Differences in Amyloid Deposition in Islets of Transgenic Mice Expressing Human Islet Amyloid Polypeptide Versus Human Islets Implanted Into Nude Mice

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Islet amyloid polypeptide (IAPP)-derived amyloid is frequently deposited in the islets of Langerhans in patients with chronic non–insulin-dependent diabetes mellitus (NIDDM). When human islets were implanted under the renal capsule in nude mice, amyloid occurred in 73% of the grafts within 2 weeks. In this study, we compare the deposition of amyloid in islets from a transgenic mouse strain expressing human IAPP (hIAPP) and in normal human islets after implantation in nude mice. The implantations were performed as follows: (1) nondiabetic recipients were given islets from transgenic mice alone, (2) human islets were implanted in the upper pole of the kidney and islets from transgenic mice were implanted in the lower pole of the kidney, (3) grafts containing a mixture of human and transgenic islets were implanted, and (4) transgenic islets and islets from nontransgenic littermates were implanted in therapeutic numbers into recipients made diabetic by a single injection of alloxan prior to implantation. The implants were removed after various periods from 4 days to 8 weeks. The implants were either fixed in Formalin, stained for amyloid, and viewed in polarized light, or processed for immunoelectron microscopy and studied after immunolabeling with specific antibodies against IAPP. We found that the course of amyloid deposition differed significantly between human islets and hIAPP-expressing mouse islets. In human islets, amyloid was mainly deposited intracellularly and only small amounts of amyloid were found extracellularly. In contrast, in islets from transgenic mice, amyloid was exclusively deposited extracellularly and deposition in this site was preceded by an aggregation of immunoreactive material along the basement membrane. These findings point to separate mechanisms for amyloid formation in these two models.

ISLET AMYLOID POLYPEPTIDE (IAPP, amylin) is a 37–amino acid polypeptide purified both from amyloid deposited in human insulinoma and from islet amyloid deposited in association with human non–insulin-dependent diabetes mellitus (NIDDM).[1-3] IAPP is the major constituent of amyloid deposited in the islets of Langerhans. Islet amyloid appears to a varying degree in 95% of individuals with NIDDM and to a minor extent in pancreata from older nondiabetic subjects.[4-5] In human islets, the expression of IAPP is restricted to β cells, where it is stored in the halo region of the insulin granules[6] and released together with insulin.[7] IAPP-derived amyloid has never been detected in rodents, and this depends on interspecies variations in the amino acid sequence of IAPP. Important differences occur at position 20 to 29, where a certain sequence in this region—GAILS—is responsible for the ability of IAPP to form amyloid fibrils.[8]

The pathogenesis of islet amyloid is not well understood. In addition to an amyloidogenic IAPP amino acid sequence, other mechanisms must be involved. However, the process has been difficult to study due to a lack of good experimental models. Recently, amyloid deposition was described in normal human islets after implantation under the renal capsule of nude mice,[9] where IAPP-immunoreactive amyloid occurred in 73% of the implants already after 2 weeks. In that study, the main portion of amyloid was present intracellularly and not extracellularly as described for pancreata recovered at autopsy.[10] However, the amount of islet amyloid varied, and in some cells the cytoplasm was virtually replaced by amyloid.

Since the amount of human material is restricted and IAPP-derived amyloid does not occur in rodents, different groups have generated transgenic mice to facilitate the study of the human IAPP molecule and the effect of IAPP-derived amyloid on the islets of Langerhans.[11-15] Recently, amyloid has been described in vivo in two of the mouse strains expressing human IAPP (hIAPP) and in association with hyperglycemia.[15,16] The time required for the first amyloid to occur in these animals exceeded 20 weeks and was usually much longer.

Therefore, we wanted to compare the development of amyloid deposits in renal subcapsular islet grafts derived from IAPP transgenic mice with grafts derived from normal human islets as a model system. We also investigated if there are any differences in the accumulation of amyloid in islets derived from female versus male mice, and also the impact of hyperglycemic stress on the grafts using alloxan-diabetic nude mice as recipients.

MATERIALS AND METHODS

Mouse Pancreatic Islets

Islets were isolated as previously described[17] from males and females of a transgenic mouse strain expressing hIAPP. The transgene consists of the rat insulin I promoter and a 7.7-kb genomic DNA fragment containing the gene coding for human preproIAPP. Female and male mice aged 30 to 40 weeks and bred to homozygosity were used. The number of hIAPP gene copies was eight after amplication of transgenic mouse DNA and human DNA with human-specific IAPP primers. For

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this, the polymerase chain reaction product was separated by agarose gel electrophoresis and stained with ethidium bromide, and the OD was measured (data not shown). Plasma levels of circulating IAPP in these animals were eight to 10 times higher than in the nontransgenic controls.

The characteristics of this strain (L1006) have been described previously.[12,18] In some experiments, islets from nontransgenic litters were used as well.

Transgenic Mouse Islet Culture

Islets from transgenic and nontransgenic mice were maintained in culture in RPMI 1640 medium containing 10% fetal calf serum for 2 days before transplantation. Islets from seven transgenic mice were isolated and cultured separately as before, and after 2 days, half of the islet material from each mouse was harvested and the remaining islets were kept in culture for an additional 10 days. These islets were embedded for electron microscopy.

Human Pancreatic Islets

Human islets from heart-beating organ donors were isolated at the Central Unit of the b-cell Transplant in Brussels and then transported by air to Uppsala. This procedure has been described in detail [19,20] The islets were kept in culture for 4 to 18 days prior to transplantation (Table 1).

Transplantation

For each transplant, an average of 200 human or transgenic mouse islets were implanted under the renal capsule of male nude mice (nu/nu; Bomholtgaard, Ry, Denmark).[9] The following experiments were performed.

(1) Transgenic islets derived from female mice were injected bilaterally in the upper pole of the kidneys of eight nude mice. The transplants were harvested from day 4 to day 28 and processed for light or electron microscopy (Table 2).

(2) In one group of 15 nude mice, diabetes was induced by intravenous injection of alloxan (75 mg/kg) 1 week before transplantation. Of these, six animals received one transplant of islets isolated from male transgenic mice. The transplant was harvested after 4 or 8 weeks (Table 3). The remaining nine animals received one transplant of islets from transgenic female or male mice in the upper pole and another transplant of islets from nontransgenic mice in the lower pole of the same kidney. The islet grafts were harvested after 2 or 8 weeks and embedded for electron microscopy (Table 3).

(3) A group of eight animals received a human islet transplant in the upper pole and a transgenic male mouse islet transplant in the lower pole of one kidney. The transplants were harvested after 2 weeks and embedded for electron microscopy (Table 4).

(4) A total of seven mice received a mixed graft containing equal amounts of human islets and islets from male transgenic mice in the upper pole of one kidney The transplants were harvested after 2 or 4 weeks and embedded for electron microscopy (Table 5).

Table 1. Characteristics of Human Pancreatic Islets Used for Transplantation

Pancreas No *	Donor Age (yr)	Days in Tissue Culture	β-Cell Fraction (%)
131	15	5	44
132	49	4	52
134	36	12	64
167	41	16	37
168	28	18	58
169	29	14	46

*Refer to human donor.

Table 2. Islets From Female Transgenic Mice Transplanted to the Upper Kidney Pole in Nude Mice and Studied at Electron Microscopic Level After Immunolabeling With hIAPP-Specific Antibodies

Implant No	Amyloid Grade	Duration of Implant (d)
1	0	4
2	0	7
3	0	7
4	4	10
5	12	14
6	8	14
7	18	21
8	14	28

Blood glucose levels were determined (Exatech glucose meter, Baxter Travenol, Deerfield, IL) in blood taken from the retroorbital plexus, and the animals were killed by cervical dislocation. The animals had free access to standard animal chow and water and were kept on a 12-hour light/dark cycle.

Light Microscopy

The implant with surrounding kidney tissue was fixed in 10% neutral buffered Formalin and embedded in paraffin. Sections of 10-μm thickness were viewed in polarized light for the presence of amyloid after staining with Congo red.

Immunoelectron Microscopy

A thin slice of the graft-bearing kidney was fixed in 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4, containing 0 1 mol/L sucrose. After fixation, the graft was excised, dehydrated in alcohol, and embedded in Unicryl (Biocell, Cardiff, UK) at 4°C. Rabbit antiserum against mouse C-peptide[21] was used at a dilution of 1·600. Antiserum against IAPP (A110)[22] was raised against rat IAPP 1-37 and shows reactivity with both hIAPP and mouse

Table 3. Transplants of Islets From Transgenic Mice in the Upper Kidney Pole of Alloxan-Diabetic Nude Mice

Implant No.*	Amyloid Grade	Duration of Implant (wk)
1	12	2
2	12	2
3	14	2
4	4	2
5	18	8
6	0	8
7	16	8
8	10	2
9	6	2
10	20	4
11	8	4
12	4	4
13	20	8
14	4	8
15	14	8

NOTE. In addition, mice no. 1-9 received a transplant from nontransgenic mice in the lower pole of the same kidney Islets were studied at the electron microscopic level after immunolabeling with hIAPP-specific antibodies.

*No. 1-7 from female donors and no. 8-15 from male donors.

Table 4. Islets From Humans and Male Transgenic Mice Implanted as Separate Grafts Into One Kidney of Nude Mice and Studied After 2 Weeks for the Presence of Amyloid at the Electron Microscopic Level After Immunolabeling With hIAPP-Specific Antibodies

Implant No.*	Upper Pole	Amyloid Grade	Lower Pole	Amyloid Grade
1	131	8	TI	10
2	131	6	TI	4
3	132	12	TI	0
4	132	8	TI	16
5	134	12	TI	10
6	134	4	TI	10
7	134	NR	TI	6
8	134	16	TI	4

Abbreviation: NR, not recovered.
*Refers to human donor.

IAPP. The IAPP antiserum A133 was raised against a synthetic peptide corresponding to position 20 to 29 of the hIAPP molecule, and this antiserum only recognizes hIAPP.[18] Both IAPP antisera were raised in rabbits and used at 1:200 dilution. The IAPP and C-peptide immunoreactions were detected by 10-nm gold particles labeled with swine anti–rabbit immunoglobulin (Biocell). The sections were contrasted with uranyl acetate and lead citrate and studied using a JEOL 1200 electron microscope (JEOL, Tokyo, Japan). To estimate the amyloid content in each implant, sections from five separate levels from each implant were investigated. From each level, 10 individual 10.000-mm^2 areas were analyzed, and the amyloid content was graded as 1 to 10 depending on the number of areas that contained amyloid. The amyloid grade corresponds to the number of amyloid-containing areas in all five levels investigated from each implant, thus giving a possible variation between 0 and 50.

RESULTS

In Vitro Culture of Transgenic Mouse Islets

Amyloid did not appear in transgenic mouse islets after 2 days in culture. In islets harvested after 12 days, amyloid was present in five of seven samples. Transgenic islets were kept in culture for 2 days prior to implantation. Therefore, we concluded that extracellular amyloid deposition in the implants occurred during the implantation period.

Transgenic Islets in Nondiabetic Recipients

Eight nondiabetic nude mice received two implants each of islets from transgenic female mice (Table 2). In each animal, the implant from the lower pole was processed for light microscopy and the upper-pole implant was processed for immunoelectron microscopy. A small amyloid deposit was found after Congo red staining in one of the implants removed after 14 days. All other implants were negative in this respect. In the implants processed for immunoelectron microscopy, the β cells appeared granulated and antisera against IAPP reacted with β-cell granules in all grafts. In implants harvested after 7 days or more, additional labeling of IAPP was observed in an amorphous substance found extracellularly along the basement membrane (Fig 1). In grafts removed after 14 and 21 days, IAPP immunoreactive amyloid-like fibrils were also found in close proximity to the β-cell membranes (Fig 2A and B). There was a significant correlation between the time and amount of electron microscopically estimated amyloid ($r = .91$, $P < .002$).

Table 5. Equal Amount of Human and Male Transgenic Mouse Islets Implanted as a Mixed Graft Into the Upper Pole of One Kidney of Nude Mice

Implant No *	Amyloid Grade	Duration of Implant (wk)
1 167/TI	6	2
2 167/TI	10	2
3 167/TI	0	2
4 167/TI	0	2
5 168/TI	4	4
6 168/TI	8	4
7 168/TI	6	4

NOTE. Islets were studied at electron microscopic level after immunolabeling with hIAPP-specific antibodies.
*Refers to human donor.

Transgenic Islets in Diabetic Recipients

Diabetes was induced in 15 nude mice (Table 3), and in all animals, blood glucose levels exceeded 21 mmol/L at the time of implantation. All animals recovered from the diabetic state by the time of graft harvest. The mean nonfasting blood glucose level 2 weeks after implantation was 5.2 mmol/L (range, 2.0 to 7.2), and in the group studied 4 weeks or more after implantation, the mean level was 4.5 mmol/L (range, 3.2 to 6.1). The fact that diabetic mice with only transgenic islets also became normoglycemic after the implantation may indicate that these particular islets are fully competent from a functional point of view.

There was no difference in immunolabeling with antiserum

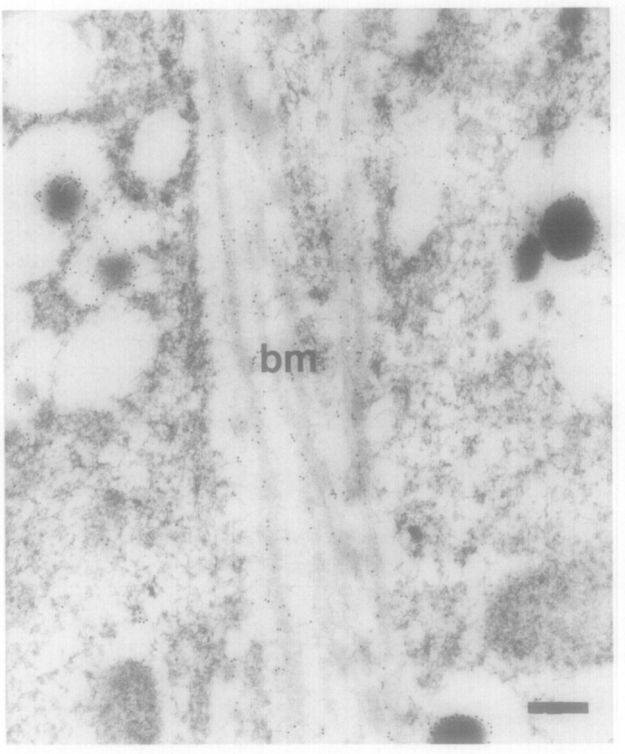

Fig 1. Immunolabeling with antibodies against IAPP appears extracellularly along the basement membrane (bm) in transgenic mouse islet 14 days after implantation (antiserum 133; bar, 500 nm).

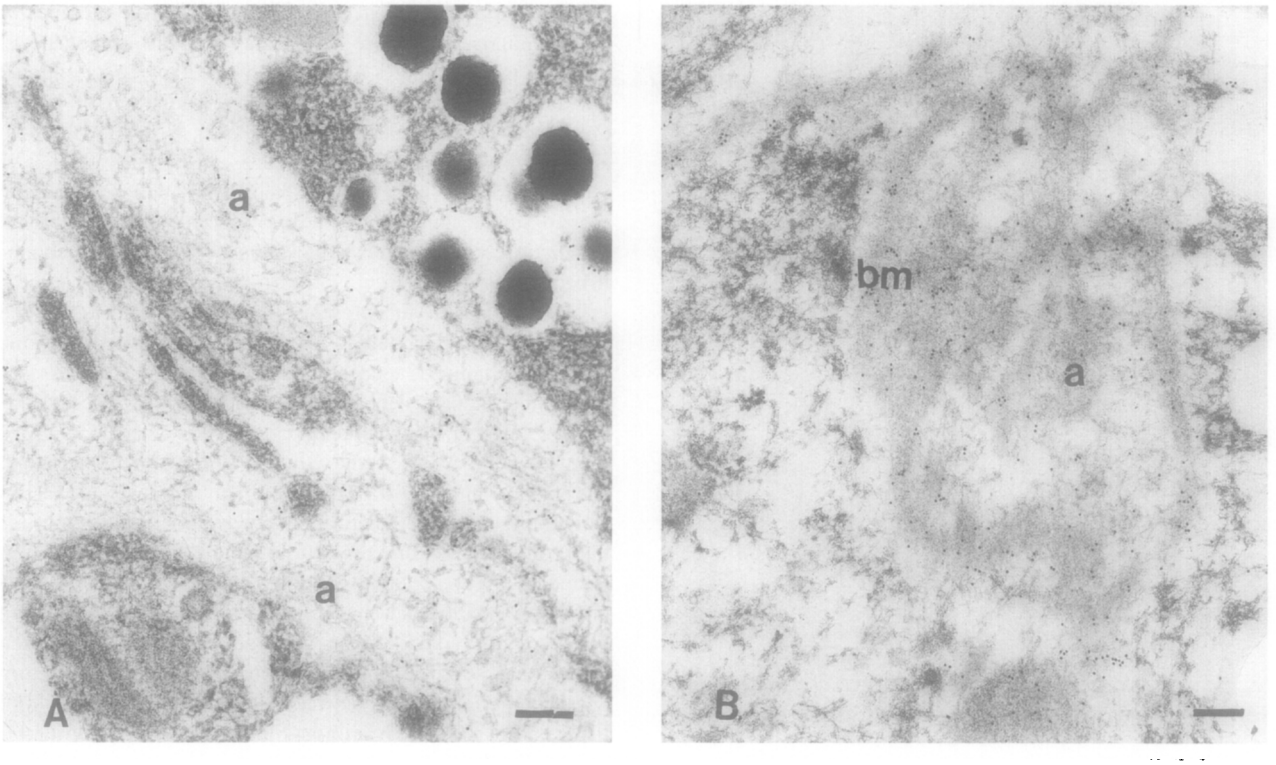

Fig 2. Extracellular amyloid (a) labeled with antiserum 110 deposited in a transgenic mouse islet 14 days after implantation. Basement membrane (bm) surrounds the amyloid in B (bar: A, 200 nm; B, 500 nm).

against IAPP between implants residing in alloxan-diabetic and nondiabetic recipients. Only the implants from transgenic animals showed immunolabeling with the human-specific IAPP antiserum and extracellular amyloid was found as in nondiabetic animals. There was no clear correlation between the time the implant resided in the recipient and the amount of amyloid deposited. The amount of amyloid was not significantly increased compared with islets implanted into nondiabetic recipients. No difference in the amount of amyloid was found between islets prepared from different sexes. The additional nontransgenic islet implant to the lower pole in mice no. 1 to 9 did not decrease the amyloid frequency in the upper-pole implants containing transgenic islets.

Grafts of Animals Receiving Both Human and Transgenic Islets

Seven of eight human islet implants were recovered (Table 4), and in four of these, extensive deposition of intracellular amyloid occurred already 2 weeks postimplantation (Fig 3). Extracellular amyloid was rare in the human islets (Fig 4). In some of the human islet implants, β cells were considerably degranulated. None of the transgenic mouse islet implants showed any intracellular amyloid, but immunoreactive IAPP associated with small deposits of amyloid appeared along the basement membrane as described before.

Mixed Islet Transplants

It is possible to differentiate between human and transgenic mouse islets, since IAPP is present in mouse but not in human δ cells. Furthermore, mouse C-peptide antiserum does not react

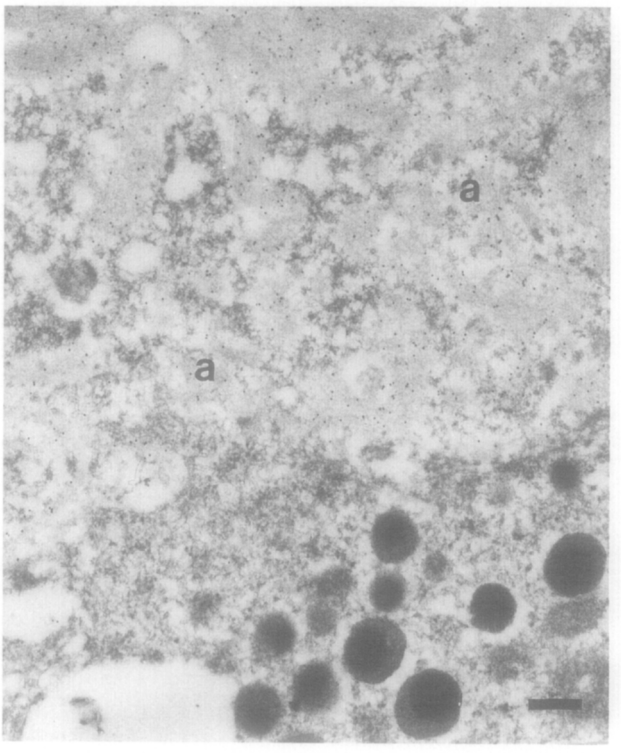

Fig 3. Intracellular amyloid (a) deposited in the β cell of a human islet implanted to the upper pole of the kidney and removed after 2 weeks (antiserum 110; bar, 500 nm).

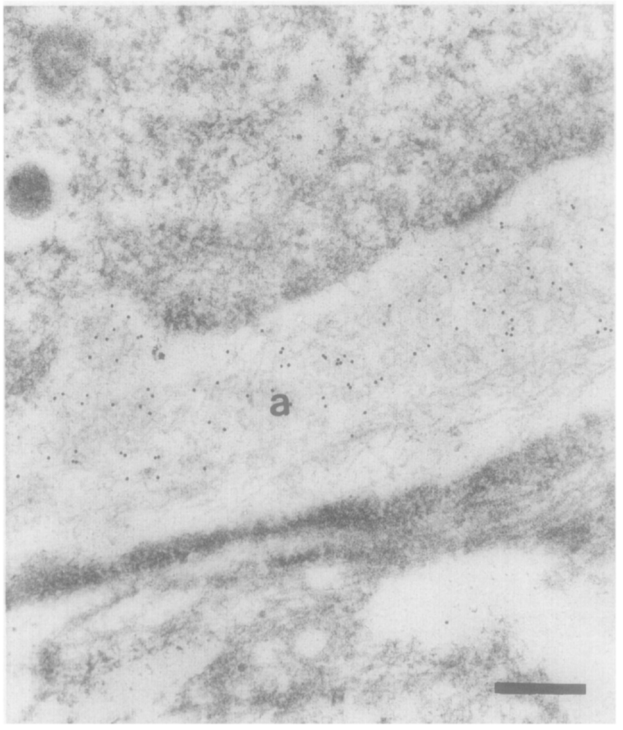

Fig 4. Extracellular amyloid (a) deposited in a human islet. The graft was removed after 2 weeks and immunolabeled with antiserum 133 (bar, 200 nm).

with human β cells. β Cells from human and mouse islets were arranged in close contact with each other (Fig 5). Intracellular amyloid was found in the human islets of the mixed implants. Despite the increased amount of IAPP produced by transgenic islets, no further amyloid seemed to be deposited extracellularly in these grafts. Human β cells appeared less degranulated in the mixed implants compared with the situation where human and mouse islets were localized separately in the kidney.

DISCUSSION

hIAPP is a strongly amyloidogenic molecule. Some degree of islet amyloidosis is found in at least some islets in greater than 95% of individuals with NIDDM, but it is also commonly found in scattered islets in the pancreas of elderly nondiabetic individuals but is uncommon in younger individuals. Islets from each human donor were used for multiple implants, and in agreement with a previous study,[9] amyloid was found to some degree in implants from all donors. The age of the donors, between 15 and 63 years (mean, 39), did not influence the rate of amyloid formation. Given the rapidly deposited amyloid in the human islet transplants, easily found by light microscopy, the sparse amount of amyloid usually only detected by electron microscopy in transplanted islets from transgenic mice was surprising. However, the same mouse strain does not spontaneously develop islet amyloidosis even at a late age.[12]

Why transplanted human islets so rapidly form amyloid while transgenic mouse islets, expressing even more hIAPP, do not is difficult to understand, but may reflect an important mechanism in amyloidogenesis. Theoretically, the presence of nonamyloidogenic mouse IAPP may hinder fibrillogenesis, although in vitro studies have not indicated any effect of equal amounts of mouse IAPP on the fibril formation of hIAPP (Westermark P, unpublished result, 1995). However, the possible in vivo impact on fibrillogenesis of the coexpression of mouse IAPP and hIAPP is not known but will be tested by crossing-in the human transgene into a recently developed IAPP knockout mouse (Gebre-Medhin S and Westermark P, unpublished results, 1998).

The findings in the present study further underscore the differences between human islets and islets from transgenic mice concerning the tendency for IAPP amyloid fibril formation. While human islets regularly exhibited pronounced intracellular amyloid, no such precipitations were detected in any of the various mouse islet grafts studied here. On the other hand, extracellular amyloid was more common in transplanted islets from transgenic mice. There are many possible explanations for these differences. Formation of amyloid fibrils has been suggested to occur as an off-pathway event in protein folding-unfolding.[23] Molecular chaperones involved in protein-folding pathways[24] may differ in humans and mice. It has also been shown that several components present in β-cell secretory granules that may differ between species strongly affect fibril formation in vitro.[6,25] Insulin and proinsulin are strong inhibitors of fibrillogenesis, while Ca^{2+} and Zn^{2+} act as promoters. Finally, oversecretion of IAPP may be important. Induction of

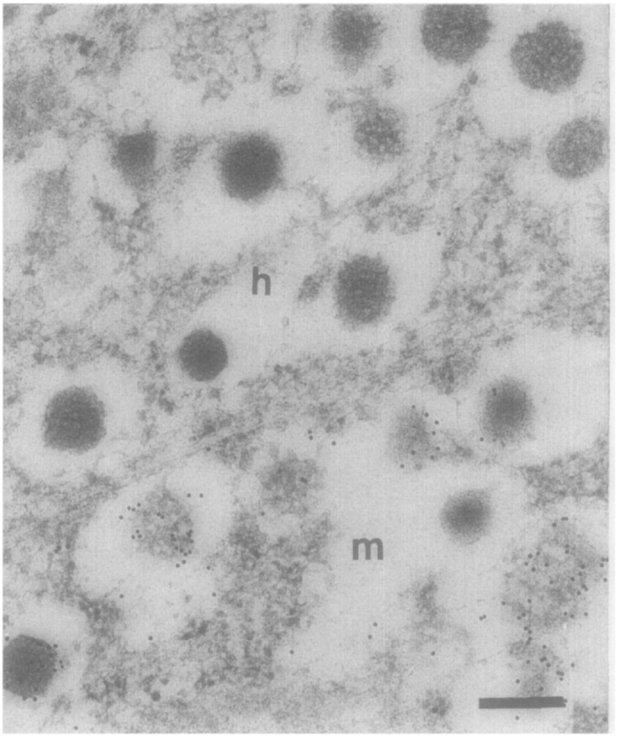

Fig 5. Immunolabeling with antiserum specific to mouse C-peptide reveals the close connection between mouse (m) and human (h) β cells in the mixed implants. The mixed graft was implanted to the upper pole of the kidney and removed after 4 weeks (bar, 500 nm).

islet amyloid has been found in some experimental models in which β cells have been stimulated to an increased release of IAPP. Thus, in a transgenic mouse strain and in the cat, islet amyloid occurred after administration of growth hormone and dexamethasone.[26,27]

The experiment with mixed islets aimed to study the possible catalytic effect of small amyloid deposits in human islets on hIAPP secreted from transgenic mouse islets. A seeding mechanism is believed to strongly accelerate the process of fibrillogenesis[28] in all types of amyloid. As soon as the first fibril has formed, elongation occurs rapidly. Such a mechanism would explain the long lag phase in most types of amyloid disorders, and could also explain the common finding of single islets with heavy amyloid deposits in a pancreas otherwise devoid of islet amyloid (unpublished observation). However, such synergistic interaction was not observed, and this observation suggests that a more complex mechanism is involved in amyloid formation in human islets.

Extracellular matrix components have been chemically and immunologically shown to be associated with other amyloid proteins.[29,30] Young et al[31] have shown that the basement membrane component heparan sulfate proteoglycan is present in islets with IAPP-derived amyloid. The increased immunolabeling with antibodies against IAPP in the vicinity of the basement membrane in transgenic mouse islets could be the result of an increased molecular interaction between IAPP and the basement membrane in this species. This interaction of IAPP might result in higher local IAPP concentrations and facilitate extracellular fibrillogenesis formation. This apparently does not occur in human islets to the same extent.

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