0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(11):5090–5098

doi: 10.1210/jc.2003-030180

Ketosis-Prone Diabetes: Dissection of a Heterogeneous Syndrome Using an Immunogenetic and β-Cell Functional Classification, Prospective Analysis, and Clinical Outcomes

MARIO MALDONADO, CHRISTIANE S. HAMPE, LAKSHMI K. GAUR, SUSANA D'AMICO, DINAKAR IYER, LISA P. HAMMERLE, DOUGLAS BOLGIANO, LUCILLE RODRIGUEZ, ARUN RAJAN, ÅKE LERNMARK, AND ASHOK BALASUBRAMANYAM

Division of Endocrinology, Department of Medicine, Baylor College of Medicine (M.M., S.D., D.I., A.R., A.B.); and Endocrine Service, Ben Taub General Hospital (M.M., L.R., A.B.), Houston, Texas 77030; Robert J. Williams Laboratory, Department of Medicine, University of Washington School of Medicine (C.S.H., L.K.G., L.P.H., Å.L.); and Puget Sound Blood Center (L.K.G., D.B.), Seattle, Washington 98195

Ketosis-prone diabetes is heterogeneous. Its causes could include novel β-cell functional defects. To characterize such defects, 103 patients with diabetic ketoacidosis were evaluated for β-cell autoimmunity and human leukocyte antigen (HLA) class II alleles, with longitudinal measurements of β-cell function and biochemical and clinical parameters. They were classified into four Aβ groups, based on the presence of glutamic acid decarboxylase (GAD)65, GAD67, or IA-2 autoantibodies (A+ or A−) and β-cell functional reserve (β+ or β−). The group distribution was: 18 A+β−, 23 A−β−, 11 A+β+, and 51 A−β+. Collectively, the two β− groups differed from the two β+ groups in earlier onset and longer duration of diabetes, lower body mass index, less glycemic improvement, and persistent insulin requirement. HLA class II genotyping showed that the A−β− group differed from the A+β− group in having lower frequencies of two alleles strongly associated with autoimmune type 1 diabetes susceptibility: DQA*03 and DQB1*02. Similarly, the A−β+ group differed from the A+β+ group in having a lower frequency of DQB1*02. Ketosis-prone diabetes comprises at least four etiologically distinct syndromes separable by autoantibody status, HLA genotype, and β-cell functional reserve. Novel, nonautoimmune causes of β-cell dysfunction are likely to underlie the A−β+ and A−β− syndromes. (*J Clin Endocrinol Metab* 88: 5090–5098, 2003)

EITHER VIRTUAL ABSENCE of insulin secretion by the pancreatic β-cell or overwhelming insulin resistance is thought to be a prerequisite for the development of diabetic ketoacidosis (DKA). Hence, DKA traditionally has been considered a complication of patients with type 1 diabetes or rare, severe, insulin resistance syndromes (1, 2). However, we and others have demonstrated that DKA commonly occurs in persons with apparently heterogeneous forms of diabetes, including those who fit clinical definitions of type 2 diabetes (3–8). The etiological bases of these forms are unknown (9), but their widespread emergence has sparked a vigorous debate into the need for a pathogenetically specific and clinically useful classification of ketosis-prone diabetic syndromes (10). Such a classification scheme might also identify subgroups of patients with potentially novel forms of β-cell dysfunction.

We hypothesized that the basis of different ketosis-prone diabetic syndromes resides in variations in two key features associated with classic, autoimmune, type 1 diabetes: presence or absence of markers of β-cell autoimmunity, and quantitative differences in β-cell function. We further hypothesized that differences in these two measures would influence clinical outcomes of ketosis-prone diabetic patients, such as long-term glycemic control and requirement for insulin treatment. To address these hypotheses, we have established a comprehensive, prospective program to characterize the subtypes of DKA-prone patients in a large, multiethnic diabetic population, using detailed clinical, serological, biochemical, and genetic measurements. We report the data from 103 such patients, who have been followed closely and assessed repeatedly for 12 months after the index episode of DKA. Our results indicate that there are four distinct forms of ketosis-prone diabetes, with important implications both for identification of novel pathogenic mechanisms of β-cell dysfunction and for predicting clinical outcomes.

Patients and Methods

Patient characteristics

The protocol was approved by the Institutional Review Boards for Human Studies of Baylor College of Medicine and the Harris County Hospital District (Houston, TX). Adult patients admitted to the Ben Taub General Hospital with a diagnosis of DKA from July 1, 1999, to January 31, 2001, were invited to participate in the study. Informed consent was obtained from all patients who elected to participate. DKA was defined by the presence of all of the following: anion gap equal to or greater than 15, blood pH less than 7.30; serum bicarbonate concentration equal to or less than 17 mmol/liter; serum glucose concentration greater than 200 mg/dl (>11 mmol/liter); serum ketones equal to or greater than 5.2 mmol/liter, by the Acetest reagent (Bayer Corporation, Pittsburgh, PA) or urine ketones moderate to large (>13.9 mmol/liter), together with the absence of concomitant conditions that might result in anion gap aci-

Abbreviations: AUC, Area under the curve; BMI, body mass index; DKA, diabetic ketoacidosis; GAD, glutamic acid decarboxylase; GST, glucagon stimulation test; HbA_{1c}, glycosylated hemoglobin; HLA, human leukocyte antigen; MODY, maturity onset diabetes of youth.

dosis or ketosis, such as pregnancy, renal insufficiency (other than a mild, reversible prerenal state), lactic acidosis, acute alcohol intoxication, or organic poison ingestion. A family history of diabetes was defined by a reported diagnosis of diabetes in a parent or sibling of the patient.

Clinical outcomes analysis. Patients admitted to Ben Taub General Hospital with DKA during the study period were interviewed within 24 h of admission. Those who met entry criteria and consented to participate underwent a detailed assessment of medical history, physical signs, precipitating causes of DKA, serum and urine biochemistry, anthropometry, and clinical course during the hospitalization. Following a standard inpatient protocol for management of DKA, the study team assumed all subsequent outpatient diabetes management of enrolled patients.

A standard outpatient diabetes management protocol was followed in a clinic dedicated to the study. Patients were placed on twice-daily NPH insulin at the time of hospital discharge, with the dose determined by the mean daily insulin requirement during the previous 2 hospital days. They were instructed to measure capillary blood glucose levels before each meal and at bedtime. Stored blood glucose data were uploaded to a computer database. The first clinic visit was 1 wk after hospital discharge. Subsequent visits were scheduled at 1- to 4-wk intervals. If mean blood glucose values during a 2-wk period attained American Diabetes Association-defined goals for fasting and/or bedtime plasma glucose levels (11), the insulin dose was reduced by 50%, and the patient was reassessed in the clinic 1 wk later. If the mean blood glucose values remained at American Diabetes Association goals at two consecutive clinic visits made 2–4 wk apart, insulin was discontinued, and the patient was monitored closely (by daily telephone contact for at least 1 wk, then less frequently). If blood glucose values increased after cessation of insulin but ketosis did not develop (as measured by plasma acetone concentration), the patient was placed on oral hypoglycemic agents and monitored periodically for hyperglycemia and ketosis. Conversely, if the patient developed ketosis on decreasing insulin dosage, the insulin regimen was intensified, and no further attempts were made to discontinue insulin in such patients. Glycosylated hemoglobin (HbA_{1c}) was measured at baseline and every 3 months.

Autoantibody analysis. Serum was analyzed for the presence of glutamic acid decarboxylase (GAD)65, GAD67, and IA-2 autoantibodies by highly sensitive and specific quantitative radioligand binding assay methods, as reported previously (12, 13), with recent modifications (14). Briefly, ^{35}S-labeled recombinant GAD65, GAD67, and IA-2 proteins were produced by *in vitro* transcription and translation of the respective cDNA preparations. The radiolabeled antigen was incubated overnight at 4 C with a 1/25th final dilution of serum. Antibody bound to the radiolabeled antigen was separated from free labeled antigen using protein A-Sepharose beads (Zymed, San Francisco, CA) in microtiter plates (14). Bound radioactivity was determined by liquid scintillation counting (1450 Microbeta Liquid Scintillation Counter, Wallac, Turku, Finland). Antibody-positive and -negative samples were included in every assay to correct for interassay variation. The World Health Organization islet cell autoantibody standard (15) was used as the positive standard for GAD65Ab and IA-2Ab. An in-house GAD67Ab-positive serum sample, as verified by immunoprecipitation, was used as the positive standard for GAD67Ab. A randomly selected control serum sample from a healthy volunteer was used as the negative standard.

Autoantibody levels were expressed as a relative index, as described previously (12–14): index = [counts per minute (cpm) of tested sample − cpm of negative standard]/(cpm of positive standard − cpm of negative standard). The upper limit of the normal range was established for each antibody and for each ethnic group specifically for this study. The upper limit was defined as the value at the 99th percentile of the level measured in each of the following healthy, nondiabetic groups of adult subjects living in Houston: 80 Caucasian-Americans, 104 Hispanic-Americans, and 83 African-Americans. All the control subjects were aged 21–70 yr and were normoglycemic persons identified during screening studies to recruit subjects for an influenza virus vaccine trial (Caucasian-Americans), a weight loss trial (Hispanic-Americans), and a hypertension prevalence study (African-Americans). Patients were classified as antibody positive (A+) if the antibody index for at least one of the measured serum antibodies exceeded the ethnic-specific 99th percentile, or antibody negative (A−) if the index for every antibody was below the 99th percentile.

Human leukocyte antigen (HLA) genotyping. DNA was isolated from peripheral blood leukocytes (16). Comprehensive HLA class II genotyping was performed by an oligoblot method using sequence-specific oligonucleotides, as described earlier (16). The polymorphic second exons of DQA1, DQB1 and DRB1, DRB3, DRB4, and DRB5 were amplified using PCR and labeled by incorporation of digoxigenin-labeled 2′-deoxyuridine 3′-triphosphate (dUTP) during the PCR. Labeled PCR products were hybridized to allele-specific probes selected from the second exon of the various loci described above, which were immobilized on nylon membranes. Positive reactions were visualized by a color precipitation reaction.

Measurement of β-cell secretory capacity. β-Cell secretory capacity was assessed in all patients in two ways: 1) fasting plasma C-peptide concentration, measured on three occasions: at the time of initial presentation with DKA (after correction of ketoacidosis), and after 6 months and 12 months close outpatient management; and 2) plasma C-peptide response to glucagon [glucagon stimulation test (GST)], measured at the time of initial presentation with DKA (after correction of ketoacidosis) and after 6 months of follow-up.

The initial measurement of fasting plasma C-peptide concentration was performed as follows. The plasma sample was obtained at 0800 h, at least 6 h after the last sc dose of regular insulin and at least 24 h after cessation of iv insulin, when the patient was nonketotic (acetone-negative in undiluted plasma). The initial GST was performed after an overnight fast, at least 10 h after the last sc dose of NPH or regular insulin. One milligram of glucagon was injected iv, and serum C-peptide concentrations were measured at 0, 5, and 10 min. The repeat measurements were performed in a similar manner, at a time when the patient had stable levels of HbA_{1c} and fasting blood glucose and was following a stable antidiabetic regimen. The tests were performed in the overnight fasting state, at least 10 h after an sc dose of NPH or regular insulin, or (if the patient had discontinued insulin) at least 10 h after a dose of metformin or thiazolidinedione, or 24 h after a dose of sulfonylurea. C-peptide concentrations were determined by highly specific RIA (Human C-Peptide RIA kit, Linco Research Inc., St. Louis, MO).

β-Cell functional reserve was defined as preserved (β+) if the peak C-peptide response to glucagon was at least 1.5 ng/dl (0.5 nmol/liter) or fasting C-peptide concentration was at least 1 ng/dl (0.33 nmol/liter). β-Cell functional reserve was defined as absent (β−) if the glucagon-stimulated or fasting C-peptide concentrations did not meet these criteria. Receiver operator characteristic analysis of these levels, together with those in 105 type 1 and 145 type 2 diabetic subjects, as well as five normal healthy controls of each relevant ethnic group (Caucasian-American, African-American, and Hispanic-American), were used to determine these cutoff values [area under the curve (AUC) value = 0.97776 for fasting C-peptide, 0.96751 for peak C-peptide response to glucagon, and 0.96089 for C-peptide/glucose ratio]. These cutoff values are very similar to those reported previously to differentiate normal subjects, type 1 diabetic patients, and type 2 diabetic patients (17, 18).

Statistical analysis

Descriptive statistics (means, SD, and SEM) were used to characterize the four groups. Contingency table analysis with likelihood ratio tests were used to assess differences in relative frequencies of categorical variables (gender, ethnicity, family history of diabetes, DKA precipitant, proportion of obese patients, ability to discontinue insulin, presence of antibodies, and category of β-cell function) among the four patient groups. When this test indicated that there were significant differences between groups, pair-wise comparisons were made to identify those differences. ANOVA was used to assess differences in continuous variables [age, age at diagnosis, years with diabetes, HbA_{1c} on admission, body mass index (BMI), glucose on admission, HbA_{1c} on follow-up, C-peptide levels, and AUC for C-peptide concentrations in the GST]. Pair-wise testing using a *post hoc* multiple comparison procedure (Tukey-Kramer) was used when the four-group comparison indicated significant group differences.

To quantify the influence of HLA genotype on the types of ketosis-prone diabetes, group comparisons were made for alleles at the DQA1, DQB1, and DRB1 loci that are known to have strong associations, either positive or negative, with autoimmune type 1 diabetes (19–23).

First, the global null hypothesis of no differences between the relative

frequencies of each allele in the patient groups was tested, with a Bonferroni adjustment at each locus. In the case of each allele for which the global null hypothesis was rejected, *a priori* pair-wise comparisons were made between autoantibody-positive and autoantibody-negative patients within the $\beta+$ and $\beta-$ groups.

Results

Patients: demographics and baseline clinical characteristics

The mean age of the 103 patients was 39 yr; 56% were males, and the ethnic distribution was 40% Hispanic-American, 44% African-American, 15% Caucasian-American, and 1% Asian-American. There was a prior diagnosis of diabetes in 60% (mean duration, 5.4 yr), whereas 40% were new onset (*i.e.* no prior diagnosis of diabetes). The mean HbA_{1c} level on admission was 13.7 ± 2.4%.

Aβ classification scheme and analysis

Patients were classified into four Aβ categorical groups based on the presence or absence of autoantibodies (A+ or A−) and of β-cell functional reserve (β+ or β−). The frequency distribution of the four groups is shown in Fig. 1.

Demographic, clinical, and anthropomorphic features of the four Aβ groups. There were no differences in mean age of patients in the four groups (Table 1). However, there were significant group differences in mean age at diagnosis of diabetes: 25 ± 17, 26 ± 12, 42 ± 12, and 39 ± 12 yr, respectively, for the A+β−, A−β−, A+β+, and A−β+ groups ($P < 0.0001$). The difference in the mean duration of diabetes in the four groups was significant in the reverse direction: 9.1 ± 10.4, 9.8 ± 8.7, 0.9 ± 3.0, and 3.0 ± 4.8 yr, respectively, for group A+β−, A−β−, A+β+, and A−β+ ($P < 0.0001$). These differences were also reflected in the distribution of new-onset diabetic patients: 17, 9, 91, and 51%, respectively, for group A+β−, A−β−, A+β+, and A−β+ ($P < 0.0001$).

The proportions of patients with a family history of type 2 diabetes were 50, 83, 82, and 88%, respectively, for group A+β−, A−β−, A+β+, and A−β+ ($P = 0.01$); and the group difference was due to the significantly lower rate of a family history in the A+β− group. There were also significant group differences in mean BMI and frequency of obesity (Table 1).

After 6 months of closely monitored clinical management by the DKA study team, the proportions of patients who no longer required insulin treatment to prevent ketoacidosis were 0, 0, 45, and 51%, respectively, for the A+β−, A−β−, A+β+, and A−β+ groups ($P < 0.0001$). The frequency of recurrent episodes of DKA during the study period in the four groups showed the opposite trend: 39, 30, 9, and 2%, respectively, in the A+β−, A−β−, A+β+, and A−β+ groups ($P < 0.0001$).

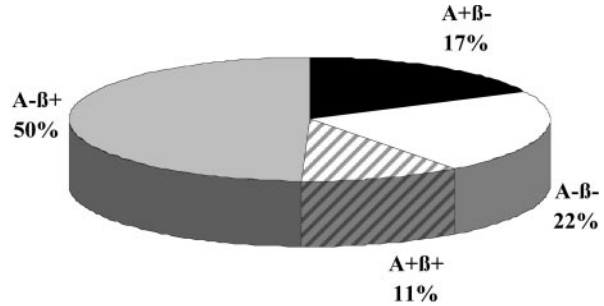

FIG. 1. Frequency distribution of patients in the four Aβ groups.

Ethnic distribution of the four Aβ groups. There was a significant difference in the distribution of ethnic groups across the four Aβ groups, largely due to a high frequency of Hispanic-Americans in the A−β+ group and a high frequency of African-American patients in the A+β− group ($P = 0.02$, Table 1).

β-Cell functional reserve of the four Aβ groups. At the time of admission for DKA, there was a significant difference in the group distribution of mean AUC of plasma C-peptide in response to glucagon stimulation, and this difference persisted after 6 months of outpatient treatment ($P < 0.0001$; Table 2 and Fig. 2A). Both β+ groups had significant improvement in β-cell function after 6 months. Similar differences were seen in mean fasting serum C-peptide levels (Table 2 and Fig. 2B).

Biochemical features and glycemic control of the four Aβ groups. There were no significant group differences in arterial blood pH or serum bicarbonate concentration at the time of index admission for DKA (Table 2). There were group differences in serum glucose concentration, calculated serum osmolality, and anion gap, and this was due to higher levels of these parameters in the A+β+ group compared with the other three groups. There were no significant group differences in mean HbA_{1c} concentration at baseline (Table 2 and Fig. 3). Differences in glycemic control between the four groups became evident after 3 months of close follow-up and management by the study team and persisted after 6 months, with mean levels of HbA_{1c} 10.3 ± 1.5, 11.5 ± 1.6, 7.1 ± 1.4, and 7.6 ± 2.1%, respectively, for the A+β−, A−β−, A+β+, and A−β+ groups ($P < 0.0001$). The HbA_{1c} levels have remained stable in all patients after 12 months of follow-up (Fig. 3).

Analysis of relative frequencies of HLA alleles within β+ and β− patient groups. Alleles at the DQA, DQB1, and DRB1 loci that are known to be strongly associated with autoimmune type 1 diabetes in multiple ethnic groups were compared within the four Aβ classes of patients. *A priori* comparisons were evaluated between A+ and A− patients within the β+ or β− groups with respect to the observed relative frequencies of the selected alleles. This was done for each allele by first testing the global null hypothesis of no differences between the relative frequencies of the allele in the four Aβ patient groups. A Bonferroni adjustment for the number of alleles evaluated at each locus (two, three, and three alleles, respectively, for DQA, DQB1, and DRB1) was applied to the *P* values reported. Table 3 shows the HLA alleles tested and the patient group frequencies evaluated in this manner. The global null hypothesis of no frequency difference was rejected for two alleles: DQA*03 ($P = 0.02$) and DQB1*02 ($P = 0.005$). For these two alleles, *a priori* pair-wise comparisons were made within each β-cell function class (*i.e.* A+β− compared with A−β−, and A+β+ compared with A+β−), and the results are described in the paragraph below.

TABLE 1. Aβ groups: clinical characteristics

	A+β−	A−β−	A+β+	A−β+	*P*
Number of patients (%)	18 (17%)	23 (22%)	11 (11%)	51 (50%)	
Age (yr)	34 ± 17	38 ± 15	43 ± 14	42 ± 13	0.1
Age at diagnosis (yr)	25 ± 17	26 ± 12	42 ± 12	39 ± 12	<0.0001[a]
Years with diabetes	9.1 ± 10.4	9.8 ± 8.7	0.9 ± 3.0	3.0 ± 4.8	<0.0001[a]
Family history of diabetes	9 (50%)	19 (83%)	9 (82%)	45 (88%)	0.01[b]
BMI (kg/m^2)	24.5 ± 3.9	23.0 ± 2.8	30.6 ± 7.6	29.4 ± 8.3	0.0003[c]
Weight category					<0.0001[a]
Lean	11 (61%)	17 (74%)	2 (18%)	17 (33%)	
Overweight	5 (28%)	6 (26%)	4 (36%)	13 (26%)	
Obese	2 (11%)	0	5 (46%)	21 (41%)	
New-onset diabetes	3 (17%)	2 (9%)	10 (91%)	26 (51%)	<0.0001[d]
Male:female ratio	1:1	1.3:1	0.6:1	1.7:1	0.4
Ethnicity					0.02[e]
African-American	13 (72%)	9 (39%)	4 (36.4%)	14 (27%)	
Hispanic-American	2 (11%)	9 (39%)	4 (36.4%)	30 (59%)	
Caucasian-American	2 (11%)	5 (22%)	3 (27.2%)	6 (12%)	
Asian-American	1 (6%)	0	0	1 (2%)	
Recurrent DKA episodes	7 (39%)	7 (30%)	1 (9%)	1 (2%)	<0.0001[f]
Insulin discontinued by 6 months	0 (0%)	0 (0%)	5 (45%)	26 (51%)	<0.0001[a]
Antidiabetic regimen at 12 months					<0.0001[a]
Insulin only	17 (94%)	23 (100%)	3 (27%)	17 (33%)	
Insulin + oral hypoglycemics	1 (6%)	0	3 (27%)	8 (16%)	
Oral hypoglycemics only	0	0	3 (27%)	21 (41%)	
Diet and exercise only	0	0	2 (19%)	5 (10%)	

[a] Pair-wise significant differences between A+β+ and A+β−; A+β+ and A−β−; A−β+ and A+β−; and A−β+ and A−β−.
[b] Pair-wise significant differences between A+β− and A−β+; and A+β− and A−β−.
[c] Pair-wise significant differences between A+β+ and A−β−; and A−β+ and A+β−; and A−β+ and A−β−.
[d] Pair-wise significant differences between A+β+ and A−β+; A+β+ and A+β−; A+β+ and A−β−; A−β+ and A+β−; and A−β+ and A−β−.
[e] Pair-wise significant differences between A−β+ and A+β−.
[f] Pair-wise significant differences between A−β+ and A+β−; and A−β+ and A−β−.

TABLE 2. Aβ groups: biochemical characteristics

	A+β−	A−β−	A+β+	A−β+	*P*
Number of patients (%)	18 (17%)	23 (22%)	11 (11%)	51 (50%)	
Serum bicarbonate on admission (mmol/liter)	8.7 ± 4.3	10.8 ± 4.3	9.6 ± 4.3	11.2 ± 4.0	0.2
Serum glucose on admission (mg/dl)	566 ± 174	510 ± 175	745 ± 301	477 ± 187	0.001[a]
Serum osmolality (mOsm/liter)	291 ± 15	292 ± 9	310 ± 25	291 ± 14	0.001[a]
Arterial pH on admission	7.16 ± 0.10	7.21 ± 0.08	7.17 ± 0.08	7.23 ± 0.09	0.06
Anion gap on admission	23.8 ± 1.4	22.8 ± 0.7	28.7 ± 1.2	22.5 ± 0.8	0.003[a]
HbA_{1c} on admission (%)	13.1 ± 2.3	14.5 ± 2.2	12.5 ± 2.3	13.8 ± 2.5	0.1
HbA_{1c} at 6 months follow-up (%)	10.3 ± 1.5	11.5 ± 1.6	7.1 ± 1.4	7.6 ± 2.1	<0.0001[b]
HbA_{1c} at 12 months follow-up (%)	10.6 ± 2.2	11.4 ± 1.9	7.7 ± 2.2	7.5 ± 2.1	<0.0001[b]
Baseline GST AUC ng/dl (over 10 min)	1.15 ± 0.16	2.68 ± 0.64	14.92 ± 1.69	19.52 ± 1.35	<0.0001[b]
6-month GST AUC ng/dl (over 10 min)	0.91 ± 0.01	1.36 ± 0.36	27.09 ± 6.40	32.93 ± 2.03	<0.0001[b]
Baseline fasting C-peptide (ng/dl)	0.14 ± 0.04	0.24 ± 0.06	1.66 ± 0.31	1.94 ± 0.13	<0.0001[b]
6-month fasting C-peptide (ng/dl)	0.14 ± 0.04	0.11 ± 0.02	2.02 ± 0.54	2.37 ± 0.17	<0.0001[b]
6-month C-peptide ≥ 0.33 (nmol/liter)	0	0	8 (73%)	48 (94%)	<0.0001[c]

Conversion factors to SI units: serum glucose × 0.055 mmol/liter; serum C-peptide × 0.331 nmol/liter.
[a] Pair-wise significant differences between A+β+ and A−β+; and A+β+ and A−β−.
[b] Pair-wise significant differences between A+β+ and A+β−; A+β+ and A−β−; A−β+ and A+β−; and A−β+ and A−β−.
[c] Pair-wise significant differences between A+β+ and A+β−; A+β+ and A−β−; A−β+ and A+β−; A−β+ and A−β−; and A+β+ and A−β+.

Analysis of patients with new-onset diabetes by Aβ categories. Forty-one subjects had new-onset diabetes as the precipitant of the ketoacidosis. Three (7%) were A+β−, two (5%) were A−β−, 10 (24%) were A+β+, and 26 (64%) were A−β+. The Aβ group differences among these 41 patients with respect to BMI, β-cell function (at baseline and follow-up), glycemic control, and insulin discontinuation were similar to the Aβ group differences noted in all 103 subjects taken together.

Paired comparisons within the β+ and β− groups

A+β− compared with A−β−. Aside from autoantibody status, the two β− groups differed in that A−β− patients had a significantly lower frequency of the autoimmune type 1 diabetes susceptibility HLA alleles DQB1*02 ($P = 0.003$) and DQA*03 ($P = 0.004$) (Fig. 4). A significantly greater proportion of A−β− patients also had a family history of diabetes compared with the A+β− group. The two β− groups were otherwise similar in most demographic, anthropomorphic, biochemical, and glycemic characteristics, both initially and after 6 and 12 months of follow-up.

A+β+ compared with A−β+. Aside from autoantibody status, the key differences between these two β+ groups were also immunogenetic. The A+β+ group had a significantly higher proportion of patients with the autoimmune type 1

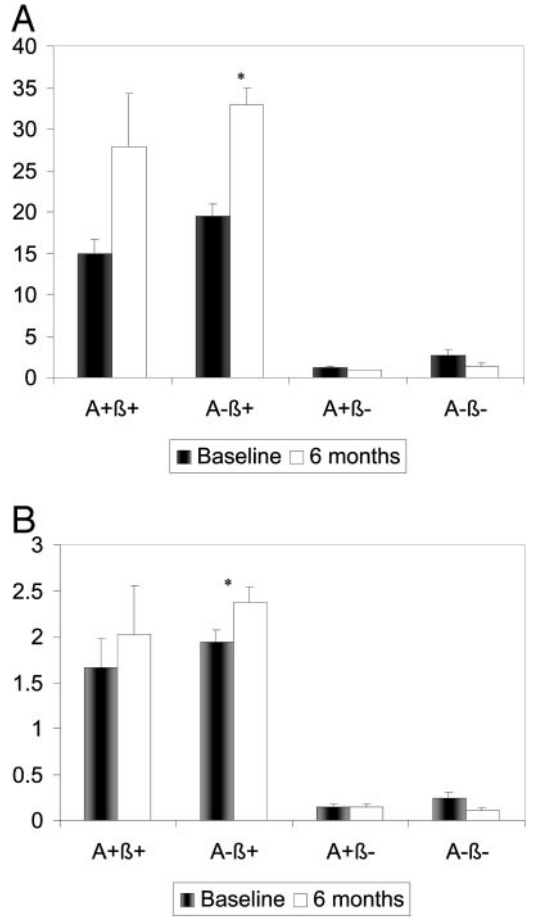

FIG. 2. A, Group comparison of β-cell functional reserve, as measured by the AUC of C-peptide response to glucagon stimulation, at baseline and after 6 months of follow-up. Values are mean ± SD. There were significant group differences at both time points between the $\beta+$ and $\beta-$ groups ($P < 0.0001$). *, $P < 0.0001$ comparing baseline to 6 months in the A$-\beta+$ group. (Conversion factor to SI units: serum C-peptide × 0.331 nmol/liter). B, Group comparison of β-cell functional reserve, as measured by fasting C-peptide levels, at baseline and after 6 months of follow-up. Values are mean ± SD. There were significant group differences at both time points between the $\beta+$ and $\beta-$ groups ($P < 0.0001$). *, $P = 0.01$ comparing baseline to 6 months in the A$-\beta+$ group. (Conversion factor to SI units: serum C-peptide × 0.331 nmol/liter).

diabetes susceptibility HLA allele DQB1*02 ($P = 0.05$; Fig. 4). There was also a significantly higher proportion of patients with new-onset diabetes in the A$+\beta+$ group. The two $\beta+$ groups were otherwise similar in most demographic, anthropomorphic, biochemical, and glycemic characteristics (with the exception of higher serum osmolality in the A$+\beta+$ group at the time of DKA), both initially and after 6 and 12 months of follow-up.

Discussion

Analysis of clinical, phenotypic, and genotypic data derived from this prospective characterization of multiethnic, heterogeneous, ketosis-prone diabetic patients indicates the presence of novel forms of β-cell dysfunction as well as a classification scheme to categorize these patients. We propose four groups based on two important features commonly used to distinguish type 1 and type 2 diabetes: presence or absence of biological markers of β-cell autoimmunity, and presence or complete absence of β-cell functional reserve. This is not meant to be rigid classification, but rather a hypothesis-testing scheme to differentiate etiologically and clinically distinct forms of ketosis-prone diabetic syndromes, and thus to uncover novel forms of β-cell dysfunction. The distinctive pathogenetic features and diagnostic implications of the four Aβ groups are discussed individually below.

A$+\beta-$ group

Patients in this group, with significantly low β-cell functional reserve together with circulating β-cell autoantibodies, are likely identical with the well-defined form of autoimmune type 1 diabetes. They had early onset diabetes and were generally lean. African-American patients predominated in this group. The results of the HLA analysis supported the contention that these patients have typical autoimmune type 1 diabetes. Irrespective of ethnicity, certain HLA allelic variants are found in high frequency in persons with autoimmune type 1 diabetes (19, 24–31). The proportion of patients with the type 1 diabetes susceptibility HLA alleles DQB1*02 and DQA*03 was significantly higher in the A$+\beta-$ group than in the three other groups, including the phenotypically similar A$-\beta-$ group. Furthermore, no A$+\beta-$ patients were positive for the protective HLA alleles DRB1*15 and DQB1*0602 (19, 32–36). All patients in this group required multiple daily insulin injections to avoid ketosis 12 months after the episode of DKA, and a significant proportion had recurrence of DKA during this period despite close monitoring by the study team.

A$-\beta-$ group

Patients in this group are likely to have diverse pathogenic mechanisms leading to ketosis-prone diabetes, including potentially novel forms of nonautoimmune β-cell failure. There were numerous similarities in clinical characteristics and β-cell functional reserve between the A$+\beta-$ and A$-\beta-$ groups (Table 2 and Figs. 2–4). At first glance, the difference between these two groups appeared to lie solely in their autoantibody status. However, HLA analysis revealed that there were also major differences between these two groups in genetic susceptibility to β-cell autoimmunity. The frequencies of one class II allele (DQB1*02), which is strongly associated with autoimmune type 1 diabetes susceptibility (24, 29, 32, 37), and of another (DQA*03), which is in linkage disequilibrium with the strong susceptibility alleles DQB1*0302 and DQB1*0301, were low in the A$-\beta-$ group compared with the A$+\beta-$ group (Fig. 4). These features make it likely that the A$-\beta-$ group consists primarily of persons with nonautoimmune mechanisms of β-cell injury, rather than persons with autoimmune type 1 diabetes whose circulating autoantibody levels have declined over time to undetectable levels (38). No A$+\beta-$ patients were positive for the protective allele DQB*0602 (33, 35, 39), whereas 9% of A$-\beta-$ patients possessed this allele. (There were no statistically significant group differences in the frequency of DQB*0602, however, probably because of the small sample sizes as well as the relatively low prevalence of the DQB*0602

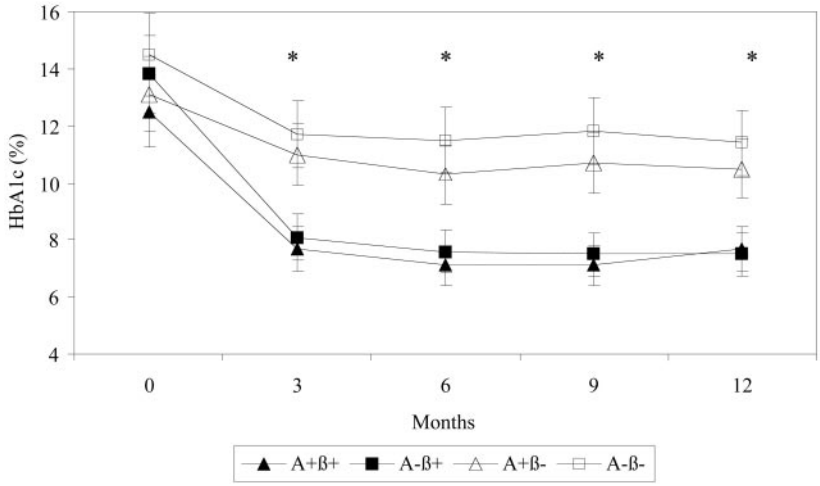

FIG. 3. Group comparison of temporal changes in mean HbA_{1c} levels over 12 months. *White squares*, A−β−; *white triangles*, A+β−; *black squares*, A−β+; *black triangles*, A+β+. Values are mean ± SD. *, $P <$ 0.0001 for the two β+ groups compared with the two β− groups.

TABLE 3. Group frequencies of class II HLA alleles known to be associated with type 1 autoimmune diabetes

Allele	A−β− (n = 23)	A−β+ (n = 51)	A+β− (n = 18)	A+β+ (n = 11)	*P*
DQA*02	1 (4%)	6 (12%)	4 (22%)	2 (18%)	>0.7
DQA*03	11 (48%)	23 (45%)	16 (89%)	5 (45%)	0.02
DQB1*02	6 (26%)	13 (25%)	13 (72%)	6 (55%)	0.005
DQB1*0302	6 (26%)	19 (37%)	8 (44%)	3 (27%)	>0.7
DQB1*0602	2 (9%)	10 (20%)	0	1 (9%)	>0.7
DRB1*03	3 (13%)	12 (24%)	5 (28%)	4 (36%)	>0.7
DRB1*04	8 (35%)	21 (41%)	10 (55%)	5 (45%)	>0.7
DRB1*15	2 (9%)	8 (16%)	0	1 (9%)	>0.7

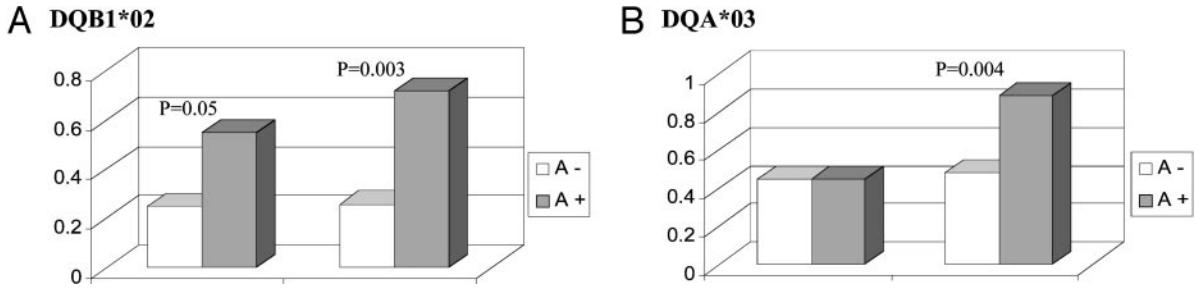

FIG. 4. Graphic comparison of frequencies of HLA class II alleles DQB1*02 (A) and DQA*03 (B) between the pair of groups within each β-cell function class. Triangles represent A+ and squares represent A− patients.

allele in the general population (40). A−β− patients also were more likely to have first-degree relatives with type 2 diabetes. The current classification scheme of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (41) would tend to place patients in the A−β− group into the clinical category of idiopathic type 1 diabetes, a category that begs further definition, as provided by the criteria presented here.

A+β+ group

Some patients in this group may represent a variant of what several reports of European cohorts have termed antibody-positive type 2 diabetes (42–44) or latent autoimmune diabetes of adults (45, 46). However, others in the A+β+ group likely represent a more aggressive form of late-onset autoimmune type 1 diabetes than described in these reports. DQB1*02 may be a marker for the more aggressive subset of A+β+, because the six A+β+ patients with DQB1*02 had higher mean HbA_{1c} (8.6 ± 2.5%) than those without DQB1*02 (6.5 ± 0.6%) after 12 months of close management ($P = 0.05$). Furthermore, five of the six patients with DQB1*02 still require insulin treatment to avoid ketosis after 12 months of follow-up, whereas insulin has been discontinued safely in four of the five A+β+ patients who lack this allele ($P = 0.03$). Although analysis of a larger cohort of A+β+ patients is needed to confirm this suggestive trend, this combination of class II HLA and autoantibody markers may represent an important diagnostic opportunity to identify A+β+ patients destined to have a more aggressive course. Because the presence of both the genetic markers and autoantibodies should precede the onset of clinical manifestations, it may be possible to identify such patients before their β-cells are irreversibly destroyed (47).

A−β+ group

This is the largest group of ketosis-prone patients, comprising the greatest number with new-onset diabetes. The frequencies of the autoimmune type 1 diabetes susceptibility

HLA alleles DQB1*02 and DQA*03 are low in this group. A−β+ patients appear clinically heterogeneous, with a wide range of BMI (Table 2). A−β+ patients have achieved good glycemic control within 6 months of follow-up, and half have been able to discontinue insulin treatment.

The causes of severe, acute β-cell dysfunction leading to DKA are likely to be diverse in this group. Half the A−β+ patients have new-onset diabetes, without a notable precipitating factor for DKA. The mean HbA_{1c} of this subgroup at presentation with DKA was 13.9 ± 2.2, indicating a relatively long period of undetected and untreated hyperglycemia. It is possible that the cause of acute β-cell failure in these patients was glucotoxicity (48–50) or lipotoxicity (51), which reversed with excellent control of glycemia after the episode of DKA. The sustained, preserved β-cell functional reserve and glycemic improvement in these patients argue against the likelihood that they have a form of type 1 diabetes with the poorly defined honeymoon period (52). In fact, all A−β+ patients have now been evaluated for more than 1 yr, and one third for more than 2 yr, and they continue to maintain uniformly excellent glycemic control (mean $HbA_{1c} \leq 7.0\%$) with adequate fasting levels of C-peptide (≥1.25 nmol/liter). The subset of A−β+ patients with previously diagnosed diabetes may comprise patients with long-standing forms of type 2 diabetes with progressive β-cell failure (53, 54) of such causes as β-cell apoptosis (55), islet cell amyloid (56), or iron infiltration (57).

Three previous studies have measured islet cell autoantibodies and β-cell function in subsets of African-American patients presenting with DKA (5–7). The patients described in these studies (*e.g.* those with "Flatbush diabetes") would fit into our two β+ groups. Consistent with our β+ group data, the mean age at diagnosis of these African-American cohorts was in the fifth decade, the mean BMI was high, only a minority had β-cell autoantibodies, and glycemic control improved markedly after intensive treatment. These similarities add support to the concept of the A−β+ group as manifesting a distinct form of ketosis-prone diabetes, but our data extend the expression of this syndrome to patients of Hispanic, Caucasian, and Asian ethnicity.

HLA genotyping was particularly helpful in distinguishing autoimmune-associated from probable nonautoimmune-associated forms of β-cell dysfunction within the class of patients with low β-cell functional reserve (*i.e.* in distinguishing the A+β− and A−β− syndromes). In the initial analysis, the class II alleles selected were those known to be strongly associated with autoimmune type 1 diabetes in multiple ethnic groups, *e.g.* the positively associated DQB1*02 and DQB1*0302 (22, 24, 27, 58–63) and the negatively associated DQB1*0602 (19, 23, 32, 33, 35, 39). In the pair-wise comparison, there was a clear difference in the relative frequencies of DQB1*02: high in the A+β− group (72%) and low in the A−β− group (26%). The frequency of DQB1*0302 showed a trend in the same direction, but did not attain significance after Bonferroni adjustment (which may not be necessary, because the association between this allele and autoimmune type 1 diabetes is well established). The protective allele DQB1*0602 (64) was absent in all patients in the A+β− group, but present in 9% of A−β− patients. DQB1*0602 is a low-frequency allele in the general population of Caucasian-Americans (5–13%) and African-Americans (4–15%) (40), hence a larger sample of patients would be necessary to have the power to detect group differences in its frequency. Interestingly, DQA*03, an allele not frequently reported to be associated *per se* with autoimmune type 1 diabetes susceptibility, also distinguished the A+β− group (89%) from the A−β− group (44%). DQA*03 is known to be in linkage disequilibrium with the strong susceptibility alleles DQB1*0302 and DQB1*0301, hence its frequency distribution is likely to represent a real difference in susceptibility to autoimmunity between the A+β− and A−β− groups.

The absence of features of autoimmune diabetes or HLA-associated susceptibility to autoimmune diabetes in the A− groups raises the possibility that they could include persons with genetic causes of β-cell dysfunction, such as syndromes of maturity onset diabetes of youth (MODY) or mitochondrial transfer RNA mutations. The MODY syndromes are characterized by Mendelian dominant inheritance due to monogenic mutations (65). Although there are at present no reported cases of subjects with documented MODY gene mutations presenting with ketoacidosis, this is certainly a possibility. Sixty-four (86%) of the patients in our A− cohort have a family history of type 2 diabetes, 45 of these with a potentially dominant mode of transmission. Screening of the extended pedigrees for linkage to the currently known MODY genes is ongoing. Diabetes associated with mitochondrial gene mutations also involves defects in glucose-stimulated insulin secretion (66). However, the absence of evidence for maternal transmission of diabetes and other typical features (*e.g.* deafness, neurologic disorders, cardiac or renal failure) make it unlikely that any of our patients harbor known mitochondrial gene mutations.

Imagawa *et al.* (67) have described a cohort of lean Japanese subjects who developed new-onset, fulminant β-cell failure of apparently nonautoimmune cause after a relatively short period of hyperglycemia ($HbA_{1c} < 8\%$). Our two A− groups do not appear to include such patients, inasmuch as all of our A− patients, including those who were of new onset, had significantly higher HbA_{1c} levels, a less fulminant course, greater BMI and higher frequency of first-degree relatives with diabetes. Furthermore, it is not clear that the Japanese patients were truly nonautoimmune, because they possessed HLA haplotypes (DRB1, DQA1, DQB1 0405,0303,0401, or DQB1 0901,0302,0303, or 0802,0401,0302) known to be associated with autoimmune type 1 diabetes (68–70).

The clinical course of the two β− groups highlights the critical importance of β-cell functional reserve in achieving effective glycemic control. Although both β− groups experienced significant (3%) decreases in HbA_{1c} and marked declines in the rate of hospital readmissions for DKA as a result of the study intervention, their chronic glycemic status remained quite poor. Other factors, such as lack of compliance with insulin treatment, could also have played a role in this outcome. We did not systematically record treatment compliance, but it is well-known that treatment noncompliance is particularly severe and glycemic control is especially difficult to achieve in type 1 diabetic patients in indigent, minority-ethnic, urban settings in the United States (42–44).

In conclusion, we have used a heterogeneous, multiethnic

cohort to demonstrate that patients presenting with DKA comprise at least four distinct diabetic syndromes that are separable by autoantibody status, HLA genotype, and quantitative assessment of β-cell function. Novel, nonautoimmune causes resulting in variable degrees of β-cell dysfunction are likely to underlie the A−β+ and A−β− syndromes. Detailed genotypic and phenotypic characterization studies of patients in these categories are ongoing, in the hope that they will specify the etiologic bases of the syndromes revealed by the present analysis. The current data are also of clinical relevance to the evaluation and prognosis of patients with ketosis-prone diabetes. β-Cell functional reserve at the time of DKA is the strongest indicator of future metabolic control, but GAD and IA-2 autoantibody status and class II HLA allelotypes can assist in classifying ketosis-prone patients and improving prediction of clinical outcomes.

Acknowledgments

We gratefully acknowledge Lynn Bekris for assistance with autoantibody analysis; Andrew Westmark for HLA genotyping; Drs. Robert Atmar, David Hyman, Valory Pavlik, and Rebecca Reeves for ethnic-specific control sera; Dr. E. O'Brian Smith for statistical advice; and the faculty and residents of the Department of Medicine, Baylor College of Medicine, and Ben Taub General Hospital, for patient referrals. We are indebted to the late Professor Robert Turner for his encouragement and support of this research.

Received February 4, 2003. Accepted July 7, 2003.

Address all correspondence and requests for reprints to: Ashok Balasubramanyam, M.D., Division of Endocrinology, BCM 719E, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030. E-mail: ashokb@bcm.tmc.edu.

This work was supported by a Juvenile Diabetes Foundation International Career Development Award, the Chao Scholars Fund, a Siegel Foundation Grant (to A.B.), an American Diabetes Association Career Development Award (to C.S.H.), and National Institutes of Health Grants DK26190 and DK53004 (to Å.L.).

References

1. **Foster DW** 1998 Diabetes mellitus. In: Fauci AS, Braunwald E, Isselbacher KJ, eds. Harrison's principles of internal medicine. 14th ed. New York: McGraw-Hill; 2060–2080
2. **Orland M** 1998 Diabetes mellitus. In: Ewald G, McKenzie C, eds. Manual of medical therapeutics. 29th ed. Boston: Little, Brown
3. **Winter WE, Maclaren NK, Riley WJ, Clarke DW, Kappy MS, Spillar RP** 1987 Maturity-onset diabetes of youth in black Americans. N Engl J Med 316:285–291
4. **Balasubramanyam A, Zern JW, Hyman DJ, Pavlik V** 1999 New profiles of diabetic ketoacidosis: type 1 *vs.* type 2 diabetes and the effect of ethnicity. Arch Intern Med 159:2317–2322
5. **Banerji MA, Chaiken RL, Huey H, Tuomi T, Norin AJ, Mackay IR, Rowley MJ, Zimmet PZ, Lebovitz HE** 1994 GAD antibody negative NIDDM in adult black subjects with diabetic ketoacidosis and increased frequency of human leukocyte antigen DR3 and DR4. Flatbush diabetes. Diabetes 43:741–745
6. **Umpierrez GE, Woo W, Hagopian WA, Isaacs SD, Palmer JP, Gaur LK, Nepom GT, Clark WS, Mixon PS, Kitabchi AE** 1999 Immunogenetic analysis suggests different pathogenesis for obese and lean African-Americans with diabetic ketoacidosis. Diabetes Care 22:1517–1523
7. **Umpierrez GE, Casals MM, Gebhart SP, Mixon PS, Clark WS, Phillips LS** 1995 Diabetic ketoacidosis in obese African-Americans. Diabetes 44:790–795
8. **Westphal SA** 1996 The occurrence of diabetic ketoacidosis in non-insulin-dependent diabetes and newly diagnosed diabetic adults. Am J Med 101:19–24
9. **Kitabchi AE, Umpierrez GE, Murphy MB, Barrett EJ, Kreisberg RA, Malone JI, Wall BM** 2001 Management of hyperglycemic crises in patients with diabetes. Diabetes Care 24:131–153
10. **Sobngwi E, Gautier JF** 2002 Adult-onset idiopathic type I or ketosis-prone type II diabetes: evidence to revisit diabetes classification. Diabetologia 45:283–285
11. **American Diabetes Association** 2002 Standards of medical care for patients with diabetes mellitus. Diabetes Care 25:S33–S48
12. **Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian WA, Li L, Karlsen AE, Boel E, Michelsen B, Lernmark A** 1994 A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia 37:344–350
13. **Falorni A, Ortqvist E, Persson B, Lernmark A** 1995 Radioimmunoassays for glutamic acid decarboxylase (GAD65) and GAD65 autoantibodies using 35S or 3H recombinant human ligands. J Immunol Methods 186:89–99
14. **Hampe CS, Hammerle LP, Bekris L, Ortqvist E, Kockum I, Rolandsson O, Landin-Olsson M, Torn C, Persson B, Lernmark A** 2000 Recognition of glutamic acid decarboxylase (GAD) by autoantibodies from different GAD antibody-positive phenotypes. J Clin Endocrinol Metab 85:4671–4679
15. **Mire-Sluis AR, Gaines Das R, Lernmark A** 2000 The World Health Organization International Collaborative Study for islet cell antibodies. Diabetologia 43:1282–1292
16. **Leech NJ, Kitabchi AE, Gaur LK, Hagopian WA, Hansen J, Burghen GA, Palmer JP, Nepom GT** 1995 Genetic and immunological markers of insulin-dependent diabetes in black Americans. Autoimmunity 22:27–32
17. **Hother-Nielsen O, Faber O, Sorensen NS, Beck-Nielsen H** 1988 Classification of newly diagnosed diabetic patients as insulin-requiring or noninsulin-requiring based on clinical and biochemical variables. Diabetes Care 11:531–537
18. **Koskinen P, Viikari J, Irjala K, Kaihola HL, Seppala P** 1985 C-peptide determination in the choice of treatment in diabetes mellitus. Scand J Clin Lab Invest 45:589–597
19. **Erlich HA, Griffith RL, Bugawan TL, Ziegler R, Alper C, Eisenbarth G** 1991 Implication of specific DQB1 alleles in genetic susceptibility and resistance by identification of IDDM siblings with novel HLA-DQB1 allele and unusual DR2 and DR1 haplotypes. Diabetes 40:478–481
20. **Cucca F, Muntoni F, Lampis R, Frau F, Argiolas L, Silvetti M, Angius E, Cao A, De Virgiliis S, Congia M** 1993 Combinations of specific DRB1, DQA1, DQB1 haplotypes are associated with insulin-dependent diabetes mellitus in Sardinia. Hum Immunol 37:85–94
21. **Hu CY, Allen M, Chuang LM, Lin BJ, Gyllensten U** 1993 Association of insulin-dependent diabetes mellitus in Taiwan with HLA class II DQB1 and DRB1 alleles. Hum Immunol 38:105–114
22. **Agardh D, Gaur LK, Agardh E, Landin-Olsson M, Agardh CD, Lernmark A** 1996 HLA-DQB1*0201/0302 is associated with severe retinopathy in patients with IDDM. Diabetologia 39:1313–1317
23. **Greenbaum CJ, Gaur LK, Noble JA** 2002 ICA+ relatives with DQA1*0102/DQB1*0602 have expected 0602 sequence and DR types. J Autoimmun 18:67–70
24. **Todd JA, Bell JI, McDevitt HO** 1987 HLA-DQ β gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 329:599–604
25. **Fletcher J, Mijovic C, Odugbesan O, Jenkins D, Bradwell AR, Barnett AH** 1988 Trans-racial studies implicate HLA-DQ as a component of genetic susceptibility to type 1 (insulin-dependent) diabetes. Diabetologia 31:864–870
26. **Michelsen B, Wassmuth R, Ludvigsson J, Lernmark A, Nepom GT, Fisher L** 1990 HLA heterozygosity in insulin-dependent diabetes is most frequent at the DQ locus. Scand J Immunol 31:405–413
27. **Mijovic CH, Jenkins D, Jacobs KH, Penny MA, Fletcher JA, Barnett AH** 1991 HLA-DQA1 and -DQB1 alleles associated with genetic susceptibility to IDDM in a black population. Diabetes 40:748–753
28. **Sanjeevi CB, Zeidler A, Shaw S, Rotter J, Nepom GT, Costin G, Raffel L, Eastman S, Kockum I, Wassmuth R, et al.** 1993 Analysis of HLA-DQA1 and -DQB1 genes in Mexican Americans with insulin-dependent diabetes mellitus. Tissue Antigens 42:72–77
29. **Thorsby E, Ronningen KS** 1993 Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type 1 (insulin-dependent) diabetes mellitus. Diabetologia 36:371–377
30. **Vandewalle CL, Decraene T, Schuit FC, De Leeuw IH, Pipeleers DG, Gorus FK** 1993 Insulin autoantibodies and high titre islet cell antibodies are preferentially associated with the HLA DQA1*0301-DQB1*0302 haplotype at clinical type 1 (insulin-dependent) diabetes mellitus before age 10 years, but not at onset between age 10 and 40 years. The Belgian Diabetes Registry. Diabetologia 36:1155–1162
31. **Ronningen KS, Spurkland A, Tait BD, Drummond B, Lopez-Larrea C, Baranda FS** 1991 HLA Class II association in insulin-dependent diabetes mellitus among Blacks, Caucasoids and Japanese. In: Tsuji K, Aizawa M, Sasasuki T, eds. HLA 1991: Proceedings of the 11th International Histocompatibility Workshop and Conference, Yokohama, Japan, 1991, pp 713–722
32. **Heimberg H, Nagy ZP, Somers G, De Leeuw I, Schuit FC** 1992 Complementation of HLA-DQA and -DQB genes confers susceptibility and protection to insulin-dependent diabetes mellitus. Hum Immunol 33:10–17
33. **Cavan DA, Jacobs KH, Penny MA, Kelly MA, Mijovic C, Jenkins D, Fletcher JA, Barnett AH** 1993 Both DQA1 and DQB1 genes are implicated in HLA-associated protection from type 1 (insulin-dependent) diabetes mellitus in a British Caucasian population. Diabetologia 36:252–257
34. **Kockum I, Sanjeevi CB, Eastman S, Landin-Olsson M, Dahlquist G, Lernmark A** 1995 Population analysis of protection by HLA-DR and DQ genes from insulin-dependent diabetes mellitus in Swedish children with insulin-dependent diabetes and controls. Eur J Immunogenet 22:443–465
35. **Pugliese A, Gianani R, Moromisato R, Awdeh ZL, Alper CA, Erlich HA, Jackson RA, Eisenbarth GS** 1995 HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM. Diabetes 44:608–613

36. **Patel SD, Cope AP, Congia M, Chen TT, Kim E, Fugger L, Wherrett D, Sonderstrup-McDevitt G** 1997 Identification of immunodominant T cell epitopes of human glutamic acid decarboxylase 65 by using HLA-DR(alpha1*0101, beta1*0401) transgenic mice. Proc Natl Acad Sci USA 94:8082–8087
37. **Horn GT, Bugawan TL, Long CM, Erlich HA** 1988 Allelic sequence variation of the HLA-DQ loci: relationship to serology and to insulin-dependent diabetes susceptibility. Proc Natl Acad Sci USA 85:6012–6016
38. **Borg H, Marcus C, Sjoblad S, Fernlund P, Sundkvist G** 2000 Islet cell antibody frequency differs from that of glutamic acid decarboxylase antibodies/IA2 antibodies after diagnosis of diabetes. Acta Paediatr 89:46–51
39. **Kockum I, Wassmuth R, Holmberg E, Michelsen B, Lernmark A** 1993 HLA-DQ primarily confers protection and HLA-DR susceptibility in type I (insulin-dependent) diabetes studied in population-based affected families and controls. Am J Hum Genet 53:150–167
40. **Marincola FM, Stroncek D, Simonis T** 1997 Caucasian *vs*. normal In: Terasaki P, Gjertson D, eds. HLA 1997. Los Angeles: Tissue Typing Laboratory; 348–349
41. **American Diabetes Association** 2002 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 25:S5–S20
42. **Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A, Nissen M, Ehrnstrom BO, Forsen B, Snickars B, Lahti K, Forsblom C, Saloranta C, Taskinen MR, Groop LC** 1999 Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. Diabetes 48:150–157
43. **Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R** 1997 UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective diabetes study group. Lancet 350:1288–1293
44. **Hagopian WA, Karlsen AE, Gottsater A, Landin-Olsson M, Grubin CE, Sundkvist G, Petersen JS, Boel E, Dyrberg T, Lernmark A** 1993 Quantitative assay using recombinant human islet glutamic acid decarboxylase (GAD65) shows that 64K autoantibody positivity at onset predicts diabetes type. J Clin Invest 91:368–374
45. **Pozzilli P, Di Mario U** 2001 Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. Diabetes Care 24:1460–1467
46. **Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR** 1993 Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 42:359–362
47. **Bingley PJ, Bonifacio E, Gale EA** 1993 Can we really predict IDDM? Diabetes 42:213–220
48. **Weir GC, Laybutt DR, Kaneto H, Bonner-Weir S, Sharma A** 2001 β-Cell adaptation and decompensation during the progression of diabetes. Diabetes 50(Suppl 1):S154–S159
49. **Robertson RP, Olson LK, Zhang HJ** 1994 Differentiating glucose toxicity from glucose desensitization: a new message from the insulin gene. Diabetes 43: 1085–1089
50. **Eizirik DL, Korbutt GS, Hellerstrom C** 1992 Prolonged exposure of human pancreatic islets to high glucose concentrations in vitro impairs the β-cell function. J Clin Invest 90:1263–1268
51. **Kraegen EW, Cooney GJ, Ye JM, Thompson AL, Furler SM** 2001 The role of lipids in the pathogenesis of muscle insulin resistance and β cell failure in type II diabetes and obesity. Exp Clin Endocrinol Diabetes 109:S189–S201
52. **Pinero-Pilona A, Litonjua P, Aviles-Santa L, Raskin P** 2001 Idiopathic type 1 diabetes in Dallas, Texas: a 5-year experience. Diabetes Care 24:1014–1018
53. **Porte Jr D, Kahn SE** 2001 β-Cell dysfunction and failure in type 2 diabetes: potential mechanisms. Diabetes 50(Suppl 1):S160–S163
54. **Polonsky KS, Sturis J, Bell GI** 1996 Seminars in medicine of the Beth Israel Hospital, Boston. Non-insulin-dependent diabetes mellitus—a genetically programmed failure of the β cell to compensate for insulin resistance. N Engl J Med 334:777–783
55. **Chandra J, Zhivotovsky B, Zaitsev S, Juntti-Berggren L, Berggren PO, Orrenius S** 2001 Role of apoptosis in pancreatic β-cell death in diabetes. Diabetes 50(Suppl 1):S44–S47
56. **Melato M, Antonutto G, Ferronato E** 1977 Amyloidosis of the islets of Langerhans in relation to diabetes mellitus and aging. Beitr Pathol 160:73–81
57. **Salonen JT, Tuomainen TP, Nyyssonen K, Lakka HM, Punnonen K** 1998 Relation between iron stores and non-insulin-dependent diabetes in men: case-control study. BMJ 317:727
58. **Bohme J, Carlsson B, Wallin J, Moller E, Persson B, Peterson PA, Rask L** 1986 Only one DQ-β restriction fragment pattern of each DR specificity is associated with insulin-dependent diabetes. J Immunol 137:941–947
59. **Bao MZ, Wang JX, Dorman JS, Trucco M** 1989 HLA-DQ β nonASP-57 allele and incidence of diabetes in China and the USA. Lancet 2:497–498
60. **Owerbach D, Gunn S, Gabbay KH** 1989 Primary association of HLA-DQw8 with type I diabetes in DR4 patients. Diabetes 38:942–945
61. **Baisch JM, Weeks T, Giles R, Hoover M, Stastny P, Capra JD** 1990 Analysis of HLA-DQ genotypes and susceptibility in insulin-dependent diabetes mellitus. N Engl J Med 322:1836–1841
62. **Khalil I, d'Auriol L, Gobet M, Morin L, Lepage V, Deschamps I, Park MS, Degos L, Galibert F, Hors J** 1990 A combination of HLA-DQ β Asp57-negative and HLA DQ alpha Arg52 confers susceptibility to insulin-dependent diabetes mellitus. J Clin Invest 85:1315–1319
63. **Ivarsson SA, Eriksson S, Kockum I, Lernmark A, Lindgren S, Nilsson KO, Sundkvist G, Wassmuth R** 1993 HLA-DR3, DQ2 homozygosity in two patients with insulin-dependent diabetes mellitus superimposed with ulcerative colitis and primary sclerosing cholangitis. J Intern Med 233:281–286
64. **Graham J, Hagopian WA, Kockum I, Li LS, Sanjeevi CB, Lowe RM, Schaefer JB, Zarghami M, Day HL, Landin-Olsson M, Palmer JP, Janer-Villanueva M, Hood L, Sundkvist G, Lernmark A, Breslow N, Dahlquist G, Blohme G** 2002 Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. Diabetes 51:1346–1355
65. **Fajans SS, Bell GI, Bowden DW, Halter JB, Polonsky KS** 1996 Maturity onset diabetes of the young (MODY). Diabet Med 13:S90–S95
66. **Maassen JA** 2002 Mitochondrial diabetes: pathophysiology, clinical presentation, and genetic analysis. Am J Med Genet 115:66–70
67. **Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y** 2000 A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. Osaka IDDM study group. N Engl J Med 342: 301–307
68. **Awata T, Kuzuya T, Matsuda A, Iwamoto Y, Kanazawa Y, Okuyama M, Juji T** 1990 High frequency of aspartic acid at position 57 of HLA-DQ β-chain in Japanese IDDM patients and nondiabetic subjects. Diabetes 39:266–269
69. **Yamagata K, Nakajima H, Hanafusa T, Noguchi T, Miyazaki A, Miyagawa J, Sada M, Amemiya H, Tanaka T, Kono N, Tarui S 1989 Aspartic acid at position 57 of DQ beta chain does not protect against type 1 (insulin-dependent) diabetes mellitus in Japanese subjects. Diabetologia 32:762–764**
70. **Lernmark A** 2000 Rapid-onset type 1 diabetes with pancreatic exocrine dysfunction. N Engl J Med 342:344–345