Hyperplasia of “Pancreatic Polypeptide”-Cells in the Pancreas of Juvenile Diabetics

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Summary. The cellular composition of the pancreatic islets of juvenile diabetics was studied, using recently developed immunocytochemical methods. B-cells were identified only in juvenile diabetics with a disease of short duration. In chronic juvenile diabetics, the islets which are classically viewed as “atrophic”, were shown to be composed of glucagon- and of somatostatin-cells. Another type of islets which commonly occurs in the pancreas of juvenile diabetics, i. e. the ribbon-like type first described by Cecil in 1911, appeared to be composed almost exclusively of “pancreatic polypeptide” (HPP)-cells. It is suggested that hyperplasia of the HPP-cells in the pancreas of juvenile diabetics results from an atypical type of islet regeneration induced by a severe and prolonged injury to the pancreatic endocrine tissue.

Key words: Pancreatic hormones, “pancreatic-polypeptide”-cells, juvenile diabetes, immunocytochemistry, hyperplasia, islet regeneration.

In older [1, 2, 3] as well as in more recent studies [4, 5, 6, 7] the atrophy of the islets of Langerhans has been pinpointed as one of the characteristic features of the endocrine pancreas in juvenile diabetics. In these patients many islets are indeed composed of small cells, with a small and dense nucleus and scanty cytoplasm (Fig. 1). Other islets present a ribbon-like arrangement of cylindrical cells, with a clear cytoplasm and a centrally located nucleus (Fig. 2). It is generally accepted that the cellular composition of both types of islet differs markedly from normal, mainly in the complete absence of B-cells. A-cells can still be demonstrated in some of the “atrophic” islets, but the identity as well as the functional significance of all other cells composing these islets remains undefined, although they have been subjected to varying speculations. Sev-

eral authors [1, 3, 5] have considered the “atrophic” cells as inactive remnants of injured islets, based on their cytologic appearance, whereas the ribbon-like islets have been interpreted as the result of atypical regeneration [1, 8].

The present work has been undertaken to identify the islets observed in the pancreas of juvenile diabetics, using recently developed immunocytochemical techniques. This study has indicated that, in contrast with previous suggestions, the islets in juvenile diabetics should not be considered as atrophic tissue, since they contain well granulated endocrine cells, which might play an important rôle in the physiopathology of diabetes. Moreover, the cellular composition of the diabetic islets was also found to alter with the progression of the disease, as was evident from our observation that with time more and more islets in the juvenile diabetics were almost exclusively composed of cells, containing “pancreatic polypeptide”, a substance originally extracted from avian pancreas [9, 10], and later from the pancreas of several mammalian species, including man [11, 12, 13, 14].

Material and Methods

Pancreatic specimens of seven insulin-dependent juvenile diabetics were studied. At least three blocks originating from different parts of the pancreas were examined in each case, except for one (74.854), in which only one block was available.

The relevant clinical and pathological data are summarized in Table 1. Four patients had died within six months after the first symptoms of diabetes, whereas the others had survived for up to 23 years. In three of the recent-onset cases, insulinitis was observed, severely affecting numerous islets in one case, and more discretely a smaller number of islets in two

Fig. 1. "Atrophic" type of islet in the pancreas of a juvenile diabetic (57.130). The islet is composed of thin cords of small cells with a small nucleus and scanty cytoplasm. Chromium hematoxylin-phloxine. $\times 390$

Fig. 2. Ribbon-like type of islet in the pancreas of a juvenile diabetic (58.112). This type of islet is composed of columnar cells with a centrally located nucleus and a clear cytoplasm. Masson's trichrome staining. $\times 560$

Table 1. Cases of juvenile diabetes

Case	Age (years)	Sex	Known duration of diabetes	Insulitis	B-cells	PP-cells
57.130	2	F	? ^a	severe	present but reduced in number	few
56.237	21	F	? ^a	discrete	present but reduced in number	numerous
58.112	30	M	? ^a	not found	present but reduced in number	numerous (focally)
69.145	37	F	? ^a	discrete	present but reduced in number	numerous (focally)
57.135	43	M	23 yrs	not found	not found	very numerous
62.228	37	M	17 yrs	not found	not found	very numerous
74.854	38	M	19 yrs	not found	not found	few (only one block)

^a The four cases of acute juvenile diabetes had died within a few days after being admitted to the hospital in diabetic coma. Diabetes had not been diagnosed previously

Fig. 3. Recent-onset juvenile diabetic (69.145). The section has been stained for insulin and glucagon, using an extension of the PAP-technique of Sternberger. The insulin-cells (I) (dark cells) are reduced in number and partly degranulated. The glucagon-cells (G) (paler cells) are increased in number. $\times 350$

others. Insulinitis was not found in any of the chronic cases. Specimens from patients without diabetes or any other symptom of pancreatic disease, were used as control material.

Paraffin sections of Bouin-fixed tissue were stained with a routine method (haemalum-erythrosine-saffron) and with classical granule staining techniques (Gomori's chromium haematoxylin and aldehyde fuchsin, Ivic's Victoria Blue, the silver impregnation techniques of Hellman-Hellerström and of Grimelius). For the immunocytochemical demonstration of the different types of islet cells, both the indirect fluorescence method of Coons and the peroxidase-antiperoxidase (PAP) method of Sternberger [15, 16] with specific antibodies to insulin, glucagon, somatostatin and to human (HPP) or bovine (BPP) pancreatic polypeptide were carried out.

All antisera were diluted in tris-buffered saline, pH 7.6. For the immuno-fluorescence technique, the following dilutions were used: guinea-pig anti-insulin serum (a gift of Dr. A.E. Lambert of the University of Louvain) 1:400, rabbit anti-glucagon serum (a gift of Dr. P. Lefèbvre of the University of Liège) 1:10, rabbit anti-somatostatin serum (a gift of Dr. M.P. Dubois, Centre de Recherches Agronomiques, Nouzilly, France) 1:150, rabbit antihuman or anti-bovine pancreatic polypeptide (a gift of Dr. R.E. Chance, Lilly Research Laboratories, Indianapolis, Ind., U.S.A.) 1:400 and 1:800 respectively. Fluorescence was demonstrated with goat-antirabbit or goat

antiguinea-pig serum labelled with fluorescein isothiocyanate (Nordic, Antwerp, Belgium).

For the PAP-technique the antisera were used at the following dilutions: anti-insulin 1:8000, anti-glucagon 1:1000, antisomatostatin 1:1000, anti-BPP 1:40.000. Rabbit antiperoxidase bound to horseradish peroxidase was prepared in our laboratory and used at a dilution of 1:300. A recently developed extension [17, 18, 19] of the PAP-method of Sternberger, allowing the cytochemical demonstration of two hormones in one section, was also applied.

Control studies were performed using antibodies which had been incubated with the respective antigens.

Results

In the pancreas of normal controls, the insulin-containing B-cells prevailed, representing 70–80% of the islet population. Second in number were the glucagon-secreting A-cells (20–25%), whereas somatostatin-cells (type III or D-cells) numbered 5 to 10%. HPP-cells were rare and did not occur in every islet.

In recent-onset juvenile diabetics all four hormone-producing cells could be demonstrated, but the B-cells were markedly reduced in number and stained poorly with the immunocytochemical methods for insulin, probably as a consequence of their degranulated state (Fig. 3). With routine granule staining, the

Fig. 4. Recent-onset juvenile diabetic (57.130). The somatostatin-cells, demonstrated by indirect immunofluorescence, appear increased in number. $\times 350$

Fig. 5. Low magnification of the pancreatic tissue in a juvenile diabetic with a disease of 17 years duration (62.228). The section has been stained with the soluble PAP-complex method of Sternberger, with a specific antibody to HPP. It shows an impressive hyperplasia of atypical islets, exclusively composed of HPP-cells (dark spots). $\times 22$

B-granules were indeed difficult to detect. In contrast, glucagon- and somatostatin-containing cells appeared increased in number (Fig. 4), but our studies do not determine whether this increase is relative or absolute. The numerical evaluation of the HPP-cells was markedly complicated by their very irregular distribution: they were found in small numbers in large areas of the pancreas, whereas they were extremely numer-

ous in other parts. Very few HPP-cells were detected in the recent-onset case with severe insulinitis (57.130), whereas they were much more numerous in two of the other cases of short duration (58.112 and 69.145), at least in some parts of the pancreas. An extreme degree of HPP-cell hyperplasia (Fig. 5) was observed in the fourth acute case (56.237) and in two of the three chronic cases (57.135 and 62.228). In these cases,

Fig. 6. Higher magnification of an HPP-islet. Note the ribbonlike pattern and the centrally located nuclei (compare with Fig. 2). Same case and same staining method as in Fig. 5. $\times 560$

Fig. 7. Low magnification of the pancreatic tissue in a juvenile diabetic (56.237). In the lower part of the figure the islets contain only HPP-cells (dark spots), whereas in the upper part glucagon was demonstrated (arrows). In another preparation, with simultaneous staining of somatostatin and glucagon, it appeared that the latter islets contained somatostatin as well. Same method as in Fig. 3. $\times 16$

many areas showed islets composed exclusively of HPP-cells (Fig. 6). Moreover, the double staining technique revealed that the distribution of these HPP-islets followed a lobular pattern: in some lobules the islets were composed of glucagon- and somatostatin-cells, whereas neighbouring lobules showed islets only composed of HPP-cells (Fig. 7). Only few islets contained the three endocrine cell types. This peculiar

distribution of pancreatic endocrine cells appears to be characteristic of the pancreas of chronic juvenile diabetics. We did not observe it to the same extent in the pancreas of maturity-onset diabetics or in other pathological conditions. In one case (74.854) only rare HPP-cells could be detected, but only one block was available for study.

A comparison with routinely stained sections

Fig. 8. This figure demonstrates the presence of HPP-cells in the epithelium of a duct (long arrows) and the development of an HPP-islet in continuity with this epithelium, (short arrows). Same staining method as in Fig. 6. Same case as in Fig. 2 (58.112). $\times 300$

suggested that the HPP-islets corresponded to the ribbon-like pattern (Fig. 2), whereas the islets composed of glucagon- and of somatostatin-cells were equivalent to those which have been classically described as "atrophic" islets (Fig. 1). On the other hand, the immunocytochemical study has confirmed the complete absence of B-cells in the pancreas of juvenile diabetics after many years duration.

As to the origin of the HPP-cells, clearcut indications were found that these cells derive from the epithelium of ducts. Not only were they often observed among the epithelial cells, but images of neoformation of HPP-islets in close contact with ducts were often encountered (Fig. 8).

Discussion

Our study has confirmed the progressive disappearance of the B-cells from the pancreas of juvenile (insulin-dependent) diabetics with disease of long duration. It has also revealed that, contrary to the classical view, the remaining and apparently "atrophic" islets, are actually composed of active endocrine cells, mostly glucagon- and somatostatin-cells. This finding is in agreement with recent work by Orci et al. [20], who studied the pancreas of two chronic juvenile diabetics by electron microscopic and immunocytochemical techniques and concluded that the islets were depleted of B-cells, but were composed of glucagon- and of somatostatin-cells.

The most surprising finding of our study, however, was the impressive hyperplasia of HPP-cells in certain areas of the pancreas of juvenile diabetics. So far, HPP-cells have not been described in the pancreas of human diabetics. "Pancreatic polypeptide" is a 36-aminoacid peptide which has been isolated from the avian pancreas [9, 10] and from the pancreas of several mammals, including man [11, 12, 13, 14]. Cells containing "pancreatic polypeptide" have been demonstrated in the pancreas of chicken [21] and of a variety of other species [22, 23, 24]. Their ultrastructural characteristics have not been clearly defined, but Larsson et al. [23] suggested that they correspond to the type V-cells of Deconinck et al. [25].

So far, the physiological role of the "pancreatic polypeptide" is poorly understood, but recent evidence suggests that it is a hormone-like substance regulating the functions of the exocrine pancreas, the liver, the gallbladder, as well as of the gastric mucosa [11, 12, 13, 26, 27, 28]. Nothing is known as yet about its possible importance in the physiopathology of diabetes, but an important hint is the observation of Floyd and Fajans [29] of increased plasma-levels of HPP in juvenile diabetics. The latter observation is consistent with our morphologic finding of a marked hyperplasia of HPP-cells in the pancreas of such patients. Although morphometric studies have not been carried out as yet, it appears unlikely to us that the observed HPP-cell hyperplasia might result either from an atrophy of the exocrine pancreas, or from the disappearance of the B-cells. In this regard, we would like to stress again that HPP-cells are rare in the

pancreas of normal young individuals. However, it appears important to point out that the HPP-cell hyperplasia is not a specific feature of the pancreas of juvenile diabetics. It is found also, although not to the same extent, in maturity-onset diabetics and in all circumstances of pancreatic injury (e.g. pancreatitis, tumours of the exocrine or endocrine tissue, angiosclerosis).

The appearance of an increased number of HPP-cells in all these conditions might be related to islet regeneration. It is likely that, in normal conditions, regeneration produces "normal" islets containing all the type of islet-cells in their classical proportion. However, it is conceivable that with a persistent injury, the regenerative capacity of the pancreas deteriorates progressively and this leads to the development of islets in which B-cells are less and less represented, whereas HPP-cells become more numerous with time. The end stage of such an evolution would be found in juvenile diabetics, especially in those with a disease of long duration, in whom numerous lobules are capable only of producing islets solely composed of HPP-cells. From their appearance, it is evident that the HPP-islets correspond to the ribbon-like type of islets, which was described many years ago by Cecil [8] as of regenerative origin. As the cells composing these islets previously failed to stain with any of the known methods for demonstration of the pancreatic hormones, their possible functional significance was completely disregarded.

In recent years, two new hormones have been detected in the pancreas: somatostatin and pancreatic polypeptide. Somatostatin has been located in the D-cells [30, 31, 32]. However, in our opinion, the D-cells, as described by Bloom in 1931 [33], represent a heterogeneous group composed of several types of endocrine cells. At the ultrastructural level it has been demonstrated [34, 35, 36] that somatostatin-cells correspond to the type III-cells of Deconinck et al. [25]. The ultrastructural identification of the PP-cells is still controversial. Larsson et al. [23] feel that they might correspond to the type V-cells of Deconinck et al. [25], whereas Heitz et al. [37] express the opinion that they are equivalent to D₁-cells (or type IV-cells of Deconinck et al. [25]). Further studies are required for a definite identification of the PP-producing cells and for the elucidation of the role of pancreatic polypeptide in the physiopathology of diabetes.

Acknowledgements. This work was supported by grant nr. 20468 of the Belgian Medical Research Council. We are grateful to Mrs. N. Buelens and Mrs. S. Pieters for their valuable technical assistance.

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Received: September 24, 1976, and in revised form: October 11, 1976

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