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Pancreatic pathology in non-insulin dependent diabetes (NIDDM)

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Abstract

NIDDM is a heterogeneous disease and subgroups of NIDDM include MODY (Maturity Onset Diabetes of the Young), Malnutrition-related diabetes (MRDM) and Fibrocalculus pancreatic diabetes (FCPD). Endocrine cell population is relatively unchanged in NIDDM: B-cells are reduced by up to 30% and A-cells increased by 10%. Islet amyloid is found in 96% of subjects occupying up to 80% of the islet associated with a reduction in B-cells. Amyloid formation is unlikely to cause diabetes but progressive accumulation increases the severity of the disease. Islet amyloid is formed from the islet amyloid polypeptide (IAPP), a normal constituent of B-cells, co-secreted with insulin. The causal factors for IAPP fibrillogenesis are unknown but abnormal synthesis or overproduction could be involved: stimulation of B-cell secretion in NIDDM by obesity, hyperglycaemia or suphonylurea therapy may promote amyloidosis and further aggravate islet pathology. A mutation of the glucokinase gene in MODY leads to diminished B-cell secretion but not amyloid formation. Diabetes and mutations of mitochondrial DNA is associated with poorly developed islet structure. Exocrine pancreatic size is reduced and there is evidence of sub-clinical chronic pancreatitis in NIDDM. In MRDM and FCPD, chronic pancreatitis and exocrine necrosis is associated with reduced insulin secretion. Unlike cystic fibrosis where islet amyloid is present in diabetic individuals, amyloid is absent from subjects with FCPD. Pathological changes in the exocrine and endocrine pancreas in NIDDM results from and contributes to the pathophysiology of insulin secretion in NIDDM.

Keywords: NIDDM pancreatic islet; Pathology; Pancreatitis; Amyloid; β-Cell mitochondrial mutations

1. Introduction

NIDDM is a heterogeneous condition without a well-defined aetiopathology: the pathophysiology includes increased peripheral insulin resistance and abnormal β-cell function. However, the contribution that each of these factors make to the disease is difficult to estimate from physiological measurements and is variable in different individuals and at different stages of the disease. Various subgroups of NIDDM have been de-

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scribed which have particular features. These include MODY (Maturity Onset Diabetes of the Young) [1], malnutrition-related diabetes (MRDM) and Fibrocalculus pancreatitis diabetes (FCPD) [2,3]. In addition, diabetes is associated with other conditions such as cystic fibrosis (CF) [4], haemachromotosis, chronic pancreatitis [5] and mutations in mitochondrial genes [6].

Many of these conditions are associated with changes in pancreatic or islet structure which can be related directly to some of the clinical symptoms, e.g. chronic pancreatitis and calcification in FCPD. However, changes in islet structure can only be assessed at post-mortem examination which does not permit correlation of islet morphology with the onset and progression of NIDDM. Furthermore, abnormalities in the biochemical function of β-cells may not result in any morphological change: these include alterations in glucose sensing due to mutations in the glucokinase gene in MODY, inadequate vascular perfusion, aberrant nervous control of insulin secretion, poor energy supply in β-cells or incomplete proinsulin processing.

2. Quantitative changes in islet cells in NIDDM

Unlike insulin-dependent diabetes (IDDM), insulin secretion is preserved in NIDDM at the onset of the disease but is inappropriate for the level of plasma glucose. Abnormalities in the numbers of islets and β-cells in patients with NIDDM has been suggested as possible contributory factors.

In the normal pancreas, the insulin containing β-cells occupy 60–80% of the islet, the non β-cells and islet capillaries filling the remainder [7]: glucagon containing α-cells, pancreatic polypeptide cells (PP cells) and somatostatin containing D-cells line the islet capillaries. In contrast to rodent islets where capillaries are primarily located at the islet perimeter, human islet capillaries penetrate into the central regions of the islet and are lined with non-β-cells. Quantitative estimates of islet cell populations in NIDDM have been made in post-mortem pancreas and compared with non-diabetic age matched subjects [7–10]. Results show that the islet β-cell population in NIDDM is similar [9,10] or reduced by up to 30% [7]. A small but significant increase in the number of α-cells has also been detected [8–10]. The significance of only a 30% reduction in β-cells in relation to substantially decreased islet function in NIDDM is unclear : a 95% partial pancreatectomy is required to induce diabetes in rodents and dogs [11] but hyperglycaemia is present in baboons when β-cell mass is reduced to 50% by streptozotocin treatment [12]. It is possible therefore, that in man and primates, functional efficiency of insulin secretion requires a larger population of β-cells than in rodents, and relatively small changes in the β-cell population can affect glucose homeostasis. However, it is likely that defective insulin secretion is induced by factors other than a reduced β-cell mass. However, the most marked change in islet structure found in NIDDM was the presence of amyloid deposits in the islets [7,13–17]. Islet amyloid was found in 0.5–80% of the islets as small perivascular deposits or larger masses occupying up to 86% of the islet [7,13]. Amyloid deposition is associated with a reduction in the endocrine cell population [7].

3. Islet amyloid and NIDDM

Islet amyloidosis in diabetes was originally described in 1901 by Opie [18] and has subsequently been shown to be a characteristic feature of NIDDM in many studies of post-mortem pancreas [13–17]. The amyloid deposits consist of amorphous fibrillar masses which lie between the islet capillaries and endocrine cells (Fig. 1a). The plasma membrane of β-cells adjacent to the amyloid have deep invaginations filled with amyloid fibrils, [19,20] (Fig. 1b). This irregularity of the β-cell membrane could impair normal function of glucose signalling and insulin release at this region of the cell. Islet amyloid has been found associated with diabetes in every ethnic group so far examined: the 60–80% prevalence in Caucasian subjects [7,16,17] is similar to that seen in Japanese [8], Asian, Indian [15] and in Pima Indians [14]. This is in comparison with a prevalence of islet amyloid of less than 20% in age-matched non-diabetic groups [17].

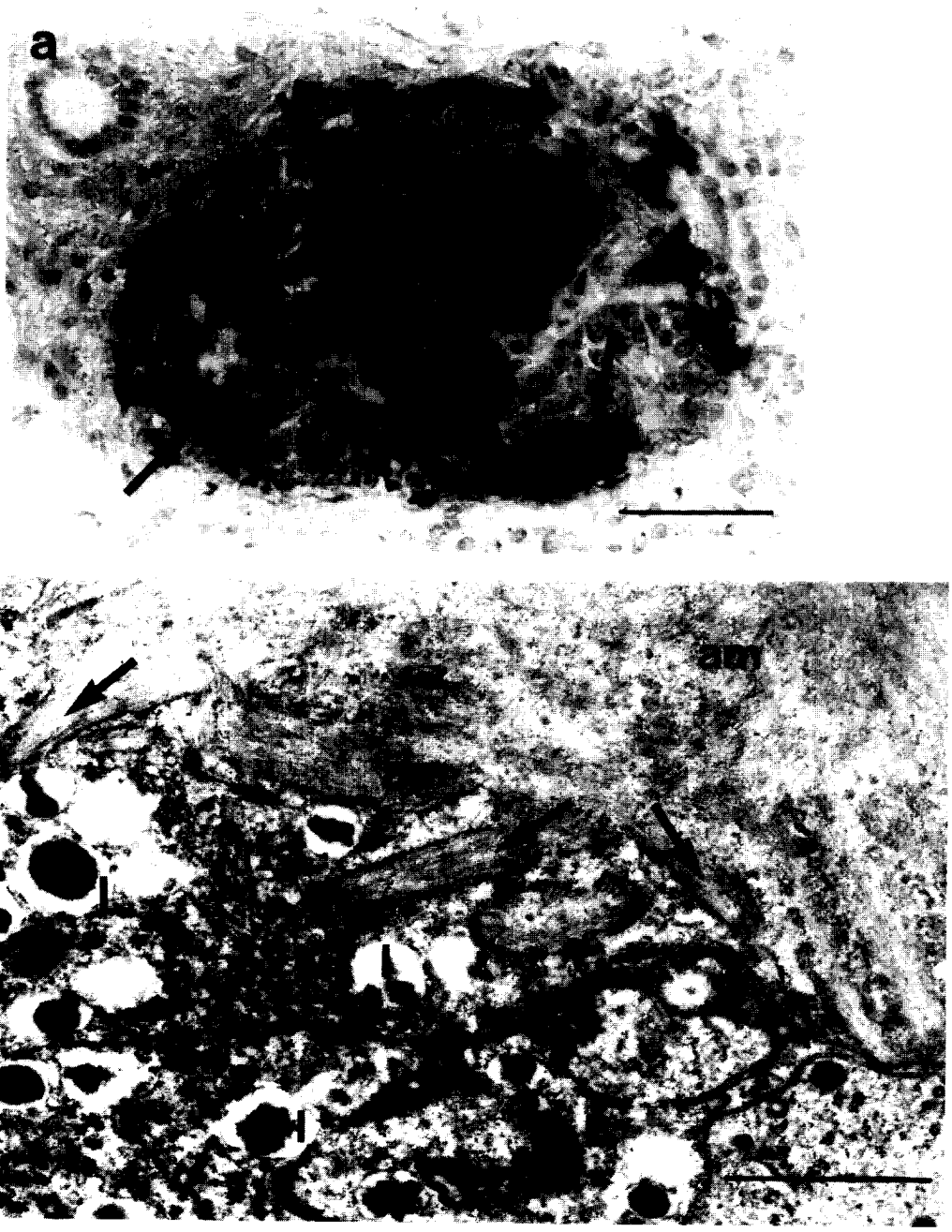

Fig. 1. (a) Amyloid deposit in an islet from patient with NIDDM. Amyloid formed from islet amyloid polypeptide (IAPP) is immunoperoxidase labelled for IAPP (arrows). Amyloid occupies approximately half of the islet and is situated between the endocrine cells (e) and islet capillaries (arrowheads) Scale Bar = 50 μm. (b) Electron micrograph of amyloid in an islet of patient with NIDDM. Beta cells (B) containing insulin granules (I) are adjacent to amyloid deposits. Fibrillar amyloid (am) is present in invaginations of the plasma membrane of the beta cell (arrows) and are likely to disrupt the function of the membrane in glucose signalling and insulin release. Scale bar = 1.0 μm.

4. Amyloidogenic factors

The factors causing islet amyloidosis are unclear. Amyloid fibrils are formed from islet amyloid polypeptide (IAPP) also known as 'amylin' [20,21]. IAPP which is a 37 amino acid polypeptide is co-localised and co-secreted with insulin from pancreatic β-cells [22,23] and is present in β-cells of all species examined so far [24]. The mechanisms responsible for the conversion of the soluble IAPP into insoluble fibrils are unknown. Possible factors for this conversion in diabetes include expression of an abnormal molecular structure of IAPP, overproduction of the normal peptide, alterations in the biosynthesis of IAPP or interactions with chaperone proteins [25]. However, abnormalities in the IAPP gene have been examined in diabetic individuals and no mutations or linkage of polymorphisms in the IAPP gene with NIDDM have been found [26,27]. Diabetes-associated amyloid is found only in man and older cats and monkeys and not in rodent models of NIDDM [28]. This is explained by species-specific alterations in the amino acid sequence in the region of the molecule, IAPP, which determines the amyloidogenic properties of $IAPP_{20-29}$. The finding of extensive islet amyloidosis in hypersecreting human insulinomas [30] and islets of Pima Indians where insulin resistance is an important feature of NIDDM [14], suggests that oversecretion of β-cell products is a factor in creating fibrils from IAPP.

The relationship of islet amyloid formation to the onset and progression of NIDDM is difficult to assess in man, since damaging pancreatic biopsies would be required. Amyloid formation precedes the onset of hyperglycaemia in monkeys and cats, and increases with the progression of the disease [31–33]. More extensive amyloid deposits are present in patients with more severe islet dysfunction as shown by the requirement for insulin therapy [34]. Macaca mulatta monkeys in captivity exhibit a diabetic syndrome with similar features to that seen in NIDDM in man; older animals become obese, insulin resistant, hyperinsulinaemic and eventually diabetic. Quantitative morphometry of pancreatic tissue from animals sacrificed at different stages in the progression of the syndrome demonstrated the relationship of the development of amyloid to the progression of the disease in this model of NIDDM [33]. Initially small perivascular amyloid deposits were present in islets of obese normoglycaemic insulin resistant animals; these amyloid deposits surrounding islet capillaries could impair transfer of glucose and insulin to and from islet circulation and contribute to glucose intolerance. Islet amyloidosis was more extensive in the diabetic animals occupying up to 80% of the islet, and β-cells were reduced by 80–90%. It appears that, in this species, amyloidosis is progressive and associated with destruction of islet cells and a minimum critical mass of β-cells is required to maintain normoglycaemia.

The lack of a convenient laboratory model has hampered research into the cause of islet amyloid formation. Recently, transgenic mice have been created which express the gene for human IAPP under control of the rat-insulin 2 gene promoter [35]. In these animals, human IAPP (hIAPP) is produced in mouse β-cells and co-secreted with insulin. The circulating concentrations of IAPP in these mice are 2–10 times higher than in non-transgenic mice [35] but the animals were not obese, hyperinsulinaemic or diabetic. These data do not support the hypothesis that over production of IAPP leads to insulin resistance and hyperglycaemia. Amorphous accumulations of IAPP were found adjacent to capillaries in islets of hIAPP transgenic mice up to the age of 86 weeks and could represent the early stages of amyloid deposition *in vivo* [36]. However, fibrillar amyloid was formed from human IAPP *in vitro* when islets isolated from hIAPP islets were cultured in the presence of glucose [37]. The extent of amyloid formation was found to be roughly equivalent to the degree of hIAPP production; fibrils were formed after 2 days culture in 16.7 mM or 28 mM glucose but required 7 days when exposed to 8 or 11 mM glucose. Fibrils were localised within and between islet β-cells suggesting that overexpression, overproduction and/or decreased clearance of IAPP from the islet intercellular spaces are contributory factors for amyloid formation.

5. Occurrence of islet amyloid

The relationship of amyloid deposition to β-cell

function in diabetes has not been confirmed but some information can be deduced from the different clinical conditions in which amyloid is found (Table 1). Human insulinomas are characterised by unregulated secretion of insulin and other β-cell products and abnormalities in the processing of proinsulin [38]. It is therefore likely that proIAPP is also abnormally processed in tumour β-cells. The presence of amyloids in islets of patients with diabetes secondary to cystic fibrosis [39] suggests that the diabetes in this condition is related to NIDDM rather than to IDDM but the causal factors for the deposits are unclear. Patients with end-stage renal failure on dialysis treatment have very high concentrations of plasma IAPP (> 20 pmol/l) which are reduced following dialysis [40]. The high prevalence of islet amyloid in these patients suggests that poor clearance of IAPP from the circulation (and therefore the islet) is associated with polymerisation of IAPP to form fibrils [41]. High levels of secretion of IAPP could also explain extensive islet amyloid deposition in a patient with severe insulin resistance induced by anti-receptor antibodies [42]. Amyloid was not found in the pancreas of an elderly patient with Maturity Onset Diabetes of the Young (MODY) whose diabetes was associated with a mutation in the glucokinase gene. The mild form of diabetes in this condition is due to a reduction in glucose sensing by the β-cell and normal insulin production; the absence of amyloid in this patient supports the hypothesis that amyloid formation is related to β-cell hypersecretion.

The hypothesis that overproduction of IAPP leads to amyloid formation in NIDDM is not supported by measurements of plasma IAPP in diabetic and non-diabetic subjects. IAPP is co-secreted with insulin in a molar ratio of insulin: IAPP 10–100:1 and is present at plasma concentrations ranging from 2–15 pmol/l in fasted subjects [23,25,43]. No dramatic elevations of plasma IAPP concentrations have been found in patients with NIDDM although some obese subjects appear to have higher circulating levels of IAPP [44,45]. However, concentrations of IAPP in peripheral blood may not be an accurate representation of changes in concentrations in the islet and a proportion of secreted IAPP could remain in the islets as fibrils in NIDDM.

6. NIDDM and pancreatitis — FCPD and MRDM

Hyperglycaemia is associated with a form of chronic exocrine disease, so-called 'Tropical Diabetes'. Fibro-Calculus pancreatitis Diabetes (FCPD) is a widespread condition in Southern India [3]; severe chronic pancreatitis develops often before the age of 35 years accompanied by formation of calcified nodules in pancreatic ducts.

Table 1
Occurrence of islet amyloid

Condition	amyloid	Possible cause
90% NIDDM subjects	+ + +	?
10% Elderly non-diabetics	+	'prediabetes'
50% Human insulinomas	+ + +	overproduction/abnormal synthesis IAPP
Diabetic cystic fibrosis	+	?
ESRF and Dialysis	+	decreased clearance IAPP
Anti insulin receptor antibodies	+ + +	hypersecretion IAPP
Pre-diabetic cats and monkeys	+ +	hypersecretion IAPP
IDDM	no	absence of β-cells
Rodent diabetes	no	lack of fibrillogenic IAPP
MODY (glucokinase mutation)	no	(normal β-cell secretion poor glucose sensing)
Pancreatitis	no	?
FCPD	no	?

ESRF = end stage renal failure with dialysis treatment
MODY = maturity-onset diabetes of young
FCPD = Fibrocalculus pancreatitis diabetes

As the exocrine tissue atrophies, islets become surrounded with fibrous tissue (Fig. 2). However, the cellular structure of the islets remains relatively unchanged and amyloid deposition does not appear to be a feature of FCPD (Clark and Yajnik, unpublished data). Malnutrition-Related Diabetes Mellitus (MRDM) is designated as a separate diabetic syndrome [2] but its clinical presentation can be very similar to that of FCPD and/or NIDDM. Many patients in different regions of the tropics suffer from diabetes related to poor nutrition and both MRDM and FCPD are typically found in lean individuals [47,48]. The most severe form of exocrine disease associated with diabetes is seen in cystic fibrosis (CF) when exocrine tissue is completely replaced by fibrous and fatty material [4]. Pancreatic islets are however intact and remain suspended in a matrix of adipose and connective tissue. The islets retain the normal complement of endocrine cells except in older CF patients who develop diabetes associated with islet amyloidosis [39]. The inter-relationships between the exocrine and endocrine pancreas is not completely clear. However, a functional interaction must exist since disease in one compartment affects the function of the other — many patients with NIDDM have reduced exocrine function [49]. It is possible that pancreatitis damages the islet vascular and nerve supply and affects islet function resulting in hyperglycaemia.

7. Mutations in mitochondrial DNA and NIDDM

Recently, defects in mitochondrial genes (mtDNA) have been related to diabetes. In particular, pedigrees with maternal transmission of NIDDM and deafness possess a mutation in mtDNA [50]. The same mitochondrial mutation is also involved in a separate clinical condition — mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) [6].

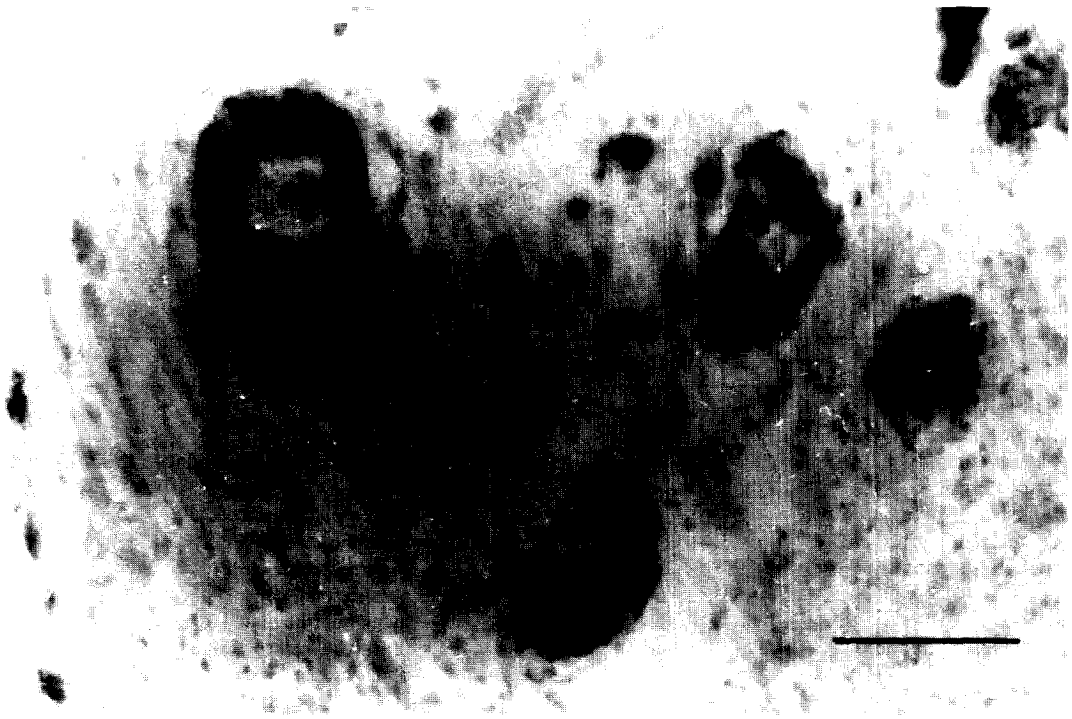

Fig. 2. Pancreatic islets in a patient with FCPD. Islets cells immunolabelled for insulin (arrows) are present in the islets. The islets are suspended in a matrix of connective tissue (ct) resulting from chronic pancreatitis and exocrine atrophy. Scale bar = 100 μm.

Glucose-stimulated insulin secretion is an ATP-dependent process and aberrant mitochondrial function in β-cells could impair insulin production. Other conditions involving more severe rearrangements and deletions of mitochondrial genes (e.g Kearns-Sayre Syndrome KSS) are also associated with glucose intolerance or diabetes [46]. In KSS, islet shape and cellular composition are not characteristic of IDDM although the clinical features are similar (young age of onset of insulin-dependent diabetes). This morphological difference could provide clues to the relationship of changes in mtDNA and islet dysfunction. Pancreatic islets in IDDM are irregular in shape and largely filled with non β-cells. In comparison, islets in KSS are devoid of β-cells but are small and regular in comparison to the apparently 'collapsed', irregular shape of islets in IDDM. This could be explained by the following hypothesis. Abnormal mitochondrial function in KSS would impair foetal and neonatal development of the normal population of β-cells: insulin secretory capacity from a reduced number of β-cells would be adequate to maintain normoglycaemia during the early years of life, but not sufficient for particular phases of growth. During these stages, the small population of β-cells would gradually be lost without major changes to the islet architecture, leaving a regular shaped islet. Further studies on tissue from patients with MELAS and other mitochondrial abnormalities are required to confirm this hypothesis.

8. Summary

Pathological changes in pancreatic islets provide evidence that impairment of β-cell function is likely to be an early event in the onset of NIDDM: small changes in the numbers of β-cells could influence the insulin secretory capacity in NIDDM. Deposition of islet amyloid is a common marker for the heterogeneous clinical features of the NIDDM syndrome. Progressive islet amyloidosis is associated with a reduction in β-cells and increased severity of the clinical features as shown by the need for insulin therapy. The aetiology of NIDDM associated with pancreatitis in 'tropical diabetes' is unclear. Mutations in mitochondrial genes could affect both the development and function of islet β-cells leading to hyperglycaemia.

The heterogeneity of the pancreatic pathology and clinical features of patients with NIDDM suggests that a variety of therapeutic agents are required: therapies directed towards potentiating β-cell secretion (of both insulin and IAPP) may promote amyloidosis and further aggravate islet pathology and early diagnosis and treatment of pancreatitis could reduce the prevalence of NIDDM in equatorial countries.

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