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Morphometric evidence for a striking B-cell reduction at the clinical onset of type 1 diabetes*, **

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Summary. The distribution and volume of the pancreatic endocrine cells were studied in a case of type 1 diabetes with a duration of approximately 7 days. Immunocytochemical techniques combined with morphometry were used. The PP-cell rich lobe, making up about 10% of the total pancreatic volume, was not included in this study. The volume density and the absolute volume of the B-cells was found to be reduced to about one third to one seventh of the values determined in four controls of similar age and/or pancreatic volume. The A-cell volume was also diminished whereas the D- and PP-cell volume remained constant. As B-cell necroses could not be detected and insulitis was in the initial stages of development it is concluded that the destruction of B-cells proceeds slowly in type 1 diabetes. In the majority of cases it probably starts years before the clinical onset of the disease.

Key words: Type 1 diabetes of recent onset – Endocrine pancreas – Immunocytochemistry – Morphometry – Time course of B-cell reduction

Total (or virtually total) and permanent loss of B-cells characterizes islet histopathology in long-lasting type 1 diabetes (Gepts 1965). Currently this phenomenon is best explained by an autoimmune process specifically directed against B-cells and possibly triggered by a virus infection (Cudworth 1978).

The view that viruses might play a role in initiating B-cell destruction seems to be supported by some recent case reports demonstrating generalized Coxsackie B virus infection in young patients with acute failure of

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insulin secretion and ketoacidotic coma (Templeton et al. 1977; Yoon et al. 1979; Champsaur et al. 1980; Ahmad and Abraham 1982).

However, other studies showed that the onset of the disease process may not be as abrupt as the onset of clinical symptoms suggest. Thus it was observed that the occurrence of islet cell antibodies may precede the clinical manifestation of type 1 diabetes by months or years (Gorsuch et al. 1981 and 1982). Further, the number of B-cells appears to be severely decreased in juvenile diabetics dying in the acute phase of the disease (Gepts and DeMey 1978).

Using the combined approach of immunocytochemical staining and morphometric analysis based on stereological methods, we investigated the qualitative and quantitative changes of the endocrine pancreas in a young patient suffering from type 1 diabetes of short duration. In particular, we attempted to answer the following questions: (1) Is there any evidence of acute B-cell necrosis at the abrupt onset of type 1 diabetes; (2) how severe is the loss of B-cell mass at the time of diagnosis; and (3) what are the implications of B-cell reduction regarding timing and pathogenesis of B-cell disappearance in type 1 diabetes.

Subjects and methods

Pancreases were obtained within 24 h after death from a 9 year old girl with diabetes and from four non-diabetic patients. The diabetic girl was admitted to hospital in severe ketoacidotic diabetic coma (blood glucose above 1,000 mg/dl) leading to circulatory failure and death within 24 h. Retrospectively it was found that she probably showed the first symptoms of diabetes 7 days before hospital admission. She proved to be slightly positive for conventional islet cell antibodies (Drs. Helmke and Federlin, Gießen, FRG), but showed no elevated antibody titers against a large scale of viruses including the Coxsackie B group. The four nondiabetic patients aged 13, 15, 34 and 40 years (control group) had similar pancreatic volumes as the diabetic patient (Table 1).

The freshly prepared pancreases were carefully dissected free of connective tissue, fat and large vessels. Their volumes were measured according to Archimedes' principle by water displacement. Samples were taken from 8 parts of each pancreas according to the scheme proposed by Malaisse-Lagae et al. (1979) and fixed in Bouin's solution.

In this series no attempt was made to separate the PP lobe from the remainder of the gland because of the difficulty in dissecting this area of the pancreas precisely. As its endocrine cell volume could therefore not be calculated, we subtracted the volume of the PP lobe from the total pancreatic volume. In a pilot study on 4 pancreases from 2 non-diabetics and 2 type 2 diabetics where the PP lobe had been successfully dissected (as demonstrated by immunocytochemistry), it comprised about 10% of the pancreatic volume. This value corresponds to those recently reported (Stefan et al. 1982; Rahier et al. 1983).

Immunocytochemistry was performed on deparaffinised serial sections (5 μm), using the indirect peroxidase technique (insulin) and the unlabeled antibody enzyme method (glucagon, somatostatin and pancreatic polypeptide). The dilution of the primary antisera was as follows: anti-insulin (porcine; Novo) 1:1,000, anti-glucagon (Milab) 1:1,000, anti-somatostatin (Novo) 1:5,000, and anti-PP (gift from Dr. R.E. Chance, Indianapolis) 1:10,000. Anti-guinea pig IgG (1:60 dilution) labeled with peroxidase and soluble peroxidase-antiperoxidase complexes (1:60) produced in rabbits were obtained from DAKO. The histochemical reaction for peroxidase was carried out using 3,3′-diaminobenzidine tetrahydrochloride (0.05% w/v) and 0.01% H_2O_2 in 0.05 M tris-HCl buffer (pH 7.6). After fixation with 1% OsO_4 in PBS (pH 7.2), the sections were dehydrated and mounted. The specificity of immunostaining was determined

by preabsorption of the antisera with the respective hormones. Further controls included the application of non-immune sera as first layer, rabbit IgG as first or third layer, and sheep IgG as second layer.

Stereologic procedures. Whole cross-sections were taken from constant parts of the pancreatic head, body and tail and were evaluated for their mesenchymal proportion and the volume of the endocrine cells according to stereological principles (Weibel 1979; Oberholzer 1983). Two magnifications were used: 1:22 for determination of the volume density of the mesenchymal part (level 1) and 1:500 for determination of the volume density of the endocrine cells (level 2). Point counting was done by projecting the sections onto a rectangular test grid with 6,581 test points and a grid distance of 320 µm (final magnification: 1:22) and 5 µm (final magnification 1:500) respectively.

Level 1. The sections evaluated for the mesenchymal proportion (fat, connective tissue, vessels) of the pancreatic tissue were stained with PAS. The test fields were totally occupied by pancreatic tissue and corresponded to the test areas. The stereological parameters were calculated according to the following formulas.

(1) $V_{V(MES/PAN)} = \frac{\Sigma P_{(MES)}}{\Sigma P_{(PAN)}}$

$V_{V(MES/PAN)}$ Volume density of the mesenchymal portion of pancreatic tissue referred to the total pancreas

$P_{(MES)}$ Hits on mesenchymal tissue

$P_{(PAN)}$ Hits on pancreatic tissue (mesenchymal and parenchymal)

(2) $V_{V(PAR/PAN)} = 1 - V_{V(MES/PAN)}$

$V_{V(PAR/PAN)}$ Volume density of the pancreatic parenchyma

(3) $V_{(MES)} = V_{V(MES/PAN)} \cdot V_{(PAN)}$ (ml)

$V_{(MES)}$ Volume of the mesenchymal part of the pancreas

$V_{(PAN)}$ Volume of the pancreas

(4) $V_{(PAR)} = V_{(PAR/PAN)} \cdot V_{(PAN)}$ (ml)

$V_{(PAR)}$ Parenchymal volume of pancreas

Level 2. The volume densities of the various endocrine cell types were obtained by evaluation of immunostained sections using the following formulas.

(5) $V_{V(x/PAR)} = \frac{\Sigma P_{(x)}}{\Sigma P_{(PAR)}}$

$P_{(x)}$ Hits on immunostained endocrine cells

$P_{(PAR)}$ Hits on pancreatic parenchyma

(6) $V_{(x)} = V_{V(x/PAR)} \cdot V_{(PAR)}$ (ml)

$V_{(x)}$ = Volume of endocrine cell type (insulin, glucagon, somatostatin, PP)

The selected fields on the sections were totally occupied by parenchymal tissue and thus identical with the test areas. Stratified random sampling of the test fields was achieved by stepwise selection of test areas along a diagonal line through the section. In each case of the control group 60 test areas with 394,860 total points over pancreatic parenchyma were evaluated. In the case of the diabetic patient 320 fields with a total of 2,105,920 test points were analysed.

In order to reach a statistical significance of 95% at a mean error of only 10% even for volume density values as low as 0.0015 (volume density of the PP-cells of the control 1, see Table 2) at least 255,723 test points lying over profiles of pancreatic parenchyma had

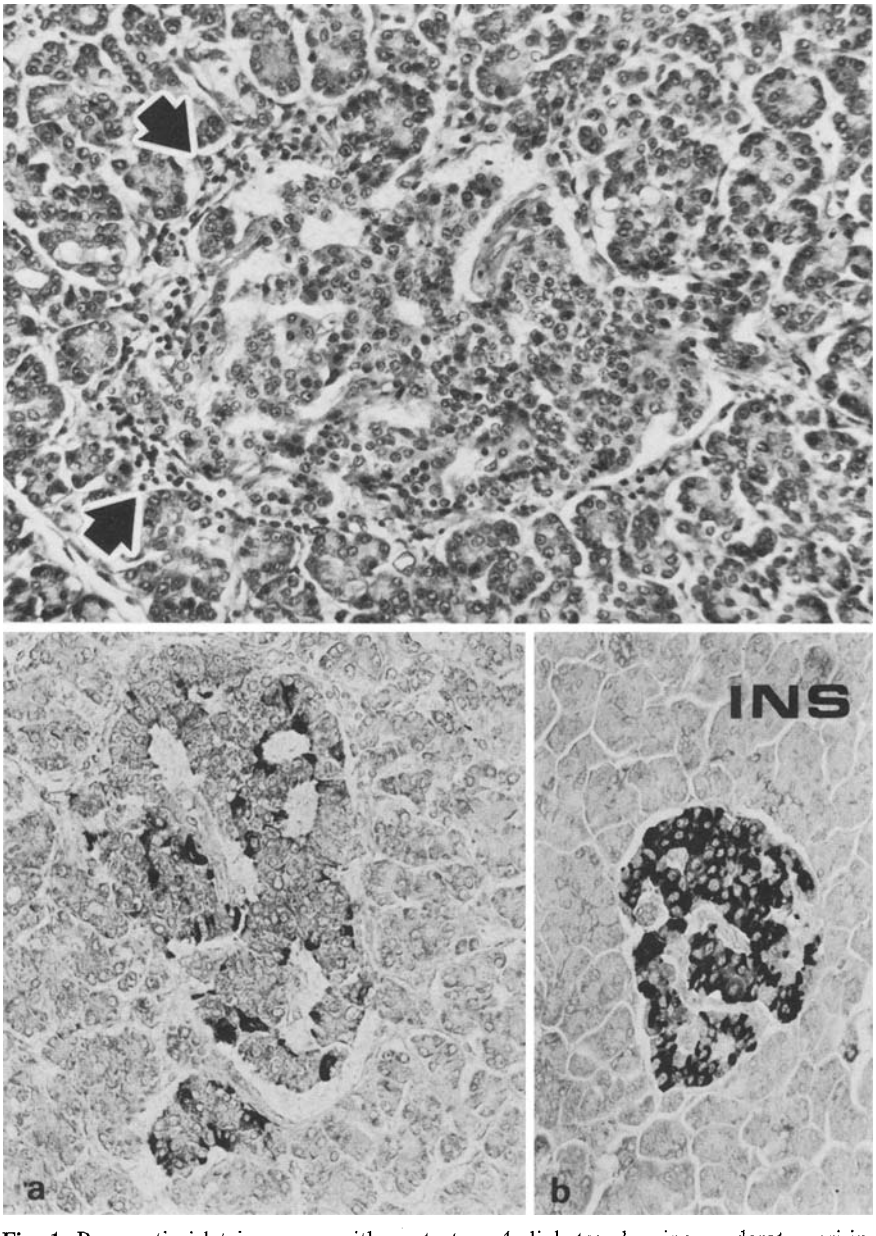

Fig. 1. Pancreatic islet in a case with acute type 1 diabetes showing moderate peri-insulitis (*arrows*). PAS × 250

Fig. 2a, b. Pancreatic islet in acute type 1 diabetes showing distinctly reduced number of B-cells (**a**). Control islet (**b**). Immunostaining for insulin (*INS*). × 250

to be evaluated (Oberholzer 1983). These calculations showed that the number of evaluated test fields comfortably exceeded the minimal number of test fields required by the above conditions. The results of the diabetic patient were compared with those of the control group by testing whether they were lying outside or within the twosided 95%-confidence interval of the values of the control group. The R/SD quotient (David 1954) was used to test the normality of the distribution of the different endocrine cell values. As the distribution was not normal (for PP producing cells, Table 2), a logarithmic transformation of all data (patient and control group) was performed in order to obtain normally distributed values. The two-sided 95%-confidence interval of the transformed data of the control group was then calculated and compared with the value of the patient. This procedure makes it possible to estimate the probability for the single values of the diabetic patient supposing that they belong to the control group. The values of the patient were considered to be different from those of the control group, if the two-sided probability was lower than 5% ($2P < 0.05$).

Results

Examination of numerous sections from all regions of the pancreas (except of the PP-lobe) from the nine-year-old diabetic patient revealed that the islets were well preserved. There was no evidence of islet cell necrosis. In general two islet populations could be recognized: (1) normal-sized or slightly hypertrophic islets, occasionally showing nuclear hypertrophy; and (2) small islets with irregular outlines and small cell nuclei. The two islet types were, as a rule, not randomly distributed, but the shrunken islets were more frequently observed in the pancreatic body and tail. Insulitis affected only a few, normal-sized islets from different regions. The lymphocytic infiltration was always discrete and in general confined to the islet periphery (Fig. 1).

Immunocytochemistry revealed that all of the normal-sized or hypertrophic appearing islets as well as the islets with insulitis contained insulin producing cells, some of which were hypertrophic. In the majority of these

Table 1. Clinical features and data on pancreatic volume in a case of type 1 diabetes and non-diabetic patients

Case	Age (years)	Sex	Pancreatic Volume[a] ml	Mesenchymal Volume ml	Parenchymal Volume ml	Cause of death
Type 1 Diabetes	9	F	58.8	24.4	34.4	Diabetic coma after diabetes of 7 days duration
Controls						
1	13	M	49.7	14.7	35.0	Cerebral haemorrhage
2	15	M	58.5	12.2	46.3	Traffic accident
3	34	M	50.4	16.4	34.0	Congenital cerebral aneurysm
4	40	F	69.3	30.5	38.8	Ulcer bleeding

[a] The volume of the PP lobe amounting to 10% of the total pancreatic volume was excluded

Table 2. Volume densities ($x-10^{-3}$) of the different cell types of the endocrine pancreas in a case of type 1 diabetes with acute clinical onset and in non-diabetic controls

	Type 1 Diabetic	Control 1	Control 2	Control 3	Control 4	Mean of controls	R/SD
Insulin	1.01 ±0.89	4.14	7.83	5.72	5.29	5.75±1.54 15.5%=VC	2.396
Glucagon	0.80 ±0.18	1.88	2.63	2.04	1.72	2.07±0.40 11.2%=VC	2.275
Somatostatin	0.79 ±0.32	0.82	1.33	0.67	0.66	0.87±0.32 21.2%=VC	2.094
PP	0.25 ±0.21	0.15	0.17	0.20	0.19	0.18±0.02 6.4%=VC	2.500[a]
Total	2.85 +1.90	6.99	11.96	8.63	7.86	8.86±2.17 14.1%=VC	2.290

VC: Relative variation coefficient (Sachs 1978)
R/SD-quotient (R: Range; SD: Standard deviation) (David et al. 1954)

[a] Distribution not normal

Table 3. 95% two-sided confidence limits for the volume densities of the endocrine cells related to the parenchyma of the pancreas in the control group

	Type 1 Diabetic	95% two-sided confidence limits
Insulin	1.01[b]	3.30– 5.75
Glucagon	0.80[b]	1.43– 2.71
Somatostatin	0.79	0.28– 1.46
PP[a]	−0.60[b]	−0.85–−0.65
Endocrine pancreas	2.85	5.41–12.31

[a] Log transformed values
[b] Values outside the confidence interval

islets B-cells were still the dominating cell type. However, some islets already showed reduced B-cell numbers (Fig. 2). B-cell granulations as assessed by the intensity of the immunoreaction in the single cells, varied considerably. There was no evidence of B-cell regeneration. B-cells were absent from the small islets which contained only A-cells and D-cells and rare PP-cells. The PP-lobe contained no B-cells. The endocrine pancreas of the non-diabetic patients did not display abnormalities.

The quantitative data are given in Table 1, 2 and 3. The mesenchymal and parenchymal volume of the pancreas from the diabetic patient was not statistically discernible from that of the other pancreases of this series (Table 1). When compared with the values seen in the controls stereologic analysis of the immunocytochemically stained section revealed a decrease in the mean volume densities $V_{V(x/PAR)}$ of B-($2P<0.05$), and to a minor degree of A-cells, in the case with the acute diabetes (Table 2 and 3). The

Table 4. Volume (ml) of the pancreatic endocrine cells[a] in a case of type 1 diabetes with acute clinical onset and in non-diabetic controls

	Type 1 Diabetic	Control 1	Control 2	Control 3	Control 4	Mean of controls	R/SD
Insulin	0.047 ±0.041 (SD) (35.5%)	0.145 (59%)	0.363 (65.4%)	0.222 (66.1%)	0.180 (67.6%)	0.228±0.096 (SD) 24.3% = VC (65%)	2.271
Glucagon	0.037 ±0.023 (28%)	0.066 (27%)	0.122 (22%)	0.080 (23.8%)	0.058 (21.8%)	0.082±0.028 19.7% = VC (23%)	2.286
Somato-statin	0.037 ±0.015 (28%)	0.029 (11.8%)	0.062 (11.2%)	0.026 (7.7%)	0.022 (8.3%)	0.035±0.018 29.7% = VC (10.2%)	2.222
PP	0.011 ±0.010 (8.5%)	0.005 (2.2%)	0.008 (1.4%)	0.008 (2.4%)	0.006 (2.3%)	0.007±0.002 16.5% = VC (1.8%)	1.500[b]
Endocrine pancreas	0.132 ±0.089 (100%)	0.245 (100%)	0.555 (100%)	0.336 (100%)	0.266 (100%)	0.351±0.142 23.4% = VC (100%)	2.183

VC: Relative variation coefficient (Sachs 1978)
R/SD-quotient (R: Range; SD: Standard deviation) (David et al. 1954)

[a] PP lobe excluded
[b] distribution not normal

Table 5. 95% two-sided confidence limits for the absolute volume of the cell types of the endocrine pancreas in the control group

	Type 1 Diabetic	95% two-sided confidence limits
Insulin	0.047[b]	0.075– 0.198
Glucagon	0.037[b]	0.038– 0.127
Somatostatin	0.037	0.002– 0.068
PP[a]	−1.959[b]	−2.360– −2.000
Endocrine pancreas	0.132	0.090–0.612

[a] Log transformed values
[b] Values outside the confidence interval

distinct variation in frequency of B- and PP-cells in different regions of the diabetic pancreas is reflected by a high SD of the volume densities of these cell types.

The B- and A-cell volume (absolute mass of insulin and glucagon cells) in the diabetic pancreas showed a reduction to about one fifth ($2P<0.05$) and one half respectively, of the mean control values ($2P<0.05$) (Table 4 and 5). The D-cell volume is within normal range (Fig. 3), whereas the volume of the PP-cells is slightly increased ($2P<0.05$).

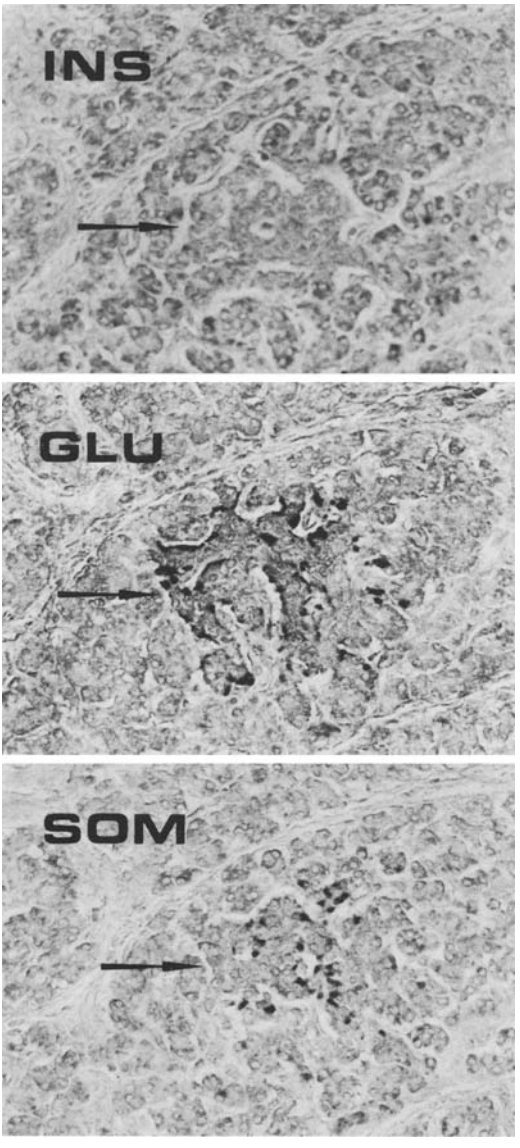

Fig. 3. Serial sections of a small-sized pancreatic islet in acute type 1 diabetes showing complete absence of B-cells and unaltered numbers of A- and D-cells. Immunostaining for insulin (*INS*), glucagon (*GLU*) and somatostatin (*SOM*). ×250

Discussion

The complete or almost complete absence of B-cells is the major characteristic of the endocrine pancreas in chronic type 1 diabetes (Gepts and DeMey 1978; Stefan et al. 1982; Rahier et al. 1983; Klöppel and Drenck 1983). The results of the present stereological study in a case of recent onset type 1 diabetes revealed that even at the time when the first symptoms of diabetes appeared the immunocytochemically demonstrable B-cells were reduced in

mass by about 80% of the values found in controls of similar age and/or similar pancreatic volume.

Our sterological evaluation of the endocrine cell mass of the pancreas was based on the principles formulated by Weibel (1979) and Oberholzer (1983) and is comparable with the methods applied in other recent studies on quantification of the islet cell population in non-diabetics, long-term type 1 diabetics and type 2 diabetics (Rahier et al. 1981 and 1983; Stefan et al. 1982). Unlike these studies, however, the PP lobe remained unconsidered in our stereological evaluation. This was because the dissected dorsal part of the pancreatic head was frequently found not to be congruent with the PP area identified by immunocytochemistry. Only in a limited number of cases did the dissected part of the pancreatic head represent the true PP region and in these cases, including non-diabetics and type 2 diabetics, the PP lobe amounted to approximately 10% of the total pancreatic volume. This value confirms recent data reported from Rahier et al. (1983), who found the percentage volume of the PP lobe remarkably constant in all pancreases except those of chronic type 1 diabetes in which the PP lobe made up 20% of the total volume. Since we excluded the PP lobe from our calculations of the total endocrine mass, our data relate only to the part of the pancreas rich in B-cells. This part, however, is largely responsible for the functional integrity of the endocrine pancreas. Thus if in the various types of diabetes the B-cells show quantitative changes, these should be best revealed in the islets rich in B-cells.

In our case with a type 1 diabetes with a duration of symptoms of approximately 7 days, there was a conspicuous decrease in B-cell number in many areas, but not in all. Morphometry was therefore done on several sections from various sites of the pancreas in order to record the reduction of B-cells as accurately as possible. This evaluation revealed that the total B-cell mass was reduced to about one third to one seventh of the values determined in four controls. Qualitatively the remaining B-cells appeared to be intact. Insulitis, the key finding in acute type 1 diabetes (LeCompte et al. 1958; Gepts 1965) affected only single islets and presented as a small peri-insular infiltrate of lymphocytes. These findings imply that the B-cell destruction underlying the disappearance of B-cells only slowly proceeds and presumably starts long before the clinical onset. Moreover, the findings imply that the critical point of B-cell reduction is approximately 80% in terms of metabolic compensation. Intervening virus infections or stress may then lead to a sudden collapse of insulin secretion. A virus being detected at that time does therefore not prove that type 1 diabetes is of viral origin, since the process has presumably been initiated long before its clinical manifestation.

A recent prospective study in HLA-identical siblings of type 1 diabetes has provided evidence that the occurrence of islet cell antibodies precedes the abrupt clinical onset by months or years (Gorsuch et al. 1981 and 1982). Similar latency periods may also emanate from the results of our studies, if we suppose that the B-cell volume in type 1 diabetes declines linearly. In Fig. 4 linearly decreasing B-cell masses of three hypothetical cases are

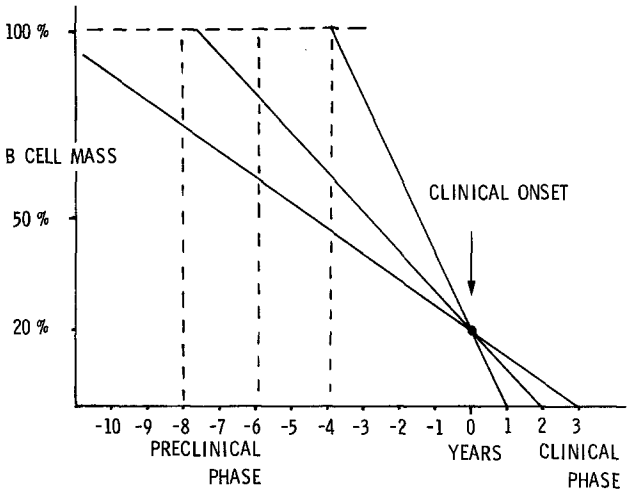

Fig. 4. Hypothetical relationship in time between the degrees of the B-cell volume in type 1 diabetes and the course of the disease. The decreasing B-cell masses of three hypothetical cases are represented by linearly descending lines. At the clinical onset of the disease it is supposed that the B-cell volume is diminished to 20% of the normal values. The rate of B-cell loss in the preclinical phase is then determined by the time passing from the onset of symptoms until the time when total insulin dependency is reached. This clinical phase lasts on the average between one to three years. The respective preclinical phases come up to four to ten years

plotted against the time course of the disease, divided into a preclinical and clinical phase. The B-cell masses, represented by the descending lines, decline at different speed, but cross in the point where the B-cell masses are reduced to approximately 20% and, according to our hypothesis, diabetes becomes clinically manifest. The rate of the progressive decline of the B-cell mass during the preclinical stage is determined by the duration of the clinical phase elapsing until total insulin dependency is reached and B-cells are absent from the pancreas. In most cases this occurs after 1–3 years. If we now retrace the B-cell mass up to the 100% level, i.e. the point where they were still unchanged, preclinical phases of 4–10 years duration are revealed. Though we are aware that progression of disease processes may not be linear but may rather follow exponential functions or advance step-wise, our working model on the time course of B-cell disappearance might give a rough idea of duration of the latency periods possibly to be taken into account in type 1 diabetes. Moreover, the model implies that a long lasting residual B-cell function corresponds with a long preclinical period while, on the other hand, rapid deterioration of residual B-cell function is preceded by a short preclinical period. This entails that in small children the disease should run a rapid course whereas in general a more gradual decline in B-cell function should be observed in adolescents. Recent reports in support of this concept show that the residual B-cell function, as determined by urinary C-peptide excretion at one year after diagnosis,

is more strikingly reduced in small children than at older age (Crossley et al. 1981; Madsbad 1983).

The morphological findings do not provide an answer to the question of which events underlie the gradual B-cell disappearance. The absence of acute B-cell necrosis from islets affected by insulitis nevertheless speaks in favour of a slowly acting autoimmune process which possibly damages the B-cells by mechanisms like peripolesis and apoptosis. Since we also found some decrease in A-cells it may be speculated that to a certain degree B-cell disappearance is associated with some loss of non-B-cells.

In conclusion, our stereologic study on the total B-cell volume in an acute type 1 diabetes suggests that the abrupt clinical onset is preceded by a long latency period, for the B-cell mass is reduced to about 80% of the values found in controls and there is obviously no massive B-cell destruction at the time of clinical manifestation.

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References

Ahmad A, Abraham AA (1982) Pancreatic isleitis with coxsackie virus B 5 infection. Hum Pathol 13:661–662

Champsaur H, Dussaix E, Samolyk D, Fabre M, Bach C, Assan R (1980) Diabetes and coxsackie B 5 infection. Lancet 1:251

LeCompte PM (1958) "Insulitis" in early juvenile diabetes. Arch Pathol 66:450–457

Crossley JR, James AG, Elliott RB, Berryman CC, Edgar BW (1981) Residual B-cell function and islet cell antibodies in diabetic children. Pediatr Res 15:62–65

Cudworth AG (1978) Type 1 diabetes mellitus. Diabetologia 14:281–291

David H, Hartley HO, Pearson ES (1954) The distribution of the ratio, in a single normal sample, of range to standard deviation. Biometrika 11:482

Gepts W (1965) Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 14:619–633

Gepts W, DeMey J (1978) Islet cell survival determined by morphology. An immunocytochemical study of the islets of Langerhans in juvenile diabetes mellitus. Diabetes 27:(Suppl 1):251–261

Gorsuch AN, Lister J, Dean BM, Spencer KM, McNally JM, Bottazzo GF, Cudworth AG (1981) Evidence for a long prediabetic period in type 1 (insulin-dependent) diabetes mellitus. Lancet 19/26:1363–1365

Gorsuch AN, Spencer KM, Lister J, Wolf E, Bottazzo GF, Cudworth AG (1982) Can future type 1 diabetes be predicted? A study in families of affected children. Diabetes 31:862–866

Klöppel G, Drenck CR (1983) Immunzytochemische Morphometrie beim Typ-1- und Typ-II-Diabetes mellitus. Dtsch Med Wochenschr 108:188–189

Madsbad S (1983) Prevalence of residual B cell function and its metabolic consequences in type 1 (insulin-dependent) diabetes. Diabetologia 24:141–147

Malaisse-Lagae F, Stefan Y, Cox J, Perrelet A, Orci L (1979) Identification of a lobe in the adult human pancreas rich in pancreatic polypeptide. Diabetologia 17:361–365

Oberholzer M (1983) Morphometrie in der klinischen Pathologie. Allgemeine Grundlagen. Springer, Berlin Heidelberg New York Tokyo

Rahier J, Wallon J, Henquin JC (1981) Cell populations in the endocrine pancreas of human neonates and infants. Diabetologia 20:540–546

Rahier J, Goebbels RM, Henquin JC (1983) Cellular composition of the human diabetic pancreas. Diabetologia 24:366–371

Rahier J, Wallon J, Loozen S, Lefevre A, Gepts W, Haot J (1983) The pancreatic polypeptide cells in the human pancreas: The effect of age and of diabetes. J Clin Endocrinol Metab 56:441–444

Sachs L (1978) Angewandte Statistik. Statistische Methoden und ihre Anwendungen. 5. Auflage. Springer, Berlin Heidelberg New York

Stefan Y, Orci L, Malaisse-Lagae F, Perrelet A, Patel Y, Unger RH (1982) Quantitation of endocrine cell content in the pancreas of nondiabetic and diabetic humans. Diabetes 31:694–700

Weibel ER (1979) Stereological methods. Practical methods for biological morphometry, vol. 1. Academic Press, London New York Toronto Sydney San Francisco

Yoon JW, Austin M, Onodera T, Notkins AL (1979) Virusinduced diabetes mellitus. Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. New Engl J Med 300:1173–1179

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