Residual β -Cell Function and Islet Cell Antibodies in Diabetic Children

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Summary

There are no prospective studies evaluating residual β -cell function and islet cell antibody status during the first 2 years after the diagnosis of diabetes in children. We report such a prospective study of 21 insulin-dependent diabetic children. The relationship of residual β -cell function during the 2 years after diagnosis, to age at diagnosis, islet cell antibody (ICA) status, histocompatibility status, and exogenous insulin is reported.

Twenty-one children were studied for at least 2 years after the diagnosis of diabetes. Blood was obtained at the time of diagnosis for the determination of ICA and histocompatibility status. ICA status was determined 12 months after diagnosis, and again at 24 months if ICA had been detected at 12 months. Residual β -cell function was assessed serially by measuring 24-hr urinary C-peptide. The 24-hr C peptide excretion was expressed relative to 24-hr creatinine excretion. This ratio is termed urinary C peptide excretion (UCP). Islet cell antibodies were measured by indirect immunofluorescence on human O-group pancreas. Fluorescein-labeled anti-C₃c was used to determine whether the islet cell antibodies were able to fix complement. A standard NIH micro-lymphocytotoxicity test was used to determine histocompatibility status at the A, B, and C loci.

Throughout the entire study period, the UCP of each diabetic was always below the mean of the group of nondiabetic children. For all diabetic children, the UCP decreased during the 24 months after diagnosis. Most children's (14 of 21) UCP had fallen below 0.20 during the 12 months after diagnosis. Twelve of 21 diabetic children were ICA positive at diagnosis. Five had unaltered titers of ICA 12 months later and four had no ICA detectable at 12 months, whereas for three the titer had fallen by at least two dilutions. The magnitude of the UCP 12 months after diagnosis correlated significantly with age at the time of diagnosis ($r = 0.557$; $P < 0.05$). Similarly, there was also a significant correlation between the UCP 24 months after diagnosis and age at diagnosis ($r = 0.446$; $P < 0.05$). There was no significant difference between insulin dose, expressed as units/kg/day at 12 and 24 months after diagnosis. There was no significant relationship between ICA status and insulin dose at 12 or 24 months. There was no discernible relationship between the evolution of ICA or UCP with histocompatibility antigens at the A, B, or C loci.

Speculation

The positive correlation between urinary C peptide and age at diagnosis of diabetes suggests that pancreatic size and potential for subsequent growth might be factors influencing the impact of a diabetogenic insult. For insulin-dependent children, the impact of the diabetogenic process is usually greatest during the 12 months after diagnosis. Potential therapeutic efforts to limit islet cell damage need to be applied early, and it is possible they may not need to be continued much beyond 12 months after diagnosis.

Assay of immunoreactive C peptide may enable residual β -cell

function to be quantitated in diabetics receiving exogenous insulin (3, 6, 7, 9). Fasting serum concentrations may be within the normal nondiabetic range (5) at the time of diagnosis of diabetes (4, 12), but decline subsequently (12, 13). Antibodies directed against pancreatic islet cells (ICA) have been detected in the serum of more than one-half of newly diagnosed insulin-dependent diabetics (8, 11). To our knowledge, there are no prospective studies evaluating residual β -cell function and ICA status during the first 2 years after the diagnosis of diabetes in children. We report such a prospective study of 21-insulin-dependent diabetic children. The relationship of residual β -cell function during the 2 years after diagnosis, to age at diagnosis, ICA status, histocompatibility status, and exogenous insulin administered is reported. Such a study provides a background for the evaluation of attempts to conserve the capacity for endogenous insulin production.

PATIENTS, PROCEDURES AND METHODS

PATIENTS

All newly diagnosed diabetic children, aged less than 14 years, admitted to Princess Mary Hospital, Auckland, New Zealand, during the period of March 1, 1976 to February 28, 1978, were eligible for inclusion in this study. Twenty-five such children were admitted to hospital during the study period. Twenty-one of these 25 children were studied for at least 2 years after the diagnosis of diabetes (Table 1). Two children were enrolled in another study which prevented them from participating in this study, and the other two children did not wish to participate in the study. Eighteen of these children attended a Children's Diabetic Clinic and were seen regularly by the same two physicians. The other three children were seen by a third pediatrician. Glycosuria was assessed three to four times daily, either with Clinitest tablets or Ketodiasix. All received insulin, usually administered as a single daily injection of a purified preparation, with individual variations as dictated by their symptoms, serial urinalyses, and growth, but not the results of this ongoing study. The dietary advice prescribed regular meals, small snacks between meals, and allowed a "free" choice of foods with restriction only on those supplying excessive amounts of concentrated and refined carbohydrate. Informed consent was obtained from the parents of all children participating in this study.

PROCEDURES

Blood was obtained at the time of diagnosis for the determination of ICA and histocompatibility status. ICA status was determined 12 months after diagnosis, and again at 24 months if ICA had been detected at 12 months. After discharge from hospital, serial 24-hr urine specimens were obtained during the ensuing 24 months. The urinary C peptide excretion (UCP) was measured in these specimens. Because the 24-hr urine specimen was collected at home, it was assumed that the children maintained their usual insulin regime, diet, and level of activity during each collection

Table 1. *Details of study patients*

Patient	Age	Sex	HLA status ¹	ICA status ²			Urinary C peptide				Insulin dose (units/kg/day)	
				0	12	24	6 months	12 months	18 months	24 months	12 months	24 months
B. T.	7.5	M		1:4	1:4	1:4	0.76	0.16	0.06	ND ³	0.30	0.55
R. P.	9.0	M	A2, 3; BW35, 7; CW6W4, -;	1:2	1:2	1:2	0.33	0.20	(0.14) ⁴	0.08	0.54	0.60
J. K.	10.5	F	A2, 3; BW35, 15; W6	1:2	1:4	1:4	0.60	NK	0.16	0.21	0.59	0.84
D. M.	11.25	M	A1, 3; B7, 8; W6	1:16	1:16	1:8	0.99	0.77	0.52	0.51	0.89	0.94
S. G.	11.5	M	A1, -; B5, 8; C(W4), -; W4, W6	1:2	1:2	0	0.85	0.13	(0.09)	0.14	0.62	1.53
C. A.	3.1	M		1:8	1:2	NA	0.55	0.08	NK	NK	0.35	NA
S. E.	3.5	F	A3, W24; BW35, 8; C(W4), -; W6	1:16	1:2	0	0.40	0.04	<0.01	<0.01	0.64	0.77
D. H.	4.75	M	A3, -; B7, -; W6	1:2	0	ND	0.34	0.12	NK	NK	0.83	NA
C. D.	5.2	M	A(W30/31), (W24); B7, W16; W6	1:2	0	ND	0.16	NK	(0.08)	NK	1.09	1.09
H. S.	5.75	F	A2, 3; B37, -; W4, W6	1:8	1:2	0	(0.51)	0.11	ND	ND	0.80	0.82
R. P.	8.25	M	A(28), 9; B7, -; W6	1:2	0	ND	0.11	0.04	0.01	<0.01	1.02	1.10
J. R.	13.2	M	A2, (3); B8, 12; C(W5), -; W4, W6	1:2	0	ND	(0.45)	0.04	ND	ND	0.34	0.72
I. M.	1.1	M		0	0	ND	NK	0.04	ND	ND	0.63	NA
D. F.	5.2	M	A2, 9; B5, (W35); W4 W6	0	0	ND	0.18	0.03	(0.02)	<0.01	1.19	0.96
E. R.	5.25	F	A1, 28; BW35, 8; C(W2), 1; W6	0	0	ND	1.03	(0.34)	(0.11)	NK	0.35	0.51
C. T.	9.1	M	A1, 2; B7, 8; W6	0	0	ND	(0.82)	0.42	0.70	0.50	0.24	0
K. T.	9.4	M	A2, 9; BW22, (27); (W4), W6	0	0	ND	NK	0.07	ND	ND	0.33	0.92
B. G.	10.7	M		0	0	ND	NK	0.08	ND	ND	0.48	NA
B. McD.	11.5	F	A1, 11; B8, 40; C(W3), -; W6	0	0	ND	(0.86)	0.38	0.38	0.35	0.33	0.30
S. H.	12.4	M	A3, W24; BW22, (37); W4, W6	0	0	ND	(0.88)	0.76	0.52	0.47	0.80	0.67
G. S.	12.7	M	A1, 9; B40, (W21); C(W4), -; W4, W6	0	0	ND	(0.66)	0.16	NK	0.12	0.73	1.1

¹ HLA status, histocompatibility status.² ICA status, islet cell antibody status.³ ND, not done (see text); NK, not known; NA, patient not available for sampling.⁴ Numbers in parentheses, values obtained close to the defined time of sampling.

period. The quantity of carbohydrate ingested during the collection period was not documented. Blood glucose was not measured within the 24-hr period, nor was 24-hr urinary glucose excretion routinely measured.

Our previous experience with serial measurements of UCP had demonstrated that once UCP fell to a very low level (0.10 to 0.15) it never recovered. Therefore, in this study once the defined UCP was below this low level, further specimens were no longer obligatory.

MATERIALS AND METHODS

Residual β -cell function was assessed serially by measuring 24-hr urinary C peptide, using a commercially available kit (Novo Research Institut, Denmark, antibody M1230). Samples were kept at 5°C during collection, and aliquots with 0.1% bovine serum albumin were stored at -20°C until assay. The 24-hr C peptide excretion (nmoles) was expressed relative to 24-hr creatinine excretion (mmoles) because of differences in size among the children. This ratio is termed UCP. The detection limit of the C peptide assay was 0.01 nmoles/liter. Inasmuch as urinary creatinine concentrations were >2 mM the lower limit of detection for the ratio UCP was 0.005. The UCP was measured in two groups of healthy nondiabetics: twenty one children, aged 5 to 16 years, and 12 young adults aged 18 to 37 years. The mean UCP for the 21 nondiabetic children was 1.47 nmoles C peptide per mmole creatinine (S.D., 0.43; range, 1.0 to 2.3). For the 12 nondiabetic young adults, the mean UCP was similar, 1.39 (S.D., 0.53; range, 1.0 to 2.5). The mean absolute 24-hr excretion for nondiabetic children was 7.0 nmoles C peptide (S.D., 2.8; range, 3.1 to 12.6), and for the nondiabetic adults, it was 21.4 (S.D., 7.3; range, 11.1 to 33.3). The UCP correlated significantly with the absolute 24-hr C peptide excretion per M^2 ($r = 0.732$; $n = 38$; $P < 0.001$).

Islet cell antibodies were measured by indirect immunofluorescence on human O-group pancreas (10). Sera were screened at a dilution of 1:2. Positive sera were titered in doubling dilutions. Fluorescein-labeled anti-C₃c was used to determine whether the islet cell antibodies were able to fix complement (1). The undiluted test serum was applied to the tissue for 20 min and then washed with phosphate-buffered saline. Undiluted normal human serum, a source of complement, was then applied for 30 min and then washed off, and the fluorescent conjugate was added for 20 min.

A standard NIH microlymphocytotoxicity test was used to determine histocompatibility status at the A, B, and C loci. For the purpose of data analysis, the 21 diabetic children were subdivided into the following groups according to ICA status:

- Group A. Stable titer: detectable ICA at the time of diagnosis, with an unchanged titer 12 months later.
- Group B. Falling titer: detectable ICA at the time of diagnosis and either no ICA at 12 months or a 2-fold or greater fall in antibody titer.
- Group C. ICA-negative: no ICA detectable at the time of diagnosis or any time subsequently.

Student's t test and linear regression analysis were used for the statistical analyses. The 0.05 level of probability was used as the criterion of significance.

RESULTS

Throughout the entire study period, the UCP of each diabetic child was always below the mean of the group of nondiabetic children (Table 1). For all diabetic children, the UCP decreased during the 24 months after diagnosis. The number of children with a UCP <0.20 (more than three standard deviations below the mean for the group of nondiabetic children) increased from the time of diagnosis (Table 1). Most children's (14 of 21) UCP had fallen below 0.20 during the 12 months after diagnosis. All children in the falling titer group (group B) had a UCP less than 0.2 by 12 months after diagnosis. The relationship between antibody status and UCP during the 24 months after diagnosis is

demonstrated in Figure 1. The UCP for the falling antibody group (group B) was significantly less ($P < 0.05$) than that for the other two groups only at 6 months after diagnosis. There were no other significant differences between these three groups.

Twelve of the 21 diabetic children were ICA positive at diagnosis. Five had unaltered titers of ICA 12 months later, four had no ICA detectable at 12 months, while for three the titer had fallen by at least two dilutions (Table 1). Ten of 12 sera that were ICA positive at diagnosis were positive with fluorescein-labeled anti-C₃c. The other two sera were weakly positive. All the ICA's that were positive at 12 months with titers greater than 1:2 also fixed complement. It was not possible to be certain whether the sera from children with weakly positive antibody titers fixed complement.

The magnitude of the UCP 12 months after diagnosis correlated significantly with age at the time of diagnosis. ($r = 0.557$; $P < 0.05$) (Fig. 2). Similarly, there was also a significant correlation between the UCP 24 months after diagnosis and age at diagnosis ($r = 0.446$; $P < 0.05$).

There was no significant difference between insulin dose, expressed as units/kg/day at 12 and 24 months after diagnosis. There was no significant relationship between ICA status and insulin dose at 12 or 24 months. There was no discernible rela-

Fig. 1. Relationship between ICA status and UCP during the 24 months after diagnosis. Group mean values $\pm$ S.E. for ICA groups A, B, and C 6, 12, 18, and 24 months after diagnosis. Three ICA subgroups: Δ , group A; $\bullet$, group B; $\circ$, group C.

Fig. 2. Relationship between age at diagnosis and UCP 12 months after diagnosis. Three ICA subgroups: Δ , group A; $\bullet$, group B; $\circ$, group C.

tionship between the evolution of ICA or UCP with histocompatibility antigens at the A, B, or C loci.

DISCUSSION

In our experience, 24-hr UCP is a more convenient way of assessing residual β -cell function in diabetic children than is fasting serum C peptide. UCP correlates significantly with fasting serum C peptide (9). This previous observation has been confirmed in our own laboratory (2). Children dislike and resist frequent blood sampling, and fasting blood specimens are inconvenient to obtain from non-hospitalized children. One of the problems inherent in the serum C peptide assay, the presence of a cross-reacting antibody-bound proinsulin (5), does not apply to urinary C peptide, and extraction before assay is unnecessary. UCP provides a cumulative estimation of residual β -cell function throughout an entire 24-hr period. Its validity as a measure of residual β -cell function has been established (7, 9). Although the degree of control of hyperglycemia might vary in the individual or from one to another, the clinical criteria of control were uniform. This, together with the frequency of observation, would make differences in groups unlikely to be due to this factor.

The frequency of positive ICA at diagnosis in our group of patients was similar to that previously reported (8, 11). Titers varied from 1:2 to 1:16, with a median of 1:4 for both the persisting and falling ICA subgroups. Most of the ICA had the ability to fix complement.

The hypothesis that the ICA reflect an active immunologic process and further destruction of β -cells leads to the expectation of a strong correlation between ICA status and residual β -cell function at 12 and 24 months after diagnosis. The lack of such a correlation for our data does not support this hypothesis. Our data suggest that the antibodies themselves are not destroying the β -cells. An explanation for the lower UCP in the falling ICA subgroups at 6 months after diagnosis may be that the severity and/or duration of the asymptomatic diabetogenic process differed from that of the persisting ICA subgroup. The ICA-negative subgroup may contain three populations: children in whom the pancreatic destructive process ceased with or without retention of significant UCP and children whose diabetes has a different etiology.

Although at diagnosis children of differing ages may have a similar mean UCP, the absolute amount of C peptide produced per day, being related to body size, may differ widely. For example, a 12-month infant with a UCP within the normal range near diagnosis (1.37) excreted a 24-hr total of 1.8 nmoles C peptide. A 12-year-old child with a UCP of 0.91 excreted 7.0 nmoles C peptide in 24 hr. Were the absolute production of C peptide to remain constant in both the children, the smaller child would be more disadvantaged as an adult. The group of 12 nondiabetic adults had a mean 24-hr excretion of 23.4 nmoles C peptide (range, 11.1 to 33.3).

Within the confines of this study, no recovery of pancreatic β -

cell function was observed. The mass of β -islets is less at a younger age. Therefore, the same diabetogenic insult would have a greater impact in a younger child. This might explain the relationship observed between age at the time of diagnosis of diabetes and subsequent residual β -cell function in our study. The UCP is a convenient and useful index of residual β -cell function, but the absolute C peptide excretion provides more useful information for the prediction of the consequences of reduced β -cell function. Extrapolation of the age dependence of degree of residual cell function suggests that in some older children islet cell damage may be sufficiently incomplete that clinical diabetes may not occur, at least in childhood. The onset of clinical disease may be delayed until pregnancy, obesity, or some other stress eventuates.

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