An Association Between Complement-Fixing Cytoplasmic Islet Cell Antibodies and Endogenous Insulin Secretion in Children with Insulin-dependent Diabetes Mellitus

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SUMMARY

Cytoplasmic islet cell antibodies and endogenous insulin secretion were studied in 184 children and adolescents having insulin-dependent diabetes mellitus (IDDM) in a cross-sectional study. The mean age of the subjects was 12.3 yr (range: 2.8–19.2 yr), and the mean duration of diabetes was 4.6 yr (range: 0.1–15.6 yr). Islet cell antibodies (ICA) were determined by both the indirect immunofluorescence (IF-ICA) and the complement-fixing (CF-ICA) methods. Forty-four patients (23.9%) were positive with respect to both IF- and CF-ICA, 54 patients (29.3%) had only IF-ICA, and 86 patients had no ICA. The patients having CF-ICA had a significantly higher endogenous insulin secretion in comparison with the patients who were only IF-ICA positive. The difference between the groups remained significant even when the age at onset of diabetes and the duration of the disease were taken into account.

This finding, revealing an association between CF-ICA and endogenous insulin secretion, suggests that complement-fixing antibodies are seen only if the beta-cell mass is sufficiently preserved. The result contradicts the hypothesis, based on studies in vitro, that CF-ICA should be involved in the selective beta-cell damage in IDDM. DIABETES 32:743–747, August 1983.

The association of cytoplasmic islet cell antibodies (ICA) with the onset of insulin-dependent diabetes mellitus (IDDM) has been confirmed in several reports, as has the presence of these antibodies before the clinical onset of the disease.[1–5] In first-degree relatives of IDDM patients, ICA are more common than in healthy controls.[3,5] However, there are no undisputed data that predict that an ICA-positive person will become diabetic. The exact role of ICA in the pathogenesis of IDDM is still an enigma. The antigen of cytoplasmic ICA is unknown and, at least in the assay procedure, ICA react with all types of islet cells.[6]

So far no relationship has been reported between ICA and the indicators of residual beta-cell function in IDDM,[7–9] but there are reports that indicate that IDDM patients have in their serum complement-dependent cytotoxic factor(s) influencing the islet cell function in vitro.[10–12] It has been suggested that complement-fixing subspecies of ICA (CF-ICA) may be more closely related to the actual damage of beta-cells.[13] The presence of CF-ICA has also been demonstrated before the diagnosis of overt IDDM.[5,14]

The purpose of the present study was to investigate whether there is any association between CF-ICA and endogenous insulin secretion in children and adolescents with IDDM.

PATIENTS AND METHODS

The study included all the 184 insulin-dependent diabetic children and adolescents (100 boys) regularly attending the Diabetes Clinic in the Department of Pediatrics, University of Oulu, Finland, during 1980. Diagnosis of diabetes had been made during the years 1964–1980. The mean age (SEM) of the patients was 12.3 (0.3) yr (range: 2.8–19.2 yr), the mean age at the onset of IDDM was 7.5 (0.3) yr (range: 0.9–16.7 yr), while the mean duration of the disease was 4.6 (0.3) yr (range: 0.1–15.6 yr). At diagnosis, 10.9% of the patients had no signs of ketosis or ketoacidosis, 32.1% had only ketosis (Ketostix, Miles Laboratories, Stoke Poges, England, in plasma positive, pH $\geq$ 7.35), 43.5% were in mild ketoacidosis (7.15 < pH < 7.35, Ketostix in plasma positive) and 13.6% in severe ketoacidosis (pH $\leq$ 7.15).

All patients were seen regularly in the Diabetes Clinic, from four to eight times in 1980. The treatment of the patients aimed at clinical well-being, absence of diabetic symptoms, normal growth, and an optimal metabolic control. The treatment consisted of highly purified insulin once or twice daily, a regulated diet, and regular physical exercise. The mean daily insulin dose (SEM) was 0.74 (0.02) U/kg.

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TABLE 1
Clinical and laboratory data in three groups of diabetic children classified on the basis of the ICA status [mean (SEM)]

	IF-ICA + CF-ICA + (I)	IF-ICA + CF-ICA − (II)	IF-ICA − CF-ICA − (III)	Statistics
Number of patients	44	54	86	
Age at onset (yr)	8.5 (0.6)	8.6 (0.5)	6.4 (0.4)	$F_{2,181} = 7.2$; $P < 0.001$ Comparison between groups: I versus III, $P < 0.05$; II versus III, $P < 0.01$
Duration of diabetes (yr)	2.7 (0.5)	3.6 (0.4)	6.4 (0.4)	$F_{2,181} = 20.3$; $P < 0.001$ Comparison between groups: I versus III, $P < 0.001$; II versus III, $P < 0.001$
Severe ketoacidosis at onset (%)	10.0	17.0	15.9	$X^2_6 = 2.5$; NS
Mean HbA_1 during 1980 (%)	13.5 (0.3)	14.3 (0.3)	14.0 (0.2)	$F_{2,181} = 1.7$; NS
Daily insulin dose (U/kg)	0.62 (0.04)	0.74 (0.04)	0.81 (0.02)	$F_{2,181} = 9.5$; $P < 0.001$ Comparison between groups: I versus II, $P < 0.05$; I versus III, $P < 0.001$

A venous blood sample for the assay of ICA, serum C-peptide, and hemoglobin A_1 (HbA_1) was obtained once from every patient at a regular visit to the Diabetes Clinic. Before that visit the patient had collected a 24-h urine sample for the measurement of urinary C-peptide excretion. In addition, serum C-peptide and HbA_1 concentrations were measured at least once more in all the patients, and an individual mean serum C-peptide and mean HbA_1 were calculated on the basis of all the determinations (N = 2–8/subject) from each subject during 1980. The patients were studied in the postprandial state between 10 a.m. and noon (i.e., between breakfast and lunch).

ICA were determined by both the conventional indirect immunofluorescence (IFL)[1] and the complement-fixation (CF) methods.[13] Cryostat sections of 5 μm from fresh human snap-frozen group O pancreas were used as a substrate throughout the experiments. The blocks of pancreas were stored at −70°C. The sera were stored at −20°C until needed and studied undiluted. Pooled human serum (NHS) diluted 1:2 with 0.9% sodium chloride was used as a source of extra complement. It was stored at −50°C in small aliquots for not more than 3 mo. Fluorescein-conjugated rabbit anti-human-IgG (Behringwerke A.G., Marburg, West Germany) diluted 1:40 was used for the detection of IF-ICA. In the CF-method, the incubation time for the test sera was 20 min. After washing with phosphate-buffered saline, NHS was applied for 20 min to assure the fixation of the complement. Fluorescein-conjugated rabbit anti-human C3c (Behringwerke A.G.) in dilution 1:40 was used as the detector antiserum. The results were read under a Leitz "Orthoplan" microscope. All microscopic analyses were done by one person (A.M.).

Serum C-peptide was measured according to Heding, using antiserum M 1230 (Novo Research Institute, Denmark).[15] Antibody-bound proinsulin was separated from C-peptide by polyethylene glycol before the assay.[16] The sensitivity of the assay was 0.02 nmol/L, and the interassay coefficient of variation was 10%. The fasting reference range was 0.10–0.40 nmol/L in nondiabetic children younger than 6 yr and 0.16–0.85 nmol/L in older children.[17] Urinary C-peptide was quantified by the same method in samples diluted 1:20. If necessary, the assay was repeated in more (1:40) or less (1:10) diluted samples. Hence, the sensitivity of the urinary C-peptide assay was 0.2 nmol/L. The 24-h urinary C-peptide excretion was expressed in relation to the body surface area. The reference range of 24-h urinary C-peptide excretion in our laboratory was 4.5–17.0 nmol/L/m^2 in nondiabetic children [N = 15, mean age (SEM) 12.4 (1.2) yr].

HbA_1 was measured by ion-exchange chromatography using a modification of the method decribed by Welch and Boucher.[18] The columns used were 0.7 × 10 cm and packed with Bio-Rex 70 resin (Bio-Rad Lab., Richmond, California) of 100–200 mesh. The reference range in nondiabetic children was 7.0–10.2% with a mean (SEM) of 8.6 (0.1)%. The interassay coefficient of variation was 7%.

For the statistical evaluation, the patients were divided into

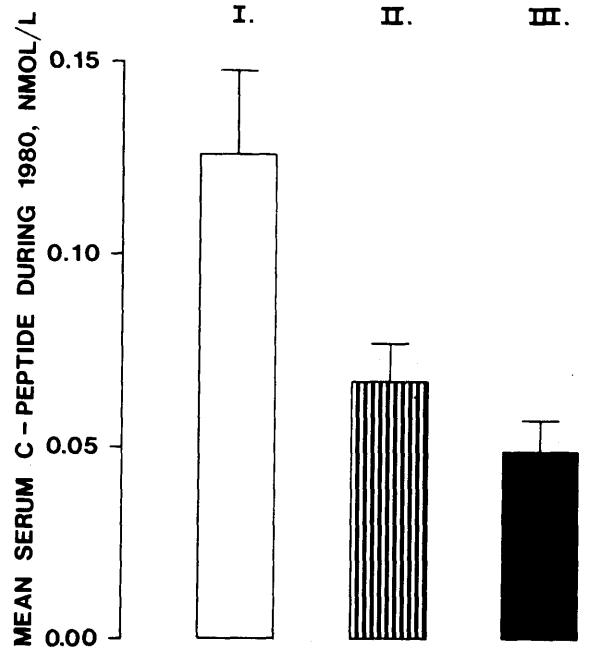

FIGURE 1. Mean serum C-peptide concentrations during 1980 in the children with both IF-ICA and CF-ICA detectable (group I), in those with only IF-ICA detectable (group II), and in those without ICA (group III). $F_{2,181} = 9.0$; $P < 0.001$. Comparison between groups: I versus II, $P < 0.05$; I versus III, $P < 0.001$; II versus III, NS.

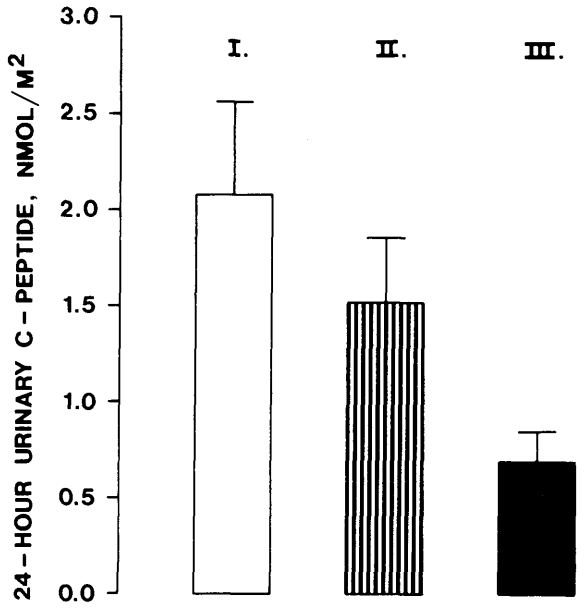

FIGURE 2. Twenty-four-hour urinary C-peptide excretions in those children with both IF-ICA and CF-ICA detectable (group I), in those with only IF-ICA detectable (group II), and in those without ICA (group III). $F_{2,181} = 5.7$; $P < 0.005$. Comparison between groups: I versus II, NS; I versus III, $P < 0.01$; II versus III, NS.

three groups on the basis of ICA: (1) those in whom both IF-ICA and CF-ICA were detectable, (2) those in whom only IF-ICA was detectable, and (3) those without any ICA.

The statistical analyses included a linear regression analysis, cross tabulation and chi-square statistics, and the parametric one-way analysis of variance. The analysis of covariance was used to test whether age at onset and duration of diabetes had any confounding effect on the variables measuring endogenous insulin secretion.[19]

RESULTS

Forty-four (23.9%) patients had both CF-ICA and IF-ICA in their serum. In 54 (29.3%) patients only IF-ICA were detectable, while 86 (46.7%) were negative with respect to ICA.

The mean (SEM) of the individual mean serum C-peptide was 0.07 (0.01) nmol/L (range: 0.00–0.62 nmol/L). The mean serum C-peptide exceeded the detection limit in 110 patients (59.8%). The mean 24-h urinary C-peptide excretion was 1.16 (0.15) nmol/m^2 (range: 0.00–15.11 nmol/m^2). One hundred eleven patients (60.3%) had a urinary C-peptide excretion higher than the detection limit. The urinary C-peptide was within the reference range in 14 patients (7.6%). There was a strong positive correlation between 24-h urinary C-peptide excretion and mean serum C-peptide ($r = 0.78$; $P < 0.001$). The mean serum C-peptide correlated positively with the age at onset of diabetes ($r = 0.48$; $P < 0.001$) and negatively with the duration of diabetes ($r = -0.47$; $P < 0.001$). There was similarly a positive correlation between 24-h urinary C-peptide and the age at onset of diabetes ($r = 0.43$; $P < 0.001$) and a negative correlation between urinary C-peptide and the duration of diabetes ($r = -0.44$; $P < 0.001$).

Table 1 presents clinical and laboratory data in the three groups of patients classified on the basis of the ICA status. Children without detectable ICA in their serum were younger at the onset of diabetes and had a longer duration than the patients in the other two groups. In the present study population those children with an early onset of diabetes had automatically a longer duration, as all the diabetic children are transferred to treatment units for adults in late adolescence. Hence, the findings of a younger age at onset and a longer duration of diabetes in the subjects without detectable ICA are in accordance with the evanescent character of ICA. There were no significant differences in the degree of ketoacidosis at onset or mean HbA_1 concentrations during 1980 between the three groups. The subjects with both CF-ICA and IF-ICA detectable had a significantly lower insulin dose than the two other groups.

The children who were positive for CF-ICA had significantly higher serum C-peptide concentrations during 1980 than the patients with only IF-ICA detectable or those who were ICA negative (Figure 1). There were also significant differences in the 24-h urinary C-peptide excretion between the three groups (Figure 2). The patients with CF-ICA detectable had a higher urinary C-peptide excretion than those with no ICA in their serum.

Table 2 shows the differences in endogenous insulin secretion between the groups after adjustment for the duration of diabetes and age at onset. Patients with both CF- and IF-ICA still had a significantly higher mean serum C-peptide concentration than the patients who were only IF-ICA positive. Similarly, the serum C-peptide concentration tended to be increased in patients with CF-ICA and IF-ICA detectable when compared with ICA-negative subjects. There were no significant differences between the three groups of patients in the 24-h urinary C-peptide excretion after the analysis of covariance, although the patients who were CF-ICA positive still had the highest absolute excretion.

TABLE 2
Endogenous insulin secretion in three groups of diabetic children classified on the basis of the ICA status, mean (SEM), adjusted by an analysis of covariance including age at onset and duration of diabetes as covariates

	IF-ICA + CF-ICA + (I)	IF-ICA + CF-ICA − (II)	IF-ICA − CF-ICA − (III)	Statistics
Number of patients	44	54	86	
Mean serum C-peptide during 1980 (nmol/L)	0.10 (0.01)	0.05 (0.01)	0.07 (0.01)	$F_{2,179} = 4.9$; $P < 0.01$ Comparison between groups: I versus II: $P < 0.01$; I versus III: $P < 0.1$
24-h urinary C-peptide (nmol/m^2)	1.63 (0.33)	1.20 (0.29)	1.14 (0.24)	$F_{2,179} = 1.6$; NS

DISCUSSION

Bottazzo et al. have suggested that CF-ICA probably reflect the active damage caused to the beta-cells, as these antibodies appear to correlate more closely than IF-ICA with the clinical onset of IDDM.[13] The present study provides evidence of the role of CF-ICA as markers of continuing beta-cell function in IDDM. The results indicate that the living beta-cell may comprise the antigen of the CF-ICA. If this is so, CF-ICA will remain detectable as long as there is an antigenic stimulation in the form of functioning beta-cells.

In the reports of Theophanides et al.[7] and Madsbad et al.[8] no association was found between ICA and residual beta-cell function. In both these studies the indirect immunofluorescence method was used for the ICA assay. Our results of diabetics with ICA detectable only by the indirect immunofluorescence are consistent with the findings of these previous studies. Crossley et al. reported a prospective study of 21 children observed for at least 2 yr from the clinical onset of diabetes.[9] They assessed the residual beta-cell function by measuring 24-h urinary C-peptide excretion. ICA were determined by both the IFL and CF methods. No correlation was observed between ICA status and residual beta-cell function. However, in their analysis of the results CF-ICA were not differentiated from IF-ICA.

In the present study the endogenous insulin secretion was decreased in all three groups of diabetic children when compared with nondiabetic children. Based on the mean values, the urinary C-peptide excretion in diabetic children was about one-eighth that in normal children. However, there were significant differences in the residual beta-cell function between the diabetic children classified according to the ICA status. Those children with both CF-ICA and IF-ICA detectable in their serum had a higher mean serum C-peptide concentration (Figure 1) and a higher daily urinary C-peptide excretion (Figure 2) than the other children.

The ICA-negative subjects were younger at the onset of diabetes and had a longer duration of the disease than the ICA-positive patients. These observations complicate the interpretation of the data on endogenous insulin secretion. From this and previous studies[20–22] we know that both a young age at onset and a long duration of diabetes are factors disfavoring the residual beta-cell function. However, after adjustment for these two parameters by an analysis of covariance, the children who were CF-ICA positive still had a significantly higher mean serum C-peptide concentration than the children with only IF-ICA. The adjusted serum C-peptide concentration in the children who were CF-ICA positive was 0.10 nmol/L. In adult IDDM patients, a plasma C-peptide concentration of 0.10 nmol/L or more has been suggested as reflecting a physiologically significant beta-cell function.[23] This limit may also be applied to diabetic children, although nondiabetic children younger than 6 yr have a lower plasma C-peptide concentration than older children.[17]

There were no significant differences in the urinary C-peptide excretion between the three groups after adjustment for the age at onset and the duration of diabetes. On the other hand, the 24-h urinary C-peptide excretion was based on a random sample collected at home, while the mean serum C-peptide concentration integrated the endogenous insulin secretion over 12 mo. Accordingly, the mean serum C-peptide concentration can be considered a more reliable indicator of the residual beta-cell function in this study especially as it has been shown that the collection of fractionated urine samples at home often remains incomplete.[24] Furthermore, the children who were CF-ICA positive received significantly lower doses of exogenous insulin than the children with only IF-ICA and those without ICA in their serum, without having a poorer metabolic control than the patients in the two other groups. This finding indicates that the observed differences in the mean serum C-peptide concentrations between the CF-ICA positive subjects and the other two groups are not only statistically but clinically significant.

Diabetic patients have in their serum cytotoxic antibodies suppressing the beta-cell function in vitro. The beta-cell inhibition is complement dependent, and it has been proposed that complement-fixing antibodies may contribute to the beta-cell destruction in diabetic patients.[10–12] Our data on an association between CF-ICA and residual beta-cell function in diabetic patients suggest that a certain degree of beta-cell persistence is linked to the occurrence of this particular type of cytoplasmic ICA. This finding is in a way paradoxical to the idea that CF-ICA should be indicators of a rapid beta-cell destruction. The reasons behind the disparity of our results with the findings in vitro are so far speculative. In order to study the causal relations between CF-ICA and beta-cell function we need a qualified prospective procedure with a reliable method for quantitative ICA assay.

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