Originals

Increased reduction in fasting C-peptide is associated with islet cell antibodies in Type 1 (insulin-dependent) diabetic patients

B. Marner¹, T. Agner², C. Binder², Å. Lernmark¹, J. Nerup², T. Mandrup-Poulsen² and S. Walldorff³

¹Hagedorn Research Laboratory and ²Steno Memorial Hospital, Gentofte

³Department of Pharmacology, University of Copenhagen, Copenhagen, Denmark

Summary. A cohort of 82 patients with Type 1 (insulin-dependent) diabetes was followed prospectively for 24 months, and 54 of them for 30 months, to study the relationship between fasting levels of immunoreactive C-peptide and titres of islet cell antibodies. After diagnosis, fasting C-peptide rose temporarily for 1–6 months of insulin therapy and declined continuously thereafter. While islet cell antibodies were present among 55% of the newly diagnosed patients, only 31% remained positive at 30 months. Their antibody titres decreased from 1:81 at diagnosis to 1:3. Only 3 patients (4%) who were islet cell antibody negative at diagnosis became positive later. The median C-peptide values among the persistently islet cell antibody positive patients decreased from 0.11 pmol/ml at 18 months, to 0.09 pmol/ml at 24 months, to 0.06 pmol/ml at 30 months compared to 0.18 ($p=0.04$), 0.15 ($p=0.05$) and 0.16

($p<0.003$) pmol/ml, respectively, for the islet cell antibody negative patients. The median slope for the latter was -0.09 compared to -0.19 for the islet cell antibody positive patients ($p=0.01$). These differences were reflected in increasing dosages of insulin, since patients remaining antibody-positive for 30 months were given 1.3–1.4 times more insulin ($p=0.01$ – 0.004) than the antibody negative patients. This study demonstrates that islet cell antibodies may be a useful marker for predicting an increased rate by which endogenous B cell function is lost in Type 1 diabetes.

Key words: Type 1 (insulin-dependent) diabetes, islet cell antibodies, fasting C-peptide, insulin dosage, prospective analysis, fasting blood sugar.

Type 1 (insulin-dependent) diabetes is associated with a disappearance of pancreatic B cells from the endocrine pancreas. In patients who have had Type 1 diabetes for several years the reduction of B cells is nearly complete [1–3]; this confirms early observations [4] of negligible levels of pancreatic insulin in long-term Type 1 diabetic patients. Newly diagnosed patients have shown levels of C-peptide, both at fasting and following stimulation [5, 6], which are either below or at the level of detection when at poor metabolic control [6, 7] while many well-regulated patients have fasting C-peptide well within the normal range [8, 9]. Although a cross-sectional analysis of the progression of Type 1 diabetes shows a decrease in residual B cell function [6], it is not clear to what extent this is true for the individual patient studied prospectively.

Several studies indicate that high residual C-peptide is correlated to a better metabolic control and lower requirements for exogenous insulin [8–10]. Since lability seems to occur in the absence of endogenous C-peptide [11], it would be important to be able to predict loss of B cell function since diminished metabolic control may

increase the risk for the development of serious morbidity [12].

Anti-islet autoimmune reactivity, usually found at clinical onset [13, 16], is also discernible in subjects who later were found to develop Type 1 diabetes [17–19]. In fact, islet cell antibodies and a concomitant decrease in B cell function may be observed as long as 3–8 years before the clinical diagnosis [18]. Previous analyses [15, 16, 20, 21] of islet cell antibodies in relation to the duration of Type 1 diabetes and residual B cell function have shown conflicting results. In the present study, we followed 82 Type 1 diabetic patients prospectively from diagnosis through 30 months of insulin therapy to determine islet cell antibody titres in relation to fasting C-peptide.

Subjects and methods

Subjects

Type 1 (insulin-dependent) diabetic patients ($n=85$, 30 females, 55 males) admitted to the Steno Memorial Hospital were studied in a prospective manner from the time of diagnosis and during 30 months

Fig. 1. Age variation among 85 Type 1 (insulin-dependent) diabetic patients studied prospectively for 30 months. The dotted bars represent age variation in the incidence of Type 1 (insulin-dependent) diabetes in Denmark [22]

of insulin therapy. The first patient was admitted in July 1979, and the last patient in November 1981. The patients were diagnosed as Type 1 (insulin-dependent) diabetic patients based on the following criteria: ketonuria, fasting blood glucose above 14 mmol/l, underweight or normal body weight and insulin considered to be ultimately required. Patients were not included in the study if their disease had been diagnosed more than 4 weeks prior to admission. The age distribution of the patients at the time of admittance (Fig. 1) was compatible with that previously reported for Denmark [22].

All patients were treated intensively with highly purified porcine insulin. Except for the visit at 30 months, they attended the outpatient clinic after an overnight fast, postponing their morning insulin dose. At the visits to the outpatient clinic after about 1, 3, 6, 9, 12, 18, 24 and 30 months of insulin therapy, blood was collected for the determination of blood glucose, plasma C-peptide (CPR), and serum islet cell cytoplasmic antibodies (ICA). The results of fasting CPR and ICA were never disclosed to the physicians. The patients were seen by no more than six different physicians and no less than two physicians. The attending physician had authority, together with the patient, to adjust the insulin dosage and composition of diet according to available information on glucose metabolism, weight changes and information from the patient.

At the 30-month followup, fasting blood samples were only available from 57 patients. At all other visits there were only a few drop-outs as indicated in the Results section.

Islet cell cytoplasmic antibodies (ICA)

ICA was determined by indirect immunofluorescence [23]. Briefly, serum samples were applied onto 2–3 µm cryostat sections of human blood group 0 pancreas (specimens H84 and H75) and incubated at room temperature for 30 min. The sections were washed three times and a 1/10 dilution of rabbit (fluorescein isothiocyanate-conjugated) anti-human IgG serum (Dakopatts, Copenhagen, Denmark) added. Following a 30-min incubation and careful washing, the samples were read by two independent observers out of five in a epi-fluorescence microscope.

All nine followup samples from each of 85 patients (i.e. more than 700 individual serum samples) were

scored blindly without information of the identity of the sample or the serum dilution to any of the observers. Only sera which gave a fluorescence reaction in the majority of islet cells were scored as positive. All samples were tested twice. A third assay was carried out if a sample was scored differently in the two different assays. All ICA positive samples were diluted (1:3, 1:9, 1:27, 1:81, etc.) to determine the end-point titre. Positive and negative quality control samples at four different dilutions, unknown to the observers, were included at random in each assay. The specificity [23] of the assay using sections from two different pancreas H75 and H84 respectively was 99%, and the sensitivity [23] 97% and 98% respectively. The interobserver variation was 10% for the five different observers scoring the samples.

Assay for C-peptide (CPR)

Fasting plasma CPR was determined by radioimmunoassay using antibody M1230 [7]. The normal range (mean $\pm$ 2 $\times$ SD) in this assay is 0.18–0.60 pmol/ml; the lower detection limit is 0.06 pmol/ml. Since it has been shown that CPR is degraded during prolonged storage, all samples collected during one month were analyzed monthly against a set of quality control samples included in every assay. The coefficient of variation between assays was 8%.

Statistical evaluation

All results are expressed as median and range since the data showed non-parametric distributions. Non-parametric statistics (Mann-Whitney U-test) were used; 5% was chosen as the level of statistical significance.

Results

Islet cell cytoplasmic antibodies

The prevalence of ICA among the 85 Type 1 diabetic patients was 55% (47/85) at the time of diagnosis; prevalence decreased to 31% (26/84) during 24–30 months of insulin therapy. There was one drop-out at 30 months. Only three patients, 9, 15 and 16 years of age, were excluded from the further analysis, since they were ICA negative at diagnosis but were found positive in three or more samples during the followup. The prevalence among the remaining 82 patients is shown in Figure 2. The higher prevalence of ICA among the female patients was not statistically different from the males. The median ICA titre among the 47 patients ICA positive at the time of diagnosis was 1:27 and decreased to undiluted serum by 30 months when only 50% ($n=24$) of these patients remained ICA positive (Fig. 3). At the time of diagnosis the ICA titres were distributed between undiluted (1:1) and 1:729, while titres above 1:27 were uncommon after 24–30 months of Type 1 diabetes (Table 1).

The median ICA titres of the 24 patients remaining ICA positive at 30 months decreased from 1:81 at diagnosis to 1:3 at 30 months ($p<0.001$) (Fig. 3). In the group of 23 patients with ICA at diagnosis but negative at 30 months 11/23, 6/22 and 1/23 remained positive at 12, 18 and 24 months of followup respectively. The median ICA titre did not differ with age (not shown). In addition, neither age nor sex influenced the rate by which the ICA titres decreased during followup.

Fig. 2. Prevalence of islet cell cytoplasmic antibodies in relation to the duration of Type 1 (insulin-dependent) diabetes. The number of patients, out of a total of 82 at diagnosis, was 72 at 1 month, 74 at 3, 80 at 6, 74 at 9, 80 at 12, 80 at 18, 82 at 24 and 81 at 30 months. All patients (●), females (▼) and males (△) are shown

Table 1. Prevalence (%) of islet cell antibodies in 82 Type 1 (insulin-dependent) diabetic patients followed prospectively for 30 months

ICA titre	Time (months)								
	0	1	3	6	9	12	18	24	30
1:1	<u>15</u>	<u>10</u>	<u>5</u>	<u>8</u>	<u>12</u>	<u>3</u>	<u>13</u>	<u>6</u>	<u>1</u>
1:3	<u>4</u>	<u>10</u>	<u>10</u>	<u>8</u>	<u>7</u>	<u>12</u>	<u>5</u>	<u>7</u>	<u>17</u>
1:9	<u>9</u>	<u>4</u>	<u>10</u>	<u>6</u>	<u>14</u>	<u>11</u>	<u>11</u>	<u>9</u>	<u>5</u>
1:27	<u>15</u>	<u>17</u>	<u>10</u>	<u>15</u>	<u>5</u>	<u>9</u>	<u>4</u>	<u>4</u>	<u>4</u>
1:81	<u>9</u>	<u>10</u>	<u>15</u>	<u>8</u>	<u>8</u>	<u>5</u>	<u>4</u>	<u>4</u>	<u>1</u>
1:243	<u>5</u>	<u>3</u>	<u>0</u>	<u>1</u>	<u>3</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>1</u>
1:729	<u>2</u>	<u>0</u>	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>

Prevalence numbers equal to or above 10% have been underlined to indicate the trend in islet cell antibody titres

Fasting C-peptide (CPR) and ICA

At the time of diagnosis, the median fasting CPR in all 82 patients amounted to 0.16 pmol/ml (range <0.06–0.45 pmol/ml) (Fig. 4). Fasting CPR increased after one month of insulin therapy to reach maximal levels at 3 months, but declined steadily thereafter. Compared to fasting CPR at 3 months (median 0.23, range 0.06–0.54 pmol/ml), fasting CPR decreased to 0.13 (range 0.06–0.49) by 24 months ($p=0.001$), and to 0.10 (range <0.06–0.44) by 30 months ($p=0.001$) (Fig. 4). The median fasting CPR in the 54 patients followed for the entire 30 months was 0.20 (range <0.6–0.42) pmol/ml at 3 months, 0.20 (<0.06–0.62) at 6, 0.21 at 9 (<0.6–0.50), 0.18 (<0.06–0.57) at 12, 0.18 (<0.6–0.46) at 18, and 0.12 (<0.05–0.49) at 24 months. These patients did not differ from the 27 from whom only post-breakfast CPR was obtained at 30 months. At 30 months the fasting CPR in the 16 patients remaining ICA positive; after an overnight fast, was 0.06 pmol/ml (range <0.06–0.17), which was lower ($p=0.01$) than the 38 ICA patients being ICA negative at 30 months (median 0.13, range <0.06–0.44). The three patients, ICA negative at onset but becoming positive during the study, had maxi-

Fig. 3. Titres of islet cell cytoplasmic antibodies (ICA) in relation to the duration of Type 1 (insulin-dependent) diabetes. Median titres are shown for all 47 patients ICA-positive at diagnosis (●), with 23 patients being ICA-negative at 30 months (□) and 24 patients being ICA-positive at 30 months (■)

Fig. 4. Fasting C-peptide (●) and insulin dose (○) in relation to the duration of Type 1 (insulin-dependent) diabetes. Median values for 81 patients at diagnosis, 72 at 1 months, 74 at 3 months, 80 at 6, 74 at 9, 81 at 12, 80 at 18, 82 at 24 and 54 at 30 months. The dotted line shows the detection limit of the C-peptide radioimmunoassay

mal CPR values of 0.14–0.26 pmol/ml at 1–9 months decreasing to 0.06 pmol/ml in all three by 24 and 30 months.

It was tested whether the patients remaining ICA positive ($n=24$) after 30 months, i.e. the patients with high ICA titres at diagnosis (Fig. 3), had levels of fasting CPR different from the patients ($n=35$) being ICA negative throughout the study (Fig. 5). This analysis demonstrated that these two groups of patients had similar levels of fasting CPR from the time of diagnosis and throughout 12 months of insulin therapy. However, at 18, 24 and 30 months of followup the pat-

Fig. 5. Fasting C-peptide in relation to the duration of Type 1 (insulin-dependent) diabetes in 35 patients being islet cell cytoplasmic antibody (ICA) negative at diagnosis and throughout the study ($\square$), and in 24 patients ICA positive at diagnosis and throughout the study ($\blacksquare$). Median values are shown. Statistical differences between the two groups were $*p=0.04$, $**p=0.05$, and $***p=0.003$. The shaded area shows the lower part of the normal range of fasting C-peptide

Fig. 6. Insulin dose in relation to the duration of Type 1 (insulin-dependent) diabetes in 35 patients being islet cell cytoplasmic antibody (ICA) negative at diagnosis and throughout the study ($\blacksquare$) compared to 24 patients being ICA positive at diagnosis and throughout ($\square$). Median values are shown along with differences between the two groups: $*p=0.01$, $**p=0.007$, $***p=0.004$

ients remaining ICA positive had significantly lower levels of fasting CPR (Fig. 5). The effect of being persistently ICA positive, i.e. a tendency of having a high titre of ICA at the time of diagnosis, was discernible already after 18 ($p=0.04$) and 24 months ($p=0.05$) of insulin therapy but was particularly pronounced at 30 months ($p=0.003$). At

Table 2. Islet cell antibody titre at the time of diagnosis and the subsequent fasting C-peptide after 30 months of Type 1 diabetes

Measurements at 30 months	Islet cell antibody titre at diagnosis		
	Negative	1:1 or 1:3	$\geq 1:9$
Group A ($n=54$)	22	10	22
Fasting C-peptide (pmol/ml)			
Median	0.16	0.08*	0.08**
Range	<0.06– 0.44	<0.06– 0.20	<0.06– 0.25
Fasting blood glucose (mmol/l)			
Median	11.2	12.5	9.4
Range	4.8 –15.1	7.6 –17.1	4.2 –22.9
Group B ($n=27$)	12	5	10
Non-fasting C-peptide (pmol/ml)			
Median	0.09	0.15	0.17
Range	<0.06– 0.49	<0.06– 0.49	<0.06– 0.62
Non-fasting blood glucose (mmol/l)			
Median	12.1	12.9	9.7
Range	4.5 –18.0	6.2 –16.7	3.6 –16.9

Difference from islet cell antibody negative patients:

$*p=0.04$; $**p=0.006$

this time post-breakfast CPR was obtained for 27 individuals, 8 of whom had ICA and 0.13 (<0.06–0.46) pmol/ml CPR (median, range), while the 12 patients being ICA negative from the time of diagnosis had 0.19 (<0.06–0.49) pmol/ml CPR ($p=0.14$). Comparing the 30-month fasting CPR of the persistently ICA negative patients with the ICA positive patients showed that the presence of ICA at the time of diagnosis was associated with a lower fasting CPR 30 months later (Table 2). The median slope of declining fasting CPR, calculated from the population of slopes of individual patients from the highest CPR value occurring during the first 1–9 months until the end of the study, was -0.09 among the ICA negative and -0.19 among the ICA positive patients ($p=0.01$).

Insulin dose and ICA

The median dose of insulin in the 82 patients was 0.35 IU/kg body weight at the 1-month visit to the outpatient clinic (Fig. 4). After a short nadir the median insulin dosage increased steadily, and by 30 months it was 1.6-fold larger than the lowest dose at 3 months. The continuous decrease in fasting CPR corresponded to an escalating dose level of insulin (Fig. 4). An analysis of the dose of insulin in the patients being either ICA negative or positive throughout the study showed that the latter were taking more insulin (Fig. 6). During the last year of followup the ICA positive patients were, in fact, found to take 1.3–1.4 times more insulin than the ICA negative patients. It was documented that blood glucose did not differ between the two groups of patients whether fasting or post-breakfast values were obtained at 30 months (Table 2).

Discussion

Although it was not felt reasonable to include the youngest children in this study due to the protocol requirement of frequent blood samplings and fasting before

the clinical visit, our group of patients are reasonably representative of the overall expected age-related distribution [22]. To our knowledge this is therefore the first rigorous prospective analysis of Type 1 diabetic patients at all ages to determine the natural history of endogenous pancreatic B cell function during the first years of insulin therapy and to test the hypothesis that ICA may affect fasting CPR levels.

The present observation of peak fasting CPR concentrations at 1–3 months following initiation of insulin therapy support earlier cross-sectional observations [6] which also demonstrate that levels of CPR below detection limits during episodes of ketoacidosis may be regained by strict metabolic control [24]. However, following the initial months of complete or partial remission reflected in levels of fasting CPR well within the normal range and decreased insulin dose (Fig. 4), fasting CPR decreased continuously. Only four patients temporarily discontinued insulin therapy during the first year. The decline in fasting CPR is reminiscent of a previous cross-sectional analysis of 381 Type 1 patients of different duration which demonstrates that only 10–20 percent of patients with Type 1 diabetes for more than 7–10 years have signs of residual B cell function [25]. It is, however, much debated whether residual B cell function correlates with improved metabolic control [8–11].

A large number of investigators have determined the presence of ICA in Type 1 diabetes and demonstrated that the prevalence is highest at the time of onset and decreases thereafter [15, 16, 20]. The present prevalence (55%) of ICA at the time of diagnosis is well within the range of previous investigations. The evanescent character of ICA has not been explained and changes in ICA titres in relation to duration of Type 1 diabetes have to our knowledge not previously been determined in a systematic fashion. Lendrum et al. [15] followed 75 Type 1 diabetic patients, in two consecutive samples only, from about 10 weeks until 3 years of insulin treatment. The ICA titre, the highest being 1:256 in 2 patients, decreased by one or more dilutions in 29 patients (39%), did not change in 10 (13%), increased by one dilution in 4 (5%) and became negative in 7 (9%). The remaining ICA negative patients remained negative at the second test [15]. In contrast to this limited study, the present investigation demonstrates a relationship between the ICA titre and duration of disease, although the relationship becomes discernible first after about one year of insulin therapy. We conclude that patients persistently ICA positive for 30 months remained positive primarily because they had a higher ICA titre at the time of diagnosis. The present data also suggest that these patients had an increased rate of diminution of fasting CPR compared to the ICA negative patients. The presence of a high ICA titre at diagnosis or within the first three months of insulin therapy may therefore be a useful predictor of a more rapid loss of endogenous B cell function. This is important since the progressive decline in fasting CPR was associated with an increased dosage

of insulin. It is emphasized that the study was blind and that adjustments of insulin dosages and dietary regimens were made by physicians who did not know the results of the CPR and ICA analyses. Future studies should include measurements of HbA_{1c}, which was not available in the present investigation, as an expression of glycaemic control.

There are several observations supporting the view that autoimmune phenomena are involved in the pathogenesis of Type 1 (insulin-dependent) diabetes [26, 27]. The mechanism of the specific eradication of the pancreatic B cells are not known and nearly all possible immune effector systems such as cytotoxic T-cells [28, 29], antibody-dependent cellular cytotoxicity [30] or complement mediated cytotoxicity [31–34] have been proposed. The fact that the endogenous fasting CPR shows a decreasing trend in all our Type 1 diabetic patients whether or not they have ICA suggests that ICA per se may not be directly involved in the B cell destruction. Rather, it is possible that the primary immune destructive process results in an outflow of pancreatic islet cell antigens to promote the production of ICA with different antigenic specificities. This view is supported by the observation that remission in Type 1 diabetic patients treated with cyclosporin A is not associated by the presence or absence of ICA [35]. The high titre of ICA in some patients may therefore reflect a more aggressive immune destruction. It is also possible that levels of fasting CPR is controlled by the major histocompatibility complex since HLA-DR4 Type 1 patients showed better preservation of B cells [36]. Studies are also in progress to further analyze the serum from our 85 diabetic patients for islet cell surface [37] and complement-dependent cytotoxic antibodies [34], as well as complement-fixing ICA [38], to test whether these autoantibodies correlate to the progression of B cell loss and the HLA-type of the patients.

We conclude that the levels of ICA during the first 30 months of Type 1 diabetes is associated with the progression of loss of endogenous insulin production and increase of dosage of insulin.

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Dr. Åke Lernmark
Hagedorn Research Laboratory
Niels Steensensvej 6
DK-2820 Gentofte
Denmark