

# Immunogenetic and Clinical Characterization of Slowly Progressive IDDM

TETSURO KOBAYASHI, MD  
KOJI TAMEMOTO, MD  
KOJI NAKANISHI, MD  
NORUHIRO KATO, MD  
MINORU OKUBO, MD

HIROSHI KAJIO, MD  
TADAO SUGIMOTO, MD  
TOSHIO MURASE, MD  
KINORI KOSAKA, MD

**OBJECTIVE**— To examine the clinical and immunogenetic heterogeneity of IDDM.

**RESEARCH DESIGN AND METHODS**— We divided 207 IDDM patients into groups based on the interval from clinical onset to initiation of insulin therapy: group A (<3 mo, acute clinical-onset group,  $n = 134$ ), group B (3–12 mo, intermediate group,  $n = 31$ ), and group C ( $\geq 13$  mo, slowly progressive group,  $n = 42$ ). Immunogenetic and clinical markers were compared between group A and group C.

**RESULTS**— The mode age of onset was higher in group C (52 yr) than group A (10 yr). Group C had a higher prevalence of islet cell antibodies (42.9%, 18 of 42) than group A (25.4%, 34 of 134,  $P = 0.05$ ). Serum C-peptide immunoreactivity assayed by radioimmunoassay in response to a 100-g oral glucose tolerance test was significantly higher in group C than in group A. Group C patients were also more likely to have a family history of NIDDM (26.1%, 11 of 42) among their first-degree relatives than group A patients (11.2%, 15 of 134,  $P = 0.039$ ). The prevalences of family history of IDDM and endocrine autoimmune diseases were not different between groups C and A. The frequency of complications of endocrine autoimmune disease was not different between group A (6.7%, 9 of 134) and group C (2.3%, 1 of 42). Significant associations with two class I major histocompatibility complex antigens (HLA-A24 and -Bw54) and one class II antigen (HLA-DR4) were observed in group A. Group A patients were associated with three diabetogenic HLA-DQ haplotypes including DQA1\*0301-DQB1\*0401, DQA1\*0301-DQB1\*0302, and DQA1\*0301-DQB1\*0303. In contrast, group C lacked the association with class I antigens, although HLA-DR4 and HLA-DQA1\*0301-DQB1\*0401 were more common in this group than in control subjects.

**CONCLUSIONS**— These results indicate that the clinical subtype with slowly progressive course (slowly progressive IDDM) has distinct findings including late-age onset, high prevalence of islet cell antibodies, preserved  $\beta$ -cell function, and high family history of NIDDM. An additive effect of class I and class II major histocompatibility complex antigens is suggested as an explanation for the acute clinical manifestations and more severe  $\beta$ -cell destruction in group A patients.

FROM THE DEPARTMENT OF ENDOCRINOLOGY AND METABOLISM, TORANOMON HOSPITAL; THE OKINAKA MEMORIAL INSTITUTE FOR MEDICAL RESEARCH, TORANOMON, MINATO-KU; AND THE INSTITUTE FOR DIABETES CARE AND RESEARCH, ASAHI LIFE FOUNDATION, MARUNOUCHI, CHIYODA-KU, TOKYO, JAPAN.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO TETSURO KOBAYASHI, MD, DEPARTMENT OF ENDOCRINOLOGY AND METABOLISM, TORANOMON HOSPITAL, 2-2-2 TORANOMON, MINATO-KU, TOKYO 105, JAPAN.

RECEIVED FOR PUBLICATION 2 DECEMBER 1991 AND ACCEPTED IN REVISED FORM 7 JANUARY 1993.

IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; ICA, ISLET CELL ANTIBODIES; CPR, C-PEPTIDE IMMUNOREACTIVITY; RIA, RADIOIMMUNOASSAY; NIDDM, NON-INSULIN-DEPENDENT DIABETES MELLITUS; EAD, ENDOCRINE AUTOIMMUNE DISEASE; MHC, MAJOR HISTOCOMPATIBILITY COMPLEX; HLA, HUMAN LEUKOCYTE ANTIGEN; OGTT, ORAL GLUCOSE TOLERANCE TEST; TMA, THYROID MICROSOMAL ANTIBODY; BW, BODY WEIGHT; HPLC, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY; JDF U, JUVENILE DIABETES FOUNDATION UNIT; CV, COEFFICIENT OF VARIATION; PCR, POLYMERASE CHAIN REACTION; RFLP, RESTRICTION FRAGMENT-LENGTH POLYMORPHISM; BMI, BODY-MASS INDEX; Hp, HAPTOGLOBIN PHENOTYPE.

Several prospective studies demonstrated that the natural history of IDDM is characterized by a heterogeneous clinical course before and after the clinical onset. Some cases are preceded by an ICA<sup>+</sup> subclinical prediabetic period, and these patients later develop acute clinical  $\beta$ -cell failure with classical symptoms of IDDM (1–3). In these cases, the persistence of ICA is usually short. Other patients are characterized by persistently positive ICA and slowly progressive deterioration of  $\beta$ -cell function through a non-insulin-dependent stage to ultimately an insulin-dependent state (4,5). This form of diabetes is clinically classified as NIDDM during the period when insulin is not required. Furthermore, some patients with fluctuating or transient ICA positivity have been reported, and  $\beta$ -cell function improved or did not deteriorate after ICA converted to negative (5–8). The specific clinical course of IDDM may be related to the immunogenetic background, including HLA (5,9), sex (10), race (9,11,12), and other predisposing factor(s) (9). However, scant knowledge is available regarding the differences in immunogenetic backgrounds and other clinical findings between acute-onset IDDM and slowly progressive IDDM.

In this study, possible heterogeneity in immunogenetic and clinical aspects was examined by comparing the findings between acute clinical-onset and slowly progressive IDDM in a large Japanese diabetic population.

## RESEARCH DESIGN AND METHODS

We studied 207 IDDM patients (103 males and 104 females; mean  $\pm$  SE age at onset,  $26 \pm 1$  yr [range 2–62 yr]; mean  $\pm$  SE duration of diabetes,  $100 \pm 7$  mo [range 0–564 mo]). Of 207 patients, 178 (86%) were recruited from a primary-care base. The remaining 29 patients were referred from other institutions, and 18 of these 29 patients were seen within 3 mo of onset. All subjects were of Japanese ethnic stock

and were residents of the Tokyo and Yokohama areas. The diagnosis of IDDM was based on the criteria of the National Diabetes Data Group (13), with the following additional requirements; daily urinary CPR excretion <6.6 nmol and an integrated serum CPR value during the 100-g OGTT ( $\Sigma$ CPR) <3.3 nM. These two C-peptide criteria for distinguishing between IDDM and NIDDM were established on the basis of previous reports (5,14,15). The 207 subjects were recruited from the IDDM patients attending the outpatient clinic for diabetes of Toranomon Hospital from December 1980 through July 1989. The 100-g OGTT and blood sampling for blood glucose, CPR, ICA, HbA<sub>1c</sub>, and TMA were conducted from 1987 to 1989. The rate of recruitment of IDDM patients in this study was 90% (207 of 230). No heavy alcohol users were among the subjects. All 207 IDDM patients were ketosis prone, and the blood glucose levels were unstable. Of the 207 IDDM patients, 12 (5.8%) had other EADs (16): 11 (5.3%) had Graves' disease and 1 (0.5%) had Hashimoto's thyroiditis. All patients underwent a 100-g OGTT and had blood glucose and serum CPR levels determined after a 12-h overnight fast. All subjects were on a diet containing >250 g of carbohydrate/day before OGTT. Blood samples were also drawn for ICA and TMA. Patients were also measured for daily urinary CPR excretion.

A family history of diabetes in first-degree relatives and a family history of EADs were collected for each patient at interview and/or by questionnaire. The questionnaire on family history included the following items: 1) Do you have any family members with diabetes? If so, what kind of treatment do or did they have? 2) Do you have any family members with disease(s) of the thyroid (i.e., swollen thyroid gland), adrenals, pituitary gland, or other hormonal glands? If so, please specify.

The subjects were divided into three groups based on the duration of diabetes before insulin therapy. In group A (acute-onset IDDM), the duration of

diabetes before insulin therapy was <3 mo; in group B (intermediate group), the duration before insulin was 3–12 mo; and in group C (slowly progressive IDDM), the duration before insulin was  $\geq$ 13 mo. This division of the subjects was based on our prospective observation of the progression of  $\beta$ -cell failure in ICA<sup>+</sup> NIDDM (5,10), which showed that all 17 patients with ICA and progressive  $\beta$ -cell failure required insulin  $\geq$ 13 mo (range 13–45 mo, mean 27 mo) after onset. Data on the duration of illness before insulin therapy were collected from the medical records ( $n = 195$ ) or at interview (group A,  $n = 6$ ; group B,  $n = 3$ ; group C,  $n = 3$ ). The data on age at onset, fasting blood glucose before insulin therapy, daily dose of insulin, maximum BW, BW at onset, the presence of complicating EAD, and the absence of other disorders including infection, hepatic disease, renal disease, and pregnancy before the initiation of insulin were obtained from medical records. The medical records used an itemized fill-in format. Classification and subdivision of the subjects were conducted independently by three investigators. The three groups were compared for age at onset, sex, fasting blood glucose level before insulin therapy, dose of insulin, prevalence of ICA, TMA, past maximum BW, BW at onset, the prevalence of complicated EADs, family history of diabetes in first-degree relatives of the patient, family history of EAD in first-degree relatives, prevalence of HLA, and serum CPR response to 100-g OGTT.

#### ICA, TMA, and serum and urinary CPR and HbA<sub>1c</sub>

Detection of ICA was performed by the indirect immunofluorescence method as described previously (5,17). The quality of the assay was class A (cutoff point, 5 JDF U; sensitivity, 90%; specificity, 92% [18]). Our laboratory participated in the second through fifth international workshops on the standardization of ICA assays. Serum CPR was measured by a sensitive RIA as described elsewhere (19). The intra-assay CV of the CPR assay at

CPR concentrations of 0.760 and 0.056 nM were, respectively, 3.4 and 9.5%. The interassay CV values at CPR concentrations of 0.760 and 0.056 nM were, respectively, 8.1 and 14.2%. With respect to 24-h urine collection for the CPR assay, only the samples with an increased creatinine level (males: 1600  $\mu$ mol [18 mg]/kg BW; females: 884  $\mu$ mol [10 mg]/kg BW) were subjected to measurement. HbA<sub>1c</sub> levels were measured by HPLC (normal range: 4.8–6.3%). The intra-assay and interassay CVs of HbA<sub>1c</sub> were 0.9 and 1.2%, respectively. TMA were tested by the Microsome Test (Fujizoki, Tokyo). TMA tests were considered positive when the titer was >1:240.

#### HLA typing

**HLA-DQ typing.** Typing of HLA-DQA1 and HLA-DQB1 alleles was performed by the previously described PCR-RFLP method (20–23). The sequences of the primers used were as follows.

For DQA1 typing:

GH26 5' GTGCTGCAGGTGTAAACTTGT  
ACCAG 3' (20)

GH27 5' CACGGATCCGGTAGCAGCGGTA  
GAGTTC 3' (20)

For DQB1 (DQw1) typing:

P1bis 5' TGTGCTACTTCACCAACGGG 3'  
(22)

DQ202 5' CAGGGCAGATCCC GCGGTACG  
CCACCTC 3' (23)

For DQB1 (DQw 2, 3, and 4) typing:

P1bis 5' TGTGCTACTTCACCAACGGG 3'  
(22)

DQ204 5' CACCTGCAGTGC GGAGCTCCA  
ACTGGTA 3' (23)

Typing for these alleles was carried out in 146 randomly selected IDDM patients and 90 normal control subjects.

**HLA typing for A, B, C, and DR antigens.** HLA typing for the A, B, C, and DR antigens was performed in 190 randomly selected IDDM patients by a standard microcytotoxicity test (24). The 203 normal control subjects, who were all of Japanese ethnic stock and lived in the Tokyo and Yokohama area, were typed for HLA.

**Table 1—Clinical characteristics of subjects divided by duration of diabetes before insulin treatment**

|                                                   | GROUP A                    | GROUP B                    | GROUP C                     |
|---------------------------------------------------|----------------------------|----------------------------|-----------------------------|
| (n)                                               | 134                        | 31                         | 42                          |
| DURATION OF DIABETES (MO)                         | 99 ± 9                     | 110 ± 21                   | 96 ± 14                     |
| SEX (M/F)                                         | 67/67                      | 12/19                      | 24/18                       |
| AGE AT ONSET (YR)                                 | 21.6 ± 1.2*<br>(2–60)      | 28.3 ± 3.0†<br>(5–62)      | 38.0 ± 2.4‡<br>(6–62)       |
| FASTING BLOOD GLUCOSE BEFORE INSULIN (mM)         | 31.2 ± 3.2§<br>(12.4–71.7) | 20.0 ± 2.5†<br>(15.7–28.1) | 13.3 ± 0.6  <br>(10.4–20.8) |
| PEAK CPR (NM)                                     | 0.17 ± 0.02                | 0.17 ± 0.03                | 0.26 ± 0.02¶                |
| 24-H URINE CPR (NMOL)                             | 4.3 ± 0.7                  | 4.4 ± 0.9                  | 5.4 ± 0.5¶                  |
| DAILY DOSE OF INSULIN (U/KG BW)                   | 0.71 ± 0.08                | 0.69 ± 0.09                | 0.70 ± 0.09                 |
| HbA <sub>1c</sub> (%)                             | 10.3 ± 0.2                 | 10.2 ± 0.3                 | 9.6 ± 0.2¶                  |
| TMA (%)                                           | 19.4 (26/134)              | 9.7 (3/31)                 | 30.9 (13/42)#               |
| OTHER EAD (%)                                     | 6.7 (9/134)                | 6.4 (2/31)                 | 2.3 (1/42) <sup>a</sup>     |
| HISTORY OF DIABETES IN FIRST-DEGREE RELATIVES (%) |                            |                            |                             |
| IDDM                                              | 7.5 (10/134)               | 6.4 (2/31)                 | 11.9 (5/42)                 |
| NIDDM                                             | 11.2 (15/134)              | 12.9 (4/31)                | 26.1 (11/42) <sup>b</sup>   |
| FAMILY HISTORY OF EAD (%)                         | 3.0 (4/134)                | 3.2 (1/31)                 | 9.5 (4/42)                  |

Data are means ± SE.

\*P &lt; 0.005 vs. group C.

†P &lt; 0.05 vs. group A.

‡P &lt; 0.01 vs. group B.

§P &lt; 0.01 vs. group C.

||P &lt; 0.05 vs. group B.

¶P &lt; 0.01 vs. group A and group B.

#P = 0.054 (2-sided), 0.027 (1-sided) vs. group B.

<sup>a</sup>P = 0.02 vs. group A.<sup>b</sup>P = 0.04 vs. group A.

### Statistical analysis

The Mann-Whitney *U* test was applied to compare values between the different groups and the Kolmogorov-Smirnov test was used to evaluate the differences of distributions. Fisher's exact test was used to compare the prevalence of ICA, TMA, family history, and HLA between different groups. The correction was accomplished by multiplying the value for *P* by the number of HLA antigens tested. The data were expressed as means ± SE.

## RESULTS

### Overall backgrounds of the IDDM subjects

The age at onset of diabetes ranged from 2 to 62 yr with a mode of 10 yr. Eighty-

eight (43%) patients had diabetes onset at ≥30 yr of age. A difference in the male/female ratio was observed between the patients who developed IDDM at <30 yr of age (49/70) and those who developed IDDM at ≥30 yr of age (53/35, *P* < 0.01). There was no apparent seasonality in diabetes onset.

### Comparison of the clinical and immunogenetic characteristics

#### Subdivided subjects

No difference was noted in the duration of diabetes among the three groups. Single fasting blood glucose values before insulin treatment in group A were significantly higher than in group C (Table 1). In group C patients, insulin was started

45 ± 15 mo (13–300 mo) after the diagnosis. At the time of this investigation, all subjects were ketosis prone (>4 episodes of ketonuria and/or ketoacidosis; mean: 9 episodes), and their fasting blood glucose levels measured at the outpatient clinic were unstable. No difference was observed in the daily insulin dose between groups A and C (Table 1). HbA<sub>1c</sub> values in group C were significantly lower than those in groups A and B.

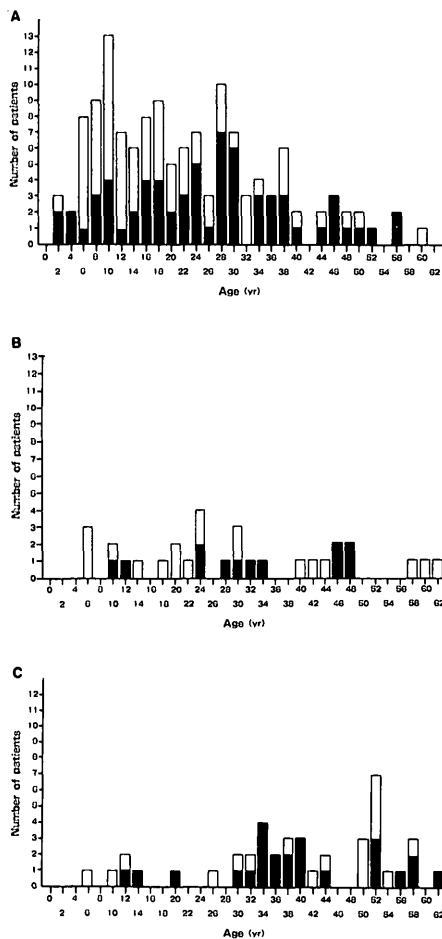
### BW, sex ratio, and age distribution

No significant differences were noted in maximum BW expressed as BMI or in the BMI at onset between groups A and C. No differences in BMI by sex within or among groups were observed (maximum BMI, males vs. females: group A, 24.0 ± 0.2 vs. 23.4 ± 0.2, NS; group B, 24.8 ± 0.6 vs. 25.9 ± 0.6, NS; group C, 24.3 ± 0.6 vs. 25.1 ± 0.6, NS. BMI at onset, males vs. females: group A, 20.5 ± 0.3 vs. 19.1 ± 0.3, NS; group B, 20.1 ± 0.6 vs. 19.6 ± 0.6, NS; group C, 21.3 ± 0.8 vs. 19.5 ± 0.7, NS). No differences were found in sex ratio between groups A, B, and C. The mean age at diabetes onset was significantly higher in group C than in group A. The modal age of onset in group A was 10 yr (Fig. 1A), and in group C it was 52 yr. The age distribution in group C was different from that in group A (*P* < 0.01 by the Kolmogorov-Smirnov test; Fig. 1c).

### TMA and family history

The prevalence of TMA was 20.3% (42 of 207). The overall prevalence of family history of diabetes in first-degree relatives of the subjects was 18.7% in group A and 38.0% in group C (*P* = 0.02). The prevalence of family history of NIDDM in group C was significantly higher than in group A (Table 1). A family history of IDDM or other EADs was not significantly different between group A (4 Graves' disease) and group C (3 Graves' disease and 1 Hashimoto's thyroiditis).

To examine the possibility that older patients were more likely to have a



**Figure 1**—Age distribution in groups A (A), B (B), and C (C). The period before insulin was <3 mo in group A, 3–12 mo in group B, and ≥13 mo in group C. (□), female; (■), male.

family history of diabetes, the incidence of a positive family history among the patients >40 yr of age was compared between groups A and C. The mean ± SE age of the patients >40 yr of age was 51.4 ± 1.4 yr in group A (n = 45) and 53.5 ± 1.2 yr in group C (n = 27, NS). The incidence of a positive family history of NIDDM in group A patients >40 yr of age was 9% (4 of 45), while it was 33% in group C (9 of 27, P = 0.03 vs. group A). In addition, the incidence of a family history of IDDM in groups A and C patients >40 yr of age was 4% (2 of 45 and 1 of 27; NS).

**Table 2**—Prevalence of ICA with duration of diabetes in groups A, B, and C

|             | DURATION OF DIABETES (MO) |            |            | OVERALL PREVALENCE |
|-------------|---------------------------|------------|------------|--------------------|
|             | 0–12                      | 13–59      | ≥60        |                    |
| GROUP A (%) | 54 (20/37)                | 32 (7/22)  | 9 (7/75)   | 25 (34/134)        |
| GROUP B (%) | 50 (4/8)                  | 40 (2/5)   | 6 (1/18)   | 23 (7/31)          |
| GROUP C (%) |                           | 52 (11/21) | 33 (7/21)* | 43 (18/42)†        |

\*P = 0.023, group A vs. group C.

†P = 0.05, group A vs. group C.

### ICA

The overall prevalence of ICA was 28.5% (59 of 207). The prevalences of ICA in group C was higher than that in group A (Table 2).

### Serum and 24-h urinary CPR

Serum CPR response represented by integrated value of serum CPR levels at six sampling points during OGTT. The 0-, 30-, 60-, 90-, 120-, and 180-min and peak values were significantly higher in group C than in groups A and B (Tables 1 and 3). No significant difference was observed in CPR by sex in group A or group C. The 24-h urinary CPR value in group C was higher than in groups A or B (Table 1).

### HLA-A, -B, -C and -DR

HLA-A24 was significantly more common in group A than in normal control

subjects (Table 4), and HLA-B52 was significantly less common. HLA-Bw54 was also increased in group A but was insignificant in group C compared with normal control subjects. HLA-Cw1 was also increased in group A, but insignificant in group C. HLA-DR2 was significantly lower in both groups A and C than in the normal control subjects (Table 5). HLA-DR4 was significantly higher in both groups A and C (Table 5). The relative risk of a heterozygous combination of HLA-DR4/DR9 was not increased in either group A or group C.

### DQA1 and DQB1

The prevalence of DQA1\*0301 was significantly increased in all three groups of patients (Table 6). DQA1\*0103 was significantly decreased in all three groups. DQB1\*0302, DQB1\*0303, and DQB1\*0401 were significantly increased in group

**Table 3**—Integrated serum C-peptide values during 100-g OGTT in terms of duration of diabetes in groups A, B, and C

|              | DURATION OF DIABETES (MO) |           |            | TOTAL              |
|--------------|---------------------------|-----------|------------|--------------------|
|              | 0–12                      | 13–59     | ≥60        |                    |
| GROUP A (NM) | 1.1 ± 0.2                 | 0.8 ± 0.2 | 0.9 ± 0.1  | 0.9 ± 0.1 (0–2.6)  |
| n            | 37                        | 22        | 75         | 134                |
| GROUP B (NM) | 0.9 ± 0.4                 | 1.7 ± 0.8 | 0.7 ± 0.2* | 0.9 ± 0.2† (0–3.2) |
| n            | 8                         | 5         | 18         | 31                 |
| GROUP C (NM) |                           | 1.1 ± 0.3 | 1.6 ± 0.2‡ | 1.4 ± 0.1‡ (0–2.4) |
| n            |                           | 21        | 21         | 42                 |

Data are means ± SE.

\*P < 0.01 group B vs. group C.

†P < 0.05 group B vs. group C.

‡P < 0.01 group A vs. group C.

Table 4—HLA-A and -B antigen frequencies in groups A, B, and C and normal control subjects

| ANTIGENS<br>HLA | GROUP A<br>(n = 122) | GROUP B<br>(n = 31) | GROUP C<br>(n = 37) | CONTROL<br>(n = 203) | RELATIVE RISK |         |         | P VALUE VS. CONTROL        |         |         |
|-----------------|----------------------|---------------------|---------------------|----------------------|---------------|---------|---------|----------------------------|---------|---------|
|                 | n (%)                | n (%)               | n (%)               | n (%)                | GROUP A       | GROUP B | GROUP C | GROUP A                    | GROUP B | GROUP C |
| A1              | 1 (1)                | 0 (0)               | 0 (0)               | 1 (0)                |               |         |         |                            |         |         |
| A2              | 51 (42)              | 15 (48)             | 20 (54)             | 74 (36)              | 1.25          | 1.63    | 1.95    | NS                         | NS      | NS      |
| A3              | 1 (1)                | 0 (0)               | 0 (0)               | 1 (0)                |               |         |         |                            |         |         |
| A11             | 17 (14)              | 4 (13)              | 6 (17)              | 36 (18)              |               |         |         |                            |         |         |
| A24             | 86 (70)              | 21 (68)             | 21 (57)             | 114 (56)             | 1.87          | 1.64    | 1.02    | 0.01                       | NS      | NS      |
| A26             | 21 (17)              | 6 (19)              | 12 (33)             | 48 (24)              | 0.67          | 0.78    | 1.61    | NS                         | NS      | NS      |
| A31             | 11 (9)               | 1 (4)               | 3 (8)               | 25 (12)              |               |         |         |                            |         |         |
| A33             | 17 (14)              | 6 (19)              | 6 (17)              | 28 (14)              |               |         |         |                            |         |         |
| B5              | 1 (1)                | 0 (0)               | 1 (3)               | 0 (0)                |               |         |         |                            |         |         |
| B7              | 18 (15)              | 6 (19)              | 3 (8)               | 10 (10)              |               |         |         |                            |         |         |
| B8              | 0 (0)                | 0 (0)               | 0 (0)               | 1 (0)                |               |         |         |                            |         |         |
| B13             | 1 (1)                | 0 (0)               | 1 (3)               | 3 (1)                |               |         |         |                            |         |         |
| B15             | 14 (11)              | 2 (6)               | 6 (15)              | 33 (14)              |               |         |         |                            |         |         |
| B16             | 3 (2)                | 0 (0)               | 2 (6)               | 1 (0)                |               |         |         |                            |         |         |
| B27             | 1 (1)                | 0 (0)               | 0 (0)               | 1 (0)                |               |         |         |                            |         |         |
| B35             | 19 (15)              | 1 (4)               | 6 (17)              | 22 (11)              |               |         |         |                            |         |         |
| B37             | 0 (0)                | 0 (0)               | 0 (0)               | 0 (0)                |               |         |         |                            |         |         |
| B39             | 1 (1)                | 0 (0)               | 4 (11)              | 16 (8)               |               |         |         |                            |         |         |
| B40             | 3 (2)                | 0 (0)               | 0 (0)               | 0 (0)                |               |         |         |                            |         |         |
| Bw42            | 0 (0)                | 0 (0)               | 0 (0)               | 0 (0)                |               |         |         |                            |         |         |
| B44             | 16 (13)              | 5 (16)              | 6 (17)              | 27 (13)              |               |         |         |                            |         |         |
| Bw48            | 6 (5)                | 0 (0)               | 2 (6)               | 7 (3)                |               |         |         |                            |         |         |
| B51             | 12 (10)              | 0 (0)               | 2 (6)               | 26 (13)              |               |         |         |                            |         |         |
| Bw52            | 10 (8)               | 1 (4)               | 4 (11)              | 45 (22)              | 0.31          | 0.11    | 0.44    | 0.001*                     | 0.01    | NS      |
| Bw54            | 50 (40)              | 9 (29)              | 9 (25)              | 36 (18)              | 3.13          | 1.90    | 1.72    | 0.001 × 10 <sup>-2</sup> † | NS      | NS      |
| Bw56            | 2 (2)                | 0 (0)               | 0 (0)               | 2 (1)                |               |         |         |                            |         |         |
| Bw58            | 0 (0)                | 0 (0)               | 0 (0)               | 1 (0)                |               |         |         |                            |         |         |
| Bw59            | 4 (3)                | 1 (4)               | 0 (0)               | 3 (1)                |               |         |         |                            |         |         |
| Bw60            | 11 (9)               | 7 (23)              | 6 (17)              | 29 (14)              |               |         |         |                            |         |         |
| Bw61            | 33 (27)              | 1 (4)               | 6 (17)              | 50 (25)              |               |         |         |                            |         |         |

Relative risk was calculated as follows: (a) × (d)/(b) × (c), where a = patients positive for antigen, b = patients negative for antigen, c = control subjects positive for antigen, and d = control subjects negative for antigen.

\*Corrected P < 0.05.

†Corrected P < 0.01.

A, whereas only DQB1\*0401 was increased in group C. DQB1\*0601 was significantly decreased in all three groups.

**DQA1-DQB1 haplotypes**

In group A, three haplotypes (DQA1\*0301-DQB1\*0401, DQA1\*0301-DQB1\*0302, and DQA1\*0301-DQB1\*0303) showed an increased prevalence, whereas only DQA1\*0301-DQB1\*0401 was significantly increased in group C (Table 7). DQA1\*0103-DQB1\*0601 was decreased in all three groups.

**CONCLUSIONS**— Scant knowledge is available on the natural history of ICA positivity among patients with NIDDM despite the large number of such patients (~10% of all Japanese NIDDM patients; 25). Recently, we reported that some NIDDM patients with persistent ICA and/or insulin autoantibodies progressed gradually to insulin dependency over several years (5,10,26). This slowly progressive clinical course is one of the characteristics observed in some Japanese IDDM patients (11,25). A comparison of

the immunogenetic and clinical findings in these slowly progressive patients with those with typical acute-onset IDDM may help identify some of the pathogenic factors contributing to this clinical subtype of IDDM.

In this clinic-based study, 20% of Japanese IDDM patients had a history of slowly progressive β-cell failure for >13 mo before the initiation of insulin treatment. Slowly progressive IDDM (group C) was characterized by a late age of onset and a different age distribution

Table 5—HLA-C and -DR antigen frequencies in groups A, B, and C and normal control subjects

| ANTIGENS | GROUP A   | GROUP B  | GROUP C  | CONTROL   | RELATIVE RISK |         |         | P VALUE VS. CONTROL         |         |         |
|----------|-----------|----------|----------|-----------|---------------|---------|---------|-----------------------------|---------|---------|
|          | (n = 122) | (n = 31) | (n = 37) | (n = 203) | GROUP A       | GROUP B | GROUP C | GROUP A                     | GROUP B | GROUP C |
| Cw1      | 66 (54)   | 14 (45)  | 14 (39)  | 70 (34)   | 2.20          | 1.56    | 1.30    | 0.004*                      | NS      | NS      |
| Cw2      | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     |               |         |         |                             |         |         |
| Cw3      | 48 (39)   | 11 (36)  | 17 (47)  | 102 (50)  | 0.63          | 0.54    | 0.89    | NS                          | NS      | NS      |
| Cw4      | 9 (7)     | 4 (12)   | 0 (0)    | 20 (10)   |               |         |         |                             |         |         |
| Cw5      | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     |               |         |         |                             |         |         |
| Cw7      | 23 (19)   | 7 (23)   | 10 (28)  | 43 (21)   |               |         |         |                             |         |         |
| Cw9      | 0 (0)     | 0 (0)    | 1 (3)    | 0 (0)     |               |         |         |                             |         |         |
| Cw11     | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     |               |         |         |                             |         |         |
| DR1      | 11 (9)    | 4 (12)   | 2 (6)    | 26 (13)   |               |         |         |                             |         |         |
| DR2      | 13 (10)   | 1 (4)    | 4 (11)   | 68 (33)   | 0.23          | 0.07    | 0.25    | 0.003 × 10 <sup>-3</sup> †  | 0.0003† | 0.006*  |
| DR3      | 0 (0)     | 0 (0)    | 0 (0)    | 0 (0)     |               |         |         |                             |         |         |
| DR4      | 88 (71)   | 21 (68)  | 23 (62)  | 81 (40)   | 3.68          | 3.16    | 2.47    | 0.002 × 10 <sup>-15</sup> † | 0.007   | 0.02    |
| DR7      | 1 (1)     | 1 (4)    | 1 (3)    | 0 (0)     |               |         |         |                             |         |         |
| DR8      | 20 (16)   | 5 (16)   | 6 (17)   | 40 (20)   |               |         |         |                             |         |         |
| DR9      | 47 (38)   | 11 (36)  | 11 (31)  | 63 (31)   | 1.36          | 1.22    | 0.98    |                             |         |         |
| DR10     | 0 (0)     | 0 (0)    | 0 (0)    | 1 (0)     |               |         |         |                             |         |         |
| DR4/DR9  | 22 (18)   | 6 (19)   | 4 (11)   | 15 (7)    | 2.76          | 3.00    | 1.52    | 0.007                       | 0.029   | NS      |

\*Corrected P &lt; 0.05.

†Corrected P &lt; 0.01.

than acute-onset IDDM as well as a higher prevalence of ICA, longer persistence of  $\beta$ -cell function, and a more common family history of NIDDM compared with acute-onset patients. An elaborate population-based study may be required to further characterize these cases.

A striking male predominance was observed among IDDM patients >30 yr of age. Some IDDM patients in group C had a long duration of diabetes before insulin therapy. Male predominance was previously reported in ICA<sup>+</sup> NIDDM (10), and these findings suggest that maleness has some influence on slowly progressive  $\beta$ -cell failure through hormonal or immunological processes. Male predominance of IDDM has also been documented in Caucasians (27) and experimental animals (28), but the finding is not consistent.

The mechanisms underlying the different clinical courses of groups A and C remain speculative. Group C patients had a higher average age of onset than

those in group A. Different clinical features based on the age at onset were reported (29). Adult-onset IDDM is characterized by a longer period before diagnosis and better preservation of residual  $\beta$ -cell function (29). The different  $\beta$ -cell mass in adults and children may possibly explain the different latencies before insulin treatment.

Genetic factors related to HLA may explain the differences in the severity of  $\beta$ -cell damage between groups A and C. Our haplotype analysis of DQA1-DQB1 provided some evidence of genetic differences between acute-onset IDDM and slowly progressive IDDM: group A patients featured three diabetogenic haplotypes (DQA1\*0301-DQB1\*0401, DQA1\*0301-DQB1\*0302, and DQA1\*0301-DQB1\*0303), whereas only DQA1\*0301-DQB1\*0401 was increased in group C (Tables 6 and 7). Thus, the difference in  $\beta$ -cell damage between groups A and C may perhaps be explained by the relative dose of DQ- $\alpha$  and - $\beta$  heterodimers encoded by the DQA1

and DQB1 genes regulating the rate of  $\beta$ -cell destruction as well as susceptibility to IDDM (30). Group A patients also had a significant increase of HLA-A24, HLA-Bw54, HLA-Cw1, and HLA-DR4, as well as a decrease of HLA-Bw52 and HLA-DR2, which are characteristic findings in Japanese IDDM patients (17). Note that group C lacked any class I (HLA-A24, HLA-Bw54, or HLA-Cw1) association, whereas group A had such an association. A study on residual  $\beta$ -cell function in IDDM has demonstrated that a high proportion of IDDM patients with no  $\beta$ -cell function had a highest relative risk of 20 for HLA-A24 (31). In contrast, IDDM patients with a long duration, who had preserved  $\beta$ -cell function, did not have a significant association with HLA-A24 (31). The class I MHC is possibly associated with  $\beta$ -cell destruction in an additive manner with class II antigens in experimental animals (32).

One of the characteristics of group C patients was a high frequency of NIDDM in first-degree relatives, espe-

Table 6—Frequencies of HLA-DQA1 and -DQB1 alleles in groups A, B, and C and normal control subjects

| HLA ALLELES     | GROUP A  | GROUP B  | GROUP C  | CONTROL  | RELATIVE RISK |         |         | P VALUE                |                       |                      |
|-----------------|----------|----------|----------|----------|---------------|---------|---------|------------------------|-----------------------|----------------------|
|                 | (n = 88) | (n = 26) | (n = 32) | (n = 90) | GROUP A       | GROUP B | GROUP C | GROUP A                | GROUP B               | GROUP C              |
| DQA1*0101/*0102 | 22 (25)  | 16 (62)  | 11 (34)  | 46 (51)  |               |         |         |                        |                       |                      |
| DQA1*0103       | 2 (2)    | 0 (0)    | 6 (19)   | 36 (42)  | 0.07          | 0.03    | 0.34    | 1 × 10 <sup>-9</sup>   | 4 × 10 <sup>-12</sup> | 0.02*                |
| DQA1*0201       | 0 (0)    | 0 (0)    | 0 (0)    | 2 (2)    |               |         |         |                        |                       |                      |
| DQA1*0301       | 84 (95)  | 24 (92)  | 30 (94)  | 56 (62)  | 12.75         | 7.29    | 9.11    | 2.8 × 10 <sup>-9</sup> | 0.028                 | 0.0049               |
| DQA1*0401       | 2 (2)    | 2 (8)    | 1 (3)    | 2 (2)    |               |         |         |                        |                       |                      |
| DQA1*0501       | 4 (5)    | 0 (0)    | 1 (3)    | 14 (16)  | 0.26          | 0.10    | 0.18    | NS                     | 0.02*                 | NS                   |
| DQA1*0601       | 4 (5)    | 0 (0)    | 0 (0)    | 4 (4)    |               |         |         |                        |                       |                      |
| DQB1*0501/*0502 | 2 (2)    | 1 (4)    | 2 (3)    | 8 (9)    |               |         |         |                        |                       |                      |
| DQB1*0503       | 2 (2)    | 0 (0)    | 1 (3)    | 10 (11)  |               |         |         |                        |                       |                      |
| DQB1*0601       | 2 (2)    | 0 (0)    | 2 (6)    | 40 (44)  | 0.03          | 0.02    | 0.08    | 4 × 10 <sup>-11</sup>  | 5 × 10 <sup>-5</sup>  | 6 × 10 <sup>-4</sup> |
| DQB1*0602       | 4 (5)    | 0 (0)    | 1 (3)    | 10 (11)  |               |         |         |                        |                       |                      |
| DQB1*0604       | 12 (14)  | 11 (42)  | 12 (38)  | 15 (17)  |               |         |         |                        |                       |                      |
| DQB1*0201       | 0 (0)    | 0 (0)    | 0 (0)    | 0 (0)    |               |         |         |                        |                       |                      |
| DQB1*0301       | 6 (7)    | 0 (0)    | 4 (13)   | 17 (19)  |               |         |         |                        |                       |                      |
| DQB1*0302       | 28 (32)  | 4 (15)   | 6 (19)   | 12 (14)  | 3.03          | 1.18    | 1.50    | 0.028                  | NS                    | NS                   |
| DQB1*0303       | 38 (43)  | 11 (42)  | 7 (22)   | 20 (23)  | 2.66          | 2.57    | 0.98    | 0.024                  | NS                    | NS                   |
| DQB1*0401       | 41 (48)  | 9 (35)   | 17 (53)  | 20 (23)  | 3.05          | 3.50    | 3.97    | 0.006                  | 0.020                 | 0.015                |
| DQB1*0402       | 6 (7)    | 4 (15)   | 1 (3)    | 6 (7)    |               |         |         |                        |                       |                      |

\*Uncorrected P value.

cially in their parents. A misclassification of the NIDDM patients into group C is unlikely, because these group C patients had a high (42.9%) prevalence of ICA despite their long duration of diabetes

and a low CPR. Recently, we found that up to 23% of the parents of IDDM probands had NIDDM, and some of these NIDDM parents had ICA and progressed slowly to IDDM (33). Thus,

some of the parents with NIDDM documented in the present survey probably had IDDM in the pre-insulin-dependence stage. An association between IDDM probands and NIDDM parents

Table 7—DQA1-DQB1 haplotype frequencies in groups A, B, and C and normal control subjects

| HAPLOTYPE           | GROUP A  | GROUP B  | GROUP C  | CONTROL  | RELATIVE RISK |         |         | P VALUE                  |         |         |
|---------------------|----------|----------|----------|----------|---------------|---------|---------|--------------------------|---------|---------|
|                     | (n = 88) | (n = 26) | (n = 32) | (n = 90) | GROUP A       | GROUP B | GROUP C | GROUP A                  | GROUP B | GROUP C |
| DQA1*0103-DQB1*0601 | 2 (2)    | 1 (4)    | 1 (2)    | 26 (29)  | 0.06          | 0.09    | 0.08    | 0.007 × 10 <sup>-3</sup> | 0.009   | 0.002   |
| DQA1*0102-DQB1*0602 | 4 (5)    | 0 (0)    | 1 (3)    | 5 (6)    |               |         |         |                          |         |         |
| DQA1*0103-DQB1*0603 | 0 (0)    | 0 (0)    | 0 (0)    | 1 (1)    |               |         |         |                          |         |         |
| DQA1*0102-DQB1*0604 | 12 (14)  | 10 (38)  | 7 (22)   | 9 (10)   |               |         |         |                          |         |         |
| 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*0301-DQB1*0301 | 2 (2)    | 0 (0)    | 2 (3)    | 1 (1)    |               |         |         |                          |         |         |
| DQA1*0501-DQB1*0301 | 2 (2)    | 0 (0)    | 1 (3)    | 9 (10)   |               |         |         |                          |         |         |
| DQA1*0601-DQB1*0301 | 0 (0)    | 0 (0)    | 0 (0)    | 4 (4)    |               |         |         |                          |         |         |
| DQA1*0301-DQB1*0302 | 27 (31)  | 3 (12)   | 6 (19)   | 7 (8)    | 5.2           | 1.5     | 2.7     | 0.0018                   | NS      | NS      |
| DQA1*0301-DQB1*0303 | 39 (44)  | 11 (42)  | 7 (22)   | 19 (21)  | 3.0           | 2.7     | 1.0     | 0.016                    | NS      | NS      |
| DQA1*0301-DQB1*0401 | 43 (49)  | 10 (38)  | 15 (47)  | 21 (23)  | 3.1           | 2.1     | 2.9     | 0.0064*                  | NS      | 0.025*  |

\*Uncorrected P value.

was reported as evidence of the heterogeneity of IDDM by Chern et al. (34). A high incidence of a family history of diabetes in ICA<sup>+</sup> late-onset diabetic patients was also reported by Groop et al. (35). The possibility cannot be ruled out that other genetic factor(s) contribute to the high prevalence of a family history of NIDDM in group C. We surveyed Hp, a putative genetic marker of NIDDM (36), and found that Hp 2-1 was significantly increased in the NIDDM parents of IDDM probands (37). This result suggests that a subgroup of NIDDM gene(s) may contribute to the development of IDDM as an additive risk factor (38).

In a Caucasian population, the familial occurrence of other EADs with IDDM has been reported (39). However, no significant difference was noted in the frequency of such diseases, a family history of such diseases, or the presence of TMA in our previous study (11). A weak genetic predisposition to autoimmunity in the Japanese population may explain this result.

**Acknowledgments**—This work was supported in part by a grant from the Ministry of Health and Welfare of Japan.

We thank Dr. Y. Iizuka (Tokyo University) and M. Furuta for assistance with statistical analysis, Dr. T. Hiraga for generous help, and M. Shibata and F. Takano for excellent secretarial work.

## References

- Gorsuch AN, Spencer KM, Lister J, McNally JM, Dean BM, Bottazzo GF: Evidence for a long prediabetic period in type 1 (insulin-dependent) diabetes mellitus. *Lancet* 2:1363-65, 1981
- Eisenbarth GS: Type 1 diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 314:1360-68, 1986
- Kida K, Gotoh Y, Kaino Y, Matsuda H, Kobayashi T: Use of human insulin (recombinant DNA) in a type 1 diabetic child with autoimmunity: a case report. *Jpn Diabetes Soc* 27 (Suppl. 2):259-62, 1984
- Irvine W, McCallum C, Gray R, Duncan L: Clinical and pathogenic significance of pancreatic-islet-cell antibodies in diabetics treated with oral hypoglycemic agents. *Lancet* 1:1025-27, 1977
- Kobayashi T, Itoh T, Kosaka K, Sato K, Tsuji K: Time course of islet cell antibodies and  $\beta$ -cell function in non-insulin-dependent stage of type 1 diabetes. *Diabetes* 36:510-17, 1987
- Spencer KM, Tarn A, Dean BM, Lister J, Bottazzo GF: Fluctuating islet-cell autoimmunity in unaffected relatives of patients with insulin-dependent diabetes. *Lancet* 1:764-66, 1984
- McCulloch DK, Klaff LJ, Schoenfeld SL, Greenbaum CJ, Benson EA, Palmer JP: Subclinical beta cell dysfunction is not always progressive among first degree relatives of type 1 diabetics: five year follow-up of the Seattle family study (Abstract). *Diabetes* 38 (Suppl. 2):90A, 1989
- Landin-Olsson M, Karlsson A, Dahlquist G, Blom L, Lernmark A, Sundkvist G: Islet cell and other organ-specific autoantibodies in all children developing type 1 (insulin-dependent) diabetes mellitus in Sweden during one year and in matched control children. *Diabetologia* 32:387-95, 1989
- Barbosa J, Rich SS: Genetics of insulin-dependent diabetes. In *Immunogenetics of Endocrine Disorders*. New York, Liss, 1988, p. 163-202
- Kobayashi T, Nakanishi K, Sugimoto T, Itoh T, Murase T, Kosaka K, Tsuji K: Maleness as risk factor for slowly progressive IDDM. *Diabetes Care* 12:7-11, 1989
- Kobayashi T, Sawano S, Itoh T, Sugimoto T, Takahashi S, Tanaka T, Suwa S: Islet-cell antibodies in insulin-dependent and non-insulin-dependent diabetics in Japan: their prevalence and clinical significance. In *Clinico-Genetic Genesis of Diabetes Mellitus*. Mimura G, Baba S, Gotoh Y, Kobberling J, Eds. Amsterdam, Excerpta Med., 1982, p. 150-60
- Urakami T, Miyamoto Y, Fujita H, Kitagawa T: Type 1 (insulin-dependent) diabetes in Japanese children is not a uniform disease. *Diabetologia* 32:312-15, 1989
- National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039-57, 1979
- Matsuda A, Kuzuya T: Urine C-peptide after recovery from diabetic ketoacidosis—an index of insulin dependency. *Diabetes Care* 5:581-84, 1982
- Gjessing HJ, Matzen LE, Feber OK, Froland A: Fasting plasma C-peptide, glucagon stimulated plasma C-peptide, and urinary C-peptide in relation to clinical type of diabetes. *Diabetologia* 32:305-11, 1989
- Pinchera A, Fenzi G: Endocrine autoimmune disease. In *Endocrinology*. 3rd ed. Degroot LJ, Cahill GF Jr, Odell WD, Martini L, Potts JT Jr, Nelson DH, Steinberger E, Winegrad A, Eds. New York, Grune & Stratton, 1979, p. 2063-83
- 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* 35:335-40, 1986
- Boitard C, Bonifacio E, Bottazzo GF, Gleichmann H, Molenaar J: Immunology and diabetes workshops: report on the third international (stage 3) workshop on standardisation of cytoplasmic islet cell antibodies. *Diabetologia* 31:451-52, 1988
- Nakanishi K, Kobayashi T, Miyashita H, Ohkubo M, Sugimoto T, Murase T, Kosaka K, Inouye K, Kono M: Relationships between islet cell antibodies, residual beta-cell function and metabolic control in patients with insulin-dependent diabetes mellitus of long duration: use of a sensitive C-peptide radioimmunoassay. *Metabolism* 39:925-30, 1990
- Maeda M, Murayama H, Ishi H, Ota M, Tsuji K, Inoko H: A simple and rapid method for HLA-DQA1 genotyping by digestion of PCR-amplified DNA with allele specific restriction endonuclease. *Tissue Antigens* 34:290-98, 1989
- 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 endonuclease. *Tissue Antigens*



- 35:20–31, 1990
22. Trucco G, Fritsch R, Giorda R, Trucco M: Rapid detection of IDDM susceptibility with HLA-DQB alleles as markers. *Diabetes* 38:1617–22, 1989
  23. 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
  24. Terasaki PI: HLA-DR joint report. In *Histocompatibility Testing*. Terasaki PI, Ed. Los Angeles, CA, UCLA Tissue Typing Laboratory, 1980, p. 506
  25. Kobayashi T: Immunology and immunogenetics of type I–diabetes in Japan. *IDF Bull* 35:34–37, 1990
  26. Nakanishi K, Kobayashi T, Sugimoto T, Murase T, Itoh T, Kosaka K: Predictive value of insulin autoantibodies for further progression of beta cell dysfunction in non-insulin-dependent diabetics. *Diabetes Res* 9:105–109, 1988
  27. Schiffrin A, Suissa S, Poussier P, Guttmann R, Weitzner G: Prospective study of predictors of  $\beta$ -cell survival in type I diabetes. *Diabetes* 37:920–25, 1988
  28. Yoon J-W, Melez KA, Smathers PA, Archer JA, Steinberg AD: Virus-induced diabetes in autoimmune New Zealand mice. *Diabetes* 32:755–59, 1983
  29. Karjalainen J, Salmela P, Ilonen J, Sercel H-M, Knip M: A comparison of childhood and adult type I diabetes mellitus. *N Engl J Med* 320:881–86, 1989
  30. Khalil I, Deschamps I, Lepage V, Al-Daccak R, Degos L, Hors J: Dose effect of cis- and trans-encoded HLA-DQ aB heterodimers in IDDM susceptibility. *Diabetes* 41:378–84, 1992
  31. Nakanishi K, Kobayashi T, Miyashita H, Okubo M, Sugimoto T, Murase T, Kosaka K, Tsuji K: Effect of class I and class II on beta-cell damage in insulin-dependent diabetes mellitus (IDDM) [Abstract]. *J Jpn Diabetes Soc* 33 (Suppl. 1): 255, 1990
  32. Allison J, Cambell IL, Morahan G, Mandl TE, Harrison LC, Miller JFAP: Diabetes in transgenic mice resulting from overexpression of class I histocompatibility molecules in pancreatic  $\beta$ -cells. *Nature Lond* 333:529–33, 1988
  33. Kajio H, Kobayashi T, Nakanishi K, Ohkubo M, Sugimoto T, Murase T, Itoh T, Kosaka K: Islet cell antibodies (ICA) and beta-cell functions in first-degree relatives of insulin-dependent diabetics: a prospective study (Abstract). *Diabetes Res Clin Pract* 5 (Suppl. 1):S255, 1988
  34. Chern MM, Anderson VE, Barbosa J: Empirical risk for insulin-dependent diabetes (IDD) in sibs. *Diabetes* 31:1115–18, 1982
  35. Groop LC, Bottazzo GF, Doniach D: Islet cell antibodies identify latent type I diabetes in patients aged 35–75 years at diagnosis. *Diabetes* 35:237–41, 1986
  36. Stern MP, Ferrell RE, Rosenthal M, Haffner SM, Hazuda H: Association between NIDDM, Rh blood group, and haptoglobin phenotype. *Diabetes* 35: 387–91, 1986
  37. Matsushita T, Tsukada T, Nakayama T, Kitamura M, Kobayashi T, Nakanishi K, Murase T: Haptoglobin phenotype in insulin-dependent diabetes and non-insulin-dependent diabetes in Japanese population (in Japanese). *Physico-chem Biol* 33:52, 1989
  38. Wagener DK, Sacks JM, Laporte RE, Macgregor JM: The Pittsburgh study of insulin-dependent diabetes mellitus: risk for diabetes among relatives of IDDM. *Diabetes* 31:136–44, 1982
  39. Bottazzo GF, Cudworth AG, Moul DJ, Doniach D, Festenstein H: Evidence for a primary autoimmune type of diabetes mellitus (type 1b). *Br Med J* 2:1253–55, 1978