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Correlation of islet cell antibodies and HLA-DR phenotypes with diabetes mellitus in adults

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Summary. In a cross-sectional study, sera of 81 adult diabetic in-patients were tested for the presence of pancreatic islet cell antibodies (ICA), both IgG and complement-fixing. All patients had been well controlled initially with oral hypoglycaemic agents and therefore had been classified as having Type 2 (non-insulin-dependent) diabetes. However, 14 were subsequently classified as Type 1 (insulin-dependent) because they became insulin-dependent within 2 months of diagnosis. Ten of these patients (71%) were ICA-positive. Sixty-seven patients had been non-insulin-dependent for at least 1 year after diagnosis. Circulating ICA were present in 18 patients and 16 of these (89%) required insulin therapy. Secondary oral hypoglycaemic agent failure developed within a mean period of 3.7 years after diagnosis. In contrast, in the ICA-negative sub-group ($n = 49$) insulin treatment became necessary in 29 patients. Secondary oral hypoglycaemic agent failure of these patients had developed after a mean period of 8.4 years, which was significantly longer than in the ICA-positive patients ($p < 0.01$). Complement-fixing-ICA were detected only in sera with an ICA-IgG titre of at least 8, and its prevalence was similar in the sub-groups tested, i.e., the Type 1 diabetic patients and the patients with secondary oral hypoglycaemic agent failure. With HLA-DR typing, a significant excess of the DR3 antigen and heterozygous DR3/DR4 phenotypes was found in ICA-positive patients with secondary oral hypoglycaemic agent failure and in the Type 1 diabetic patients, which was comparable with the frequencies reported in juvenile-onset Type 1 diabetes. The heterozygous DR3/W6 phenotype was significantly increased in the ICA-positive patients when compared with 13 ICA-negative patients. Thus, the presence of ICA and an excess of certain HLA-DR phenotypes identify a sub-group within the adult diabetic population with secondary oral hypoglycaemic agent failure which can be regarded as a retarded form of Type 1 diabetes.

Key words: Islet cell antibody, complement-fixing islet cell antibody, HLA-DR phenotypes.

Cytoplasmic antibodies to pancreatic islet cells (ICA) have been described in adult diabetic patients treated with oral hypoglycaemic agents and are associated with a high probability of becoming insulin-dependent within the near future. Considering the heterogeneity of diabetes, ICA-positive, apparently non-insulin-dependent diabetes may represent a retarded pathogenesis of Type 1 (insulin-dependent) diabetes [1, 2]. This is supported by the observation that ICA-positive patients, even though initially controlled with oral hypoglycaemic agents, expressed a significant prevalence of HLA-DR3 and heterozygous DR3/DR4 phenotypes comparable with the frequencies found in juvenile onset Type 1 diabetes [3–5].

Subjects and methods

Subjects

Eighty-one patients (aged 30–75 years, mean ± SEM age 50 ± 1 years) were initially well controlled with diet or additional oral hypoglycaemic agents and, therefore, had been classified as having Type 2 (non-insulin-dependent) diabetes. They were referred either to the diabetic-clinics of Düsseldorf or Quakenbrück because of recent poor metabolic control. Serum samples obtained on admission were tested for ICA-IgG and complement-fixing ICA (CF-ICA). Classification was made on a clinical basis without knowledge of the ICA results. Type 1 diabetes was assumed, if satisfactory metabolic control could be achieved by insulin therapy, otherwise patients were classified as having Type 2 diabetes. Metabolic control was judged by recent weight change, ketonuria, haemoglobin A_1 concentration, and fasting and post-prandial blood glucose values. In addition, basal immunoreactive insulin and maximal immunoreactive insulin response after β-cell stimulation with intravenous tolbutamide (1 g) alone or in combination with an oral glucose challenge (100 g) were determined. Secondary oral hypoglycaemic agent failure was diagnosed when satisfactory metabolic control could no longer be maintained with a strict diet and oral hypoglycaemic agents. These patients had persistent post-challenge blood glucose > 11.1 mmol/l, haemoglobin A_1 concentration > 8.5%, and were suffering from glycosuria and occasional ketonuria. Their endogenous immunoreactive insulin secretion upon β-cell stimulation was also markedly decreased. Twenty-nine patients were recruited for retrospective HLA-typing and the calculated HLA-DR-antigen frequencies were compared with the values of 100 randomly selected unrelated healthy control subjects.

Table 1. Classification and clinical and laboratory data from 81 adult apparently Type 2 diabetic patients according to their ICA status

	Type 1 diabetic patients ($n=14$)		Patients with insulin-dependency secondary to oral hypoglycaemic agent failure ($n=45$)		Type 2 diabetic patients ($n=22$)		Control subjects ($n=20$)
	ICA-positive ($n=10$)	ICA-negative ($n=4$)	ICA-positive ($n=16$)	ICA-negative ($n=29$)	ICA-positive ($n=2$)	ICA-negative ($n=20$)	
Immunoreactive insulin (mU/l)	6±1	Not tested	8±2	14±5	14±6	17±2	7±2
Maximal immunoreactive insulin[a] (mU/l)	4±1	Not tested	3±1	10±3	52±37	57±15	40±7[b]
Time since diagnosis (years)	<0.2	<0.2	3.7 (1–15)[c]	8.4 (1–17)[c]	1.3 (0.5–2)	4.8 (0.5–15)	
Age at diagnosis (years)	44.3 (30–62)	47.3 (38–71)	46.1 (30–75)	52.3 (31–75)	39.0 (34–44)	52.0 (30–74)	

Immunoreactive insulin results expressed as mean ± SEM; age expressed as mean with range in parentheses.
[a] maximal additional secretion upon β-cell stimulation with intravenous tolbutamide or in combination with oral glucose challenge.
[b] obtained after 5 min; [c] $p<0.01$

Methods

ICA-IgG and CF-ICA were detected by indirect immunofluorescence, using cryostat sections of human pancreas [6–8]. HLA-DR1, 2, 3, 4, 5, W6, 7, W8 and W10 specificities were recognized on peripheral B-lymphocytes [9]. Statistical significances were calculated using the χ^2 test and the Wilcoxon-Mann-Whitney U-test.

Results

Clinical classification

Fourteen of the 81 patients required insulin therapy within 2 months of diagnosis, and this group was termed adult-onset Type 1 diabetes. Sixty-seven patients, who had been well controlled with diet or additional oral hypoglycaemic agents for at least 1 year, were categorised as Type 2 diabetic patients. Of these, 45 developed secondary oral hypoglycaemic agent failure and 22 remained satisfactorily controlled with diet or additional oral hypoglycaemic agents. The patients requiring insulin therapy suffered from β-cell deficiency, as demonstrated by low basal immunoreactive insulin values and failure of insulin secretion upon stimulation with intravenous tolbutamide. In contrast, the Type 2 diabetic sub-groups had low basal immunoreactive insulin values but responded with markedly increased levels upon stimulation with tolbutamide alone or combined with oral glucose challenge (Table 1).

High association of ICA and Type 1 diabetes

ICA-IgG were observed in one (0.7%) and CF-ICA in none of 150 control sera obtained from healthy subjects. ICA were found in 10 (71%) of the 14 adult Type 1 diabetic patients. In 18 out of the 67 apparent Type 2 diabetic patients, ICA were detected, and 16 of this sub-group (89%) had developed secondary oral hypoglycaemic agent failure, while only two remained non-insulin-dependent. Of the 49 ICA-negative patients, 29 required insulin therapy. The time interval between diagnosis of diabetes and development of insulin dependency was significantly shorter in the ICA-positive than in the ICA-negative sub-groups ($p=0.01$; mean ± SEM 3.7 ± 0.9 and 8.4 ± 1.1 years, respectively; Table 1). ICA were detected in 50% of the ICA-positive sera and were strongly associated with an ICA-titre of at least of 8; 12 out of 16 of such high-titre sera contained CF-ICA. There was no difference in prevalence of CF-ICA in the sera of either adult Type 1, secondary oral hypoglycaemic agent failure, or Type 2 diabetic sub-groups.

HLD-DR phenotype frequencies in ICA-positive Type 1 diabetic patients

Sixteen of the 29 patients typed for HLA-DR antigens were ICA-positive, comprising 11 diabetic patients classified as secondary oral hypoglycaemic agent failures and five with adult Type 1 diabetes. The remaining 13 were ICA-negative and comprised eight with secondary oral hypoglycaemic agent failure and five with Type 2 diabetes. The HLA-DR frequencies of the two ICA-positive and two ICA-negative sub-groups, respectively, were combined because there were no differences in DR frequencies among the corresponding sub-groups. In the ICA-positive population, DR3, and heterozygous DR3/DR4 and DR3/W6 phenotype frequencies, were significantly increased (Fig. 1; 75%, 31.3% and 25% versus 30%, 6% and 3% in the control group, respectively), whereas DR2 and DR5 phenotype frequencies were significantly decreased (6.3 and 0% versus 31 and 27% in the controls, respectively). In contrast, the ICA-negative sub-group had antigen frequencies similar to the control population.

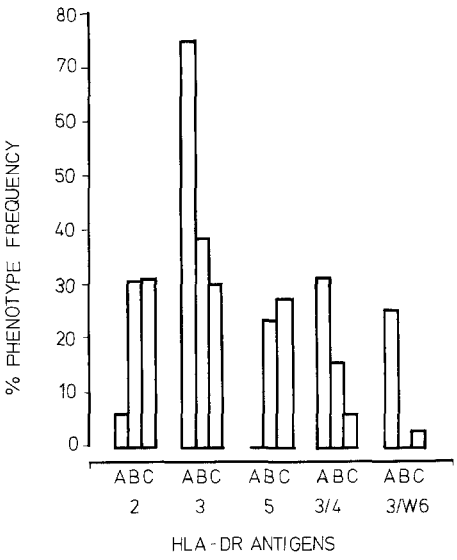

Fig. 1. HLA-DR phenotype frequencies in 16 ICA-positive (A), 13 ICA-negative (B) diabetic patients and 100 control subjects (C). When comparing the frequencies of the ICA-positive and ICA-negative sub-groups, significant differences were found with respect to the DR3, DR5 and DR3/W6 frequencies ($p < 0.05$). When comparing the values of the ICA-positive sub-group with those of the control population, significant deviations were found at DR3 ($p < 0.0005$), DR5 ($p < 0.02$), DR3/DR4 ($p < 0.001$) and DR3/W6 ($p < 0.0006$). The differences between the ICA-negative sub-group and the control values were not significant

Discussion

The present results of a cross-sectional investigation confirm and extend the data of other studies [1, 2] on the value of ICA as an in vitro test predicting the likelihood of forthcoming secondary oral hypoglycaemic agent failure in apparent Type 2 diabetic subjects. Of 18 ICA-positive patients, 16 (89%) developed secondary oral hypoglycaemic agent failure after a mean period of 3.7 years. This incidence is similar to the data resulting from a prospective study of Irvine et al. [1]. By acturial statistics, these authors calculated that the probability of becoming insulin-dependent within 5 years is 86% for ICA-positive patients controlled initially with oral hypoglycaemic agents. The prevalence of 71% for ICA-positive patients in the adult Type 1 diabetic group is similar to the ICA prevalence in juvenile Type 1 patients as originally described by Lendrum et al. [10]. Our observation that there is a positive correlation between CF-ICA and the titre of ICA confirms recently published results in juvenile Type 1 diabetic subjects [11]. However, we did not find that the presence of CF-ICA is more strongly associated with subsequent insulin dependency (results not shown). These data support recent observations by Mustonen et al. [12], but contrast with other reports [2, 7].

In our study, ICA-positive patients with adult Type 1 diabetes or with secondary oral hypoglycaemic agent failure expressed HLA-DR phenotype frequencies similar to those with juvenile-onset Type 1 diabetes [3–5], whereas the ICA-negative sub-groups failed to do so. Thus, the notion that ICA-positive diabetes controlled with oral hypoglycaemic agents represents a clinically and pathogenically retarded form of Type 1 diabetes [1] is supported. The significance of the observed positive association of DR3/W6 heterozygous phenotypes and presence of ICA will be evaluated further in a prospective study in apparent Type 2 diabetes.

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