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Quantitative immunocytochemical study of islet cell populations in diabetic calmodulin-transgenic mice

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Abstract The present study describes the changes in the endocrine pancreas of severely diabetic calmodulin-transgenic mice using light microscopic immunocytochemical and morphometric techniques. A marked reduction in the number and volume of islets, together with distortion of their normal architecture, was found in diabetic mice. In addition, the volume density of both endocrine tissue and B-cells was decreased. An irregular distribution of non-B-cells was also observed in diabetic animals. The volume density and the percentage of A-cells appeared increased. However, when quantified per area unit, the number of all the islet cell types diminished, although only the decrease in B-cell number was statistically significant. The decrease in B-cell mass might account for the diabetic state developed in this animal model.

Key words Pancreatic islet cells · Quantitative immunocytochemistry · Transgenic mice · Experimental diabetes

Introduction

Several animal models of spontaneous or induced diabetic syndromes have been employed to study the pathogenesis of insulin-dependent diabetes mellitus (IDDM). Various methods have been used to produce the experimental disease, the procedure preferently employed being the administration of selective toxic chemicals such as alloxan and streptozotozin.

In 1989, a new animal model with induced IDDM was reported by Epstein et al. [3]: the authors produced transgenic mice carrying a calmodulin minigen regulated by the rat insulin II promoter. Thus, a high content of calmodulin and its mRNA were elicited in B-cells, together with a rapidly progressive loss of a significant proportion of the insulin-secreting cell mass. Calmodulin is an intracellular mediator of many actions of calcium, such as the regulation of insulin secretion; control of calcium homeostasis is critical for both development and maintenance of the B-cell specific function. Deleterious effects of elevated calmodulin on the function and viability of B-cells were described initially in a study by Epstein et al. [3], where the diabetic syndrome appeared early, followed a progressive course and was characterized by reduced pancreatic content and serum levels of insulin.

The histopathological changes present in the endocrine pancreas of humans and experimental animals with IDDM are mainly characterized by a marked decrease in B-cell mass. However, changes in the other islet cell populations might be relevant because of the well-known hormonal interrelationships that occur in such a complex organ [17].

The aim of the current study was to provide further information concerning the quantitative morphological changes observed in the different islet cell populations of diabetic calmodulin-transgenic mice, attempting to clarify the pathogenesis of the disease in this new model.

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Material and methods

The preparation of a minigen construct for calmodulin expression in pancreatic B-cells as well as the production of transgenic mice have been extensively described in a previous report [3].

In this study we employed four male 5-week-old transgenic mice of the OVE 27 line and four normal male age-matched mice as controls. Fasting blood glucose levels above 200 mg/dl were used as a criterion to classify the animals as diabetic. For this pur-

pose the animals were bled through the retroorbital plexus under light ether anaesthesia and glucose levels were measured using commercial strips (Haemoglucotest 20–800, Boehringer Mannheim). After the bleeding procedure the animals were sacrificed by cervical dislocation and samples of pancreas tail were carefully dissected, fixed in Bouin's fluid and embedded in paraffin for light microscopical studies. Several series of sections (thinner than 5 μm) were obtained from different levels of the blocks. Each of the sections from a given series was mounted on a separate slide to stain adjacent sections for immunocytochemical identification of insulin-, glucagon-, somatostatin- and pancreatic polypeptide-secreting cells (B-, A-, D- and PP-cells respectively) [15]. For this procedure we have employed our own guinea pig polyclonal anti-insulin serum (diluted 1:8000), whereas polyclonal rabbit sera against glucagon (1:200), somatostatin (1:120) and pancreatic polypeptide (1:5000) were kindly supplied by Dr. Lise Heding (Novo Nordisk), Dr. Suad Efendic (Department of Endocrinology, Karolinska Institute) and Dr. Ronald Chance (Eli Lilly) respectively. Controls for serological specificity were performed by preabsorbing a given antiserum with an excess of the related antigen. The sections were counterstained with haematoxylin.

Morphometrical analysis was carried out by the point-counting method [19] using an 8×8 mm grid (256 squares and 289 intersections) mounted in the eyepiece of the microscope. Seventy-two islets, 3,109 insular cells and 165,594 intersections were surveyed for the control group, whereas 43 islets, 805 insular cells and 161,680 intersections were recorded for the diabetic group. The volume occupied by a given parameter per unit volume of tissue (volume density: V_{vi}) was estimated by obtaining the ratio between the number of total intersections (excluding connective tissue) and the number of intersections enclosed within the profiles of a given parameter. By this method we obtained the V_{vi} of both the exocrine and the endocrine pancreas, as well as the V_{vi} of B-, A-, D- and PP-cells.

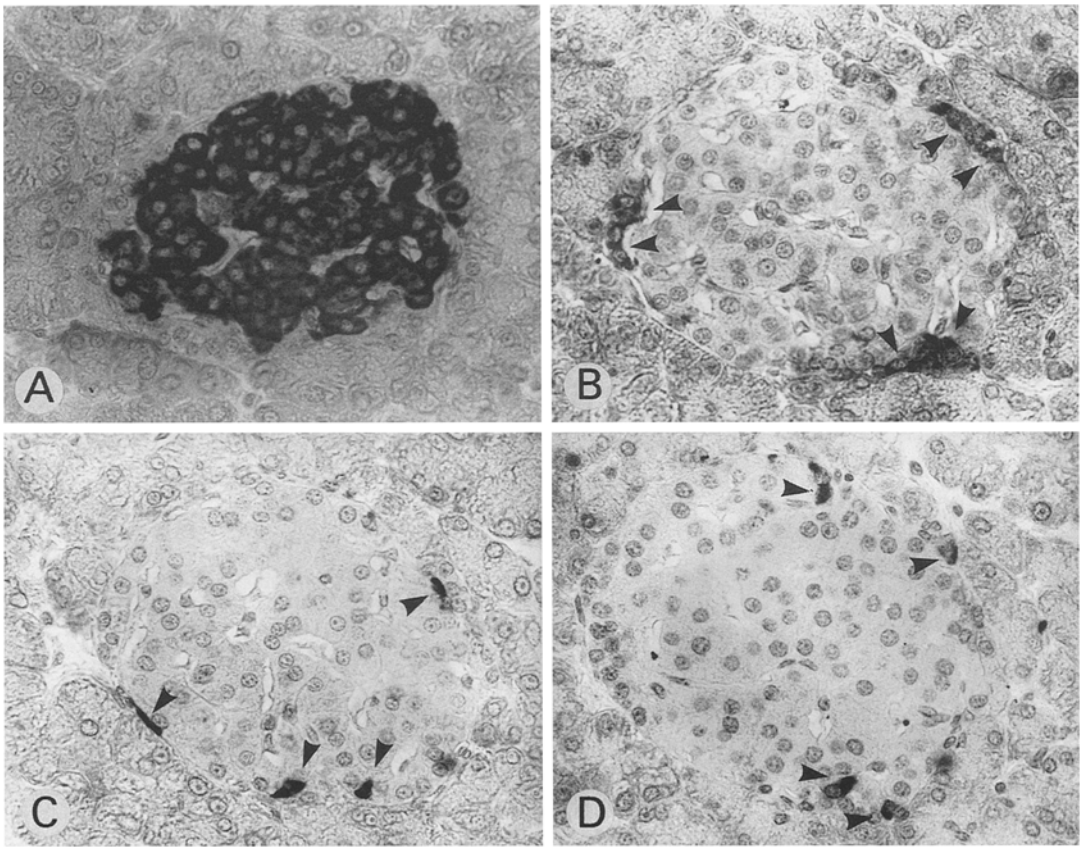
The absolute number of each type of cell and its corresponding percentage of the total were determined by counting cell nuclei [18]. The number of islets and of insular cells per 10^6 μm^2 (15,800 intersections) were also calculated. The approximate size of B-cells was obtained from the ratio between the area occupied by these cells and their absolute number. Finally, islet volume (μm^3) was estimated by the method first proposed by Reaven et al. [12].

Results

Fasting blood glucose concentrations were within normal ranges in control mice (113±13 mg/dl) whereas markedly hyperglycaemic levels were observed in transgenic mice (376±12 mg/dl).

When the pancreases were analysed under light microscopy, striking differences were found between control and diabetic mice. While a normal morphological appearance was observed in control animals (Fig. 1), severe lesions of the endocrine pancreas were detected in all the transgenic mice (Fig. 2). They consisted in a marked reduction in the number and size of pancreatic islets as well as distortion of the typical architecture in about 20% of them (Fig. 3). In many islets, the non-B-cells exhibited an irregular distribution in contrast to the peripheral arrangement observed in the normal islets (Fig. 2).

Fig. 1A–D Serial sections of the same pancreatic islet from a control mouse for immunocytochemical identification of insulin- (**A**), glucagon- (**B**), somatostatin- (**C**) and pancreatic polypeptide- (**D**) secreting cells. *Arrowheads* indicate specifically stained non-B-cells. Immunoperoxidase study counterstained with haematoxylin, × 385



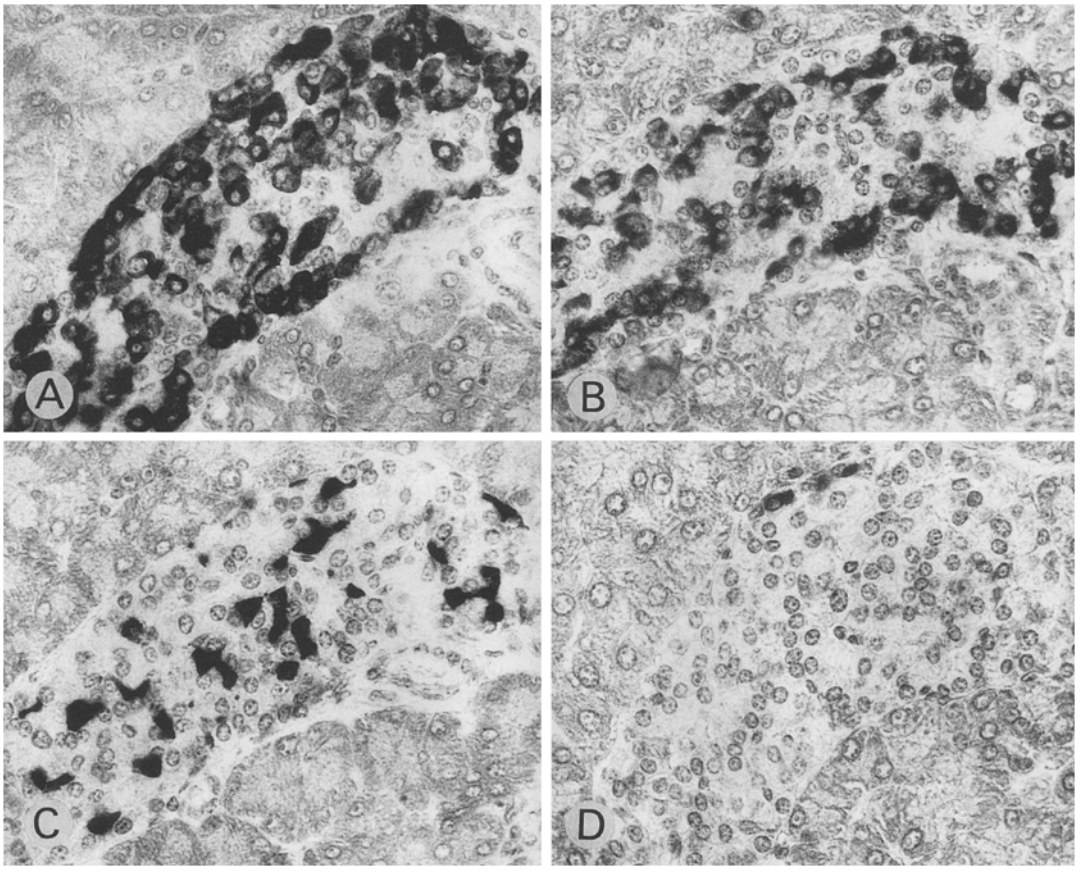


Fig. 2A–D Serial sections of the same pancreatic islet from a diabetic mouse for immunocytochemical identification of insulin- (**A**), glucagon- (**B**), somatostatin- (**C**) and pancreatic polypeptide- (**D**) secreting cells. Notice the abnormal distribution of non-B-cells in (**B**) and (**C**). Immunoperoxidase study counterstained with haematoxylin, × 385

The morphometric analysis of the pancreas showed significant differences between control and transgenic animals (Tables 1–3). Both the volume of the pancreatic islets and their number per 10^6 μm^2 diminished in diabetic mice. The V_{vi} of both endocrine tissue and B-cells significantly decreased in diabetic mice compared to that of control animals ($P<0.05$ and 0.01 respectively). This diminution corresponds to 74.2% and 54.8% respectively. A parallel decrease in the percentage of B-cells and in the number of B cells per 10^6 μm^2 was also detected.

Both the V_{vi} and the percentage of A-, D- and PP-cells increased in diabetic mice. However, this increment attained significance only in the case of A-cells. However, the number of the three types of non-B-cells per10^6 μm^2 showed a decrease of almost 50% in the diabetic mice. This decrease, however, was not significantly different when compared with control animals. Finally, the ratio between the area occupied by B-cells and their absolute number was higher in diabetic (168.84±42.92) than in control (99.7±18.58) mice, although not statistically significant.

Table 1 Volume and number of islets in control and transgenic mice (each value represents the mean±SEM, the number of animals is shown between parentheses)

	Islet volume ($\mu m^3 \times 10^4$)	Islet number (per 10^6 μm^2)
Control (4)	72±15	5±0.4
Transgenic (4)	24±1.5	2.5±0.3
P value	<0.05	<0.01

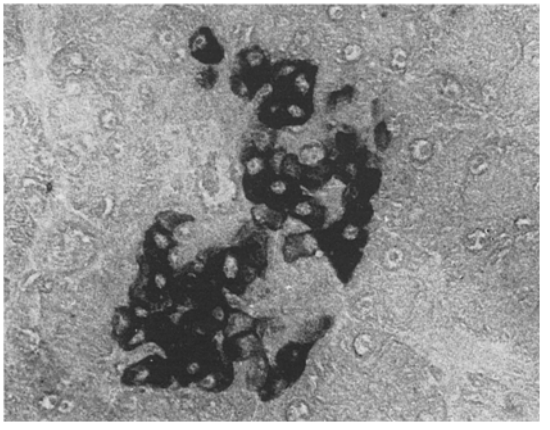


Fig. 3 An islet from a diabetic mouse showing disturbed architecture, in a section stained for immunocytochemical identification of insulin. Immunoperoxidase technique, × 385

Table 2 Volume density of endocrine pancreas and islet cells in control and transgenic mice (layout as for Table 1, *NS* not significant)

	Endocrine pancreas ($\times10^{-2}$)	B-cells ($\times10^{-1}$)	A-cells ($\times10^{-1}$)	D-cells ($\times10^{-1}$)	PP-cells ($\times10^{-1}$)
Control (4)	4.07±0.76	6.93±0.82	2.04±0.35	0.81±0.36	0.22±0.10
Transgenic (4)	1.05±0.23	3.80±0.56	4.53±0.57	1.36±0.15	0.30±0.05
P value	<0.005	<0.025	<0.01	NS	NS

Table 3 Percentage and number of islet cells in control and transgenic mice (layout as for Table 1)

	% islet cells/total islet cells				Number of islet cells/10^6 μm^2			
	B-cells	A-cells	D-cells	PP-cells	B-cells	A-cells	D-cells	PP-cells
Control (4)	68.82 ±8.25	21.20 ±3.76	7.96 ±3.55	2.02 ±0.97	440 ±95.39	120 ±18.52	43 ±16.40	11 ±4.66
Transgenic (4)	39.82 ±6.58	44.08 ±5.55	13.15 ±1.78	2.95 ±0.34	68 ±21.97	68 ±8.25	16 ±7.02	5 ±1.73
P value	<0.05	<0.025	NS	NS	<0.025	NS	NS	NS

Discussion

The calmodulin-induced diabetic syndrome developed by Epstein et al. [3] in transgenic mice has been reported in detail. In a preliminary immunohistochemical study of pancreatic islets from two animals, the authors also described a marked reduction in the number of B-cells accompanied by a relative increase of A- and D-cells. In the present report, the changes occurring in pancreatic islets and in their cell population were specifically and quantitatively evaluated in a larger series of control and diabetic mice. The different parameters studied provide a wide morphological survey of the endocrine pancreas at an advanced stage of this diabetic syndrome.

The group of diabetic mice currently studied showed a conspicuous decrease in the number and size of pancreatic islets, which is a common characteristic of human [4, 5, 11, 14] and animal [5, 13] IDDM. We have also seen the alteration in the architecture of some islets as described by Epstein et al. [3]. The low V_{vi} of B-cells in the endocrine pancreas as well as the decrease in B-cell number, either expressed as a percentage of the islet cell types or calculated per area unit of pancreatic tissue, made evident the decrease in B-cell mass. Most of the B-cells from diabetic mice also showed weaker specific staining of the secretory material than those of controls; data which agrees with the biochemical findings described by Epstein et al. [3]. The higher ratio between the area of B-cells and their absolute number found in transgenic mice, though not statistically significant, might represent a possible hypertrophy of individual B-cells to compensate the decrease in insulin-secreting cell mass. The decrease in B-cell mass currently shown together with the lower pancreatic insulin content reported by Epstein et al. [3] may explain the diabetic state present in the calmodulin-transgenic mice.

The values of B-cell size obtained in normal mice are comparable to those described in normal rats by Bonner-Weir [2] employing electron microscopy. Such agreement between data obtained using two different measurement devices – light and electron microscopy – validates our data. The abnormal non-peripheral distribution of non-B-cells found in some of the BIC (Baminsch Cann) islets has also been reported in other animal models and in humans with IDDM [1, 11].

In our diabetic mice both V_{vi} and the percentage of non-B-cells increased but this change was only significant in the case of A-cells. Similar – though not identical – changes have been already described in human chronic IDDM [5] and in animal models with spontaneous [1, 6, 7] or induced [8, 9] diabetic syndrome. However, a decrease in A- and D-cell number in the BB rat [16] and in D-cell mass in the Chinese hamster [10] has also been reported.

A uniform decrease in the number of all of the different types of islet cells was also observed (Table 3). It can thus be assumed that the ratio between islet cell degradation:neoformation is altered to favour the former process. The magnitude of such a decrease was uneven: while the B-cell number dropped to 15%, the non-B-cells underwent an almost 50% diminution. The fact that only the decrease in the number of B-cells was significant suggests that in these diabetic transgenic mice, diminution of the endocrine mass of the pancreas mainly affects the B-cell sector. This alteration is the major cause of the diabetic state in our experimental model.

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