A 12-Year Prospective Study of the Relationship Between Islet Antibodies and β-Cell Function At and After the Diagnosis in Patients With Adult-Onset Diabetes

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To clarify the relationships between islet antibodies (islet cell antibody [ICA], GAD antibody [GADA], and IA-2 antibody [IA-2A]) versus the progression of β-cell dysfunction, we have followed a group of diabetic patients from their diagnosis at 21–73 years of age. Patients with ICA had high levels of GADA and/or IA-2A at diagnosis and a more severe β-cell dysfunction 5 years after diagnosis than those with only GADA in low concentrations. The aim of the current 12-year follow-up study was to examine the further progression of β-cell dysfunction in relation to islet antibodies at and after diagnosis. Among 107 patients, complete β-cell failure 12 years after diagnosis was restricted to those with islet antibodies at diagnosis (16 of 21 [77%] with multiple antibodies and 4 of 5 [80%] with only GADA). In contrast, among antibody-negative patients, fasting P-C-peptide levels were unchanged. Most GADA-positive patients (22 of 27 [81%]) remained GADA positive after 12 years. Associated with decreasing fasting P-C-peptide levels (0.85 nmol/l [0.84] at diagnosis vs. 0.51 nmol/l [0.21] 12 years after diagnosis, $P < 0.05$), ICA developed after diagnosis in 6 of 105 originally antibody negative mostly overweight patients. In conclusion, multiple islet antibodies or GADA alone at diagnosis of diabetes predict future complete β-cell failure. After diagnosis, GADA persisted in most patients, whereas ICA development in patients who were antibody negative at diagnosis indicated decreasing β-cell function.
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Antigen-unspecific islet cell antibodies (ICAs) and antigen-specific GADA (glutamic acid decarboxylase 65 antibody) are markers of islet autoimmunity present in most patients at the diagnosis of type 1 diabetes (1). In patients clinically believed to have type 2 diabetes, ICA and GADA predict future insulin dependency, a condition often referred to as latent autoimmune diabetes in adults (LADA) (2–6). Like GADA, protein tyrosine phosphatase–like protein antibodies (IA-2 antibodies [IA-2As]) are antigen-specific islet antibodies. IA-2As are detected in a high frequency at diagnosis in type 1 diabetic children (7), whereas the frequency is lower in adult-onset type 1 diabetic patients (8,9) and in LADA patients (10). Recently, we reported that, if combined with ICA or GADA, IA-2A in high levels predict β-cell failure. On the other hand, different from IA-2A, GADA positivity in low levels alone indicates a slowly progressive β-cell dysfunction during the first 5 years after diagnosis (11). Whether low GADA levels are associated with complete β-cell failure when the duration of diabetes increases is not known. After diagnosis, probably in parallel with β-cell destruction (12,13), islet antibodies disappear in type 1 diabetic children when the duration of diabetes increases, ICAs more rapidly than IA-2As and GADAs (14). It is unclear how long after the diagnosis of diabetes islet antibodies remain in adults and whether there are differences between the three antibodies in this respect. Associated with future low C-peptide levels, development of GADA or ICA after the diagnosis of diabetes has been described in young adults (15,16). Whether development of islet antibodies after diagnosis occurs in elderly diabetic patients needs to be clarified.

The aims of this 12-year follow-up study were to *1*) evaluate whether patients with only GADA at diagnosis of diabetes still have preserved β-cell function 12 years after diagnosis, *2*) clarify whether the association between islet antibody positivity at diagnosis and future β-cell dysfunction varies with age, *3*) follow the frequencies and levels of islet antibodies after diagnosis in patents with adult-onset diabetes, and *4*) follow the putative de novo development of islet antibodies after diagnosis.

RESEARCH DESIGN AND METHODS

Patients. All new consecutively diagnosed adult diabetic patients (>20 years of age, $n = 231$) between September 1985 and August 1987 in the city of Malmö, Sweden, were included in this prospective study (4,11,17,18). A total of 12 years after diagnosis of diabetes, 147 patients were invited to a follow-up study (67 of the 231 patients had died and 17 had moved from the city), and 130 of the 147 (88%) eligible patients accepted. Blood samples with volumes sufficient for measurements of all three islet antibodies were obtained from 213 patients at diagnosis. Among the 213 patients, blood samples for fasting P-C-peptide measurements were obtained after an overnight fast from 196 patients at diagnosis, from 150 patients after 3 years, from 148 patients after 5 years, and from 107 patients after 12 years. The 107 patients who completed

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GADA, GAD antibody; IA-2A, IA-2 antibody; ICA, islet cell antibody; JDF, Juvenile Diabetes Foundation; LADA, latent autoimmune diabetes in adults.

TABLE 1
Islet antibodies present at diagnosis in patients with adult-onset diabetes

	Entire material	≥65 years of age at diagnosis	Completed the 12-year follow-up
n	213	73	107
3 Antibodies			
ICA + GADA + IA-2A	25 (12)	8 (11)	15 (14)
2 Antibodies			
ICA + GADA	8 (4)	1 (1)	4 (4)
ICA + IA-2A	4 (2)	1 (1)	0
GADA + IA-2A	1 (0.5)	1 (1)	1 (1)
Total 2 antibodies	13 (6)	3 (4)	5 (5)
1 Antibody			
ICA	1 (0.5)	0	1 (1)
GADA	7 (3)	1 (1)	5 (5)
IA-2A	20 (9)	11 (15)	8 (7)
Total 1 antibody	28 (13)	12 (16)	14 (13)
0 Antibodies	147 (69)	50 (68)	73 (68)
Any antibody			
ICA	39 (18)	10 (14)	20 (19)
GADA	42 (20)	11 (15)	25 (23)
IA-2A	50 (23)	20 (27)	24 (22)

Data are *n* (%).

the 12-year follow-up were 54 years of age (median), interquartile range 21, range 21–73, at diagnosis; 45 (42%) of the patients were women. During the entire follow-up study, patients and physicians were ignorant of the results of antibody and C-peptide measurements. The study was approved by the Ethics Committee at Lund University.

Assay methods. GADA and IA-2A were determined by radioligand binding assays (19,20). The GADA assay was based on ^{125}I-labeled human recombinant GAD65. Both the sensitivity and specificity of this GADA assay were 100% when compared with a ^{35}S-GADA assay evaluated in the Diabetes Autoantibody Proficiency Testing Program for GADA (no. 2, 24 samples tested). A value >1.9 units/ml (97.5% percentile of 199 nondiabetic control subjects) was considered abnormal (11). The IA-2A assay was based on ^{35}S-methionine–labeled human recombinant in vitro transcribed-translated intracellular domain of IA-2. The cDNA used for the transcription-translation reaction coded for amino acids 606–979 corresponding to nucleotides 1889–3010 of the mRNA in Genbank (accession no. L18983). The sequence was checked, confirming the sequence described for IA-2, except for a G2072A substitution (20). The IA-2A results are presented as an index. In the latest Diabetes Autoantibody Proficiency Testing Program for IA-2A (no. 3, 24 samples tested), this IA-2A assay performed with 100% sensitivity and 100% specificity. An IA-2A index >1.1 (>97.5% percentile of 198 nondiabetic control subjects) was considered abnormal (11). ICAs were determined by a prolonged immunofluorescence assay. In the latest Diabetes Autoantibody Proficiency Program (no. 13, 20 samples tested), this ICA assay performed with 100% sensitivity and 100% specificity. In the current study, the detection limit was 3 Juvenile Diabetes Foundation (JDF) units for the used pancreas, i.e., an ICA value ≥3 JDF units was considered abnormal.

Fasting P-C-peptide was used as a measure of endogenous β-cell function. The detection limit was 0.10 nmol/l for the assay used (21). Undetectable f-P-C-peptide level was used as a marker of complete β-cell failure. Comparisons with an intravenous glucose-glucagon infusion test have previously shown that an undetectable f-P-C-peptide level is a reliable sign of complete β-cell failure (11). HbA_{1c} was determined by a high-performance liquid chromatography method (22). Reference values for healthy individuals were 3.90–5.30%.

Statistical analyses. Nonparametric Kruskal-Wallis and Mann-Whitney tests were used to evaluate differences between groups and Friedman and Wilcoxon's signed-rank tests to evaluate paired differences. Fisher's exact test was used to evaluate frequency differences between groups. $P < 0.05$ was considered significant. If not otherwise stated, data are presented as median (interquartile range).

RESULTS

Islet antibody status at diagnosis of diabetes. The patients included in the 12-year follow-up study ($n = 107$) did not differ from the entire patient material ($n = 213$) with regard to islet antibody status (Table 1).

Islet antibody status at follow-up

Patients with two or three islet antibodies at diagnosis of diabetes. Among patients with two or three islet antibodies at diagnosis, antibody levels were significantly lower after 12 years compared with levels at diagnosis ($P = 0.001$ for IA-2A, $P = 0.02$ for GADA, and $P = 0.01$ for ICA) (Fig. 1). In keeping with this, the frequency of positivity for all three islet antibodies decreased from 25 of 67 (37%) in patients antibody positive at diagnosis to 5 of 34 (15%) in patients still antibody positive after 12 years ($P = 0.02$) (Tables 1 and 2). Nevertheless, among patients with all three islet antibodies at diagnosis, 13 of 15 (87%) were positive for at least one antibody (always GADA) after 12 years. Among patients with two antibodies at diagnosis, three of five remained positive for two antibodies, and four of five (80%) were positive for at least one antibody (always GADA) after 12 years (Table 3). No patient with two antibodies at diagnosis developed additional islet antibodies after diagnosis. Figure 1 shows that among the different antibodies, the frequencies of ICA and IA-2A positivity ($P < 0.0001$), but not GADA, decreased after diagnosis. The frequency of GADA positivity remained high after 12 years (17 of 20, 85%).

Among patients with two or three islet antibodies at diagnosis, β-cell failure (undetectable fasting P-C-peptide) was present in 20 of 27 (74%) patients after 5 years (Fig. 1). In six of the remaining seven patients, however, fasting P-C-peptide levels decreased between 5 and 12 years after diagnosis. Indeed, in four of six patients complete β-cell failure had occurred after 12 years. Hence, almost all patients with two or three islet antibodies at diagnosis (16 of 20 [80%]) had complete β-cell failure 12 years after diagnosis. Moreover, in the four patients with preserved β-cell function, fasting P-C-peptide levels decreased significantly from diagnosis to after 12 years (0.43 nmol/l [0.94] vs. 0.31 nmol/l [0.50], $P = 0.002$) when they also showed

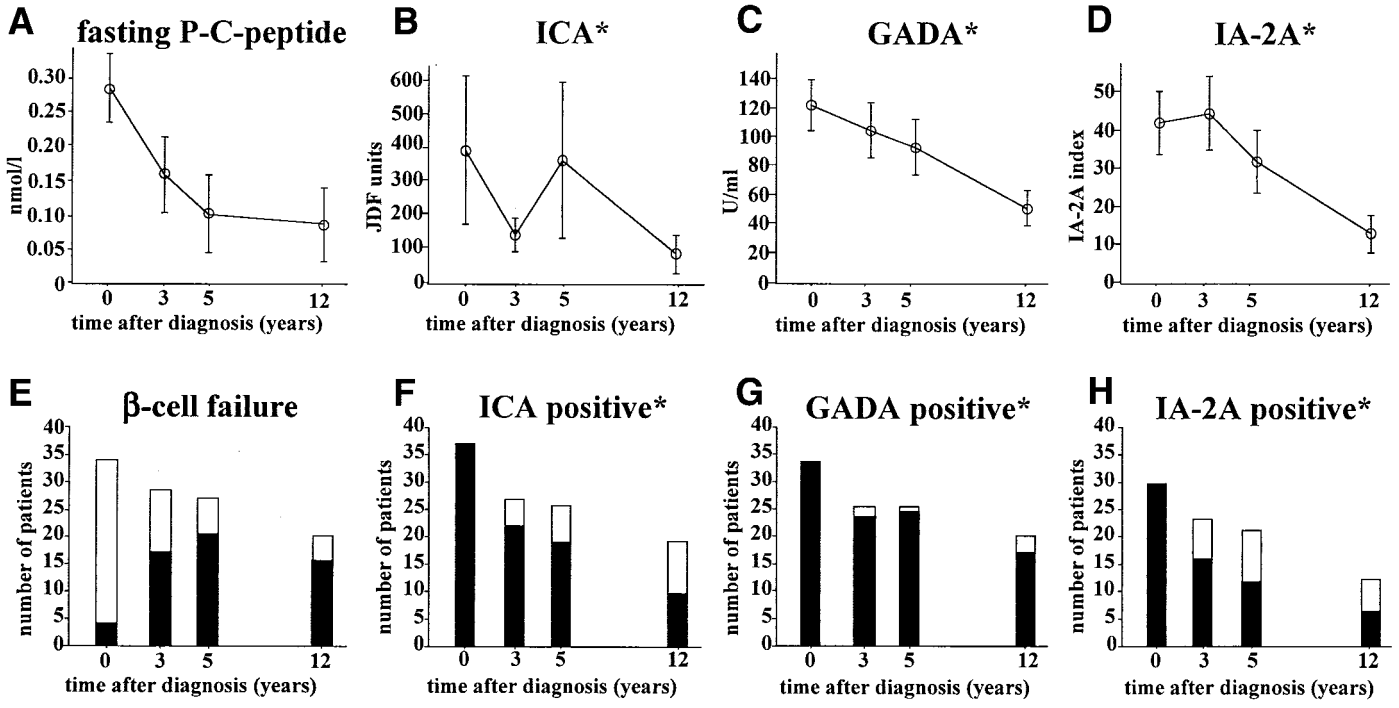

FIG. 1. Adult-onset diabetic patients with ≥2 islet antibodies at diagnosis. *Upper panel*: levels of fasting plasma C-peptide (*A*), ICA (*B*), GADA (*C*), and IA-2A (*D*) (mean ± SE) at and after the diagnosis of diabetes. *Lower panel*: numbers of patients with (■) and without (□) complete β-cell failure (fasting plasma C-peptide <0.10 nmol/l) (*E*), ICA (*F*), GADA (*G*), and IA-2A (*H*) at and after the diagnosis of diabetes. *Among patients positive for that antibody at diagnosis.

lower fasting P-C-peptide levels than antibody-negative patients or patients with only IA-2A (0.77 nmol/l [0.54], $P < 0.05$). Among these four patients, two with ICA and GADA and one with ICA, GADA, and IA-2A had fasting P-C-peptide levels of 0.11–0.34 nmol/l after 12 years. The forth patient, who had GADA (35.7 units/ml) and IA-2A (borderline positive with index 1.2) at diagnosis but was antibody negative at follow-up, had fasting P-C-peptide levels of 1.04 nmol/l after 12 years. None of the four patients with ICA and IA-2A at diagnosis were followed-up after 12 years; however, one patient tested 5 years after diagnosis had β-cell failure on that occasion.

Of 20 patients, 5 (25%) with two or three antibodies at diagnosis were 65 years of age or older at diagnosis, and 4 of 5 (80%) had developed complete β-cell failure after 12 years.

Patients with one antibody at diagnosis. Among follow-up patients with only GADA ($n = 6$) or only ICA ($n = 1$) at diagnosis of diabetes, GADA levels increased after diagnosis in five of seven patients. All five with increasing GADA levels remained GADA positive after 12 years, whereas the two patients who did not have an increase in GADA levels were GADA negative both after 5 and 12 years, respectively (Fig. 2). Among patients who remained GADA positive, three developed additional antibodies (one IA-2A after 3 years, one ICA after 5 years, and one ICA after 5

TABLE 2
Islet antibody status at follow-up in patients with adult-onset diabetes with three islet antibodies at diagnosis

	Diagnosis	3 year	5 year	12 year
*n**	25	20	19	15
3 Antibodies				
ICA + GADA + IA-2A	25 (100)	13 (65)	10 (53)	5 (33)†
2 Antibodies				
ICA + GADA	0	2 (10)	4 (21)	2 (13)
ICA + IA-2A	0	1 (5)	0	0
GADA + IA-2A	0	1 (5)	2 (11)	2 (13)
1 Antibody				
ICA	0	0	0	0
GADA	0	3 (15)	3 (16)	4 (27)
IA-2A	0	0	0	0
0 Antibodies	0	0	0	2 (13)
Any antibody				
ICA	25 (100)	16 (80)	14 (74)	7 (47)†
GADA	25 (100)	19 (95)	19 (100)	13 (87)
IA-2A	25 (100)	15 (75)	12 (63)	6 (40)†

Data are *n* (%). **n* = number of patients available for testing; †$P < 0.0001$ vs. diagnosis.

TABLE 3
Islet antibody status at follow-up in patients with adult-onset diabetes with two islet antibodies at diagnosis

	Diagnosis	3 years	5 years	12 years
*n**	14	8	8	5
3 Antibodies				
ICA + GADA + IA-2A	0	0	0	0
2 Antibodies				
ICA + GADA	9 (64)	5 (55)	5 (63)	3 (60)
ICA + IA-2A	4 (29)	0	0	0
GADA + IA-2A	1 (7)	0	0	0
1 Antibody				
ICA	0	1 (13)	0	0
GADA	0	0	1 (13)	1 (20)
IA-2A	0	1 (13)	0	0
0 Antibody	0	1 (13)	2 (25)	1 (20)
Any antibody				
ICA	13 (93)	6 (75)	5 (63)	3 (60)
GADA	10 (71)	5 (63)	6 (75)	4 (80)
IA-2A	5 (36)	1 (11)	0	0

Data are *n* (%). **n* = number of patients available for testing.

years and then IA-2A after 12 years). Although the patient with only ICA at diagnosis was ICA negative after 12 years, the patient had developed GADA at the 3-year follow-up and continued to be GADA positive after that. All but one of the patients with only IA-2A at diagnosis were negative after 3 and 5 years; one of them was again IA-2A positive after 12 years (index 9.7).

Among six patients with only GADA at diagnosis, the frequency of complete β-cell failure had increased from one of six (17%) patients 5 years after diagnosis to four of five (80%) patients after 12 years. In the remaining patient, the C-peptide level had decreased temporarily from 1.40 nmol/l at diagnosis to 0.55 nmol/l 3 years after diagnosis and then increased to 1.09 nmol/l after 5 years, showing 1.08 nmol/l after 12 years. In the patient with only ICA, the fasting P-C-peptide level had decreased from 0.41 nmol/l at diagnosis to 0.11 nmol/l after 12 years (Fig. 2). None of the patients with only IA-2A developed β-cell failure during the follow-up.

Patients without islet antibodies at diagnosis. After diagnosis, 21 of the 105 patients who were islet antibody–negative at diagnosis became islet antibody–positive (Fig. 3). Only 1 of these 21 patients was, however, consistently antibody positive (IA-2A positive after 3 and 5 years and ICA positive but IA-2A negative after 12 years). Only two additional patients were antibody positive in two consecutive tests (one was GADA positive after 3 and 5 years and one was ICA positive after 3 and 5 years). In total, 6 patients developed ICA, 3 GADA, and 13 IA-2A. In the six patients who developed ICA after diagnosis, the levels of ICA were significantly lower than in the patients who were ICA positive at diagnosis (2.5 JDF units [6] vs. 54.5 JDF units [130]), $P = 0.004$).

None of the patients who were islet antibody–negative at diagnosis developed complete β-cell failure during the 12-year period. Indeed, 12 years after diagnosis, fasting P-C-peptide levels in islet antibody–negative patients were not significantly different compared with the levels at diagnosis. On the other hand, the six patients islet antibody–negative at diagnosis who developed ICA after diagnosis (ICA converters) showed significantly decreasing fasting P-C-peptide levels after diagnosis ($P < 0.05$) (Fig. 4). In contrast to ICA converters, patients developing IA-2A or GADA did not show decreasing fasting P-C-peptide levels after diagnosis (0.75 nmol/l [0.66] at diagnosis vs. 0.80 nmol/l [0.49] after 12 years).

Clinical characteristics. Among patients with GADA and/or ICA at diagnosis, there were clinical differences at diagnosis between patients started on insulin at diagnosis and those started on insulin later. Among patients with multiple antibodies, those not started on insulin at diagnosis ($n = 16$) were significantly older and had higher BMI but, in fact, similar fasting C-peptide levels compared with patients started on insulin treatment at diagnosis (Table 4). Among patients with one antibody, the patient started on insulin treatment at diagnosis had undetectable fasting C-peptide at diagnosis, whereas the five patients started on insulin later had significantly higher C-peptide and lower HbA_{1c} and GADA levels at diagnosis compared with patients with multiple antibodies who were started on insulin at diagnosis (Table 4). Among patients who developed ICA after diagnosis ($n = 6$), none was started on insulin treatment at diagnosis and five of six (83%) had BMI >28 kg/m^2 at diagnosis.

The frequency of future β-cell failure was low among patients with only diet treatment at diagnosis (2 of 66 [3%]). In contrast, among patients started on pharmacological treatment at diagnosis, the frequency of future β-cell failure was clearly higher ($P < 0.0001$), although there was no significant difference in this context between those started on oral antihyperglycemic treatment (9 of 26 [35%]) versus those started on insulin treatment (9 of 15 [60%]) ($P = 0.19$).

During the follow-up, the frequency of insulin treatment increased from 21 of 47 (45%) patients at diagnosis to 30 of 33 (91%) patients after 12 years in patients with GADA and/or ICA at diagnosis of diabetes ($P < 0.0001$). The two GADA-positive patients (one with only GADA and one with GADA and IA-2A) with high C-peptide at follow-up and one patient with ICA and GADA with 0.34 nmol/l fasting C-peptide after 12 years were not on insulin treatment after 12 years. Although the frequency of insulin

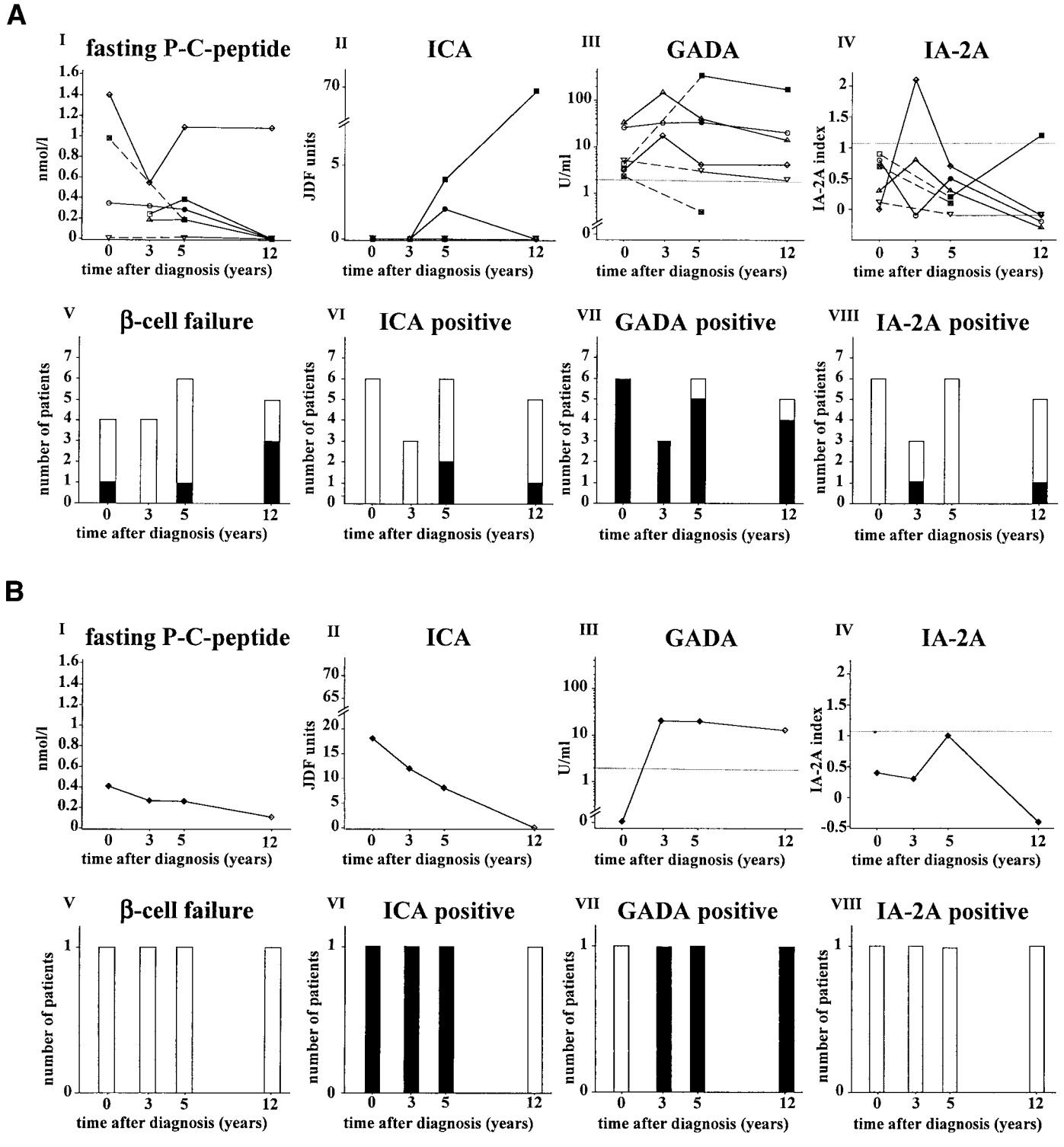

FIG. 2. Adult-onset diabetic patients with only GADA (n = 6) (*A*) or only ICA (n = 1) (*B*) at diagnosis. *Upper panels*: individual levels of fasting plasma C-peptide (*I*), ICA (*II*), GADA (*III*), and IA-2A (*IV*) at and after the diagnosis of diabetes. Symbols indicate individual patients. ■, ICA positivity; – – –, not tested after 3 years; ····, cutoff for abnormality. *Lower panels*: number of patients with (■) and without (□) complete β-cell failure (fasting plasma C-peptide <0.10 nmol/l) (*V*), ICA (*VI*), GADA (*VII*), and IA-2A (*VIII*) at and after the diagnosis of diabetes. Among GADA-positive patients, two of six became ICA positive 5 years after diagnosis (*A*), whereas the ICA-positive patient became GADA positive 3 years after diagnosis (*B*).

treatment also increased in patients without antibodies or with only IA-2A at diagnosis, from 11 of 167 (7%) patients at diagnosis to 31 of 94 (33%) patients after 12 years ($P <$ 0.0001), the frequency of insulin treatment after 12 years was clearly lower in patients without antibodies or with only IA-2A versus patients with GADA and/or ICA ($P <$ 0.0001). Among the patients who developed ICA after diagnosis, insulin treatment increased from none of six (0%) patients (both at diagnosis, after 3, and after 5 years) to four of six (67%) patients after 12 years.

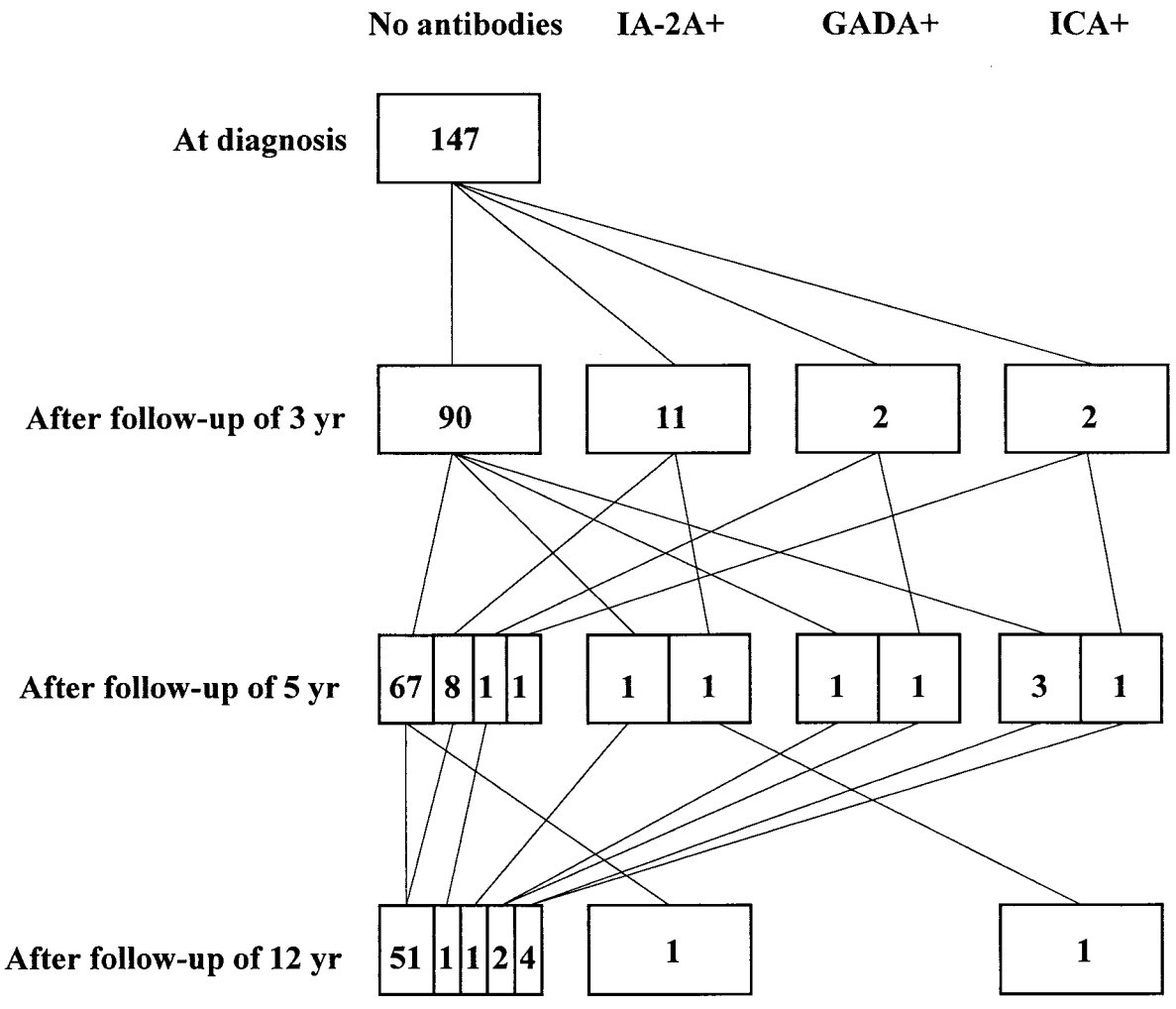

FIG. 3. The development of islet antibodies after diagnosis in patients without islet antibodies at diagnosis of adult-onset diabetes. Figures indicate numbers of patients.

DISCUSSION

In the current 12-year prospective study of patients with adult-onset diabetes, almost all of those with GADA and/or ICA at diagnosis of diabetes had developed complete β-cell failure (undetectable fasting P-C-peptide) 12 years after diagnosis. This was seen in all ages. In this context, patients with isolated GADA positivity had a more slow development toward β-cell failure than patients with multiple antibodies. Patients with isolated GADA positivity had some preserved function 5 years after diagnosis of diabetes; however, most of them (80%) had developed β-cell failure 12 years after diagnosis. Indeed, β-cell failure 12 years after diagnosis was restricted to patients who were autoantibody positive at diagnosis. Development of antibodies after diagnosis was rare. Nevertheless, ICA, but not GADA or IA-2A, developing after diagnosis in ~5% of the originally islet antibody–negative patients, predicted a decrease in fasting P-C-peptide values. Among patients antibody positive at diagnosis, the frequencies of ICA and IA-2A decreased 50% from diagnosis to 12 years after diagnosis, whereas almost all patients with GADA at diagnosis remained GADA positive during the entire study period of 12 years.

β-cell function in patients with only GADA or with two or three antibodies at diagnosis. Islet antibodies at diagnosis are considered the gold standard in the classification of type 1 diabetes, reflecting the autoimmune pathogenesis of the disease (8). This concept was confirmed in the current prospective study by our observation that β-cell failure was restricted to antibody-positive patients. In addition, as in children (23), the antibody pattern in patients with adult-onset diabetes predicted the rate of progression in β-cell dysfunction. Positivity for GADA alone at diagnosis predicted a slower decline in β-cell function than positivity for two or three antibodies. Nevertheless, although patients with only GADA had a slowly progressive β-cell failure, 12 years after diagnosis of diabetes, these patients showed complete β-cell failure as frequently as those with two or three antibodies at diagnosis. As most of our patients with two or three antibodies had ICAs, we can also confirm that ICA is associated with rapid decrease of C-peptide levels (24). Furthermore, the few patients with GADA, ICAs, or both at diagnosis that had remaining β-cell function at the 12-year follow-up mostly had low fasting P-C-peptide levels 12 years after diagnosis. This indicates that antibody positivity also

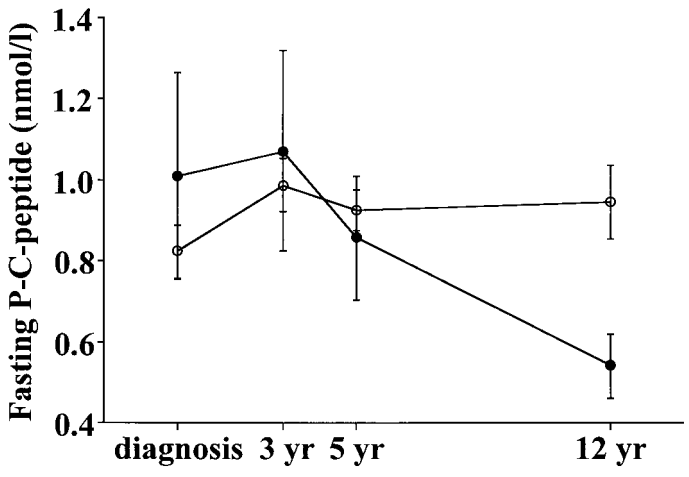

FIG. 4. β-cell function at and after the diagnosis of adult-onset diabetes in patients continuously antibody negative (○, $n = 57$) and patients antibody negative at diagnosis who developed ICA after diagnosis (●, $n = 6$). Symbols indicate mean and horizontal lines indicate SE. Fasting P-C-peptide levels were unchanged in continuously antibody-negative patients but decreased among those who developed ICA after diagnosis ($P < 0.05$).

identified type 1 diabetes in these patients, although in a less aggressive form.

Insulin treatment is common in patients with a long duration of type 2 diabetes (25,26). In agreement, 12 years after diagnosis, insulin treatment had been initiated in one-third of our antibody-negative patients, but this was not associated with complete β-cell failure in any of these patients. In fact, fasting P-C-peptide levels were unchanged 12 years from the diagnosis of diabetes in antibody-negative patients and in patients with IA-2A only. This argues that all patients without antibodies or with only IA-2A had type 2 diabetes rather than idiopathic (nonautoimmune) type 1 diabetes.

Recently, the importance of GADA in the prediction of β-cell function in elderly patients (>65 years of age at diagnosis) was questioned (27). In the current study, however, the value of GADA in the prediction of β-cell failure in elderly patients was as high as in young adults.

Frequencies and levels of islet antibodies after diagnosis. Compared with children, GADA and ICA persisted longer after diagnosis in the adult patients of the current study. In children, less than half remained GADA positive and less than one-fifth remained ICA positive 7–11 years after diagnosis (14), compared with almost all remaining GADA-positive patients and about half remaining ICA positive in our adult patients 12 years after diagnosis. IA-2A, on the other hand, remained positive in about the same frequency (50%) in adults as in children. The long duration of islet antibody positivity after the diagnosis of diabetes in the current study supports the results of a recent study of young adults (15). Decochez et al. (15) reported 94% positive for at least one islet antibody 4 years after diagnosis of diabetes, corresponding well with our results (91% after 5 years and 85% after 12 years). Accordingly, our study indicates that in the classification of diabetes, antibody measurements may be useful up to 12 years after diagnosis. In contrast to the general decrease in GADA levels, most patients with only GADA at diagnosis showed increasing GADA levels and development of additional antibodies after diagnosis. This indicates that these patients may have been at an earlier stage of the disease process at diagnosis compared with patients with two or three antibodies. Increased levels of antigen-specific antibodies after diagnosis were also observed by Decochez et al. (15).

De novo development of antibodies after diagnosis. Patients who were islet antibody–negative at diagnosis and later converted to ICA positivity, but not to GADA or IA-2A, showed decreasing fasting P-C-peptide levels at follow-up. Isolated ICA positivity at diagnosis of diabetes was only found in one patient (who actually later developed GADA). As detected in low levels in our patients developing autoimmunity after the diagnosis of diabetes,

TABLE 4
Characteristics at diagnosis in antibody-positive patients (GADA, ICA, or both) with adult-onset diabetes started on insulin treatment at diagnosis versus those started later

	Insulin at diagnosis		Insulin started later	
	1 antibody	≥2 antibodies	1 antibodies	≥2 antibodies
n	1	19	5	16
Age (years)	30	30 (29)	54 (31)	58 (23)*
Range		21–73	30–63	30–75
C-peptide (nmol/l)	<0.10	0.19 (0.15)	0.41 (0.49)†	0.22 (0.24)
Range		<0.10–0.61	0.35–1.00	<0.10–0.74
BMI (kg/m^2)	19.1	21.0 (3.5)	25.7 (4.7)‡	23.9 (4.2)§
Range		16.2–27.3	20.0–27.8	18.4–31.1
HbA_{1c} (%)	12.2	10.7 (2.9)	5.4 (2.2)‖¶	9.5 (1.4)
Range		4.8–15.7	5.1–9.3	6.5–11.6
No. of antibodies	1	3 (1)	1 (0)	3 (0)
IA-2A (index)#	—	35.2 (89.2) ($n = 13$)	—	34.6 (62.2) ($n = 15$)
GADA (index)#	4.9 ($n = 1$)	82.1 (128.9) ($n = 17$)	15.1 (25.1)†¶ ($n = 4$)	85.2 (169.5) ($n = 15$)
ICA (JDF units)#	—	28 (48) ($n = 19$)	18 (0) ($n = 1$)	66 (123) ($n = 16$)

Data are median (interquartile range) unless otherwise indicated. *Versus insulin at diagnosis/≥2 antibodies, $P = 0.004$; †versus insulin at diagnosis/≥2 antibodies, $P = 0.01$; ‡versus insulin at diagnosis/≥2 antibodies, $P = 0.03$; §versus insulin at diagnosis/≥2 antibodies, $P = 0.005$; ‖versus insulin at diagnosis/≥2 antibodies, $P = 0.02$; ¶versus insulin later/≥2 antibodies. $P = 0.02$; #among positive for those antibodies.

ICA may be directed against antigens other than GAD65 and IA-2 (28,29), perhaps not yet identified. The fact that most of these patients were overweight at diagnosis of diabetes and had had diabetes for several years until development of ICAs suggests that these patients primarily may have had type 2 diabetes. The decline in β-cell function and initiation of insulin treatment in these ICA converters, after or in parallel with the development of ICAs, suggests that these patients also had, or would develop, type 1 diabetes. A planned follow-up will show whether β-cell function will decline further. Nevertheless, our observation argues that patients may have manifestations of both type 2 and type 1 diabetes, as recently reported in Finland (30). The lower frequency of antibody converters in the current long-term follow-up study than that reported in young adults during the first years after diagnosis of diabetes (15,16) suggests that antibodies developing soon after diagnosis of diabetes are transient.

Clinical implications. The current study shows that GADA measurements may be performed many years after the diagnosis of diabetes with preserved sensitivity. In addition, ICA development many years after diagnosis may argue for repeated antibody measurements after diagnosis. Most strongly, however, our study argues for the importance of conducting islet antibody measurements at diagnosis in most patients with adult-onset diabetes; islet antibody positivity very specifically identified all patients with future β-cell failure. Previously, insulin dependency defined type 1 diabetes (31). The new American Diabetes Association classification from 1997 recognized that insulin treatment at diagnosis is not the same as insulin dependency (32). Our finding, that most antibody-positive patients developing β-cell failure did not receive insulin treatment at diagnosis, further supports the opinion that insulin treatment at diagnosis is not a reliable criterion for type 1 diabetes. Patients with multiple antibodies started on insulin later were older and had a higher BMI but as low fasting C-peptide values and as high antibody levels compared with patients started on insulin at diagnosis. This indicates that it was the "typical" type 2 phenotype that led to a clinical misclassification and a delay in insulin treatment. The subgroup of patients with GADAs or ICAs not started on insulin at diagnosis had higher fasting C-peptide levels than antibody-positive patients started on insulin at diagnosis. These patients may be considered to have slow-onset diabetes or LADA. This is further supported by the fact that, in contrast to patients with multiple antibodies, β-cell function was often partially preserved 3 and 5 years after diagnosis in most of the patients classified as having slow-onset diabetes/LADA. Indeed, the few patients multiple antibody–positive at diagnosis with low but preserved β-cell function 12 years after diagnosis may be considered as having slowly progressive autoimmune type 1 diabetes. In this context, it might be relevant to refer to the general belief that ~15% of type 1 diabetic patients are islet antibody negative (32). Our study is an argument against that opinion. In the current 12-year prospective study, only patients with islet antibodies developed β-cell failure.

We previously recommended that when antibodies against GAD and IA-2A are measured at diagnosis, antibody titers may be considered to identify slow (low titers) or rapid progression (high titers) to β-cell failure (11). We believe that these recommendations are still appropriate. However, antibody measurements are costly. Because islet antibody measurements now should be conducted in most patients with adult-onset diabetes, cost-effectiveness needs to be optimized. Therefore, although with the cost of 10% sensitivity, a primary screening for GADA followed by a second test for IA-2A in GADA-positive patients with low GADA levels in order to improve the prediction of a fast progression to β-cell failure may be suggested.

Conclusions. In this 12-year follow-up, complete β-cell failure has developed in almost all (and incomplete β-cell failure in most of the other) islet antibody–positive patients, whereas a lack of GADA or ICA or with IA-2A in low levels indicated preservation of β-cell function. GADA remained in almost all antibody-positive patients, and ICA developing after diagnosis was associated with decreasing β-cell function.

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