Distinct Genetic and Immunological Features in Patients With Onset of IDDM Before and After Age 40

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OBJECTIVE — Young age at onset is a relevant parameter associated with a rapid progression of IDDM. Our major aim was to define differences between IDDM patients with age at diagnosis >40 years and adult IDDM with onset at a younger age.

RESEARCH DESIGN AND METHODS — The correlation between islet-related antibodies (islet cell antibodies [ICAs] and antibodies [Abs] to GAD and the tyrosine phosphatase IA2), T-cell responses to GAD peptides and HLA class II isotypes was investigated in 23 IDDM patients 12–38 years of age at onset (group 1), 24 patients with IDDM >40 years of age at onset (group 2), and 12 healthy control subjects. ICAs were measured by indirect immunofluorescence, and GAD-Ab and IA2-Ab were measured by immunoprecipitation tests. T-cell responses against GAD peptides, which had been identified as typical for IDDM, were tested by 5-day proliferation assays. HLA class II alleles were typed by polymerase chain reaction.

RESULTS — ICAs and GAD-Abs were more prevalent in IDDM patients than in control subjects ($P < 0.001$), but only IDDM group 1 had IA2-Abs ($P < 0.001$ compared with IDDM group 2 and control subjects). Moreover, antibody combinations differed between IDDM patients of groups 1 and 2. T-cell responses to GAD peptides were seen in 67% of IDDM group 1 and in 71% of IDDM group 2 ($P < 0.02$ compared with control subjects). IDDM patients of group 1 were more frequently $DR4^+/DQ8^+$ and less frequently $DR2^+/DQ0602^+$ compared with IDDM patients of group 2 ($P < 0.05$).

CONCLUSIONS — Our data provide strong evidence for humoral and cellular autoimmunity in adult IDDM patients with onset both before and after 40 years of age. However, late-onset differs from young-onset IDDM with respect to Ab profiles, especially a lack of IA2-Ab, and HLA class II types. These findings have consequences for the diagnostic strategy for identifying slow-onset IDDM in individuals after 40 years of age.

IDDM is probably a T-cell–mediated autoimmune disease (1). Most patients with IDDM are diagnosed in childhood or young adulthood before the age of 35–40 years. However, there is clearly a subgroup of IDDM patients diagnosed after 40 years of age, which is characterized by a longer symptomatic period before diagnosis, better preservation of residual β-cell function, and lower frequencies of insulin autoantibodies and HLA-DR3/4 heterozygosity compared with IDDM patients with onset at childhood or adolescence (2). This group may comprise ~30% of all patients with IDDM. In the above-mentioned study, the presence of islet cell antibodies (ICAs) was not related to the age of diagnosis, thus suggesting an autoimmune process as the cause of the disease also in IDDM patients with late onset. At present it is not clear whether or not IDDM patients with onset at later adult life and "classical IDDM" with onset at younger age differ with respect to the features of autoimmune response such as its time course and target antigens. Independent from a somewhat slower progression to insulin dependency, the diagnosis of IDDM in adulthood is made by the clinical characteristics such as ketonuria, polyuria, and weight loss just as in IDDM with onset at younger age, but additional immunological data may be required in the cases with late onset.

Irvine et al. (3), Groop et al. (4), and Di Mario et al. (5) described patients with NIDDM exhibiting metabolic and immunological features of IDDM. It was pointed out by several groups that this form of diabetes was characterized by early secondary failure to diet and oral drug therapy and that these patients may be better identified by GAD-antibodies (Abs) positivity and a reduced stimulated C-peptide. This diabetes form was called latent autoimmune diabetes in adults (LADA (6,7). The clinical identification of LADA patients can be difficult, since development of insulin dependency is sometimes slow and other clinical characteristics of IDDM such as nonobesity are not always present (8). At present it is unclear whether LADA is just a variant of slowly progressing IDDM in adulthood or a unique identity of diabetes. A better clinical and immunological description of IDDM patients with onset of the disease in adulthood may help to define characteristics that may be typical for autoimmunity to islet cells in older age and may therefore be expected also to characterize LADA patients. Discrimination between NIDDM and LADA may have therapeutic implications as insulin therapy may save some residual β-cell function in the autoimmune type of diabetes (9,10). Therefore, safe diagnostic markers are highly warranted to identify LADA patients and devise well-controlled strategies for specific therapy as exemplified in the ongoing Latent Insulin-Dependent Diabetes in Adults (LIDIA) Trial in Europe. This study recruits NIDDM patients between age 35 and 60 where exercise and diet fail to control glucose metabolism according to the criteria

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Ab, antibody; ICA, islet cell antibody; LADA, latent autoimmune diabetes in adults; PBMC, peripheral blood mononuclear cell.

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of the European Association for the Study of Diabetes. ICA- and GAD-Ab–positive patients are included and treated either with oral drugs or insulin. Stimulated C-peptide will be compared between groups to look for a potential benefit of early insulin therapy in Ab-positive NIDDM patients.

Islet cell–specific autoantibodies are well established in the prediction of classical IDDM in childhood or younger adults. Cytoplasmic ICAs, insulin autoantibodies, Ab to GAD, and Ab to the tyrosine phosphatase IA2 are widely used for this purpose (11–14). Recently it was shown in a 10-year follow-up study from the diagnosis of diabetes that GAD-Abs are able to predict insulin dependency also in NIDDM patients (15). Similarly, ICAs can predict secondary failure to oral therapy with hypoglycemic agents in NIDDM (3,4,16). Therefore the islet-related antibodies ICA, GAD-Ab, and possibly IA2-Ab may represent good candidates for the screening of patients with NIDDM to identify patients with future insulin dependency.

Furthermore, genetic risk markers for the development of IDDM such as HLA class II alleles have been reported to predict secondary failure to oral hypoglycemic agents in NIDDM patients (17–20). However, these markers are less prevalent in patients with late-onset IDDM than in patients with onset of IDDM at a younger age. In the present study, we tested both IDDM risk alleles (HLA DR3 and 4 as well as DQ 0302) and IDDM "protective" alleles (DR2 and DQ 0602) for frequencies in patients with onset of IDDM at both a young and older age.

We also included T-cell assays to peptides of a putative autoantigen in IDDM (GAD 65) to improve the battery of immunological testing in patients with IDDM onset at an older age. Because IDDM is precipitated by autoreactive T-cells, the latter assays may be helpful in the identification of relevant disease-related autoimmune reactions.

RESEARCH DESIGN AND METHODS

— A consecutive series of IDDM patients consulting a clinical practice were recruited and divided into two groups according to their age of onset: before and after the age of 40 years. Children before the age of 12 years were excluded for ethical reasons in that 40 ml of blood were needed for testing. Furthermore, patients with pancreatitis or glucocorticoid therapy were excluded. All patients were <2 years from diagnosis of IDDM and most of them metabolically well or moderately well controlled as estimated by HbA_{1c} levels <8.5% at the time of testing. One blood sample was taken in the practice and used for antibody assays, HLA alleles determination, and T-cell assays.

IDDM patients were insulin treated from diagnosis or within the 1st year of diagnosis. The diagnosis had been made on the basis of clinical (ketonuria, polyuria, and weight loss) and laboratory criteria (blood sugar and HbA_{1c} levels); insulin deficiency in cases without ketonuria was confirmed by reduced C-peptide levels either basal (<0.25 nmol/l) or stimulated 6 min after intravenous injection of 1 mg glucagon (<0.5 nmol/l). Volunteers without a family history of diabetes were recruited as a healthy control group with a mean age of 27.3 years (range 23 to 36 years). Table 1 gives age, duration of diabetes, BMI (weight in kilograms divided by the square of the height in meters), frequency of ketonuria, basal or stimulated C-peptide levels, HbA_{1c}, and fasting blood sugar levels at diagnosis for all patients tested.

Antibody assays

Autoantibodies to the intracytoplasmic domain of tyrosine phosphatase like protein IA-2 (IA2-Ab) or full-length GAD65 (GAD-Ab) were determined as described previously (13). Briefly, [35S] methionine radiolabeled IA-2 (10,000 cpm) or GAD65 (20,000 cpm), produced by in vitro transcription and translation were incubated with 5 µl serum on 96-well microtiter plates. Immunocomplexes were adsorbed with protein A sepharose, washed, and counted in a liquid scintillation counter. Antibody levels were expressed in arbitrary units with a cut-off of 7 GAD-Units and 3 IA-2-Units, respectively, which exceeds the mean ± 4 SD of 100 healthy control subjects. In the second GAD Antibody Proficiency Program, the GAD-Ab assay achieved values of 100% for sensitivity, specificity, validity, and consistency (Lab ID #116). The IA-2 antibody assay was evaluated in the IDW (Immunology of Diabetes Workshops) Combined Autoantibody Workshop demonstrating a diagnostic sensitivity for IDDM of 72.5% and a specificity of 96%.

Cytoplasmic ICAs were detected by the indirect immunofluorescence test on unfixed cryostat sections of human pancreas from an organ donor with blood group 0 as described (21). The detection limit of our assay was 2.5 JDF-U. In the 11th IDW ICA Proficiency Program 1996, our laboratory achieved values of 100% for sensitivity, specificity, validity, and consistency (Lab ID #116).

HLA typing

Genomic DNA was isolated from nucleated blood cells using a fast extraction method (QIAmp Blood Kit, Qiagen, Chatsworth, CA). HLA class II alleles were typed by DNA amplification with sequence-specific primers (PCR-SSP), using commercial SSP-sets of DYNAL (Oslo, Norway). The typing was performed following the general protocol for DYNAL-SSP sets (June 1994).

T-cell assays

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation and plated at 2×10^5 cells per well in a 96-well round-bottomed plate. Each proband was tested for proliferation to a set of six GAD 65 peptides (amino acid sequences are given in Table 2) identified in previous T-cell proliferation experiments as typical for the stimulation of PBMC from IDDM patients (22–24). Per peptide 12 wells were plated with 10 µg/ml peptide, 12 wells without peptide as negative controls, and 6 wells with phytohemagglutinin and 6 wells with tetanustoxoid served as positive controls. After 4 days incubation at 37°C in 5% CO_2 humidified air, the wells were pulsed with 0.5 µCi ^{3}H-thymidine and 6 h later harvested and counted using a Betaplate counter. Positive T-cell responses were detected using a computer program (22) calculating the number of positive wells (> means + 2 SD of negative control wells) with peptide and comparing with the number of positive wells in negative control wells for statistical significance. Only responses with $P < 0.01$ compared to the negative control are reported.

Statistical analysis

When testing group differences, the Fisher's exact probability test was applied for comparing frequencies (e.g., Ab or T-cell proliferation positive patients). The t test was used to compare quantitative differences between the groups (e.g., age or BMI). $P < 0.05$ was considered significant.

RESULTS

Age, duration of diabetes, BMI, and metabolic control

The mean age of IDDM patients of group 1

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Table 1— *Relevant clinical and laboratory data in 47 patients with IDDM*

No.	Age (years)	Duration (months)	BMI* kg/m²	Ketonuria*	C-Peptide* (nmol/l)	HBA_{1c}* (%)	FBS* (mmol/l)	ICA (JDF-U)	GAD-Ab (AU)	IA2-Ab (AU)	T-cells	HLA DRB1	HLA DQB1
IDDM patients below 40 years of age (IDDM group 1)													
1	27	1	23	−	0.16	12.3	NA	40	80	—	+	0101/0401	0501/0302
2	27	3	18	+	—	9.5	12.8	40	59	—	+	0401	0302
3	19	2	22	+	—	14.6	14.3	10	30	—	+	0301	0201
4	11	1	19	+	—	12.9	16.4	—	—	—	+	0301/0404	0201/0302
5	16	1	20	−	0.22	10.5	13.1	160	114	64	+	0401	0302
6	24	1	19	−	0.14	11.1	12.9	20	52	—	+	0301	0201
7	34	9	28	−	0.19	10.7	NA	10	—	5	−	0701/1301	0202/0603
8	31	6	28	−	0.48†	9.6	10.9	40	97	—	+	0701/0401	0303
9	17	7	20	+	—	18.2	14.5	—	—	—	+	0401/1302	0302/0604
10	27	5	22	−	0.06	13.9	17.8	40	53	90	NT	0101/0401	0501/0302
11	27	24	26	+	—	NA	18.8	640	95	100	+	0402/1104	0302/0301
12	15	1	22	+	—	NA	22.4	—	—	—	+	0301/0404	0201/0302
13	17	1	19	+	—	12.3	44.7	80	105	97	+	0301/0901	0201/0303
14	18	1	18	+	—	14.9	29.6	160	52	53	+	0301/0401	0201/0302
15	17	12	20	−	0.53†	9.8	12.0	40	30	—	−	0301/1302	0201/0604
16	38	6	28	+	—	14.3	15.3	10	92	—	−	0301/0401	0201/0302
17	23	1	23	−	0.11	NA	12.2	160	50	75	NT	1301/0801	0603/0402
19	28	5	25	+	—	12.9	15.9	—	—	—	+	1101/0401	0301/0501
20	24	1	18	−	0.27	10.3	16.0	320	22	94	+	0401/0701	0202/0302
21	24	1	22	+	—	NA	19.0	40	11	—	−	0402/1301	0302/0603
22	37	1	23	+	—	11.1	NA	40	80	—	−	0401/0701	0202/0302
23	37	8	20	−	0.22	13.9	17.8	80	96	—	−	0701/1302	0604/0202
IDDM patients above 40 years of age (IDDM group 2)													
1	61	3	30	+	—	13.8	18.9	—	20	—	−	0801/1103	0301/0402
2	50	14	27	+	—	14.9	14.0	—	—	—	+	1501/0701	0602/0303
3	60	2	27	+	—	12.4	19.5	+‡	111	—	+	0101/0501	0501/0201
4	49	1	32	+	—	14.7	17.2	5	—	—	+	0101/1101	0501/0301
5	55	1	29	+	—	12.8	16.5	—	—	—	−	1501/1103	0602/0301
6	45	1	25	−	0.4†	13.7	14.4	—	—	—	−	0301/0401	0201/0301
7	40	1	29	−	0.48†	10.5	12.8	—	—	—	−	1501	0602
8	44	12	31	−	0.37†	13.8	14.8	5	—	—	+	0101/0501	0501/0602
9	45	1	23	+	—	NA	20.5	40	85	—	+	0301/1302	0201/0609
10	60	6	28	+	—	10.4	13.5	20	31	—	+	0301	0201
11	46	24	26	+	—	10.1	12.7	—	—	—	+	1601/0405	0502/0302
12	65	6	30	+	—	11.5	12.6	—	15	—	+	1201/1301	0301/0603
13	46	19	NA	+	—	10.7	16.4	—	68	—	−	0401/0701	0202/0302
14	55	21	25	−	0.38†	10.1	14.4	20	—	—	−	0401/0101	0302/0501
15	51	6	29	−	0.45†	9.8	12.4	5	—	—	+	1101/1305	0301
16	46	9	24	−	0.44†	12.1	14.9	—	—	—	+	1501/0701	0602/0303
17	45	22	30	+	—	13.5	NA	—	—	—	+	1101/1301	0603/0301
18	67	1	23	−	0.22	9.5	10.8	—	—	—	+	0401/1101	0302/0301
19	41	12	24	−	0.36†	9.8	13.7	1024	162	—	+	0701	0202
20	56	1	31	NA	0.48†	10.7	11.1	10	—	—	+	0301/0701	0201/0202
21	57	1	NA	+	—	10.5	12.6	—	—	—	+	1101	0301
22	46	2	27	+	—	9.5	12.5	—	—	—	−	0101/0801	0501/x
23	46	1	26	−	0.13	10.9	14.8	10	—	—	+	1302/0701	0604/0202
24	43	24	21	+	—	11.1	15.9	5	—	—	+	0401/1201	0301

*Data at diagnosis; †glucagon (1 mg) stimulated; ‡not titrable because of high ANF; FBS, fasting blood sugar; IA2-Ab, antibodies to the tyrosinphosphatase IA2; AU, arbitrary unit; NT, not tested; NA, not available.

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was 24.1, the mean age of group 2 was 50.2 ($P < 10^{-6}$ compared with group 1). The duration of IDDM in the patients of the two groups was not significantly different (5.3 compared with 7.9 months). The frequency of ketonuria was not significantly different between IDDM patients of both groups. In cases without ketonuria insulin deficiency was confirmed by either basal C-peptide levels <0.25 nmol/l or intravenous glucagon (1 mg) stimulated C-peptide <0.55 nmol/l. IDDM patients of group 1 had a lower BMI

Table 2—*Amino acid sequences of the GAD 65 peptides used in this study*

Number in sequence	Amino acids
161–175	MHCQTTLKYAIKTGH
177–191	RYFNQLSTGLDMVGL
505–519	CFWYYIPPSLRTLEDN
521–535	ERMSRLSKVAPVIKA
533–547	IKARMMEYGTTMVSY
247–266	NMYAMMIARFKMFPEVKEKG

compared with patients of group 2 (22.1 compared with 27.1, $P < 10^{-5}$). When compared with an age-matched group of NIDDM patients with similar HbA_{1c} levels (data not shown), IDDM patients of group 2 had a lower BMI (27.1 compared with 31.0, $P < 0.02$). The HbA_{1c} and fasting blood sugar levels did not differ between both groups of IDDM patients at diagnosis (Table 1) or at the time of testing.

Antibody levels

All 12 healthy control subjects were negative for ICAs, GAD-, and IA2-Abs. Of patients in the IDDM group 1, 19 of 23 had ICAs, 17 of 23 GAD-Abs, and 9 of 23 IA2-Abs. Patients from the IDDM group 2 exhibited a reduced frequency of ICAs (11 of 24, $P < 0.01$ compared with IDDM group 1) and GAD-Abs (7 of 24, $P < 0.003$ compared with IDDM group 1). Interestingly, 0 of 24 IDDM patients of group 2 had IA2-Abs ($P < 0.001$ compared with IDDM group 1 patients).

Of patients in the IDDM group 1, 19 of 23 were ICA-positive, and all of these 19 patients had either GAD-Ab or IA2-Ab. In contrast, 11 of 23 IDDM patients from group 2 had ICAs; seven of these 11 patients were ICA positive without the presence of GAD-Ab or IA2-Ab ($P < 0.001$ compared with IDDM group 1). Similarly, of 16 GAD-Ab–positive IDDM patients from group 1, all were ICA positive, but three of seven GAD-positive IDDM patients of group 2 were ICA negative ($P < 0.02$ compared with IDDM group 1).

Altogether 19 of 23 patients with IDDM and diabetes onset before 40 years of age (IDDM group 1, $P <$ 10–6 compared with healthy control subjects) and 14 of 24 patients with onset after age 40 (IDDM group 2, $P < 0.0001$ compared with healthy control subjects) were positive for one of the three antibodies tested (Table 1).

T-cell responses to GAD peptides

T-cell responses to the control antigens tetanustoxoid and phytohemagglutinin did not differ between the two groups of IDDM patients or between IDDM patients and healthy controls. T-cell responses to the selected GAD peptides were seen in 14 of 21 IDDM patients from group 1, 17 of 24 IDDM patients from group 2, and four of 12 healthy control subjects ($P < 0.06$ compared with IDDM group 1 and $P < 0.02$ compared with IDDM group 2).

There was no single peptide of the selected GAD 65 peptides that was specific for any of the groups tested (Table 3). Some of the peptides (e.g., 161–175, 177–191, and 533–547) were recognized by T-cells from more than 20% of IDDM patients (Table 3).

HLA class II alleles

HLA class II alleles for all groups investigated are shown in Table 1. The IDDM risk allele HLA DQ 0302 was found in 14 of 23 IDDM patients from group 1 compared with four of 24 IDDM patients from group 2 ($P < 0.002$). The IDDM protective allele HLA DQ 0602 was detected in 0 of 23 IDDM patients from group 1 and 5 of 24 IDDM patients from group 2 ($P < 0.03$). Interestingly, four of the five IDDM patients with a HLA DQ 0602 allele were ICA-negative; the remaining DQ 0602–positive patient had a very low ICA level (5 JDF-U).

In the IDDM group 1 patients, 16 of 23 were $DR4^+$, whereas in group 2 only 6 of 24 IDDM patients were $DR4^+$ ($P < 0.02$). Furthermore, 8 of 23 IDDM patients from group 1 had a DR3 allele compared with 4 of 24 IDDM patients from group 2 ($P < 0.05$). The IDDM protective allele DR2 was found in none of 23 IDDM patients from group 1 compared with 5 of 24 IDDM patients from group 2 ($P < 0.03$).

CONCLUSIONS — It is now widely recognized that IDDM is a disease with an onset not exclusively restricted to children and young adults. There is a subgroup of IDDM patients, diagnosed as IDDM, in adults even >40 years of age that differs from IDDM with onset at childhood or adolescence. Our study has investigated consecutive cohorts of IDDM patients who consulted a clinical practice, were diagnosed as IDDM by clinical criteria, and differed with respect to the age of diabetes onset. Both groups of IDDM patients were similar according to duration of diabetes, amount of insulin necessary for treatment, and HbA_{1c} levels either at diagnosis or after treatment. IDDM patients with disease onset after age 40 differ from those with onset before age 40 by a higher BMI. This excludes obesity as a consistent marker for non-insulin dependency, which is in agreement with a previous report by Gottsäter et al. (8). However, IDDM patients with onset after 40 years of age still had a lower BMI when compared with NIDDM patients of comparable age.

Both groups of IDDM patients showed evidence for autoimmune abnormalities such as islet-related antibodies at high frequencies (>80% for group 1 vs. >50% for group 2). Immunological assays and the study of the genetic background revealed marked differences between both groups. Only IDDM patients with onset of diabetes at a younger age had IA2-Ab, were more frequently HLA DR4/DQ0302-, and less often DR2/DQ0602-positive when compared with IDDM patients with disease onset after 40 years of age.

For ethical reasons, we excluded children before the age of 12 from our study so that IDDM with onset at adolescence or young adulthood was compared with cases of IDDM with onset at age after 40. Our study differs from that of Karjalainen et al. (2) in Finland in that the latter study compares childhood (age 1–18 years at onset) IDDM with adult IDDM (above 20 years of age at onset). Therefore, the differences we found for late-onset IDDM (above 40 years of age at onset) compared to "classical" juvenile onset IDDM may be in fact more pronounced than those seen in the Finnish study.

Our group of IDDM patients with disease onset after 40 years of age may reflect the final outcome of LADA patients, since these patients developed IDDM later in life and showed evidence for autoimmunity.

Table 3—*Frequency of T-cell responses to single GAD 65 peptides*

Peptide	IDDM 1	IDDM 2	Control subjects
161–175	**5/22**	**5/23**	1/12
177–191	**6/22**	2/23	1/12
505–519	2/22	4/23	2/12
521–535	0/22	2/23	0/12
533–547	**5/22**	4/23	1/12
247–266	1/22	3/23	0/12

Responses representing >20% of IDDM patients are shown in bold.

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The identification of LADA patients may be of clinical relevance, since preservation of the residual β-cell function by early insulin treatment was shown to be effective, at least in first-degree relatives of IDDM patients (9), and preliminary data from a small cohort of LADA patients (10) suggest that this is also true for the latter group. The ongoing LIDIA trial, a large European randomized study, may be able to clarify this point. One main problem of such trials is the selection of NIDDM patients at risk to progress to insulin dependency. Previous studies have shown that ICAs and GAD-Abs are effective in predicting insulin dependency in NIDDM patients (3,4,6–8). Nothing is known so far regarding the relevance of IA2-Ab for this purpose. Our study may contribute some clues to this matter because *1)* ICAs and GAD-Abs were confirmed as good markers for autoimmunity also in IDDM patients after 40 years; *2)* IA2-Abs were not present in this patient group and therefore do not seem to be useful in the screening for latent IDDM in older age groups; and *3)* Ab-screening in this age group needs a combination of ICAs and GAD-Abs, since neither of the antibodies alone was able to detect all Ab-positive individuals. This latter point is in marked contrast to results in younger IDDM patients where a combination of GAD-Ab and IA2-Ab may be able to replace ICA determination in the future (14,24a).

The differences in antibody profiles between IDDM patients with disease onset before and after the age of 40 may reflect different immune reactivities. The absence of IA2-Ab could point to a reduced importance of this antigen in IDDM patients with onset at older age. Furthermore, the presence of ICAs without the detection of GAD-Ab and IA2-Ab may reflect the importance of additional, as yet unknown, antigens hidden in the antigen mixture ICA for this patient group. Similarly, the detection of GAD-Ab in ICAs-negative sera was seen only in a fraction of IDDM patients with disease onset after 40 years and may be explained by different epitope specificities of GAD-Ab in older and younger IDDM patients.

T-cell assays are an additional option to detect autoimmune reactivity and possibly a better marker for destructive immunity compared with antibodies, reflecting the fact that IDDM is a T-cell–mediated disease. Therefore we included in our diagnostic program a T-cell assay detecting GAD 65-peptide specific T-cells (22). Indeed GAD-peptide specific T-cells were more common in both age groups of IDDM patients compared with healthy control subjects. It is not clear at present whether this result reflects a disease-specific marker or just an HLA-linked effect due to the HLA dependence of the presentation of peptides to T-cells (22–24). Interestingly, the single GAD peptides recognized by IDDM patients of both groups differed only slightly despite obvious HLA heterogeneity in both groups (Table 3). Furthermore, we were able to detect T-cell responses to GAD peptides in all four Ab-negative IDDM patients of group 1, possibly defining a TH1-dominated immune response with low antibody and good T-cell responses. Unfortunately, T-cell assays are not practical at present for clinical purposes as these assays are not standardized and relatively large amounts of blood are needed.

IDDM patients with disease onset before 40 years of age exhibited the expected frequencies of HLA alleles in that they were commonly HLA DR 4/ DQ0302 positive and DR2/ DQ0602 negative. However, this was not the case for IDDM patients with onset after 40 years of age. The latter group of patients had fewer HLA risk alleles when compared with IDDM patients with younger age of onset, and some of them were positive for IDDM-protective HLA alleles DR2/DQ0602. The latter patients may represent a subgroup of nonautoimmune IDDM patients, since four out of five DQ0602^{+} patients were Ab-negative and the fifth case had a very low ICA level (5 JDF-U).

In conclusion, our study describes immunogenetic differences between IDDM patients with disease onset before and after age 40. These differences may be explained at least partially by the heterogeneity of the IDDM group with diabetes onset after 40 years of age. There was a subgroup of patients without evidence of humoral autoimmunity and with HLA alleles that usually protect from IDDM (25). Our results may help clinicians search for latent autoimmune IDDM in adults in that screening for ICAs and GAD-Abs but not IA2-Abs is necessary to identify those patients who may benefit from early insulin therapy by preservation of residual β-cell function.

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