Virchows Archiv A Pathol Anat (1992) 421:339–344

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistology of islet amyloid polypeptide in diabetes mellitus: semi-qantitative studies in a post-mortem series*

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Received March 14, 1992 / Received after revision May 29, 1992 / Accepted June 1, 1992

Summary. Immunoreactivity for islet amyloid polypeptide (IAPP) in the islets of Langerhans of non-insulin-dependent diabetic patients and non-diabetic patients of a non-selected post-mortem series was studied with a new polyclonal IAPP antibody. Out of 133 patients examined, 124 exhibited immunoreactivity for IAPP. Immunoreactivity was localized intra- and extracellularly and was limited to the islets of Langerhans. No extracellular immunoreactivity was observed in amyloid-negative cases. Co-localization of insulin and IAPP in the same islet-cells was verified by double staining with monoclonal insulin and polyclonal IAPP antibodies. Of 100 patients with non-insulin-dependent diabetes mellitus (NIDDM) and islet amyloid, 98 exhibited IAPP-positive deposits and 71 exhibited intracellular immunoreactivity. Evaluation of intracellular immunoreactivity and degree of islet amyloid deposition in cases of overt NIDDM revealed an inverse relationship, in that intracellular IAPP immunoreactivity were reduced in patients with developing islet amyloid deposition. Our data are consistent with the hypothesis of primary β-cell dysfunction leading to amyloid formation, with subsequent disturbance of β-cell homeostasis.

Key words: Islet amyloid – Islet amyloid polypeptide – Immunohistochemistry

Introduction

In non-insulin-dependent diabetes mellitus (NIDDM) amyloid deposits in islets of Langerhans are a common, though non-specific, feature (Bell 1952; Ehrlich and Ratner 1961; Seifert 1959; Westermark 1973). The main protein of these deposits has recently been defined and named as islet amyloid polypeptide (IAPP) or amylin (Cooper et al. 1987; Westermark et al. 1987a). The relationship between IAPP-derived islet amyloid and NIDDM is currently of interest. There is, however, still no pathophysiological explanation for this phenomenon (Johnson et al. 1991; Porte and Kahn 1989; Steiner et al. 1991).

Recent immunohistochemical studies have shown different intracellular IAPP immunoreactivity in pancreatic β-cells among patients with or without islet amyloid (Berends et al. 1990; Narita et al. 1992; Toshimori et al. 1991; Westermark et al. 1987b). While strong intracellular immunoreactivity is found in cases without amyloid, immunoreactivity is reduced in β-cells with surrounding amyloid deposits (Westermark et al. 1987b).

The aim of our study was to examine the amyloid deposits in pancreatic islets of patients with NIDDM retrospectively, in a non selected post-mortem series from a General Hospital. We wished to see whether they were IAPP-derived. Using a new polyclonal IAPP antibody, the extra- and intracellular IAPP immunoreactivity in a series of patients with NIDDM was recorded and evaluated semi-quantitatively. In addition, we have investigated the relationship between intra- and extracellular IAPP immunoreactivity in diabetic patients with amyloid deposits.

Materials and methods

Of 2217 patients autopsied between 1986 and 1991, we selected 133 patients with or without clinically overt NIDDM and with or without histologically proved islet amyloid irrespective of age, sex and cause of death (Table 1).

Clinical data on individual cases concerning NIDDM were obtained from hospital records and autopsy reports and included elevated plasma glucose concentration, pathological oral glucose tolerance test, and glucose in the urine. No differentiation was permitted on therapy. Patients without diabetes mellitus had one or several negative tests for glucose in the urine.

Pancreatic tissue was routinely obtained during autopsy from pancreatic tail. The specimens were fixed in 10% buffered formalin and embedded in paraffin. The sections were stained for amyloid using Congo red (Puchtler et al. 1962) and also with haematoxylin and eosin.

* Supported by the Johanna and Fritz Buch-Gedächtnisstiftung (Hamburg, FRG)

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Table 1. Clinical and histological characteristics of the diabetic and non-diabetic patients

	Number of patients	Age at death (years)	Sex (M/F)
NIDDM with islet amyloid	100	80.6 (48–93)	38/62
NIDDM without islet amyloid	11	79.5 (63–95)	3/ 8
no DM with amyloid	10	71.3 (47–90)	5/ 5
no DM without amyloid	12	74.1 (53–86)	5/ 7

NIDDM, Non-insulin dependent diabetes mellitus; DM, diabetes mellitus

The synthetic peptide P_{131} was automatically synthesized with a model 430A peptide synthesizer (Applied Biosystems, Forster City, Calif., USA; according to recommendations given by the supplier). The peptide was isolated by reversed phase C_{18} column chromatography on TSK ODS-120T, 6×250 mm (Pharmacia, Freiburg, FRG) using a gradient of A: 0.1% trifluoroaceticacid (TFA) and B: 0.1% TFA and 80% acetonitrile (reagents from Merck, Darmstadt, FRG). The peptide P_{131} comprises the amino acid sequence from positions 7 to 24 of the human insulinoma IAPP: CATQRLANFLVHSSNNFG (Johnson et al. 1989b). This peptide is easily tranformed into amyloid in vitro and shows Congo red binding and green birefringence (data not shown; see Glenner et al. 1988).

In order to prepare antibodies two rabbits were subcutaneously injected with 250 µg of a conjugate of P_{131} and bovine thyroglobulin (Sigma, Deisenhofen, FRG) with SPDP (*N*-succinimidyl 3-(2-pyridyldithio) proprionate; Pharmacia, Freiburg, FRG) dissolved in 250 µl 0.9% sodium chloride and emulsified with 250 µl complete Freund's adjuvant (Behring Werke, Marburg, FRG). Monthly booster injections were given in incomplete Freund's adjuvant. The rabbits were bled 1 week after each injection. Only one rabbit developed anti-P_{131} antibodies which showed immunohistochemical cross-reaction with the IAPP in formalin-fixed paraffin sections. This antiserum was used for this study.

Immunohistochemical studies were performed using the peroxidase-antiperoxidase or avidin-biotin (ABC) (Vector Laboratories, Burlingame, Calif., USA) methods. Deparaffinized sections were initially incubated with the primary antibodies (amyloid A murine monoclonal antibody, amyloid F antiserum, IAPP antiserum, amyloid lambda light chain antiserum, amyloid kappa light chain antiserum and beta$_2$-microglobulin antibody) for 30 min at room temperature in a moist chamber, except for the IAPP antiserum, which was incubated overnight at room temperature. The procedure and antibody dilutions were the same as described by Wullbrandt et al. (1990). The ABC method was performed with IAPP antiserum (diluted 1:1500). The reaction was visualized with 3,3-diaminobenzidine-tetrahydrochloride (DAB). The counterstain was haematoxylin.

Five cases with diabetes mellitus and islet amyloid and five cases without diabetes mellitus and without amyloid were double stained. Double staining was performed with islet amyloid polypeptide antiserum (see above) and monoclonal insulin antibody from mouse (diluted 1:20; Immunotech, Marseille, France) using the Dako Doublestain kit system 40 (Dakopatts, Hamburg, FRG). The reaction was visualized with DAB (IAPP) and fast red (insulin). The counterstain was again haematoxylin.

Negative controls were performed using keratin antibodies (diluted 1:100; Dianova, Hamburg, FRG) instead of IAPP antiserum, omitting the first antibody. Sections of organs such as spleen, liver and kidney did not show any reaction and could be taken as negative antigen controls. Positive controls were evaluated by comparison of Congo red-stained sections with adjacent immunohistochemical stained sections.

Adjacent sections of pancreatic tissue from each patient were used for staining with Congo red, and with the ABC method for the demonstration of IAPP. All sections were studied in a special Leitz microscope with tension-free optics. The diagnosis of amyloid was made by the presence of green birefringence in polarized light. Histological evaluation was performed with regard to autolysis, number of islets with amyloid deposits, degree of amyloid deposition, number of islets with extracellular IAPP immunoreactivity, number of islets with intracellular IAPP immunoreactivity and intensity of intracellular IAPP immunoreactivity. Congo red and immunohistochemical staining patterns demonstrated the IAPP immunoreactivity of all amyloid deposits.

A grading of the results was performed subsequently on autolysis (minimal, moderate, severe), intracellular IAPP immunoreactivity (negative, weak, intermediate, strong), and on the degree of amyloid deposition: disseminated (some small, dotted or patchy foci), confluent (large curved deposits) and convoluted (extensive, garland-like) deposits.

The degree of islet amyloid deposition in the whole section was evaluted by counting the islets with disseminated, confluent and convoluted amyloid deposits and by subsequent division into three grades: cases with predominantly disseminated (grade I), confluent (grade II), and convoluted (grade III) deposits per section viewed, regardless of total values. The number of islets with amyloid deposits and the number of islets with intracellular IAPP immunoreactivity was catalogued into three groups: less than five islets, six to ten islets, and more than ten islets.

Table 2 summarizes the number of islets with amyloid deposits in cases with overt NIDDM.

Table 2. Number of amyloid-positive islets in diabetic patients

Number of islet with amyloid per section	1–5	6–10	>10
Number of patients (%)	15 (15)	18 (18)	67 (67)

Results

Of 133 patients examined in this study (Table 1), 124 exhibit immunoreactivity for IAPP. The immunoreactivity is limited to islets of Langerhans; none is found in exocrine pancreas. Immunoreactivity for IAPP is localized intracellularly and extracellularly (Figs. 1, 2). Extracellular immunoreactivity is limited to amyloid deposits. IAPP-positive deposits of amyloid were found between the basement membranes of islet cells and the capillaries (Fig. 3).

The distribution pattern of intracellular and extracellular IAPP immunoreactivity is summarized in Table 3. Nine of 11 autopsied patients with clinically overt NIDDM and without islet amyloid exhibit intracellular immunoreactivity for IAPP. No case in this group exhibits extracellular immunoreactivity for IAPP. Six of 10 patients with amyloid deposits in pancreatic tissue without diabetes mellitus exhibit IAPP-immunoreactive amyloid. Four patients had generalized amyloidosis. Amyloid deposition in these 4 cases is localized at different sites and can easily be distinguished from IAPP amyloid. Generalized amyloidosis with deposits in the interstitium and vessel walls of the islets of Langerhans does not stain for IAPP. In contrast, immunohistochemical staining of these 4 cases yielded amyloid of the lambda light chain (2 cases), kappa light chain (1 case), and amyloid A (1 case) (Table 4). Seven of 10 non-diabetic patients with amyloid exhibit intracellular IAPP immuno-

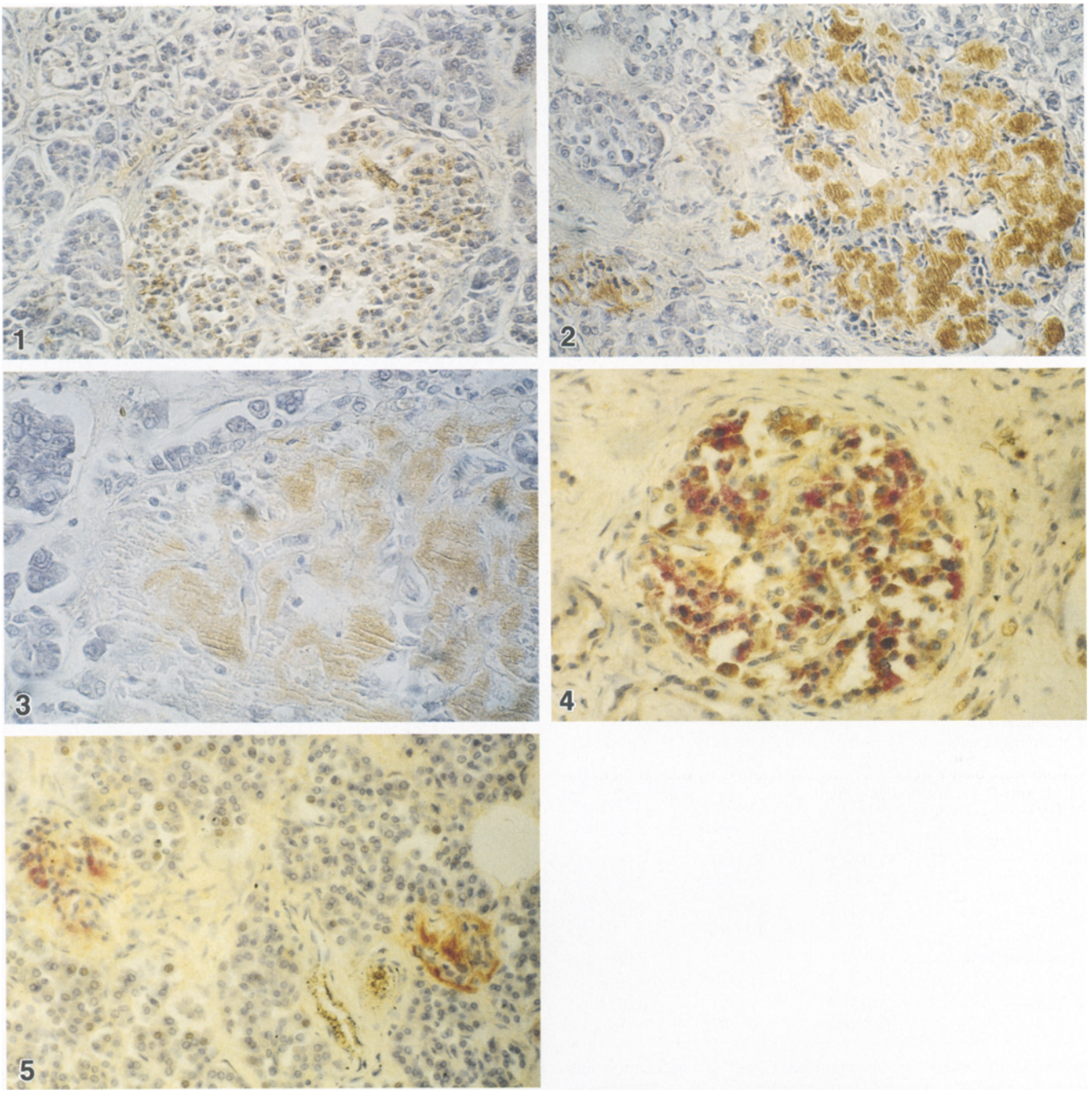

Fig. 1. Intracellular immunoreactivity for islet amyloid polypeptide (IAPP) in islets of Langerhans from a patient with clinical signs of non-insulin dependent diabetes mellitus and IAPP amyloid. IAPP antiserum, ABC, haematoxylin; ×280

Fig. 2. IAPP-immunoreactive amyloid deposits in islets of Langerhans from a patient with clinical signs of non-insulin dependent diabetes mellitus and IAPP amyloid. IAPP antiserum, ABC, haematoxylin; ×280

Fig. 3. IAPP-positive deposits of amyloid between basement membranes of islet cells and capillaries from a patient with clinical signs of non-insulin dependent diabetes mellitus and IAPP amyloid. IAPP antiserum, ABC, haematoxylin; ×450

Fig. 4. Co-localization of insulin (*red*) and IAPP (*brown*) in the same islet cells from a patient with clinical signs of non-insulin dependent diabetes mellitus and IAPP amyloid. IAPP antiserum, monoclonal insulin antibody, double stain, haematoxylin; ×350

Fig. 5. Negative extracellular and positive intracellular insulin immunoreactivity in two islets of Langerhans from a patient with non-insulin dependent diabetes mellitus and IAPP amyloid. For staining patterns see Fig. 4. IAPP antiserum, monoclonal insulin antibody, double stain, haematoxylin; ×280

reactivity. Eight of 12 patients with no clinical sign of diabetes mellitus and without islet amyloid exhibit intracellular immunoreactivity for IAPP. No extracellular IAPP immunoreactivity is observed.

Ten cases with and without diabetes mellitus were double stained with IAPP antiserum and monoclonal insulin antibody. All cases exhibit insulin and IAPP immunoreactivity. Insulin and intracellular IAPP immuno-

Table 3. Distribution of intra- and extracellular islet amyloid polypeptide (IAPP) immunoreactivity

IAPP immunoreactivity	NIDDM with islet amyloid *n* (%)	NIDDM without islet amyloid *n* (%)	No DM with amyloid *n* (%)	No DM without amyloid *n* (%)
Extracellular	98 (98)	0 (0)	6 (60)	0 (0)
Intracellular	71 (71)	9 (82)	7 (70)	8 (67)

Table 4. Immunohistochemical differentiation of generalized amyloidosis in non-diabetic patients

Patient no.	IAPP intracellular	IAPP extracellular	AA	AL	AK	AF	AB
1	+	−	−	+++	−	−	−
2	++	−	−	−	++	−	−
3	−	−	++	−	−	−	−
4	+	−	−	+++	−	−	−

AA, Amyloid A; AL, immunoglobulin lambda light chain amyloid; AK, immunoglobulin kappa light chain amyloid; AF, amyloid of transthyretin origin; AB, amyloid of beta$_2$-microglobulin origin; −, +, ++, +++, relative immunoreactivity, negative to strong immunoreactivity

Table 5. NIDDM with islet amyloid: intracellular IAPP immunoreactivity in comparison to the degree of IAPP-positive islet amyloid per section viewed

Intensity of intracellular immunoreactivity	Degree of IAPP-positive islet amyloid		
	Grade I *n* (%)	Grade II *n* (%)	Grade III *n* (%)
Negative	8 (31) *3 (27)*[a]	11 (42) *5 (46)*[a]	7 (27) *3 (27)*[a]
Positive	47 (66) *22 (63)*[a]	20 (28) *11 (31)*[a]	4 (6) *2 (6)*[a]
− Weak	31 (62) *16 (57)*[a]	15 (30) *10 (36)*[a]	4 (8) *2 (7)*[a]
− Intermediate	11 (73) *3 (75)*[a]	4 (27) *1 (25)*[a]	0 (0) *0 (0)*[a]
− Strong	5 (83) *3 (100)*[a]	1 (17) *0 (0)*[a]	0 (0) *0 (0)*[a]

Predominantly islets with disseminated (grade I), confluent (grade II) and convoluted (grade III) amyloid deposits per section viewed

[a] Consideration of autolysis: 51 cases were excluded due to moderate and severe autolysis

Table 6. NIDDM with islet amyloid: distribution of cases with microdeposits corresponding to the number of islets with intracellular immunoreactivity per section viewed

Number of islets with intracellular immunoreactivity	Number of cases with microdeposits *n* (%)
0	2 (6)
1–5	1 (3)
6–10	3 (8)
>10	29 (83)

Table 7. NIDDM with islet amyloid and microdeposits: intracellular IAPP immunoreactivity in comparison to the degree of IAPP-positive islet amyloid per section viewed

Intensity of intracellular immunoreactivity	Degree of IAPP-positive islet amyloid		
	Grade I *n* (%)	Grade II *n* (%)	Grade III *n* (%)
Negative	0 (0)	2 (67)	1 (33)
Positive	26 (81)	4 (13)	2 (6)
− Weak	16 (76)	3 (14)	2 (10)
− Intermediate	7 (88)	1 (12)	0 (0)
− Strong	3 (100)	0 (0)	0 (0)

Predominantly islets with disseminated (grade I), confluent (grade II) and convoluted (grade III) amyloid deposits per section viewed

Table 8. NIDDM with islet amyloid: number of islets with intracellular IAPP immunoreactivity in comparison to the number of islets with amyloid deposits per section viewed

Number of islets with intracellular IAPP immunoreactivity	Number of islets with islet amyloid		
	1–5 *n* (%)	6–10 *n* (%)	>10 *n* (%)
0	1 (4) *1 (9)*[a]	3 (12) *0 (0)*[a]	22 (84) *10 (91)*[a]
1–6	2 (22) *1 (20)*[a]	0 (0) *0 (0)*[a]	7 (78) *4 (80)*[a]
6–10	0 (0) *0 (0)*[a]	2 (17) *1 (12)*[a]	10 (83) *7 (88)*[a]
>10	13 (26) *7 (32)*[a]	12 (24) *4 (18)*[a]	25 (50) *11 (50)*[a]

[a] Consideration of autolysis: 51 cases were excluded due to moderate and severe autolysis

reactivity are co-localized in the same islet cells (Fig. 4). Insulin-immunoreactive cells display close proximity to extracellular IAPP-immunoreactive amyloid deposits. No insulin immunoreactivity is found within islet amyloid (Fig. 5).

Of the 100 autopsied patients with the clinical signs of NIDDM and islet amyloid, 98 have IAPP-immunoreactive amyloid deposits. Thirty-six autopsied patients with NIDDM and amyloid deposits have additional microdepositis, of which 35 proved IAPP-immunoreactive. Intracellular immunoreactivity is found in 71 cases.

Three of 100 cases could not be evaluated. Two of these exibit advanced autolysis and strong non-specific

binding properties. Simultaneous comparison of Congo red staining and IAPP immunoreaction of adjacent sections verify slight immunoreactivity in islet amyloid, but the difference is too small to count in these 2 cases. In the third case IAPP immunoreactivity of microdeposits cannot be distinguished from the intracellular immunoreactivity.

Comparison of the intracellular IAPP immunoreactivity (negative, weak, positive, strong) with the degree of islet amyloid deposition on the whole section exhibits an inverse relationship in 97 cases with clinically overt NIDDM and islet amyloid (Table 5). This inverse correlation is unchanged after assessment of autolysis, when 51 cases with moderate or severe autolysis are eliminated (Table 5, italics).

Thirty-five patients with NIDDM exhibit microdeposits in addition to different degrees of islet amyloidosis, that is to say apart from islets with disseminated, confluent or convoluted deposits. The comparison of intracellular IAPP immunoreactivity and the degree of islet amyloid deposition in these 35 cases exhibits the same inverse relationship (Tables 6, 7) as shown above.

A comparison between the number of islets with amyloid deposits and the number of islets with intracellular IAPP immunoreactivity viewed per section fails to reveal a strong inverse relationship in 97 cases with NIDDM and islet amyloid (Table 8).

Discussion

The detection of a previously unknown pancreatic islet polypeptide, identified as IAPP in islet amyloid deposits and β-cells, has opened up new perspectives for the investigation of the close association of NIDDM with islet amyloid (Johnson et al. 1991; Porte and Kahn 1989; Steiner et al. 1991).

Johnson et al. (1991) summarized the three possible potential roles of IAPP in pathogenesis of diabetes as follows: formation of islet amyloid with resultant damage to and replacement of β-cells, local effects on the secretion of insulin, and hormonal effects on peripheral tissue. The formation of islet amyloid with consecutive damage to and replacement of β-cells is corroborated by the observation of a reduced islet volume in diabetic patients due to formation of amyloid (Westermark and Wilander 1978). Local effects on the secretion of insulin induced by progredient formation of amyloid are confirmed by the observation of a significant correlation between severity of amyloid deposition and clinical feature of NIDDM (Schneider et al. 1980) with changes in glucose concentration in serum and urine, dietary control and the necessity of administering additional insulin. Hormonal effects of IAPP on peripheral tissue are controversial, as experimental observations may differ from in vivo conditions (Johnson et al. 1991; Steiner et al. 1991). IAPP may inhibit insulin secretion and glycogen synthesis in muscles or may exhibit vasodilating and serum-calcium-lowering effects (for review, see Steiner et al. 1991).

The causal relationship between IAPP and the development of NIDDM has not been established. Common hypotheses about the pathogenesis of NIDDM presume a primary β-cell dysfunction with an increased release of IAPP and pro-insulin rich granules (Porte and Kahn 1989). This release may constitute the precondition of amyloid formation, a hypothesis borne out by the observations of Johnson et al. (1989a), who described an increased IAPP immunoreactivity in pancreatic β-cells of cats with an impaired glucose tolerance. Involvement of a primary abnormal form of IAPP has been ruled out (for review, see Cooper et al. 1989). Increasing formation of amyloid itself might aggravate β-cell dysfunction. Schneider et al. (1980) confirm a significant correlation between the degree of amyloid deposition and the clinical feature of NIDDM. Aggravated β-cell dysfunction might be associated with the decreasing intracellular IAPP immunoreactivity in β-cells (Westermark et al. 1987a). If developing IAPP-immunoreactive amyloid deposition in pancreatic β-cells exerts influence on intracellular IAPP immunoreactivity, an inverse relationship should be demonstrable between the degree of amyloid deposition and intracellular IAPP immunoreactivity.

We have confirmed that amyloid deposits in pancreatic islets of patients with NIDDM are IAPP-derived in 98 of 100 cases. Separation of intra- and extracellular immunoreactivity is feasible and the immunoreactivity of IAPP is limited to pancreatic islets. Double staining with IAPP antiserum and monoclonal insulin antibodies exhibits co-localization of IAPP and insulin in the same islet cells. No insulin immunoreactivity is found in islet amyloid. Ninety-four (72%) of 130 cases exibited intracellular immunoreactivity for IAPP, 71 of which included patients with NIDDM and islet amyloid. These data are in good agreement with recent data (Berends et al. 1990).

The correlation between intracellular IAPP immunoreactivity and the degree of amyloid deposition in 97 patients with overt NIDDM and islet amyloid exhibits an inverse relationship. Cases with no intracellular immunoreactivity for IAPP exhibited all degrees of amyloid deposition with a peak at grade II (Table 5). Cases with strong intracellular immunoreactivity exhibited predominantly amyloid deposition grade I, and no grade III. These data remain unchanged after evaluation of autolysis (Table 5, italics). The inverse relationship between intracellular immunoreactivity and the degree of amyloid deposition remains unchanged by the appearance of microdeposits in 35 cases with NIDDM and islet amyloid. Apart from islets with disseminated, confluent and convoluted amyloid deposits, these cases exhibit minimal lesions. A comparison between the number of islets with amyloid deposits and the number of islets with intracellular IAPP immunoreactivity per section viewed did not reveal a strong inverse relationship (Table 8). These data suggest the inverse relationship is due to the relative extent of amyloid deposition, not to the presence of islet amyloid alone or to the quantity of affected islets per section viewed.

We did not estimate the number of immunoreactive β-cells, because this variable might be influenced by the reduction of total β-cell mass in NIDDM with islet amy-

loid and would not exhibit the effect of amyloid on the homeostasis of vital β-cells (Clark et al. 1988; Westermark et al. 1987b).

With the help of a new IAPP antiserum the present study affirms previous observations. There is a reduced intracellular IAPP immunoreactivity in islet cells of patients with NIDDM and amyloid. In addition to this, we confirm an inverse relationship between intracellular immunoreactivity and the degree of islet amyloid in NIDDM irrespective of the total amount of affected islets. The correlation between intracellular IAPP immunoreactivity and the degree of islet amyloid irrespective of the total amount of affected islets has not been reported, in recent immunohistochemical studies (Berends et al. 1990; Clark et al. 1990; Johnson et al. 1989a; Toshimori et al. 1991; Westermark et al. 1987b).

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