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Formation of amyloid in human pancreatic islets transplanted to the liver and spleen of nude mice

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ABSTRACT

In previous studies we have shown that apparently normal human islets, transplanted under the renal capsule of nude mice, frequently and rapidly develop amyloid deposits derived from the β-cell hormone islet amyloid polypeptide (IAPP). In the present study, we show for the first time that human islets, transplanted into the liver or spleen of nude mice, also develop islet amyloid rapidly. Ultrastructural studies of such islets showed that the first aggregation of IAPP takes place within the β-cells and that extracellular deposits show up later in the amyloid formation process. We also found that the amount of amyloid formed in human islet grafts placed under the kidney capsule increased with extended (26 weeks) observation time. Moreover, prolonged in vitro culture (14 days) prior to the implantation under the renal capsule seemed to enhance the formation of amyloid in the grafted islets. Since aggregated IAPP has been shown to be toxic to β-cells, the finding of amyloid deposits in transplanted islets offers a possible explanation to the frequent loss of function of islets transplanted into diabetic patients.

INTRODUCTION

Deposition of islet amyloid is the most consistent morphological alteration found present in type 2 diabetes and is seen, to some degree, in the pancreas in more than 95% of individuals with this disease (1). Although islet amyloid was described more than 100 years ago (2, 3), its possible importance for the development of diabetes has only recently become accepted. Several studies have shown that deposition of islet amyloid is associated with a decreased number of islet β-cells (4–6). Also, many remaining β-cells are morphologically injured, since their cell membranes are penetrated by bundles of amyloid fibrils when in contact with amyloid (7).

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Islet amyloid is an aggregation product of the β-cell hormone islet amyloid polypeptide (IAPP, amylin) (8–10). The reason why the 37 amino acid residue peptide IAPP forms fibrils in type 2 diabetes is incompletely understood, but in vitro synthetic human IAPP (hIAPP) has an intrinsic property to assemble into amyloid-like fibrils (11, 12). This is unlike mouse IAPP, which is lacking this property. Therefore, islet amyloidosis does not occur in wild-type mice. Given the strong tendency of IAPP to aggregate *in vitro*, one may raise the question whether inhibitors against aggregation exist *in vivo.* Insulin or proinsulin may be such physiological inhibitors (13).

There are several lines of evidence that islet amyloidosis is important for the development of a β-cell lesion in type 2 diabetes. First, individuals who have failed on oral antidiabetic drugs have a more severe islet amyloidosis than those who respond well to these drugs (1,14). Second, in a spontaneous monkey form of diabetes, islet amyloid develops before diabetes becomes manifest (15). Third, several groups have generated hIAPP-overexpressing transgenic mice and when these animals develop islet amyloidosis, this is followed by diabetes (16, 17).

We have previously shown that amyloid derived from IAPP develops rapidly and in a high percentage in apparently normal human islets transplanted under the renal capsule of nude mice (18). There was no difference whether the recipient animals were hyperglycemic or not. Islet transplantation in human patients is usually performed by infusion of a large number of donor islets via the portal vein (19). Therefore, we have studied whether the site of implantation in nude mice affects development of islet amyloidosis. Further, given our finding that amyloid deposits occur rapidly in transplanted islets, we have studied the effects of long term transplantation on the development of amyloidosis. Finally, we have investigated whether in vitro culture of islets prior to transplantation generates increased amyloid deposits.

MATERIAL AND METHODS

Details of the preparation of human pancreatic islets have recently been described (18, 20, 21). Briefly, islets were isolated from the pancreata of organ donors at the Central Unit of β Cell Transplant in Brussels. After a couple of days in culture they were sent to Uppsala where they were cultured for another 1–5 days in culture medium RPMI 1640 containing 5.6 mmol/l glucose. Transplantation of islets under the renal capsule of non-diabetic nude mice (Bomhaltgaard, Ry Denmark) has also been described previously (21). Implantation of human islets into the liver and spleen of nude mice was carried out essentially as described before with mouse islets (22). The animals were fed standard chow and had access to drinking water ad libitum. The animals were killed by cervical dislocation and organs of interest were removed immediately and fixed for light or electron microscopy.

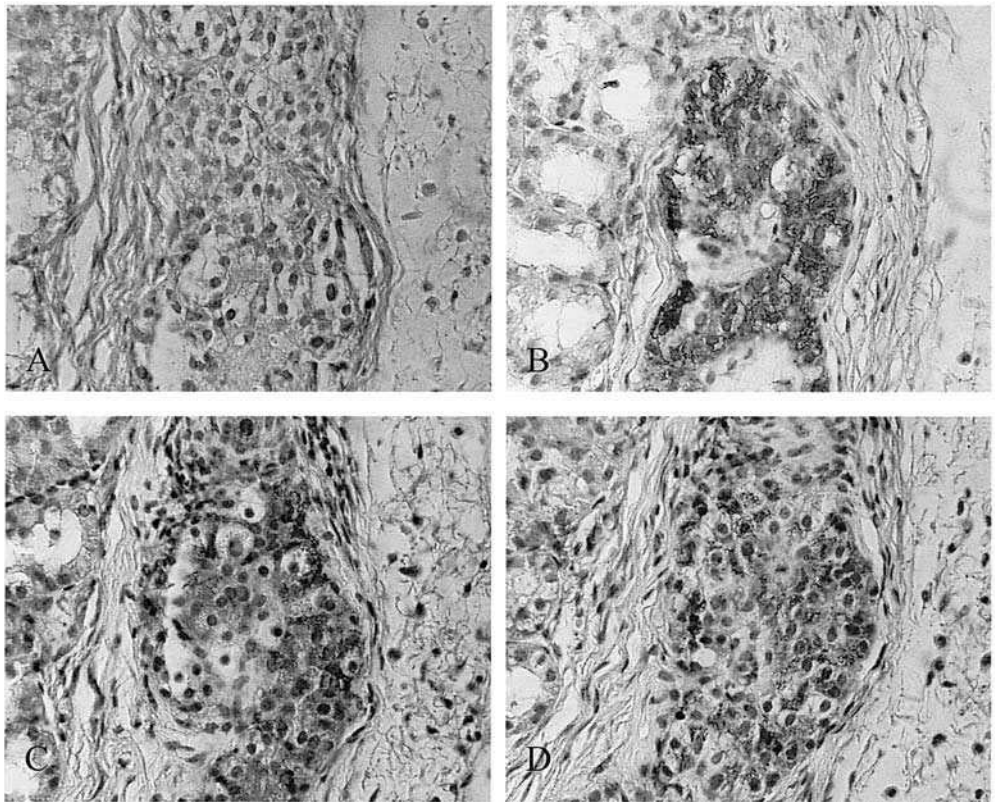

Fig. 1. Sections of a human islet transplant placed under the renal capsule of a nude mouse 4 weeks post-transplantation stained with Congo red (A). Multiple amyloid deposits (red) are evident. Insulin, IAPP and C-terminal proIAPP flanking peptide immunoreactivity is demonstrated in B, C and D, respectively. (X 500)

Microscopy

Whole spleen, liver and kidneys were fixed over night in 4% buffered neutral formaldehyde solution. Whole spleens and liver lobes were embedded in paraffin. Islets grafted under the renal capsule were visible at ocular examination and therefore, the tissue was cut out before embedding. About 5 mm thick sections were mounted on glass slides for H&E staining, alkaline Congo red staining (23) or for immunohistochemistry.

Immunohistochemistry

The presence of IAPP immunoreactivity was detected with rabbit antiserum A110 raised against mouse/rat IAPP. This antiserum crossreacts completely with human IAPP (24). Rabbit antiserum A133, which is specific to human IAPP (16), was also used. Antiserum to the C-terminal flanking peptide of proIAPP was raised in a rabbit by the use of a synthetic peptide corresponding to amino acid residues 52–67 of

proIAPP (25). Insulin antiserum was purchased from DAKOpatts (Täby, Sweden). The biotin-avidin detection system with 3,3'-diaminobenzidine tetrahydrochloride as substrate was used for visualization of the immunohistochemical reaction product.

Electron microscopy

Small pieces of the subcapsular renal islet transplants were fixed in a mixture of 2% paraformaldehyde and 0.25% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, and embedded in Unicryl (British Biocell, Cardiff, UK). Ultrathin sections, mounted on formvar-coated nickel grids, were immunolabelled with rabbit IAPP antisera A133 or A110, diluted 1:200 as described (26). Bound antibodies were visualized with 10 nm protein A-gold labeled swine anti-rabbit antibodies (British Biocell) and the sections were studied in a Jeol 1200 electron microscope (Jeol, Tokyo, Japan).

Effects of in vitro *culture of human islets*

In order to study whether pre-culture of human islets enhanced amyloid formation, islets from two donors were either cultured for two weeks and then implanted under the renal capsule of nude mice or were implanted directly. The islets were cultured in medium RPMI 1640 containing 5.6 mmol/l glucose for 14 days prior to transplantation. Cultured islets at day 0 and day 14, and islet transplants were all fixed and sectioned for light and electron microscopy as described above.

Effects of time on amyloid occurrence

In order to study the possible progression of islet amyloid deposition, four nude mice received human islets from two different donors under the renal capsule. They all had access to normal diet and water ad libitum and were killed after 26 weeks. The transplants were fixed and embedded for light microscopy.

RESULTS

Transplantation sites

Kidney. The appearance of amyloid in human islets transplanted under the renal capsule of non-diabetic nude mice has previously been described (18). The finding of predominantly small, weakly stained and apparently intracellular congophilic deposits was verified in the present study (Fig. 1A). All of the 7 studied animals had amyloid deposits in their islet transplants 4 weeks post-transplantation. The islets, at transplantation, on an average contained 58% β-cells and were from 4 different donors with a mean age of 38 years. Labeling of the transplants with antibodies against insulin resulted in an even staining of a majority of cells (Fig. 1B). In contrast, immunolabelling with antibodies against IAPP or the C-terminal flanking peptide of proIAPP showed a more irregular cellular labeling (Fig. 1C and D).

Liver. Four to five weeks after transplantation islets were identified in liver sec-

Table 1. Amyloid in human islets transplanted to the liver

Mouse No.	Amyloid (pos/total)	Human donor pancreas No. donor	Age of % (years)	β-cell
1	1/3*	3	40	42
2	2/2	3	40	42
3	3/5	3	40	42
4	2/5	4	25	55
5	0/4	4	25	55
6	0/0	5	50	25
7	1/3	6	27	55
8	3/5	7	46	50
9	1/1	7	46	50
10	1/3	8	25	68

* Number of islets with amyloid/total number of identified islets

tions of 9 of the10 implanted animals. The number of islet sections varied from 1 to 5 in each individual mouse. Amyloid, exhibiting affinity for Congo red and typical green birefringence after this staining, was found in 8 of the 9 livers in which islets were found (Table 1) (Fig. 2A). 31 islets were recovered in the sections and of these, 14 (45%) contained amyloid deposits. The islets with amyloid came from 6 different donor pancreata (aged 25–50 years) and on an average they contained 50 % β-cells. The amyloid occurred as distinct and mainly extracellular areas. The deposits often had a globular shape but thin streaks, obviously following a surface, were also found. Furthermore, the deposits were almost always multiple with many spots dispersed throughout the islets. Although there were often some lymphocytes in the tissue surrounding the islets, no inflammation was seen associated with the amyloid.

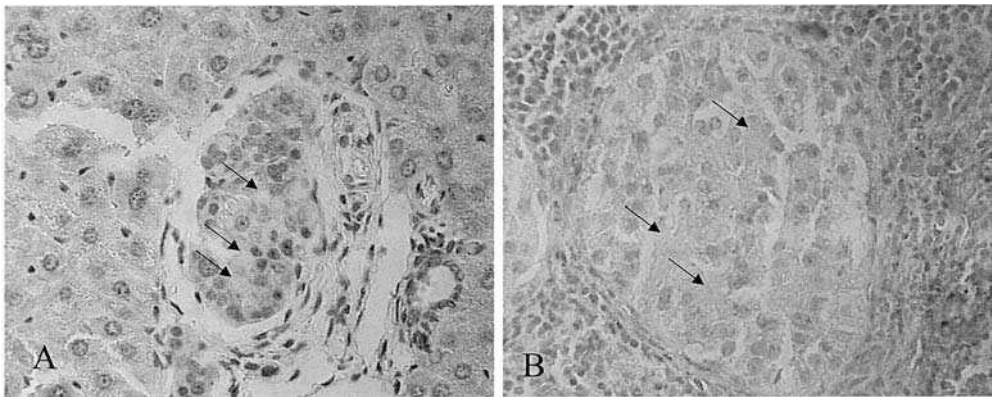

Fig. 2. Congo red staining of a liver section containing one human islet (A) and a splenic section, containing human islets (B). There are several extracellular amyloid deposits (arrows). (X 500)

Spleen. In three mice, human islets prepared from two different donors (21 and 60 years old; β-cell content 61 and 60%) were transplanted into the splenic parenchyma. Islet tissue was found in all tissue sections examined. In all of these (12/12), amyloid deposits were found (Fig. 2B). The amyloid had the same appearance as described above for human islets implanted into the liver.

Effects of prolonged transplantation time

In general, subcapsular renal islet transplants, which were harvested after 2 weeks, contained small amyloid deposits. Such deposits were only weakly stained with Congo red and showed an extremely faint green birefringence. Much of this early amyloid was intracellular. In contrast, amyloid in islets, which had been prepared from two different donors (46 and 53 years old; β-cell contents 46 and 60%) and had been kept under the renal capsule for 6 months, was apparently extracellular. All four islet grafts contained amyloid. The amount varied and was less pronounced in one graft, while three grafts contained scattered and rather large deposits. These deposits had affinity for Congo red and exhibited a comparably strong green birefringence. Apparent intracellular amyloid was not seen in any of these four transplants.

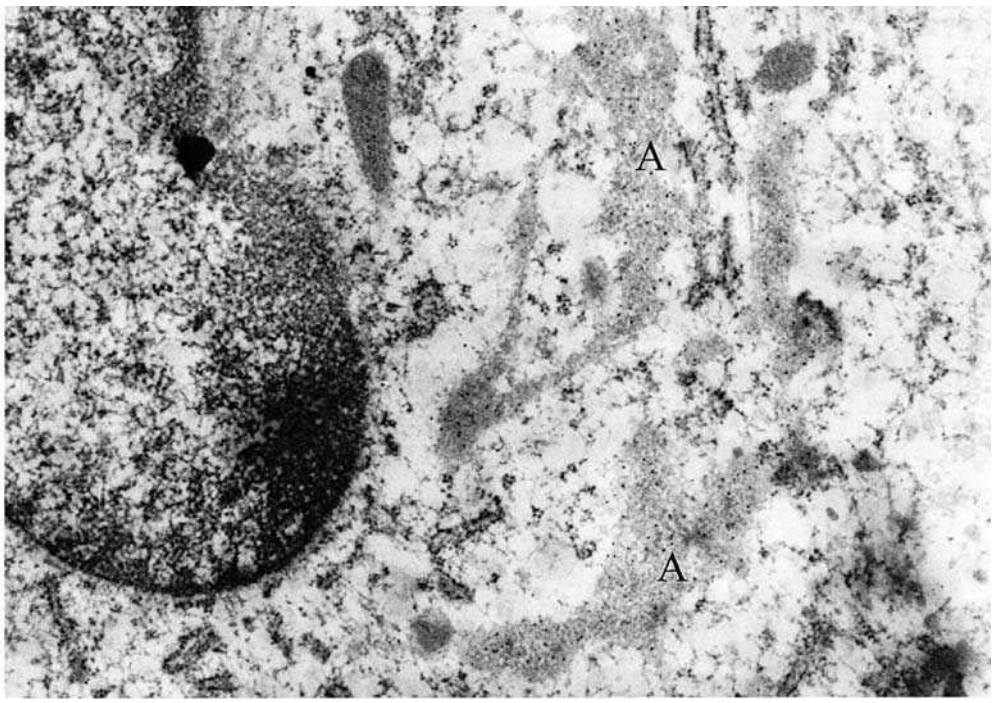

Fig. 3. IAPP immunoreactive material with faint fibrillar appearance, within a β-cell in a subcapsular human islet transplant. The compartment containing the IAPP material has not been defined. The cell seems to be disintegrating.

Table 2. Occurrence of amyloid in transplanted islets some of which had been cultured *in vitro* before transplantation

	Duration of culture in Uppsala		Islet transplant day 14	
	Day 0	Day 14	Non-cultured	Cultured 14 d
Donor 1	0	0	1+	2+*
Donor 2	0	0	2+	3+

*The amount of amyloid was determined semiquantitatively based on light and electron microscopy.

Ultrastructure of intracellular IAPP aggregation

Aggregation of IAPP within β-cells, which had resided in the kidney for a short period of time (14 d), was seen as a network of fine wavy fibrils, often thinner than mature amyloid fibrils. Quite often, the fibrillar nature of the material was not very obvious. The immunoreactive material sometimes formed larger areas in the cytoplasm but the cellular compartment was impossible to determine. In some almost completely degranulated cells, an IAPP immunoreactive material was found in irregular, sometimes tubular and branching structures. Although the exact nature of these structures could not be determined, they resembled endoplasmic reticulum and/or Golgi vesicles (Fig. 3). Many cells with IAPP deposits were almost completely degranulated. Cells, in or at which no amyloid was present, had the strongest IAPP immunoreactivity in the periphery of the granule cores. The edge of these cores was often associated with radiating fibrillar material. Such material was, however, frequently immunoreactive for IAPP.

Effects of culture of human islets prior to transplantation

By light and electron microscopical means islets from the two donors (58 and 33 years old; β-cell contents 38 and 60%) were found to be devoid of amyloid material at days 0 or 14 of in vitro culture. The effect of culture on the formation of amyloid in vivo was examined by implantation of the two different preparations of islets (non-cultured and 2-wk-cultured) into nude mice. Transplants of islets, which had been cultured for 14 days, contained considerably more amyloid than islets implanted without preceding culture (Table 2). Electron microscopically, virtually all of this amyloid was found intracellularly in almost completely degranulated β-cells.

DISCUSSION

Clinical transplantation of human islets has always been performed by infusion of the isolated islets through the portal vein. This procedure usually leads to a significant production of insulin in a recipient essentially devoid of own insulin-producing cells (19). However, a fairly constant problem has been a decline in the insulin production, at least in experimental studies (27). The reason for this is not clear.

We have previously shown that IAPP-amyloid develops in human islets transplanted under the renal capsule of nude mice (18, 26). Also in this study we found that very frequently, such islets develop IAPP-derived amyloid, first found intracellularly but later as extracellular deposits. The present study also shows that amyloid develops to the same extent in islets transplanted into the spleen or to the liver through the portal vein. This is a potentially important finding, since one putative mechanism in the declining insulin production of grafted human islets would be a β-cell injury through formation of IAPP-amyloid. Moreover, it is possible that this model is relevant for investigations of the pathogenesis of islet amyloidosis in a broad sense.

It has often been claimed that islet amyloid probably is of no causative importance in a decreasing insulin production, since there are individuals with type 2 diabetes with little islet amyloidosis. However, recent studies have indicated that mature amyloid fibrils probably not are causative for the cell death but rather immature IAPP oligomeric aggregation products (sometimes called protofibrils) (28). Such aggregation products do not bind Congo red well enough to be demonstrated at the light microscopical level.

The pathogenesis of islet amyloid is not clear. In order to shed some light on this process, transgenic mice overexpressing hIAPP have been produced. Such animals do, however, not generally develop islet amyloidosis (29–31) even when mated with *ob*/m+ mice or made hyperglycemic by partial pancreatectomy (32). Hyperglycemia, per se, does not induce islet amyloidosis, unless there is a very pronounced overexpression of hIAPP. On the other hand, IAPP-amyloid develops when hIAPP-transgenic islets are kept in culture (32, 33). We found no amyloid in the nontransplanted human islets, that had been cultured for 14 days. However, 14 days after transplantation such islets contained more amyloid compared to those, which had been transplanted without preceding prolonged *in vitro* culture. Further studies are needed in order to clarify further this finding. If indeed amyloid deposits are formed also in clinically grafted human islets the present data should be interpreted to suggest that the time in culture before the implantation should be kept at a minimum. It should be kept in mind that one reason for the extraordinary success of the Edmonton group might be their short time of *in vitro* storage (less than 6 hours) before injecting the human islets (19).

Formation of amyloid is a nucleation-dependent process (34) and seeding a solution of amyloid protein with preformed fibrils greatly enhances fibrillogenesis (35, 36). We have proposed earlier that the first amyloid, formed intracellularly, should act as a nidus for further, extracellular deposition (24, 37). Consequently, we had expected that the early amyloidogenesis occurring in subcapsular renal islet transplants should have led to very heavy deposits after 6 months. Although the deposits after that time tended to be larger and mainly extracellular, heavy deposition as seen in type 2 diabetes, was not found. Whether this depends on a reduced expression of IAPP has to be studied further.

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