6. Kobayashi T, Itoh T, Kosaka K, Sato K, Tsuji K: Time course of islet cell antibodies and β-cell function in non-insulin-dependent stage of type 1 diabetes mellitus. *Diabetes* 36:510–517, 1987
7. Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y, the Osaka IDDM Study Group: A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. *N Engl J Med* 342:301–307, 2000
8. Nakanishi K, Kobayashi T, Miyashita H, Ohkubo M, Sugimoto T, Murase T, Kosaka K, Inouye K, Kono M: Relationships among islet cell antibodies, residual β-cell function, and metabolic control in patients with insulin-dependent diabetes mellitus of long duration: use of a sensitive C-peptide radioimmunoassay. *Metabolism* 39:925–930, 1990
9. Schölin A, Björklund L, Borg H, Arnqvist H, Björk E, Blohmé G, Bolinder J, Eriksson JW, Gudbjörnsdottir S, Nyström L, Östman J, Karlsson AF, Sundkvist G: Islet antibodies and remaining β-cell function 8 years after diagnosis of diabetes in young adults: a prospective follow-up of the nationwide diabetes incidence study in Sweden. *J Internal Med* 255:384–391, 2004
10. Reijonen H, Concannon P: Genetics of type 1 diabetes. In *Joslin's Diabetes Mellitus.* 14th ed. Kahn CR, Weir GC, King GL, Jacobson AM, Moses AM, Smith RJ, Eds. Philadelphia, Lippincott Williams & Wilkins, 2005, p. 355–369
11. Rønningen KS, Spurkland A, Tait BD, Drummond B, Lopez-Larrea C, Baranda FS, Menendez-Diaz MJ, Caillat-Zucman S, Beaurain G, Garchon H-J, Ilonen J, Reijonen H, Knip M, Boehm BO, Rosak C, Löliger C, Kühnl P, Ottenhoff T, Contu L, Carcassi C, Savi M, Zanelli P, Neri TM, Hamaguchi K, Kimura A, Dong RP, Chikuba N, Nagataki S, Gorodezky C, Debaz H, Robles C, Coimbra HB, Martinho A, Ruas MA, Sachs JA, Garcia-Pachedo M, Biro A, Nikaein A, Dombrausky L, Gonwa T, Zmijewski C, Monos D, Kamoun M, Layrisse Z, Magli MC, Balducci P, Thorsby E: HLA class II associations in insulin-dependent diabetes mellitus among blacks, Caucasoids, and Japanese. In *HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference*, *Yokohoma*, *Japan*, *6–13 November 1991*. Tsuji K, Aizawa M, Sasazuki T, Eds. Oxford, U.K. Oxford University Press, 1992, p. 713–722
12. Yasunaga S, Kimura A, Hamaguchi K, Rønningen KS, Sasazuki T: Different contribution of HLA-DR and genes in susceptibility and resistance to insulin-dependent diabetes mellitus (IDDM). *Tissue Antigens* 47:37–48, 1996
13. Nakanishi K, Kobayashi T, Murase T, Naruse T, Nose Y, Inoko H: Human leukocyte antigen-A24 and -DQA1*0301 in Japanese insulin-dependent diabetes mellitus: independent contributions to susceptibility to the disease and additive contributions to acceleration of β-cell destruction. *J Clin Endcrinol Metab* 84:3721–3725, 1999
14. Fennessy M, Metcalfe K, Hitman GA, Niven M, Biro PA, Tuomilehto J, Tuomilehto-Wolf E, the Childhood Diabetes in Finland (DiMe) Study Group: A gene in the HLA class I region contributes to susceptibility to IDDM in the Finnish population. *Diabetologia* 37:937–944, 1994
15. Noble JA, Valdes AM, Bugawan TL, Apple RJ, Thomson G, Erlich HA: The HLA class I A locus affects susceptibility to type 1 diabetes. *Hum Immunol* 63:657–664, 2002
16. Honeyman MC, Harrison LC, Drummond B, Colman PG, Tait BD: Analysis of families at risk for insulin-dependent diabetes mellitus reveals that HLA antigens influence progression to clinical disease. *Mol Med* 1:576–582, 1995
17. Robles DT, Eisenbarth GS, Wang T, Erlich HA, Bugawan TL, Babu SR, Barriga K, Norris JM, Hoffman M, Klingensmith G, Yu L, Rewers M, the Diabetes Autoimmunity Study in the Young (DAISY): Identification of children with early onset and high incidence of anti-islet autoantibodies. *Clin Immunol* 102:217–224, 2002
18. Ikegami H, Makino S, Yamato E, Kawaguchi Y, Ueda H, Sakamoto T, Takekawa K, Ogihara T: Identification of a new susceptibility locus for insulin-dependent diabetes mellitus by ancestral haplotype congenic mapping. *J Clin Invest* 96:1936–1942, 1995
19. Hattori M, Yamato E, Itoh N, Senpuku H, Fujisawa T, Yoshino M, Fukuda M, Matsumoto E, Toyonaga T, Nakagawa I, Petruzzelli M, McMurray A, Weiner H, Sagai T, Moriwaki K, Shiroishi T, Maron R, Lund T: Cutting edge: homologous recombination of the MHC class I K region defines new MHC-linked diabetogenic susceptibility gene(s) in nonobese diabetic mice. *J Immunol* 163:1721–1724, 1999
20. Schlesselman JJ, Stolley PD: Research strategies. In *Case-Control Studies.* Schlesselman JJ, Ed. New York, Oxford University Press, 1982, p. 7–26
21. Nakanishi K, Kobayashi T, Inoko H, Tsuji K, Murase T, Kosaka K: Residual β-cell function and HLA-A24 in IDDM: markers of glycemic control and subsequent development of diabetic retinopathy. *Diabetes* 44:1334–1339, 1995
22. Ota M, Seki T, Fukushima H, Tsuji K, Inoko H: HLA-DRB1 genotyping by modified PCR-RFLP method combined with group-specific primers. *Tissue Antigens* 39:187–202, 1992
23. Ota M, Seki T, Nomura N, Sugimura K, Mizuki N, Fukushima H, Tsuji K, Inoko H: Modified PCR-RFLP method for HLA-DPB1 and -DQA1 genotyping. *Tissue Antigens* 38; 60–71, 1991
24. Nomura N, Ota M, Tsuji K, Inoko H: HLA-DQB1 genotyping by a modified PCR-RFLP method combined with group-specific primers. *Tissue Antigens* 38:53–59, 1991
25. Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwai Y: Microdroplet testing for HLA-A, HLA-B, HLA-C, and HLA-D antigens. *Am J Clin Pathol* 69:103–120, 1978
26. Moribe T, Kaneshige T, Inoko H: Complete HLA-A DNA typing using the PCR-RFLP method combined with allele group- and sequence-specific amplification. *Tissue Antigens* 50:535–545, 1997
27. Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T: Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In *HLA 1991: Proceedings of the Eleventh International Histocompatibility Workshop and Conference*, *Yokohoma*, *Japan*, *6–13 November 1991*. Tsuji K, Aizawa M, Sasazuki T, Eds. Oxford, U.K. Oxford University Press, 1992, p. 1065–1220
28. Nakanishi K, Kobayashi T, Miyashita H, Okubo M, Sugimoto T, Murase T, Hashimoto M, Fukuchi S, Kosaka K: Exocrine pancreatic ductograms in insulin-dependent diabetes mellitus. *Am J Gastroenterol* 89:762–766, 1994
29. Kobayashi T, Tamemoto K, Nakanishi K, Kato N, Okubo M, Kajio H, Sugimoto T, Murase T, Kosaka K: Immunogenetic and clinical characterization of slowly progressive IDDM. *Diabetes Care* 16:780–788, 1993
30. Cox DR, Oakes D: *Analysis of Survival Data.* London, Chapman and Hall, 1984, p. 91–111
31. Sugihara S, Sakamaki T, Konda S, Murata A, Wataki K, Kobayashi Y, Minamitani K, Miyamoto S, Sasaki N, Niimi H: Association of HLA-DR, DQ genotype with different β-cell functions at IDDM diagnosis in Japanese children. *Diabetes* 46:1893–1897, 1997
32. Imagawa A, Hanafusa T, Uchigata Y, Kanatsuka A, Kawasaki E, Kobayashi T, Shimada A, Shimizu I, Maruyama T, Makino H: Different contribution of class II HLA in fulminant and typical autoimmune type 1 diabetes. *Diabetologia* 48:294–300, 2005
33. Janeway CA, Travers P, Walport M, Schlomchik M: *Immunobiology. The Immune System in Health and Disease.* 5th ed. New York, Garland Publishing, 2001
34. Raddrizzani L, Sturniolo T, Guenot J, Bono E, Gallazzi F, Nagy ZA, Sinigaglia F, Hammer J: Different modes of peptide interaction enable HLA-DQ and HLA-DR molecules to bind diverse peptide repertoires. *J Immunol* 159:703–711, 1997
35. Nakanishi K, Komatsu Y, Kogawa N, Matsushita H: Analysis of eluted peptides from type 1 diabetes-susceptible HLA class II molecules identified novel islet protein, heparin/heparan sulfate-interacting protein. *Biochem Biophys Res Commun* 329:356–361, 2005
36. Graves PM, Norris JM, Pallansch MA, Gerling IC, Rewers M: The role of enteroviral infections in the development of IDDM: limitations of current approaches. *Diabetes* 46:161–168, 1997
37. Imagawa A, Hanafusa T, Makino H, Miyagawa J-I, Juto P: High titres of IgA antibodies to enterovirus in fulminant type-1 diabetes. *Diabetologia* 48: 290–293, 2005
38. Kobayashi T, Nakanishi K, Sugimoto T, Itoh T, Murase T, Kosaka K, Tsuji

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/6/1862/660880/zdb00606001862.pdf by guest on 21 May 2024

K: Maleness as risk factor for slowly progressive IDDM. *Diabetes Care* 12:7–11, 1989
39. Imagawa A, Hanafusa T, Uchigata Y, Kanatsuka A, Kawasaki E, Kobayashi T, Shimada A, Shimizu I, Toyoda T, Maruyama T, Makino H: Fulminant type 1 diabetes: a nationwide survey in Japan. *Diabetes Care* 26:2345–2352, 2003
40. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW: C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve β-cell function: report of an ADA workshop, 21–22 October 2001. *Diabetes* 53:250–264, 2004

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/6/1862/660880/zdb00606001862.pdf by guest on 21 May 2024