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Immunohistochemical localization of factor X-like antigen in pancreatic islets and their tumours

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Summary. The authors have investigated by immunohistochemistry the distribution of factor X-like antigen in normal pancreatic islets and in a series of 46 pancreatic endocrine tumours. It was found that both glucagon-producing (A) cells and pancreatic polypeptide-producing (PP) cells are immunoreactive for the antigen. Benign glucagonomas and PP-omas presented the highest concentrations of immunoreactive material whose intracellular distribution was consistent with localization within cell secretory granules. Some benign insulinomas also presented factor X immunostaining in spite of the absence of the antigen in normal insulin-producing B cells. Although malignant tumours usually exhibited very low or no immunostaining, two of three malignant glucagonomas showed scattered, intensely immunoreactive cells. The factor X-like antigen identified in this study was found to differ from chromogranin A and B. The possible implications of the present findings for coagulative disorders associated with glucagonomas or diabetes are discussed.

Key words: Factor X – Pancreatic endocrine tumours – Islet A cells – Islet PP cells – Immunocytochemistry

Introduction

Factor X is a vitamin K-dependent serine protease that has a cardinal role in the coagulation process, acting to convert prothrombin to thrombin after appropriate activation. Although the major source of factor X is the liver, a previous immunofluorescence investigation (Betterle et al. 1982a) revealed that glucagon-producing (A) cells of pancreatic islets also contain, and propably produce, factor X. In the same study, however, no immunoreactivity was found in two other types of islet cells, the insulin-producing (B) cells and the somatostatin-producing (D) cells.

The present report extends previous observations on normal islet cell populations to the pancreatic polypeptide-producing (PP) cells. In addition, the immunoreactive expression of factor X by pancreatic endocrine tumours is also described. Our data indicate that this coagulation factor, or a protein immunologically related to it, is also stored in the PP cells and that it is frequently produced by pancreatic endocrine tumours. These include some neoplasms composed of cells which do not reveal factor X-immunoreactivity in normal conditions.

Materials and methods

Specimens of grossly and histologically normal human pancreatic tissue were obtained at surgery in patients operated for insulinomas, or at post-mortem examination shortly after removal of heart and kidneys for transplantation purposes. Samples of both the pp-poor pancreatic tail (of dorsal embryological origin) and PP-rich lobe of the pancreatic head (of ventral origin, Malaisse-Lagae et al. 1979) were included. Tissues were fixed in Bouin's fluid for 18–24 h, dehydrated and embedded in paraffin.

Tissue specimens of 46 endocrine pancreatic tumours obtained at surgery from 37 patients and at autopsy from 2 patients were processed similarly. The tumours are listed in Table 1. They were classified on the basis of their hormonal production, identified by immunohistochemistry using polyclonal antibodies against insulin, glucagon, somatostatin, PP, gastrin and vasoactive intestinal peptide as described previously (Bordi et al. 1979, 1987). In addition, most neoplasms were also investigated immunohistochemically for the expression of a series of neuroendocrine markers, including chromogranin A (CgA), neuron specific enolase, PGP 9.5, HISL-19, and prealbumin as reported in detail elsewhere (Bordi et al. 1988). All malignant tumours showed lymph node and/or liver metastases, except two, in which vascular invasion and infiltration of peripancreatic fat could be documented. Benign glucagonomas and PP-omas were found in patients with multiple endocrine neoplasia syndrome type I, except one glucagonoma, which was an independent satellite tumour in a patient with larger insulinoma, and one PP-oma incidentally found at autopsy.

Immunohistochemical investigation was carried out using polyclonal antiserum raised in rabbit against human factor X (Diagnos-

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Table 1. Immunohistochemical demonstration of factor X-like antigen in a series of 46 pancreatic endocrine tumours. Relation with chromogranin A (CgA) immunostaining

Type of tumour	Number of tumours				
	Total	Immunoreactive for factor X[a]	Relation factor X/CgA[b]		
			=	+	−
Benign tumours					
Insulinoma[c]	13	5 (3)	5	1	7
Glucagonoma	5	5 (5)	5		
PP-oma	4	4 (4)	3	1	
Non-functioning	2	2 (2)	1	1	
Total	24	16 (14)	14	3	7
Malignant tumours					
Insulinoma	6	2 (1)	1	1	4
Glucagonoma	3	2 (0)			3
Gastrinoma	3	2 (0)			3
VIP-oma	2	0			2
PP-oma	3	0	2		1
Non-functioning	5	1 (0)	2		3
Total	22	7 (1)	5	1	16

[a] Numbers in parentheses refer to tumours in which the majority of tumour cells were immunoreactive for factor X. Occasional positive cells were not considered

[b] Roughly equal proportion of cells immunoreactive for factor X and CgA is indicated by =; + indicates a predominance of factor X immunoreactive cells; − indicates a predominance of CgA immunoreactive cells

[c] Include two cases of mixed insulinoma-somatostatinoma, both unreactive for factor X

tica Stago, Asnieres, France; Lot 71096). The antiserum has been extensively investigated and found to be monospecific in several immunological systems (Girolami et al. 1985a, b). The working dilution of the antiserum was 1:160 with overnight incubation at 4° C. The immunoreaction was visualized with the avidin-biotin complex procedure (Vectastain ABC Kit, Vector Laboratories, Burlingame, Calif., USA) using diaminobenzidine tetrahydrochloride as a peroxidase substrate. To exclude cross-reactivity of factor X antiserum with PP, sections of PP-rich pancreatic lobes were incubated with factor X antiserum preadsorbed with pure bovine pancreatic polypeptide (BPP) (provided by Dr. Ronald E. Chance, Ely Lilly Co., Indianapolis, Ind., USA; 1 nmol/100 µl of diluted antiserum). This antigen concentration was found to abolish binding of BPP antiserum to PP-containing cells. Furthermore, to avoid possible cross-binding of factor X antiserum to molecules of CgA and CgB, the antiserum was tested with two-dimensional immunoblotting on enriched fractions of CgA and CgB obtained from human phaeochromocytoma tissue (courtesy of Dr. A. Zannini, Milan, Italy). The methodological details of this procedure have been described elsewhere (Pelagi et al. 1989).

Results

As previously reported (Betterle et al. 1982a), islets of Langerhans in PP-poor pancreatic regions revealed an intense, selective factor X-like immunoreactivity of peripherally located glucagon-containing A cells (Fig. 1).

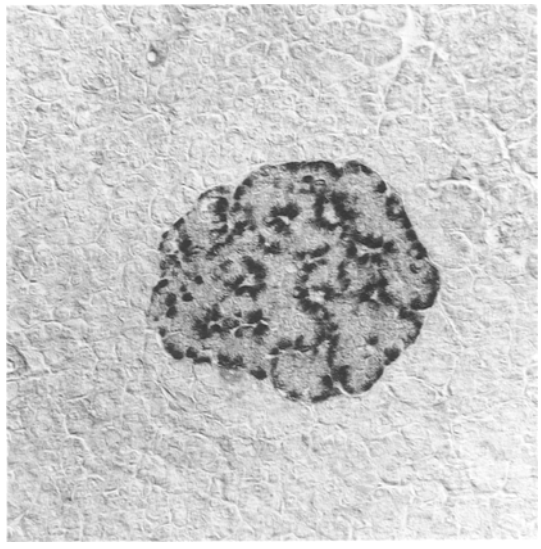

Fig. 1. Factor X-like immunostaining of peripherally located glucagon cells in a pancreatic islet of dorsal embryological origin. ABC immunoperoxidase; interference contrast optics, ×190

The PP-containing cells of PP-rich islets in the pancreatic lobe of ventral embryological origin were also found to be immunostained for factor X, although with slightly less intensity than the A cells (Fig. 2a–d). Such immunoreactivity of PP cells was unaffected by previous adsorption of antiserum against factor X with pure PP antigen (Fig. 2e).

Among the pancreatic endocrine neoplasms examined, benign glucagon cell tumours (none of which presented the typical clinical syndrome) revealed the heaviest factor X immunoreactivity. Immunostaining often occurred in virtually all tumour cells and showed distinct polarization in the basal cytoplasmic poles (Fig. 3). Benign PP-producing tumours also presented factor X-like immunoreactivity in the majority of tumour cells (Fig. 4). In both types of tumours the staining largely overlapped that shown by neuroendocrine markers of granular type (Bordi et al. 1988) such as CgA. In contrast 8 of 13 benign insulinomas were virtually non-reactive, including two cases with a mixed population of insulin and somatostatin cells. Two cases showed only a minority (10–20%) population of positive cells, whereas the remaining three cases presented positive immunoreactivity to most tumour cells. Immunoreaction for factor X was more intense than that for CgA in only one benign insulinoma and was similar (Fig. 5) in five neoplasms. The remaining seven tumours showed more abundant CgA than factor X immunoreactive cells, the latter being absent in four insulinomas showing CgA immunostaining in the majority of their cells. Diffuse factor X immunoreactivity was also found in two non-functioning tumours in spite of the very low, occasional occurrence of hormone-containing cells (mostly glucagon and PP).

In malignant endocrine tumours of the pancreas immunohistochemical expression of factor X was generally

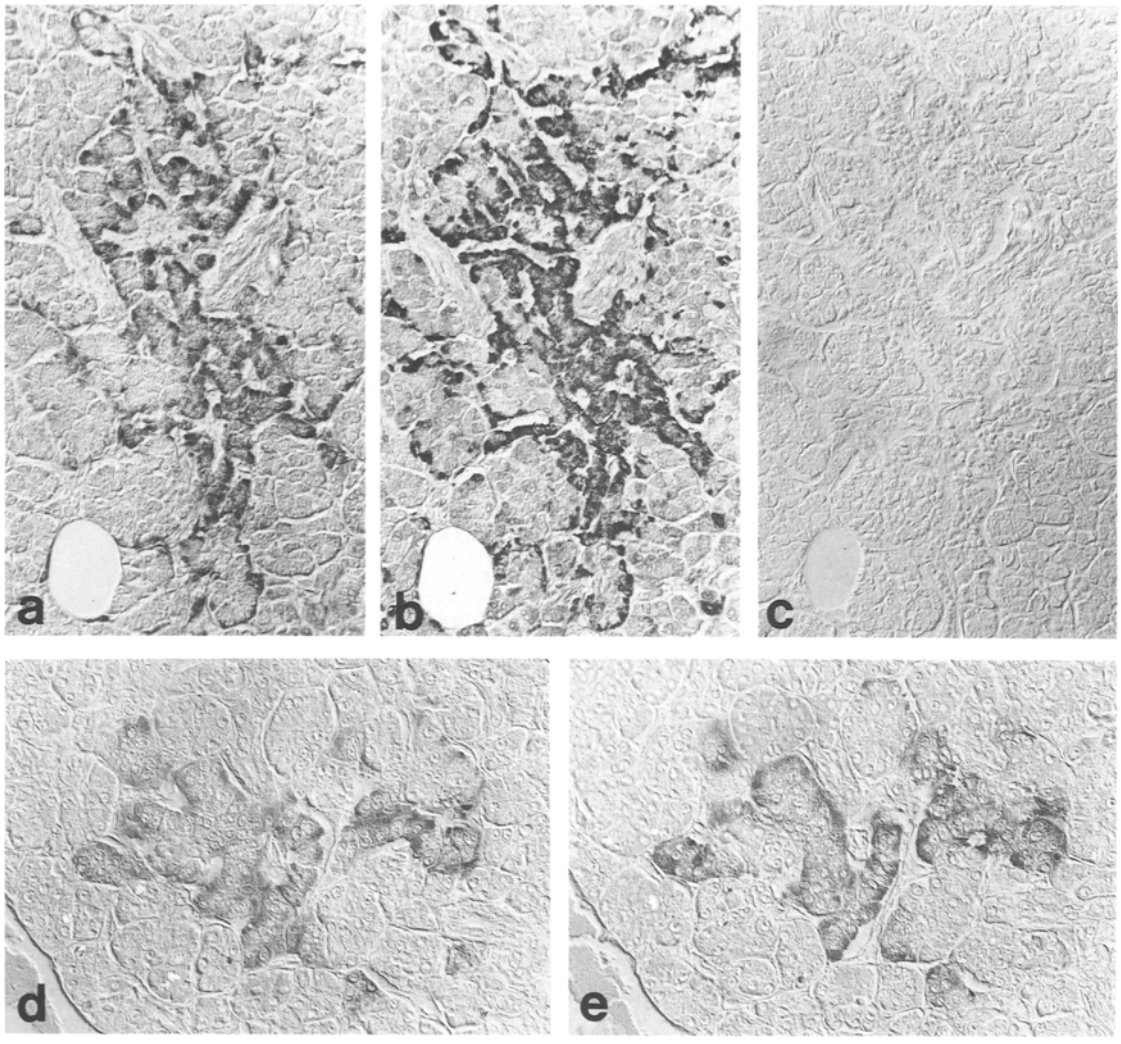

Fig. 2. Factor X-like immunostaining (**a**, **d**) of the cells composing the typical, irregularly shaped islets of the ventral embryological origin, mostly composed of PP-immunoreactive cells in consecutive section (**b**). Preadsorption of respective antisera with pure PP abolishes the binding of antiserum against PP (**c**) but not of that against factor X (**e**). ABC immunoperoxidase; interference contrast optics; **a, b, c** ×140; **d, e** ×220

low or absent. It may be noted, however, that a pronounced immunostaining of virtually all tumour cells was found in a localized neoplasm whose malignant nature was recognized only by perineural infiltration and an isolated lymph node micrometastasis. The remaining tumours were mostly represented by more aggressive and extensively metastasizing neoplasms, thus reflecting a lower degree of cell differentiation. In spite of this, two of three malignant glucagonomas associated with the full clinical expression of the syndrome exhibited a minority (5–10%) of cells with heterogeneous but often conspicuous content of factor X immunoreactive material (Fig. 6).

Discussion

The present results confirm previous observations that a factor X-like antigen is contained in A cells of normal human pancreatic islets (Betterle et al. 1982a). In addition, they show that the same antigen is also expressed by the PP-containing cells, which are mostly concentrated in the pancreatic lobe of ventral pancreatic origin (Malaisse-Lagae et al. 1979). Factor X-like immunoreactivity is not inhibited by previous absorption of the specific antiserum with purified PP in a concentration capable of abolishing the binding of the anti-PP antiserum. This finding indicates lack of cross-reactivity of the anti-factor X antiserum with PP. Thus, pancreatic PP cells should be added to the list of possible sources of factor X in man.

Our immunohistochemical study also shows that a factor X-like antigen may be produced by pancreatic endocrine tumours. The immunoreactive material mostly accumulated toward the basal cytoplasmic pole of tumour cells facing blood sinusoids. This finding is consistent with the intracellular distribution of secretory granules, indicating that factor X immunoreactivity is

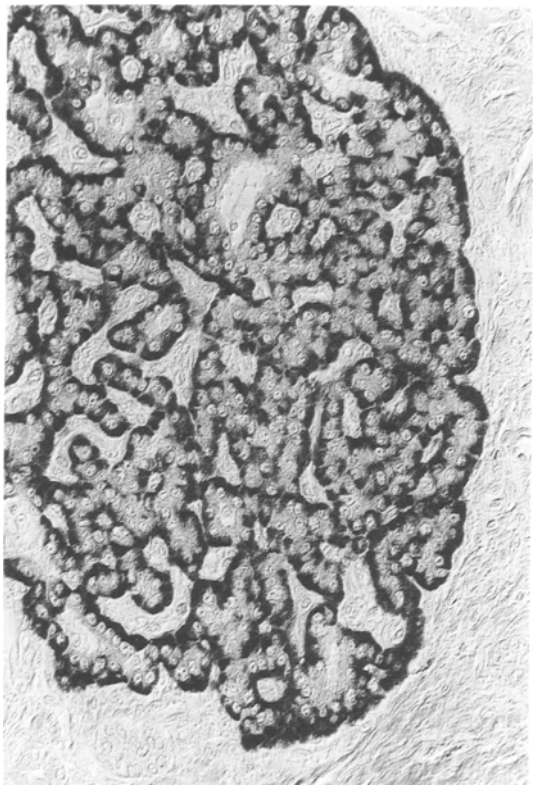

Fig. 3. Intense factor X-immunostaining of virtually all tumour cells in a benign glucagonoma with clear basal polarization of immunoreactive material consistent with its localization in cytoplasmic secretory granules. ABC immunoperoxidase; interference contrast optics, × 245

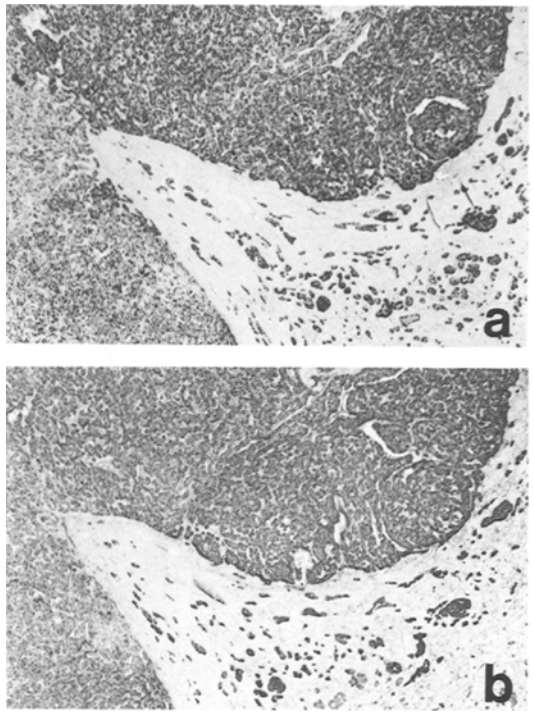

Fig. 4. Benign PP-oma showing diffuse factor X-like immunoreactivity (**a**) closely corresponding to the distribution of PP (**b**). ABC immunoperoxidase, × 25

mostly localized within the granules of tumours cells and likely co-secreted with them. It is pertinent to note that, in pancreatic endocrine tumours, this "granular" pattern of immunostaining was found to identify a subgroup of non-hormonal neuroendocrine markers. Amongst these are CgA, HISL-19, PHE5, and prealbumin, in sharp contrast with the diffuse cytoplasmic staining characteristic of the non-granular, cytosolic markers, such as neuron specific enolase and PGP 9.5 (Bordi et al. 1988). Indeed, close correspondence between the immunolocalization of factor X and that of CgA was found in serial sections of many tumours. However, the expression of the two substances was entirely dissociated in other neoplasms. Furthermore, no cross-reactivity was found when the factor X antiserum used in the present study was tested with CgA and CgB in immunoblotting preparations (courtesy of Dr. A. Zannini, Milan, Italy).

Factor X-like immunoreactivity of pancreatic endocrine tumours demonstrated peculiar variations from one tumour type to another. Among benign neoplasms a strong, diffuse immunostaining was revealed by glucagonomas and, with a slight lesser extension, PP-omas. In addition, such immunoreactivity was found in 5 of 13 insulinomas, being expressed by the majority of tumour cells in three. This finding is in contrast with the absence of immunoreaction for factor X in normal insulin-producing B cells. A similar discrepancy between neoplastic and non-neoplastic B cells, however, was already noted in the expression of prealbumin (Bussolati et al. 1984). Our series of pancreatic endocrine tumours does not include neoplasms purely composed of somatostatin D cells. However, no factor X immunoreactivity was found in two benign insulin-producing tumours showing a conspicuous D cell component, a finding consistent with the lack of immunodetectable factor X in normal D cells (Betterle et al. 1982a).

The immunohistochemical expression of factor X by malignant endocrine tumours of the pancreas was largely less diffuse than in benign neoplasms; often no immunoreactivity was found. However, immunoreactive cells were present in two of three malignant glucagonomas, although remarkably reduced in number in comparison with the benign counterpart of these tumours. The low number of these cells in malignant glucagonomas does not necessarily indicate low production of factor X-like substance but is possibly associated with increased secretion due to a defective cell mechanism of release control. The glucagon secretory behaviour in benign and malignant glucagon-producing tumours is illustrative in this regard considering the close analogies between the distribution of glucagon immunoreactive cells and that of factor X immunoreactive cells in the two groups of neoplasms (Bordi et al. 1979). Glucagon secretion is consistently high in malignant glucagonomas in spite of the low number of immunoreactive cells, whereas it may be normal in benign tumours showing plenty of glucagon-containing cells (Bordi et al. 1979).

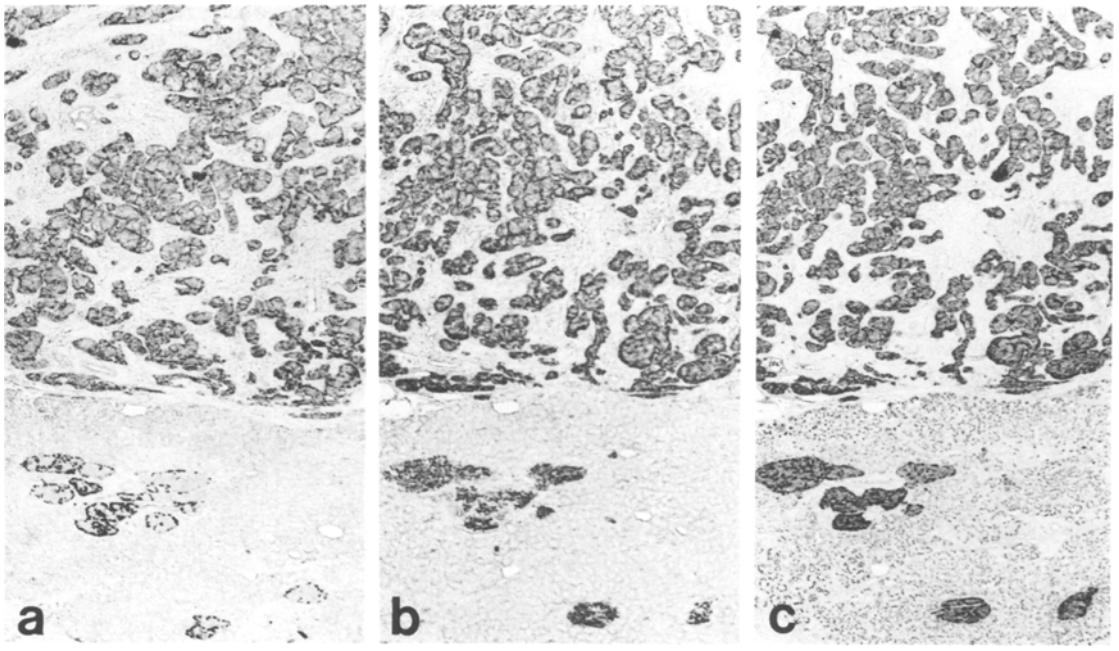

Fig. 5. Diffuse factor X-like immunoreactivity (**a**) in a benign insulinoma featuring a similar pattern of staining for insulin (**b**) and for chromogranin A (**c**). ABC immunoperoxidase, ×45

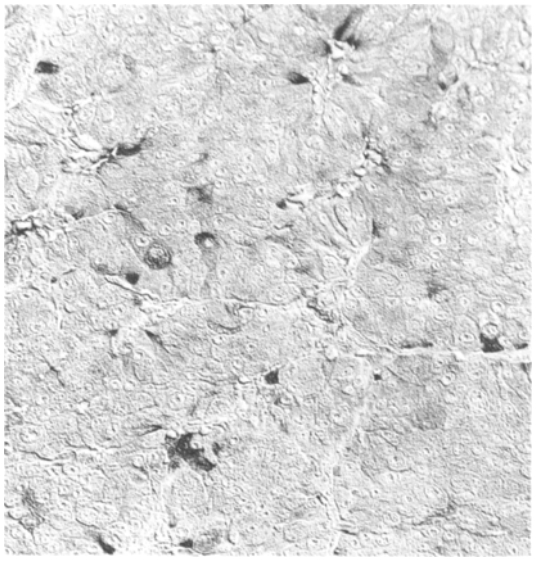

Fig. 6. Scattered factor X-immunoreactive cells in a malignant glucagonoma. ABC immunoperoxidase; interference contrast optics, ×295

In this regard it is worth noting that in the glucagonoma syndrome venous thrombosis has been described (Friesen 1982), a finding possibly dependent on elevated factor X release by tumour cells. Unfortunately, studies on circulating levels of this factor have not been performed so far in patients with the glucagonoma syndrome, nor they have in patients with PP-oma or other islet cell tumours for which, however, there are no data indicating coagulation disorders.

In conclusion, the present observation may have clinical significance. It documents that one factor of the coagulation cascade occurs in, and is probably synthesized in, pancreatic islets. Our results also encourage the study of potential coagulation disturbances in patients with islet cell tumours and, possibly, also with diabetes (Betterle et al. 1982b).

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