Association of HLA-DQ Genotype in Autoantibody-Negative and Rapid-Onset Type 1 Diabetes

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OBJECTIVE — Some type 1 diabetic patients have a distinct phenotype characterized by the absence of pancreatic autoantibodies and fulminant clinical symptoms at onset, including marked hyperglycemia, severe diabetic ketoacidosis, and normal to near-normal HbA_{1c} levels with complete destruction of β-cells. However, little is known about genetic factors of this distinct subtype of diabetes (fulminant autoantibody-negative type 1 diabetes).

RESEARCH DESIGN AND METHODS — We analyzed HLA-DQ genotypes in fulminant autoantibody-negative type 1 diabetes ($n = 22$) and autoantibody-positive type 1 diabetes (immune-mediated type 1 diabetes, $n = 78$) recruited from a cohort between 1980 and 2000.

RESULTS — Fulminant autoantibody-negative type 1 diabetes had a significantly high prevalence of the HLA-DQA1*0303-DQB1*0401 haplotype in a homozygous manner (RR 39) or in a heterozygous manner with the HLA-DQA1*0302-DQB1*0303 haplotype (RR 13). In contrast, autoantibody-positive type 1 diabetic patients had a high prevalence of the HLA-DQA1*0302-DQB1*0303 haplotype in a homozygous manner (RR 10) or in a heterozygous manner with the HLA-DQA1*0303-DQB1*0401 haplotype (RR 12).

CONCLUSIONS — Pathogenic roles of genotypic combinations of specific HLA-DQ haplotypes in a homozygous manner are suggested as causative mechanisms of aggressive β-cell damage in a subtype of autoantibody-negative type 1 diabetes with fulminant clinical features.

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Some proportion of patients with type 1 diabetes lack the humoral and cellular markers of autoimmunity, involving pancreatic β-cells even at the onset. This subtype of type 1 diabetes can be categorized as type 1A diabetes without anti-islet autoantibodies, or idiopathic type 1B diabetes, using the criteria of the American Diabetes Association (1). A distinct clinical subtype of type 1 diabetes was recently described as fulminant autoantibody-negative type 1 diabetes in Japanese diabetic patients (2–4). Furthermore, a few reports suggest the presence of the same subtype of type 1 diabetes in Caucasian diabetic patients (5–7). The characteristic features at onset of this subtype of type 1 diabetes (2–4) include: *1*) abrupt onset and fulminant symptoms, including marked hyperglycemia and severe diabetic ketoacidosis and normal to near-normal HbA_{1c} levels, with complete destruction of β-cells; *2*) the absence of islet cell autoantibodies, including islet cell antibodies (ICAs), GAD autoantibodies (GADAbs), insulinoma-associated protein 2/islet cell antigen 512 autoantibodies (IA-2Abs), and insulin autoantibodies (IAAs); and *3*) the involvement of exocrine pancreas, with elevated serum levels of pancreatic enzymes, including elastase 1, amylase, and lipase.

We reported a case with fulminant autoantibody-negative type 1 diabetes with remarkable mononuclear cell infiltration to the endocrine pancreas (insulitis) as well as exocrine pancreas, suggesting that immunological mechanisms and immunogenetic predisposition would contribute to β-cell destruction in this subtype of autoantibody-negative type 1 diabetes (4). However, the immunogenetic background remains unclear (8). In this present study, we examined HLA-DQA1 and -DQB1 genes in patients with fulminant autoantibody-negative type 1 diabetes.

RESEARCH DESIGN AND METHODS

— A total of 117 newly diagnosed type 1 diabetic patients were sequentially recruited for the study during 1980–2000. All patients met the criteria of the American Diabetes Association for type 1 diabetes (1). The duration from onset of diabetic symptoms to hospitalization was within 90 days in all cases. Patients who had a period of remission that lasted for ≥6 months after the diagnosis had been made were excluded (9). According to a previous report (3), the study subjects were divided into three groups based on the presence of diabetes-related autoantibodies (including ICAs, GADAbs, IA-2Abs, and IAAs) and on the HbA_{1c} levels at onset, as follows: group 1 included those patients with autoantibody-negative type 1 diabetes with low HbA_{1c} levels (≤8.3%) at onset (fulminant autoantibody-negative type 1 diabetes), group 2 included those with autoantibody-negative type 1 diabetes with high

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Abbreviations: GADAb, GAD autoantibody; HNF-1α, hepatocyte nuclear factor-1α; IA-2Ab, insulinoma-associated protein 2/islet cell antigen 512 autoantibody; IAA, insulin autoantibody; ICA, islet cell antibody; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

HbA_{1c} levels (>8.3%) at onset (nonfulminant autoantibody-negative type 1 diabetes), and group 3 included those with autoantibody-positive type 1 diabetes (Table 1). In all group 1 patients, blood samples for assays for pancreatic autoantibodies, including ICAs, GADAbs, IA-2Abs, and IAAs, were obtained at least twice within 10 and 30 days after the onset of diabetes. Exclusion criteria were the presence of mitochondrial DNA mutation (A/G) at 3,243 bp and/or hepatocyte nuclear factor-1α (HNF-1α) gene mutation. Seven patients were not examined for HLA-DQ gene analysis, and one patient was excluded because of the presence of mitochondrial DNA mutation (A/G) at 3,243 bp. HLA-DQ gene analysis was performed in the remaining 109 patients. The normal control subjects (43 men and 41 women, age 69 ± 6 years [means ± SD], $n = 84$) were selected according to the following criteria: no past history of urinary glucose or glucose intolerance, a normal 75-g oral glucose tolerance test (OGTT), age >60 years, and no family history of diabetes. All study subjects were the unrelated Japanese, and they gave their written consent to participate in the study after being informed of its nature. The study protocol was approved by the ethical committee of Toranomon Hospital, and investigation was performed in accordance with the guidelines expressed in the Declaration of Helsinki.

Clinical characteristics and laboratory examinations

At the onset of overt diabetes, all patients were hospitalized. Their clinical characteristics were recorded, and plasma glucose, HbA_{1c}, arterial pH, serum elastase 1, serum amylase, and serum lipase were measured within 2 days after initial diagnosis. ICA was detected by indirect immunofluorescence method, as previously described (10). The cutoff point of our assay is 5 Juvenile Diabetes Foundation units (sensitivity 90%, specificity 92%). GADAbs and IA-2Abs were assayed as previously reported (11,12). The sensitivity and specificity of GADAb assay were 80 and 100%, respectively, in the Second International GADAb Workshop (11). The IA-2Ab assay was evaluated in the third proficiency IA-2Ab test organized by the Research Institute for Children, and the results showed 100% sensitivity and 100% specificity (12). IAA was assayed as previously described (13). The

Table 1—*Clinical features at the onset in Ab-negative and Ab-positive type 1 diabetes*

	Ab-negative type 1 diabetes ($n = 31$)		Ab-positive type 1 diabetes, group 3	*P*		
Clinical characteristics	Fulminant, group 1	Nonfulminant, group 2		Group 1 vs. group 2	Group 1 vs. group 3	Group 2 vs. group 3
n	22	9	78			
Onset age (years)	36 ± 15 (21–65)	31 ± 12 (15–53)	34 ± 16 (2–71)	NS	NS	NS
Sex (M/F)	15/7	6/3	40/38	NS	NS	NS
BMI (kg/m^2)	20.7 ± 2.6 (16.7–28.7)	18.6 ± 1.6 (17.0–21.7)	18.6 ± 2.4 (13.8–24.4)	0.0208	0.0013	NS
Duration of diabetic symptoms (days)	4 ± 3 (1–10)	57 ± 40 (2–90)	50 ± 33 (1–90)	0.0013	<0.0001	NS
Plasma glucose (mmol/l)	46.8 ± 21.5 (16.9–102.0)	27.9 ± 17.2 (11.3–71.1)	26.0 ± 12.4 (11.9–70.9)	0.0096	<0.0001	NS
HbA_{1c} (%)	6.7 ± 1.2 (4.3–8.3)	12.7 ± 2.2 (8.6–15.5)	11.1 ± 2.8 (5.1–19.1)	<0.0001	<0.0001	NS
Arterial blood pH	7.10 ± 0.15 (6.91–7.34)	7.25 ± 0.11 (7.15–7.35)	7.29 ± 0.12 (7.02–7.39)	NS	0.0024	NS
ΣC-peptide (nmol/l)	0.065 ± 0.143 (0.000–0.540)	2.313 ± 1.477 (0.000–4.866)	2.606 ± 1.659 (0.000–7.404)	<0.0001	<0.0001	NS
Pancreatic enzymes						
Elastase 1 (ng/dl) (22–221)*	888 ± 771 (125–2,900)	100 ± 49 (59–154)	177 ± 253 (17–1,900)	0.0017	<0.0001	NS
Amylase (IU/l) (111–336)*	2,463 ± 3,235 (182–10,930)	150 ± 47 (98–235)	292 ± 734 (92–6,299)	0.0010	<0.0001	NS
Lipase (units/l) (25–170)*	862 ± 1,755 (59–5,815)	78 ± 51 (20–138)	100 ± 52 (19–273)	0.0101	0.0002	NS

Data are means ± SD (range). See the text for more detailed definition of each group of subjects. Ab, diabetes-related autoantibodies, including ICAs, GAD autoantibodies, IA-2Abs, and insulin autoantibodies; ΣC-peptide, integrated values of C-peptide at 0, 30, 60, 90, and 120 min during 75-g OGTT. *Normal range.

Table 2—*Frequencies of HLA-DQA1 and -DQB1 alleles in Ab-negative and Ab-positive type 1 diabetes*

	Ab-negative type 1 diabetes		Ab-positive type 1 diabetes, group 3 (n = 78)	Nondiabetic control subjects (n = 84)	P vs. control subjects (and RR)		
DQ Alleles	Fulminant, group 1 (n = 22)	Nonfulminant, group 2 (n = 9)			Group 1	Group 2	Group 3
DQA1*0101	1 (5)	0 (0)	8 (10)	31 (37)	0.034 (0.1)	0.042* (0.2)	0.001 (0.2)
DQA1*0102	5 (23)	4 (44)	20 (26)	29 (35)	—	—	—
DQA1*0103	2 (9)	2 (22)	7 (9)	29 (35)	0.029* (0.2)	NS	0.001 (0.2)
DQA1*0201	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
DQA1*0301	2 (9)	3 (33)	12 (15)	12 (14)	—	—	—
DQA1*0302	8 (36)	5 (56)	39 (50)	16 (19)	NS	NS	5×10^{-4} (4.3)
DQA1*0303	15 (68)	3 (33)	37 (47)	27 (32)	0.049 (4.5)	NS	NS
DQA1*0401	1 (5)	0 (0)	4 (5)	8 (10)	—	—	—
DQA1*0501	0 (0)	0 (0)	3 (4)	10 (12)	—	—	—
DQA1*0601	0 (0)	0 (0)	1 (1)	0 (0)	—	—	—
DQB1*0501	1 (5)	0 (0)	7 (9)	13 (15)	—	—	—
DQB1*0502	1 (5)	0 (0)	0 (0)	8 (10)	NS	NS	0.009* (0.1)
DQB1*0503	0 (0)	0 (0)	1 (1)	10 (12)	NS	NS	0.013* (0.1)
DQB1*0601	2 (9)	1 (11)	6 (8)	28 (33)	0.037* (0.2)	NS	9×10^{-4} (0.2)
DQB1*0602/0603	2 (9)	2 (22)	2 (3)	14 (17)	NS	NS	0.047 (0.1)
DQB1*0604	3 (14)	3 (33)	19 (24)	15 (18)	—	—	—
DQB1*0201	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
DQB1*0301	0 (0)	1 (11)	9 (12)	15 (18)	—	—	—
DQB1*0302	2 (9)	3 (33)	14 (18)	12 (14)	—	—	—
DQB1*0303	8 (36)	5 (56)	39 (50)	16 (19)	NS	NS	6×10^{-4} (4.3)
DQB1*0401	15 (68)	2 (22)	34 (44)	17 (20)	9×10^{-4} (8.4)	NS	0.026 (3.0)
DQB1*0402	1 (5)	0 (0)	2 (3)	11 (13)	NS	NS	0.025* (0.2)

Data are n (%). See the text for more detailed definition of each group of subjects. Ab, diabetes-related autoantibodies, including ICAs, GAD autoantibodies, IA-2Abs, and insulin autoantibodies. *Uncorrected P value.

sensitivity and specificity of IAA assay were both 100% in the Fourth International IAA Workshop (13). A 75-g OGTT was performed for each patient 3–4 weeks after obtaining steady glycemic control by insulin therapy. Serum C-peptide was measured by a sensitive radioimmunoassay as previously described (14). HLA-DQA1 and -DQB1 were analyzed using PCR with a restriction fragment–length polymorphism method as previously described (15). The samples genotyped as DQA1*03 were further tested using a Dynal SSP kit (Dynal, Oslo, Norway). Detection of mitochondrial DNA mutation (A/G) at 3,243 bp and HNF-1α gene mutation was performed as previously described (16,17).

Statistical analysis

A Mann-Whitney U test and Fisher's exact test were applied to compare the values of clinical features between the different subgroups of type 1 diabetes. Frequencies of HLA-DQA1 and -DQB1 alleles and HLA-DQA1-DQB1 haplotypes in patients were compared using Fisher's exact test. The haplotype of HLA-DQA1-DQB1 genes was deduced from population analysis as described previously (18).

RESULTS

Clinical features of patients in groups 1–3

The clinical features at the onset of diabetes of three groups including group 1 (fulminant autoantibody-negative type 1 diabetes), group 2 (nonfulminant autoantibody-negative type 1 diabetes), and group 3 (autoantibody-positive type 1 diabetes) are noted in Table 1. Among group 3 patients, ICA was positive in 79% (62 of 78), GADAb in 76% (59 of 78), IA-2Ab in 68% (53 of 78), and IAA in 13% (10 of 78). All patients in group 1 and group 2 were negative for ICA, GADAb, IA-2Ab, and IAA. Group 1 patients had significantly shorter duration of hyperglycemic symptoms before diagnosis (Table 1). Plasma glucose levels at the onset of diabetes in group 1 patients were significantly higher than those in group 3 patients. In contrast, the levels of HbA_{1c} at the onset of diabetes in group 1 patients were significantly lower than those in group 3 patients. The degree of acidosis in group 1 patients was more severe than that in group 3 patients. C-peptide response to 75-g oral glucose in group 1 was the lowest among the three groups. Of the group 1 patients, 95% (21 of 22) had elevated levels of serum pancreatic enzymes (Table 1). All features of group 1 are compatible with those in previously reported studies (2–4).

Type 1 diabetes–susceptible HLA-DQA1 and -DQB1 alleles

The allele frequencies of DQA1*0303 and DQB1*0401 were significantly higher in group 1 than nondiabetic control subjects (Table 2). The frequencies of DQA1*0302, DQB1*0303, and DQB1*0401 were significantly higher in group 3 than in control subjects. In group 2, the frequencies of any diabetes-susceptible HLA-DQ alleles did not differ from control subjects.

Table 3—*Frequencies of HLA-DQA1-DQB1 haplotypes in Ab-negative and Ab-positive type 1 diabetes*

DQA1-DQB1 haplotypes	Ab-negative type 1 diabetes: Fulminant, group 1 (*n* = 22)	Ab-negative type 1 diabetes: Nonfulminant, group 2 (*n* = 9)	Ab-positive type 1 diabetes, group 3 (*n* = 78)	Nondiabetic control subjects (*n* = 84)	*P* value vs. control subjects (and RR): Group 1	Group 2	Group 3
DQA1*0101-DQB1*0501	1 (5)	0 (0)	7 (9)	13 (15)	—	—	—
DQA1*0101-DQB1*0502	0 (0)	0 (0)	0 (0)	8 (10)	NS	NS	0.009* (0.1)
DQA1*0102-DQB1*0502	1 (5)	0 (0)	0 (0)	0 (0)	—	—	—
DQA1*0101-DQB1*0503	0 (0)	0 (0)	1 (1)	10 (12)	NS	NS	0.013* (0.1)
DQA1*0103-DQB1*0601	2 (9)	1 (11)	6 (8)	28 (33)	0.037* (0.2)	NS	0.001 (0.2)
DQA1*0102-DQB1*0602/0603	2 (9)	1 (11)	1 (1)	14 (17)	NS	NS	0.016 (0.1)
DQA1*0103-DQB1*0602/0603	0 (0)	1 (11)	1 (1)	0 (0)	—	—	—
DQA1*0102-DQB1*0604	3 (14)	3 (33)	19 (24)	15 (18)	—	—	—
DQA1*0201-DQB1*0201	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
DQA1*0501-DQB1*0201	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
DQA1*0303-DQB1*0301	0 (0)	1 (11)	5 (6)	8 (10)	—	—	—
DQA1*0501-DQB1*0301	0 (0)	0 (0)	3 (4)	10 (12)	—	—	—
DQA1*0601-DQB1*0301	0 (0)	0 (0)	1 (1)	0 (0)	—	—	—
DQA1*0301-DQB1*0302	2 (9)	3 (33)	12 (15)	12 (14)	—	—	—
DQA1*0401-DQB1*0302	0 (0)	0 (0)	2 (3)	0 (0)	—	—	—
DQA1*0302-DQB1*0303	8 (36)	5 (56)	39 (50)	16 (19)	NS	NS	0.001 (4.3)
DQA1*0303-DQB1*0401	15 (68)	2 (22)	34 (44)	17 (20)	0.001 (8.4)	NS	0.040 (3.0)
DQA1*0303-DQB1*0402	0 (0)	0 (0)	0 (0)	3 (4)	—	—	—
DQA1*0401-DQB1*0402	1 (5)	0 (0)	2 (3)	8 (10)	—	—	—

Data are *n* (%). See the text for more detailed definition of each group of subjects. Ab, diabetes-related autoantibodies, including ICAs, GAD autoantibodies, IA-2Abs, and insulin autoantibodies. *Uncorrected *P* value.

Type 1 diabetes–resistant HLA-DQA1 and -DQB1 alleles

The allele frequencies of DQA1*0101, DQA1*0103, and DQB1*0601 were significantly lower in group 1 than control subjects (Table 2). The frequencies of DQA1*0101, DQA1*0103, DQB1*0502, DQB1*0503, DQB1*0601, DQB1*0602/0603, and DQB1*0402 were significantly lower in group 3 as compared with control subjects. The frequency of DQA1*0101 was significantly lower in group 2 than control subjects.

Type 1 diabetes–susceptible HLA-DQA1-DQB1 haplotypes

The frequency of DQA1*0303-DQB1*0401 haplotype was markedly higher in group 1 than in control subjects (Table 3). In contrast, the frequencies of DQA1*0302-DQB1*0303 and DQA1*0303-DQB1*0401 haplotypes were significantly higher in group 3 than in control subjects. In group 2, the frequencies of any HLA-DQ haplotypes did not differ from control subjects.

Type 1 diabetes–resistant HLA-DQA1-DQB1 haplotypes

The frequency of the DQA1*0103-DQB1*0601 haplotype was significantly lower in group 1 than in control subjects (Table 3). The frequencies of DQA1*0101-DQB1*0502, DQA1*0101-DQB1*0503, DQA1*0103-DQB1*0601, and DQA1*0102-DQB1*0602/0603 haplotypes were significantly lower in group 3 than in control subjects. In group 2, the frequencies of any HLA-DQ haplotypes did not differ from control subjects.

The frequencies of the genotypic combinations of HLA-DQA1-DQB1 haplotypes

More interestingly, group 1 as opposed to group 3 had a significantly higher prevalence of the HLA-DQA1*0303-DQB1*0401 haplotype in a homozygous manner than nondiabetic control subjects, with an RR of 38.7 (Table 4). The prevalence of homozygous HLA-DQA1*0303-DQB1*0401 haplotype in group 1 patients was significantly higher than that in group 3 patients (Table 4). The frequency of the combination of HLA-DQA1*0303-DQB1*0401 and DQA1*0302-DQB1*0303 haplotypes was also higher in group 1 patients than in control subjects. In contrast, group 3 patients had a higher prevalence of DQA1*0302-DQB1*0303 haplotype in a homozygous manner or a heterozygous manner with DQA1*0303-DQB1*0401 haplotype than control subjects (Table 4).

CONCLUSIONS — We demonstrated that HLA-DQA1*0303-DQB1*0401 haplotype is strongly associated with diabetes in group 1 (fulminant autoantibody-negative type 1 diabetes) in a homozygous manner or in a heterozygous manner with DQA1*0302-DQB1*0303 haplotype. According to previous reports (15,19), each of these HLA-DQA1-DQB1 haplotypes was also related with group 3 (autoantibody-positive type 1 diabetes) patients (Table 3). However, the manner of the genotypic combinations of these haplotypes in group 3 patients was quite different from that in group 1 (autoantibody-negative and rapid-onset type 1 diabetes) patients (Table 4). The frequency of homozygous combination of the HLA-DQA1*0303-DQB1*0401 haplotype was markedly high (RR 38.7) in patients with fulminant autoantibody-negative type 1 diabetes, whereas the frequency of this combination was not higher in patients with autoantibody-positive type 1 diabetes compared with nondiabetic control subjects (Table 4). These results raise the fol-

Table 4—*Frequencies of the combinations of HLA-DQA1-DQB1 haplotypes in Ab-negative and Ab-positive type 1 diabetes*

DQA1*-DQB1*/ DQA1*-DQB1*	Ab-negative type 1 diabetes: Fulminant, group 1 (n = 22)	Ab-negative type 1 diabetes: Nonfulminant, group 2 (n = 9)	Ab-positive type 1 diabetes, group 3 (n = 78)	Nondiabetic control subjects (n = 84)	P value vs. control subjects (and RR): Group 1	Group 2	Group 3
0303-0401/0303-0401	7 (32)	0 (0)	6 (8)	1 (1)	1×10^{-4} (38.7)§	NS	NS
0303-0401/0302-0303	3 (14)	0 (0)	10 (13)	0 (0)	0.016 (13.3)	NS	0.001 (12.4)
0302-0303/0302-0303	1 (5)	1 (11)	15 (19)	2 (2)	NS	NS	8×10^{-4} (9.8)
0303-0401/0303-X	0 (0)	0 (0)	2 (3)	1 (1)	—	—	—
0303-0401/0302-X	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
0302-0303/0303-X	0 (0)	0 (0)	0 (0)	2 (2)	—	—	—
0302-0303/0302-X	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
0303-X/0303-X	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
0303-X/0302-X	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
0302-X/0302-X	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
0303-0401/Y-X	5 (23)	2 (22)	16 (21)	15 (18)	—	—	—
0302-0303/Y-X	4 (18)	4 (44)	14 (18)	12 (14)	—	—	—
0303-X/Y-X	0 (0)	1 (11)	3 (4)	8 (10)	—	—	—
0302-X/Y-X	0 (0)	0 (0)	0 (0)	0 (0)	—	—	—
Y-X/Y-X	2 (9)	1 (11)	12 (15)	43 (51)	4×10^{-4} (0.1)	0.046 (0.1)	2×10^{-6} (0.2)

Data are *n* (%). See the text for more detailed definition of each group of subjects. Ab, diabetes-related autoantibodies, incuding ICAs, GAD autoantibodies, IA-2Abs, and insulin autoantibodies. §P = 0.014 for the comparison with group 3, DQB1*X ≠ DQB1*0401 or DQB1*0303, DQA1*Y ≠ DQA1*0303 or DQA1*0302.

lowing questions on the pathogenesis of fulminant autoantibody-negative type 1 diabetes: *1)* Is β-cell destruction in fulminant autoantibody-negative type 1 diabetes caused by a common mechanism with autoantibody-positive type 1 diabetes that is related with an autoimmune mechanism? 2) If not, are the genotypic combinations of specific HLA-DQ haplotypes related with other mechanisms rather than autoimmunity (i.e., virus infection or chemical agent exposure) that are responsible for β-cell destruction in fulminant autoantibody-negative type 1 diabetes?

Subjects with the HLA-DQA1*0303-DQB1*0401 haplotype, which is closely related with fulminant autoantibody-negative type 1 diabetes, have a higher RR for type 1 diabetes than those with the HLA-DQA1*0302-DQB1*0303 haplotype in Japanese and Chinese populations (19,20). This suggests that the disease susceptibility of the HLA-DQA1*0303-DQB1*0401 haplotype is stronger than that of the HLA-DQA1*0302-DQB1*0303 haplotype. The HLA-DQA1*0303-DQB1*0401 haplotype has close linkage disequilibrium with the HLA-DR*0405 allele, which confers strong susceptibility to type 1 diabetes in Caucasian, Japanese, Chinese, and other ethnic groups (19). Furthermore, the HLA-DQA1*0303-DQB1*0401 haplotype has close linkage disequilibrium with HLA-A24 in the Japanese population (18). The class I HLA-A24 gene promotes pancreatic β-cell destruction in an additive manner in the patients with type 1 diabetes–susceptible HLA class II genes (21). In contrast, the HLA-DQA1*0302-DQB1*0303 haplotype, which is related with autoantibody-positive type 1 diabetes, has linkage disequilibrium with the HLA-DR*0901 allele, which is grouped in neutral/susceptible allele to type 1 diabetes because of low RR (19). The combined effect of strong diabetogenic HLA-A, -DR, and -DQ heterodimers on β-cell destruction through an immunological mechanism will explain the aggressive clinical course of autoantibody-negative and rapid-onset type 1 diabetes. Furthermore, it appears that the susceptibility of an individual to type 1 diabetes is correlated with the number of such susceptible HLA molecules found in each individual; this is called the dose effect (15,22). Therefore, it may be possible that the HLA-DQA1*0303-DQB1*0401 haplotype in a homozygous (double-risk) manner may confer strong susceptibility and cause aggressive immunological response in autoantibody-negative and rapid-onset type 1 diabetes.

The immune response to echovirus 9 infection can cause type 1 diabetes without autoantibodies by effecting the Th1/Th2 paradigm, which causes Th1 cells to become dominant (7). Drastic and massive destruction of β-cells within a few days (4) with resultant excessive pancreatic antigen exposure in fulminant autoantibody-negative type 1 diabetes may induce immunological tolerance, which fails to produce pancreatic autoantibodies. In a few patients with fulminant autoantibody-negative type 1 diabetes, GADAb was negative at the onset, and several weeks later, GADAb became positive at high titer levels (A. Shimada, Keio University, personal communication). Secondary diabetogenic virus infections may be mediated with specific class II as well as class I molecules. Epstein-Barr virus, which is one of the causative viruses of diabetes with β-cell tropism, utilizes a specific HLA-DQ genotype as a coreceptor during entry to the target cells (23). Specific class II HLA-DQ molecules may work to accelerate virus entry with subsequent rapid destruction of β-cells in fulminant autoantibody-negative type 1 diabetes.

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References

1. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
2. Kobayashi T: Immunology and immunogenetics of type 1 diabetes in Japan. *IDF Bull* 35:34–37, 1990
3. Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y: 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
4. Tanaka S, Kobayashi T, Momotsu T: A novel subtype of type 1 diabetes mellitus. *N Engl J Med* 342:1835–1837, 2000
5. Warshaw AL, Feller ER, Lee KH: On the cause of raised serum-amylase in diabetic ketoacidosis. *Lancet* 30:929–931, 1977
6. Foulis AK, Francis ND, Farquharson MA, Boylston A: Massive synchronous B-cell necrosis causing type 1 (insulin-dependent) diabetes–a unique histopathological case report. *Diabetologia* 31:46–50, 1988
7. Vreugdenhil GR, Schloot NC, Hoorens A, Rongen C, Pipeleers DG, Melchers WJ, Roep BO, Galama JM: Acute onset of type I diabetes mellitus after severe echovirus 9 infection: putative pathogenic pathways. *Clin Infect Dis* 31:1025–1031, 2000
8. Lernmark Å: Rapid-onset type 1 diabetes with pancreatic exocrine dysfunction. *N Engl J Med* 342:344–345, 2000
9. Yamada K, Nonaka K: Diabetic ketoacidosis in young obese Japanese men (Letter). *Diabetes Care* 19:671, 1996
10. Kobayashi T, Sugimoto T, Itoh T, Kosaka K, Tanaka T, Suwa S, Sato K, Tsuji K: The prevalence of islet cell antibodies in Japanese insulin-dependent and non-insulin-dependent diabetic patients studied by indirect immunofluorescence and by a new method. *Diabetes* 35:335–340, 1986
11. Kobayashi T, Nakanishi K, Murase T, Kosaka K: Small doses of subcutaneous insulin as a strategy for preventing slowly progressive β-cell failure in islet cell antibody-positive patients with clinical features of NIDDM. *Diabetes* 45:622–626, 1996
12. Masuda M, Powell M, Chen S, Beer C, Fichna P, Rees Smith B, Furmaniak J: Autoantibodies to IA-2 in insulin-dependent diabetes mellitus: measurements with a new immunoprecipitation assay. *Clin Chim Acta* 291:53–66, 2000
13. Maruyama T, Kasuga A, Ozawa Y, Nagata A, Abiko F, Suzuki Y, Saruta T: Glutamic acid decarboxylase65 (GAD65) antibodies and insulin auto-antibodies in Japanese patients with non-insulin-dependent diabetes mellitus. *Endocr J* 44: 43–51, 1997
14. 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
15. 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
16. Kobayashi T, Nakanishi K, Nakase H, Kajio H, Okubo M, Murase T, Kosaka K: In situ characterization of islets in diabetes with a mitochondrial DNA mutation at nucleotide position 3243. *Diabetes* 46: 1567–1571, 1997
17. Tanaka S, Kobayashi T, Tomura H, Okubo M, Nakanishi K, Takeda J, Murase T: A novel dominant-negative mutation of the hepatocyte nuclear factor-1α gene in Japanese early-onset type 2 diabetes. *Horm Metab Res* 32:373–377, 2000
18. Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T: Allele frequencies and haplotype frequencies for HLA and complement loci in various ethnic groups. In *HLA 1991*. Tsuji K, Aizawa M, Sasazuki T, Eds. Oxford, U.K., Oxford University Press, 1992, p. 1065–1220
19. She JX: Susceptibility to type 1 diabetes: HLA-DQ and DR revisited. *Immunol Today* 17:323–329, 1996
20. Awata T, Kuzuya T, Matsuda A, Iwamoto Y, Kanazawa Y: Genetic analysis of HLA class II alleles and susceptibility to type 1 (insulin-dependent) diabetes mellitus in Japanese subjects. *Diabetologia* 35:419–424, 1992
21. 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
22. Khalil I, Deschamps I, Lepage V, al-Daccak R, Degos L, Hors J: Dose effect of cis- and trans-encoded HLA-DQαβ heterodimers in IDDM susceptibility. *Diabetes* 41:378–384, 1992
23. Haan KM, Longnecker R: Coreceptor restriction within the HLA-DQ locus for Epstein-Barr virus infection. *Proc Natl Acad Sci U S A* 97:9252–9257, 2000