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Comparative study of seven neuroendocrine markers in pancreatic endocrine tumours

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Summary. A comparative immunocytochemical investigation was performed on a series of 59 pancreatic endocrine tumours using a panel of seven markers for neuroendocrine neoplasms: neurone specific enolase (NSE), PGP 9.5, chromogranin A (CgA), PHE5, prealbumin (Pa), HISL-19, and alpha-subunit of human chorionic gonadotropin (α-HCG). Most markers can be separated into two groups characterized by an identical immunoreactive cellular compartment and substantial overlapping in the immunohistochemical results. The first group comprises soluble cytoplasmic proteins such as NSE and PGP 9.5 and is characterized by a diffuse, homogeneous staining of the cell cytoplasm that is not related to the type of hormone produced or the degree of cell differentiation. The second group includes antigens located in the cell secretory granules such as CgA, PHE5, Pa and HISL-19 and is characterized by a heterogenous, often polarized cell staining. The latter markers strongly react with benign glucagonomas and PP-omas and, in contrast with those of the former group, are stricly neuroendocrine-specific. However, they often are less effective in staining insulinomas and malignant tumours. An additional, distinctive and useful characteristic of the HISL-19 antibody was its ability to label the Golgi complex also in tumours with absent granular staining. Finally, α-HCG was found in 9 of 16 malignant tumours (mostly glucagonomas and insulinomas) and in 4 of 43 benign neoplasms (all insulinomas). The latter finding is not in accordance with the reputed specificity of the α-HCG expression by pancreatic endocrine tumours as a marker for tumour malignancy.

Key words: Islet cell tumours – Immunohistochemistry – Neuroendocrine markers – Human chorionic gonadotropin, alpha subunit

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Introduction

In the seventies the application of immunohistochemistry to the study of islet cell neoplasms was primarily concerned with the characterization of the hormones produced by the tumours. This line of research had a crucial role in extending the classification of pancreatic endocrine tumours, previously restricted to insulinomas and gastrinomas, and led to the definition of new entities such as glucagonomas, somatostatinomas, tumours producing pancreatic polypeptide or PP-omas, and those producing vaso-active intestinal peptide or VIP-omas (for review see Creutzfeldt 1977; Solcia et al. 1981; Heitz 1984; Bordi 1986).

In the present decade, in contrast, new endocrine tumour types have not been identified in the pancreas with the exception of the interesting but rare neoplasms producing growth hormone releasing factor and causing acromegaly (Thorner et al. 1982; Kovacs et al. 1984). However, a new impulse to the immunohistochemical research was generated by the identification in the islet cells and their tumours of a variety of non-hormonal antigens common to most cells of the diffuse neuroendocrine system. These neuroendocrine markers included neurone specific enolase (NSE) (Tapia et al. 1981), chromogranin A (CgA) (Lloyd and Wilson 1983; Lloyd et al. 1984b), the alpha subunit of the human chorionic gonadotropin (α-HCG) (Heitz et al. 1983), prealbumin (Pa) (Bussolati et al. 1984), PGP 9.5 (Rode et al. 1985), HISL-19 (Srikanta et al. 1986; Krisch et al. 1986), 7B2 (Suzuki et al. 1986), PHE5 (Riddel et al. 1987), and synaptophysin (Chejfec et al. 1987).

A few studies of islet cell tumours correlated the immunohistochemical features of two different markers (NSE and CgA, Lloyd et al. 1984b; Nash and Said 1986; NSE and PGP 9.5, Rode et al. 1985; NSE and HISL-19, Krisch et al. 1986; CgA

Table 1. Antibodies and immunohistochemical procedures used in the study of the present series of pancreatic endocrine tumours

Antigen	Type of antibody[a]	Code no.	Source	Working dilution	Visualization procedure[b]
Chromogranin A	MC	LK2H10	Dr RV Lloyd, Ann Arbor, Mich, USA	1:400 1:400	IGSS[c] ABC[d]
Alpha subunit, human chorionic gonadotropin	PC	AFP 310784	National Pituitary Program, NIH, Bethesda, Md USA	1:800 1:400	IGSS[c] ABC[e]
Neurone specific enolase (NSE)	MC	K545	Dakopatts, Copenhagen, Denmark	1:1 (kit)	PAP[f]
PGP 9.5	PC		Dr RJ Thompson, Cambridge, UK	1:100	ABC[e]
HISL-19	MC		Dr G Eisenbarth, Boston, Mass USA	1:4000	IIP[g]
Prealbumin	PC		Dr. G Bussolati, Turin, Italy	1:1000	ABC[e]
PHE5	MC		Dr. G DeChirico, Orthodiagnostic, Milan, Italy	1:400	ABC[d]

[a] MC = monoclonal; PC = polyclonal
[b] Peroxidase activity with all procedures employed was demonstrated using 3,3′-diaminobenzidine (DAB) as a substrate
[c] Immunogold-silver staining (AuroProbe LM Kit, Janssen Life Science Products, Beerse, Belgium; distributed by Orthodiagnostic, Milan, Italy)
[d] Avidin-biotin-complex (Code no K 355; Dakopatts, Copenhagen, Denmark) after incubation with biotylinated rabbit anti-mouse immunoglobulin (Dakopatts Code no E 354) diluted 1:200
[e] Avidin-biotin-complex (Vectastain ABC Kit Standard, Code no PK-4000; Vector Laboratories, Inc, Burlinghame, Ca, USA)
[f] Peroxidase-anti-peroxidase complex (Dako PAP Kit System 20, Code no K545; Dakopatts, Copenhagen, Denmark)
[g] Indirect immunoperoxidase technique using peroxidase conjugated rabbit anti-mouse immunoglobulin followed by peroxidase conjugated swine anti-rabbit immunoglobulin (both diluted 1:100, Dakopatts, Copenhagen, Denmark; courtesy of Dr K Krisch, Vienna, Austria)

and synaptophysin, Chejfec et al. 1987). In the present study we have compared the immunohistochemical findings of a panel of seven antigens in a series of 59 pancreatic endocrine tumours. In particular the expression of the different markers was correlated with the hormone production and the clinical behaviour of the neoplasms.

Material and methods

Fifty-nine pancreatic endocrine tumours collected from 30 patients were investigated. Forty-three tumours were classified as benign on the basis of their histological features and of the absence of metastases or recurrences at the autopsy (2 cases) or in the follow-up (mean: 8.7 years; range: 3–22 years; 2 patients lost). Of the benign tumours 11 were single neoplasms (8 insulinomas, 2 PP-omas and 1 glucagonoma), 2 (insulinoma plus glucagonoma) were associated in the same patient whereas the remaining ones (1 insulinoma, 10 glucagonomas, 11 PP-omas, 4 mixed and 4 non functioning tumors) were collected from 3 patients with multiple endocrine neoplasia (MEN-I). The 16 malignant neoplasms presented lymph node and/or liver metastases in all cases except two in which, however, unequivocal vascular invasion and infiltration of the peripancreatic fat was apparent. They were collected from 15 patients and included a tumour recurring in a remote site 8 years after the original operation and exhibiting radical changes in its cytological and functional characteristics (Bordi et al. 1988) that justify a separate analysis. From the functional point of view the neoplasms were classified according to the symptom-causing hormone and/or to the majority islet cell population shown by immunocytochemistry. Tumours discovered as incidental findings at autopsy or during operation and showing only occasional immunoreactive islet cells but otherwise clearly recognizable as islet cell tumour on the basis of their histological appearance were classified as non-functioning tumours. Tumour tissues were fixed in Bouin's fluid in all cases except five in which 10% formalin was used. After dehydration the specimens were routinely embedded in paraffin. Serial sections were cut at 5 μm. The routine staining procedure included haematoxylin-eosin and the Grimelius (1968) silver method. The hormonal characterization of the tumours was achieved by immunohistochemistry with polyclonal antisera against insulin, glucagon, somatostatin, pancreatic polypeptide (PP), gastrin and vasoactive intestinal peptide (VIP) as described elsewhere (Bordi et al. 1979, 1987).

The antibodies against neuroendocrine markers used in the present study and the respective immunohistochemical procedures are summarized in Table 1. The control of the specificity of the reactions was performed by preadsorption of the antiserum with an excess of the respective antigen or by substitution of the primary antiserum with a non-immune serum from the same species.

The neuroendocrine markers used were defined as follows:

Chromogranin A (CgA) is one of a family of highly acidic glycoproteins isolated from the soluble fraction of chromaffin granules. They comprise three immunologically distinct groups, A, B, and C, whose molecular weight is 76–78, 120 and 84–86 kD, respectively. CgA is the major Cg component of the pancreatic islets. It is mostly located to the glucagon-producing A cells and, ultrastructurally, it is confined to the secre-

Table 2. Immunohistochemical results of seven neuroendocrine markers in a series of 59 pancreatic endocrine tumours

Type of tumour	No.	Number of tumours presenting immunoreactivity for								
		NSE[a]	PGP 9.5[a]	CgA[b]	PHE5[b]	Pa[b]	HISL-19			α-HCG
							Tot.	Gr[b]	Cl[c]	
Benign tumours										
Insulinoma	10	10	10 (1)	10 (5)	10 (4)	6 (3)	10	9 (5)	8	4
Glucagonoma	12	12	12 (1)	12	12	12 (1)	12	12	10	0
PP-oma	13	13 (2)	13 (3)	13	13 (1)	13 (3)	13	13	0	0
Mixed type[d]	4	4	4	4	4	4	4	4	2	0
Non-functioning	4	4	4 (1)	4 (3)	4 (3)	4 (2)	4	4 (2)	4	0
Total	43	43 (2)	43 (6)	43 (8)	43 (8)	39 (9)	43	42 (7)	24	4
Malignant tumour										
Insulinoma	5	5	5 (1)	5 (3)	5 (3)	2 (2)	5	2 (1)	5	3
Glucagonoma	3	3 (1)	3 (1)	3 (1)	3 (1)	2	3	3 (1)	3	3
Gastrinoma	2	2	2 (1)	2 (1)	2	2 (2)	2	2 (1)	2	1
VIP-oma	1	1	1	1	1	0	1	0	1	0
PP-oma	1	1	1	1 (1)	1 (1)	1 (1)	1	1 (1)	0	0
Non-functioning	4	4	4 (1)	2 (1)	4 (1)	2 (1)	4	4 (2)	4	2
Total	16	16 (1)	16 (4)	14 (7)	16 (6)	9 (6)	16	12 (6)	15	9

Abbreviations: NSE = neurone-specific enolase; CgA = chromogranin A; Pa = prealbumin; HISL-19: Gr = granular staining pattern, Cl = cluster staining pattern; α-HCG = alpha subunit of human chorionic gonadotropin

[a] Numbers in parentheses refer to tumors with positive but weak immunostaining

[b] Numbers in parentheses refer to tumors with sparse immunoreactive cells

[c] Cluster type pattern of staining was evaluated in tumour cells with reduced or absent granular staining. The figure may be slightly underestimated for masking was possible in a few tumours with heavy granular staining

[d] Includes clinically silent tumours with mixed major cell populations (glucagon and PP cells, 3 cases; glucagon and insulin, 1 case).

tory granules (Varndell et al. 1985; Angeletti 1986; Rindi et al. 1986; Grube et al. 1986).

Neurone Specific Enolase (NSE) are the gamma subunit-containing forms of the glycolytic enzyme enolase, which were labeled "neurone specific" since they were originally regarded as to be restricted to the neurons. However, NSE was further demonstrated in the cells of the diffuse neuroendocrine system and, though generally in markedly lower amounts, also, in some non-neural, non-neuroendocrine cell types. All islet cells are immunoreactive for NSE (Bishop et al. 1982; Marangos 1985; Pahlman et al. 1986).

The α-subunit of human chorionic gonadotrophic (α-HCG), is apparently devoid of any specific hormonal activity and is common to all glycoprotein hormones. It is produced ectopically by neuroendocrine tumours of various organs (pancreas, gastrointestinal tract, lung). It is not present in normal islet cells although it is found in normal and, particularly, hyperplastic endocrine cells of the gastrointestinal tract (Kahn et al. 1977; Heitz et al. 1983; Fukayama et al. 1987).

Prealbumin (Pa) is a major plasma protein with 55 kD MW that is mainly synthesized in the liver. It has thyroxine-binding or vitamin A-transporting properties. In the pancreatic islets Pa has been localized in the A glucagon-producing cells but it is immunologically distinct from glucagon (Bussolati et al. 1984). Moreover, Pa was also found to be expressed by a large number of neuroendocrine tumours (Bussolati et al. 1984; Miller et al. 1984). On the basis of this finding Pa, which is not per se a neuroendocrine marker, is included in the present study.

The PGP (protein gene product) 9.5 is a 27 kD MW soluble protein isolated from the human brain and accounting for 1–2% of total soluble cerebral proteins. The PGP 9.5 is functionally and immunologically distinct from NSE (for review see Thompson and Day 1988).

The monoclonal antibody (mab) HISL-19 was generated from human islet cells. In immunoblotting experiments HISL-19 mostly binds two proteins of 120 and 67 kD MW, biochemically and immunologically distinct from CgA, CgB, and CgC. All islet cell types are stained by the HISL-19 *mab* although a more intense reaction is often apparent in the peripherally located A cells. Ultrastructurally, the immunoreactive protein is located both in the secretory granules and in the Golgi complex (Srikanta et al. 1986; Krisch et al. 1988).

The *mab* designated PHE5 was generated from human pheochromocytoma tissue. In immunoblot experiments PHE5 blotts to a 90 kD protein distinct from CgA (Riddel et al. 1987).

Results

The distribution of the different types of pancreatic endocrine tumours investigated in the present study is given in Table 2. Figure 1 comparatively

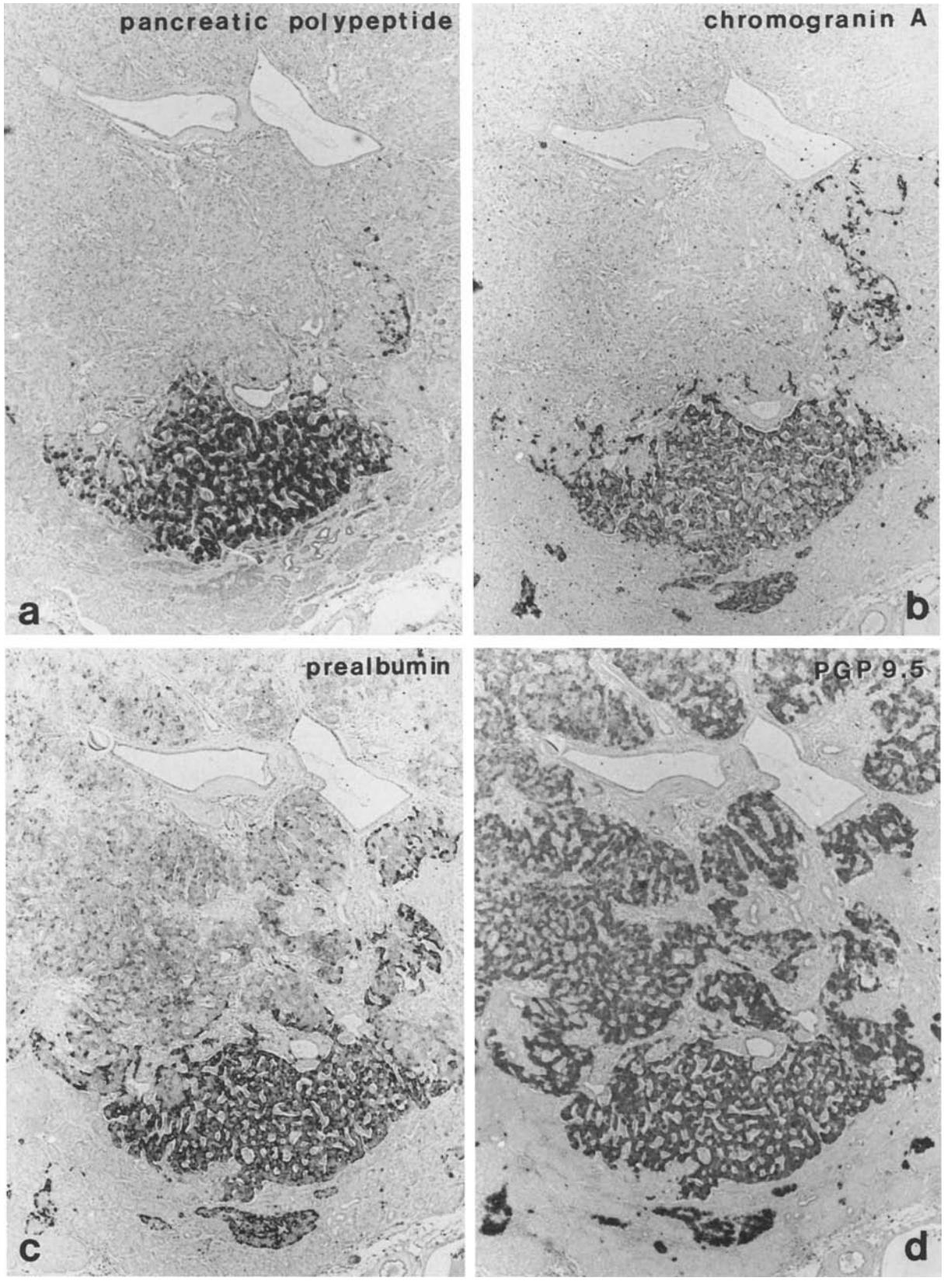
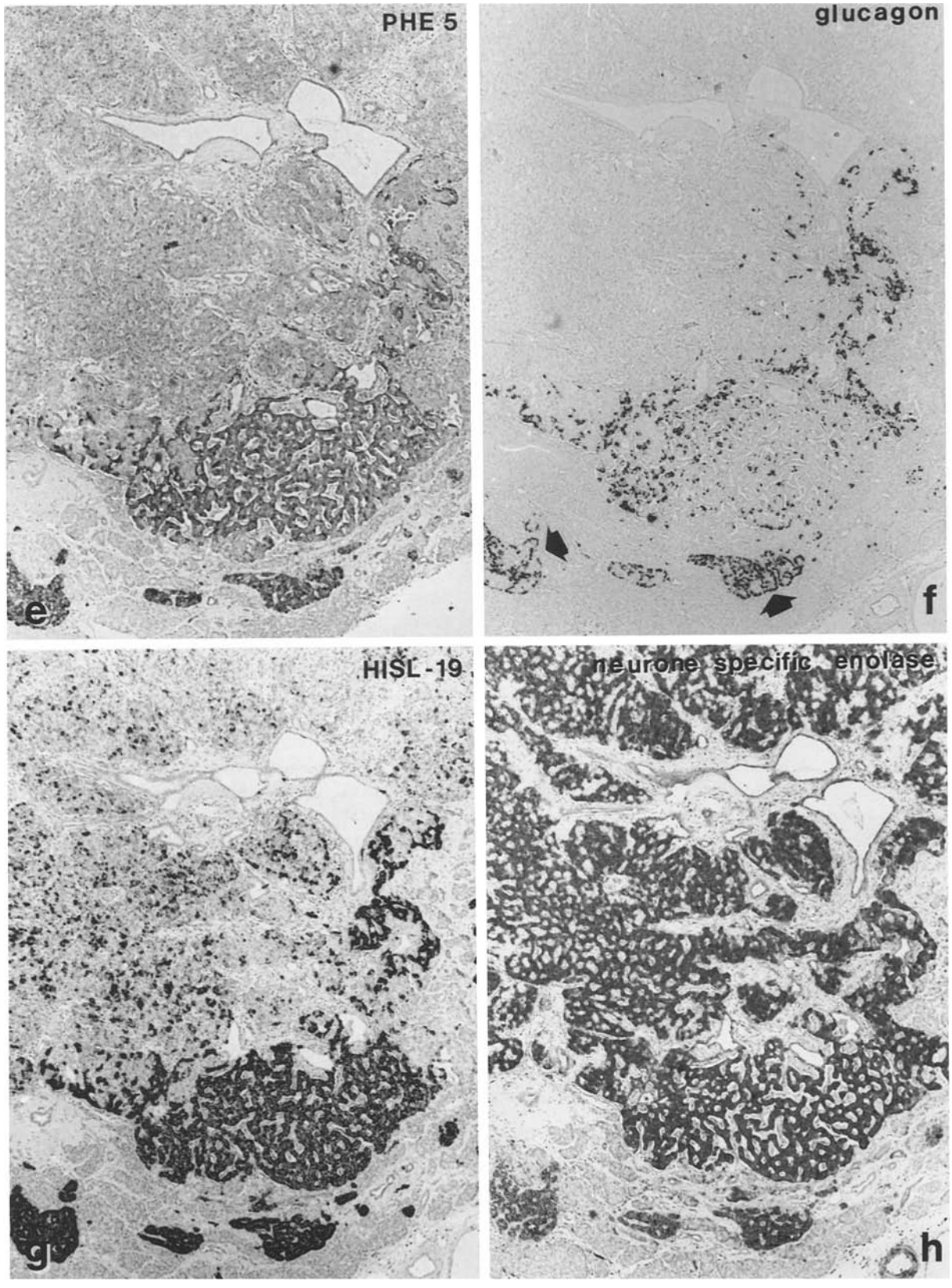

pancreatic polypeptide
a
chromogranin A
b
prealbumin
c
PGP 9.5
d

Fig. 1 a–h


PHE 5
e
glucagon
f
HISL-19
g
neurone specific enolase
h

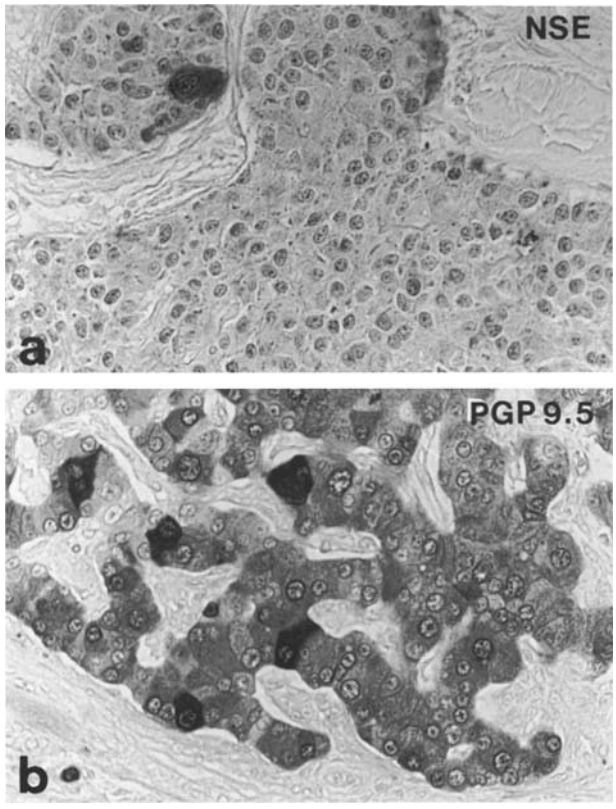


Fig. 2a, b. Large "ganglioid" cells in an insulinoma **a** and a glucagonoma **b** showing heavier immunostaining than adjacent tumour cells for neurone specific enolase and PGP 9.5, respectively (avidin-biotin-complex peroxidase. ×270)

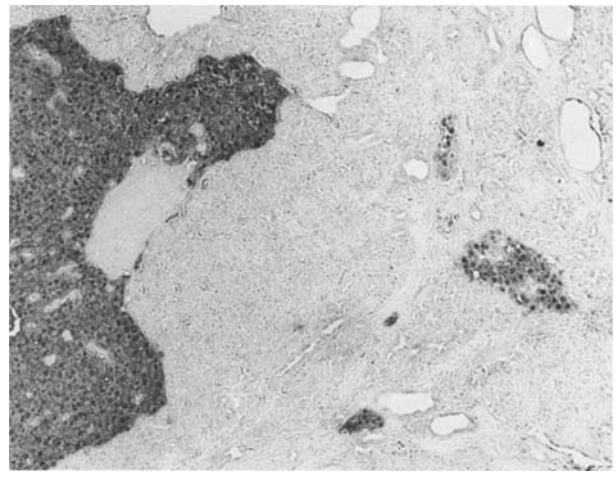


Fig. 3. Diffuse cytoplasmic immunostaining of a benign insulinoma (on the *left*) for PGP 9.5. The reaction is heavier in tumour cells than in the cells of a normal islet (on the *right*). The nuclear staining (no counterstaining in this slide) is confined to endocrine cells, either in the tumour or in the islet (avidin-biotin-complex peroxidase. ×100)

illustrates the immunohistochemical findings presented by the different neuroendocrine markers in relation with the hormone content of tumour cells.

The study clearly reveals two basic immunocytochemical patterns in most markers investigated. The first pattern is seen with the soluble cytoplasmic proteins NSE and PGP 9.5 and is characterized by a homogeneous cytoplasmic staining of virtually all neoplastic cells (Figs. 1–3). In consecutive sections the immune reactions of the two markers overlap substantially, their degree of staining being medium to intense in most cases especially with NSE. Variations in staining intensity from one tumour to another are unrelated to either the hormone content or the degree of cell differentiation. In approximately fifty per cent of tumours, either benign or malignant and both in primary or in metastatic location, a distinctive heavier staining is shown by occasional, scattered cells. These cells present large and vesicular nuclei and abundant cytoplasm which confer them a characteristic "ganglioid" appearance (Fig. 2). A varying degree of nuclear staining is frequently observed in tumour cells as well as in extratumoural islet cells. The PGP 9.5 immunoreactivity of extratumoural islets (available for examination only in benign tumours) is lower than that of insulinomas (Fig. 3) but heavier than that of glucagonomas and PP-omas, a finding indirectly indicating a higher content of PGP 9.5 in tumour cells producing insulin than in those producing other hormones. Acinar and other non-endocrine structures are consistently unreactive with the exception of nerve fibers and gangliar cells, which present a heavier immunostaining with PGP 9.5 than with NSE.

The second immunocytochemical pattern is consistent with the intracellular distribution of secretory granules being characterized by polar accumulation in the cell side facing the basal lamina

Fig. 1a–h. Serial sections of a pancreatic endocrine tumour with uneven hormone content. As shown in **a** and **f** pancreatic polypeptide (PP) and glucagon containing cells are segregated to a peripheral area of the neoplasm whereas no other types of hormone-producing cells could be found. Markers for granular antigens (**b, c, e, g**) mostly concentrate in the hormone containing cell ribbons. In contrast, markers for non-granular, soluble cytoplasmic antigens (**d, h**) uniformly stain all regions of the tumour and are independent of the cell hormonal content. In addition to typical granular staining HISL-19 (**g**) also binds the Golgi apparatus of numerous non-granulated cells. Extratumoural islets contain glucagon (*arrows* in **f**) but not PP cells. (Immunohistochemical procedures: avidin-biotin-complex peroxidase except **b**, immunogold-silver staining, and **h**, indirect immunoperoxidase. ×52.5)

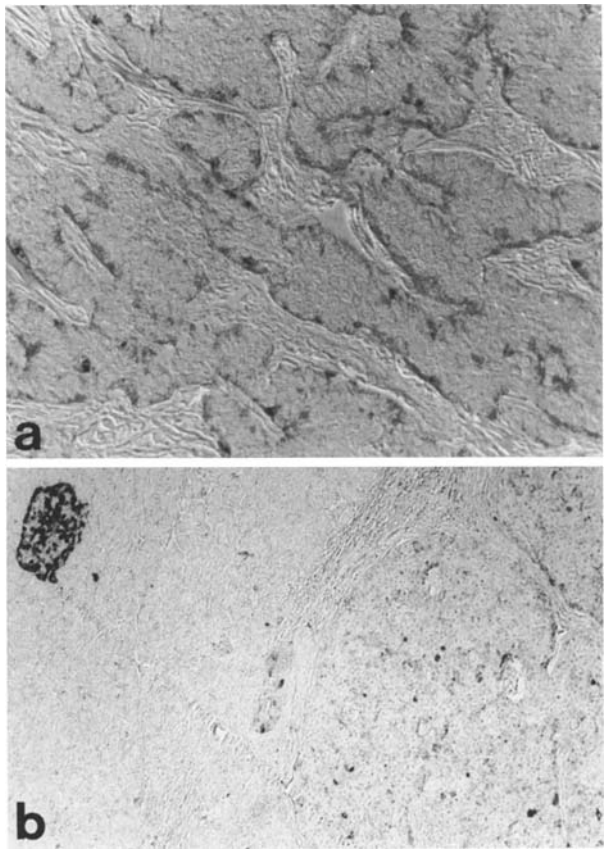


Fig. 4a, b. Immunostaining of benign insulinomas for chromogranin A. **a** A delicate, and sometimes inconsistent decoration of the granule containing poles of the tumour cells can be appreciated. **b** Only occasional sparse positive cells are present in another tumor (on the *right*). The strong reaction of the extratumour islet is mainly due to the glucagon producing cells. (Immunogold-silver staining; **a**, interference contrast optics. ×180; **b**, ×70)

and by frequent uneven concentration in contiguous cells (Figs. 4–6). This pattern is presented by CgA, PHE5, Pa and, in part, HISL-19. In consecutive sections it is found in virtually 100% of neoplastic cells immunoreactive for glucagon (Fig. 5), PP and gastrin while it is usually absent in areas of tumours lacking immunodetectable hormones (Fig. 1). Only 5 (50%) benign insulinomas show a majority of cells (roughly, 55 to 70%) stained by granular markers while in the other 5 immunoreactive cells range from 5 to 25% of the total tumour cell population (Fig. 4b). In both instances the intensity of the immunoreaction is generally lower than that shown by benign tumours producing other hormones. In parallel with their content in cells storing immunodetectable hormones, malignant tumours are usually characterized by fewer and sparse cells with granular staining. Of interest are the findings of one malignant insulinoma in which biopsies were collected with a time interval of 8 years: this case allows us to document the association between the tumour cell de-differentiation observed with passing of time and a striking decrease in the content of granular markers (Fig. 6a, c).

When compared in consecutive sections (if present) the immunostaining of the different granular markers overlaps (Fig. 1). However, some variations in the number of tumours and the frequency of cells positively reacting with the individual markers are observed (Table 2). In particular, PHE5-immunoreactive cells occur in all neoplasms and are more abundant than the CgA-immunoreactive cells in 17% of benign tumours. The latter

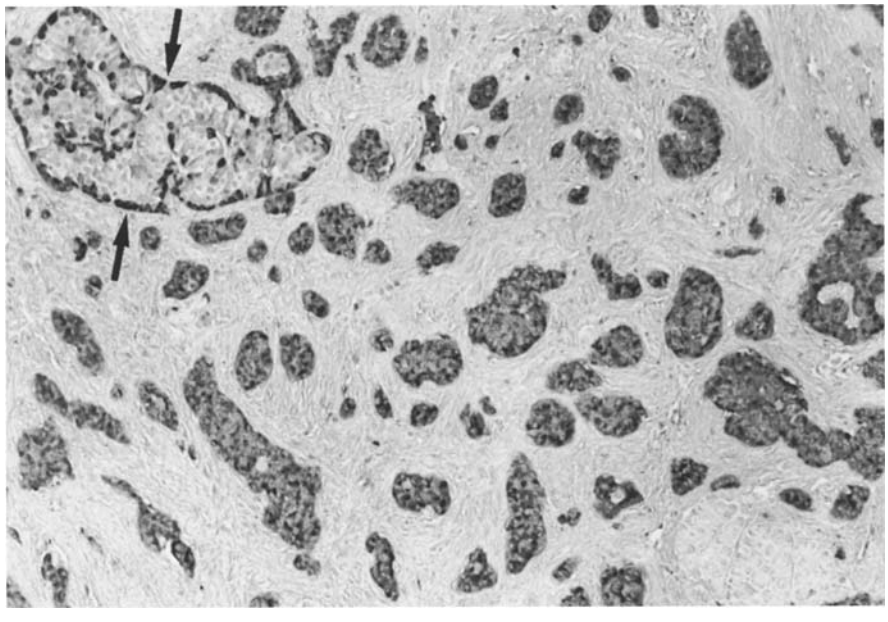


Fig. 5. Diffuse staining for prealbumin of the tumour cells in a benign glucagonoma and in the peripheral glucagon A cells of a trapped islet (*arrows*). (Avidin-biotin-complex peroxidase. ×115)

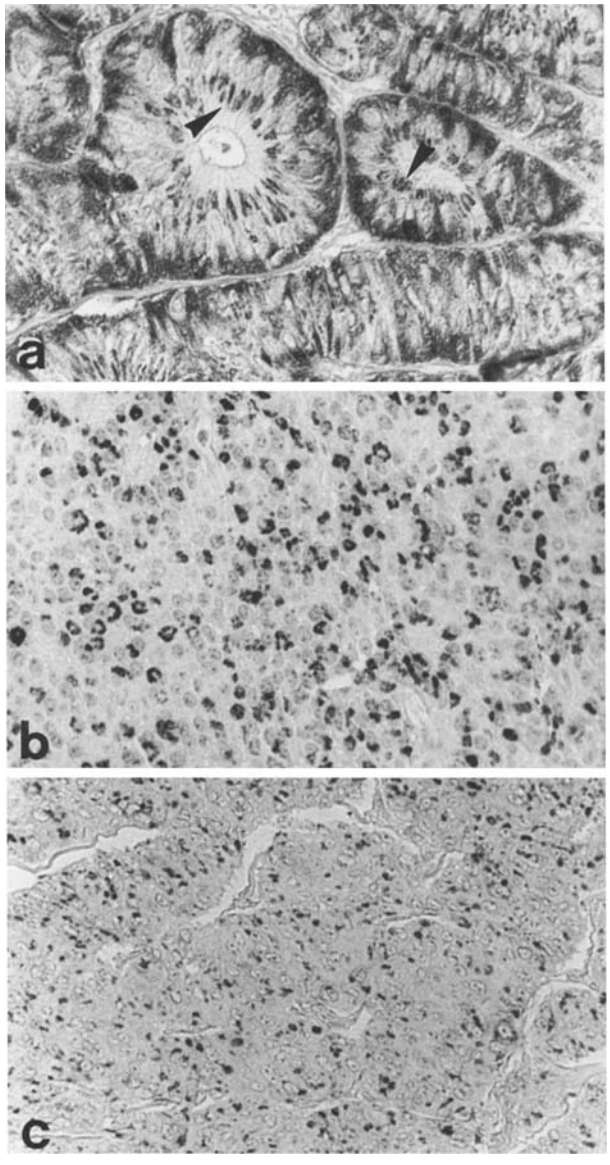


Fig. 6a–c. Different patterns of immunostaining for HISL-19. **a** Both basally located secretory granules and the supranuclear Golgi complex (*arrowheads*) are immunoreactive in a carcinoma with gland-like structures. **b** Only the Golgi complexes often showing a crescent- or ring-like appearance, are labelled in a benign insulinoma. **c** Less differentiated tumour tissue of the same case illustrated in **a** removed 8 years later and showing disappearance of granular staining and persistence of Golgi-related immunoreactive clusters. (Indirect immunoperoxidase with nuclear counterstaining. ×270)

cells are absent in two carcinomas. Immunostaining for Pa is lacking in 4 benign insulinomas and in 7 carcinomas; moreover, it is often more pronounced in benign PP-omas than in benign glucagonomas.

In addition to its reaction with the secretory granules, the HISL-19 *mab* also labels the Golgi apparatus of most tumour cells (Fig. 6). The Golgi-type staining is most often prominent in those tumours in which granular staining is inconspicuous. It is well represented also in tumour cells in which no hormone content was appreciable (Fig. 1) whereas it is usually absent in PP-omas, both benign or malignant. When both patterns of HISL-19 immunoreaction are considered, immunoreactive cells are present in all tumours of the present series. The immunohistochemical findings of the HISL-19 *mab* in the present series of tumours are detailed elsewhere (Bordi et al. 1988).

Immunostaining for α-HCG is not specifically related to that of other neuroendocrine markers. Immunoreactive cells are observed in 9 of 16 carcinomas (56.2%) (Fig. 7a, c) being more frequently found in malignant insulinomas and glucagonomas (6 of 8, i.e. 75%) than in other carcinomas (3 of 8, i.e. 37.5%). They are also found in 4 of 43 benign tumours (9.3%), all associated with the insulinoma syndrome (Fig. 7b, d). In the latter neoplasms α-HCG containing cells usually are less abundant than in malignant tumours with the exception of one case (Fig. 7b), a tumour measuring 1.5 cm in diameter and lacking any histological characteristic of malignancy. Cytologically this tumour shows a mixed population of insulin and PP-cells, roughly in the same proportion, together with less frequent somatostatin cells. The patient is well and without signs of recurrence 6 years after the operation. The other three patients are also well 16, 3 and 4 years after surgery, respectively. The α-HCG immunoreactive cells are demonstrated by either the immunogold-silver staining or the ABC-peroxidase technique with comparable results, although a slight increase of immunostained cells is sometimes observed with the former procedure. In both cases the staining is abolished by preadsorption of the antiserum with purified α-HCG.

Discussion

In the present study a panel of specific mono- and poli-clonal antibodies against antigens recently proposed as markers for neuroendocrine neoplasms is evaluated in a series of pancreatic endocrine tumours. In spite of well demonstrated structural and immunological differences, most neuroendocrine markers can be grouped into two classes on the basis of the identity of the immunoreactive cell compartment and the substantial overlapping of the immunohistochemical results: 1) markers identifying a soluble cytoplasmic antigen with diffuse staining of the tumour cells; and 2) markers reacting with antigen(s) located in the secretory

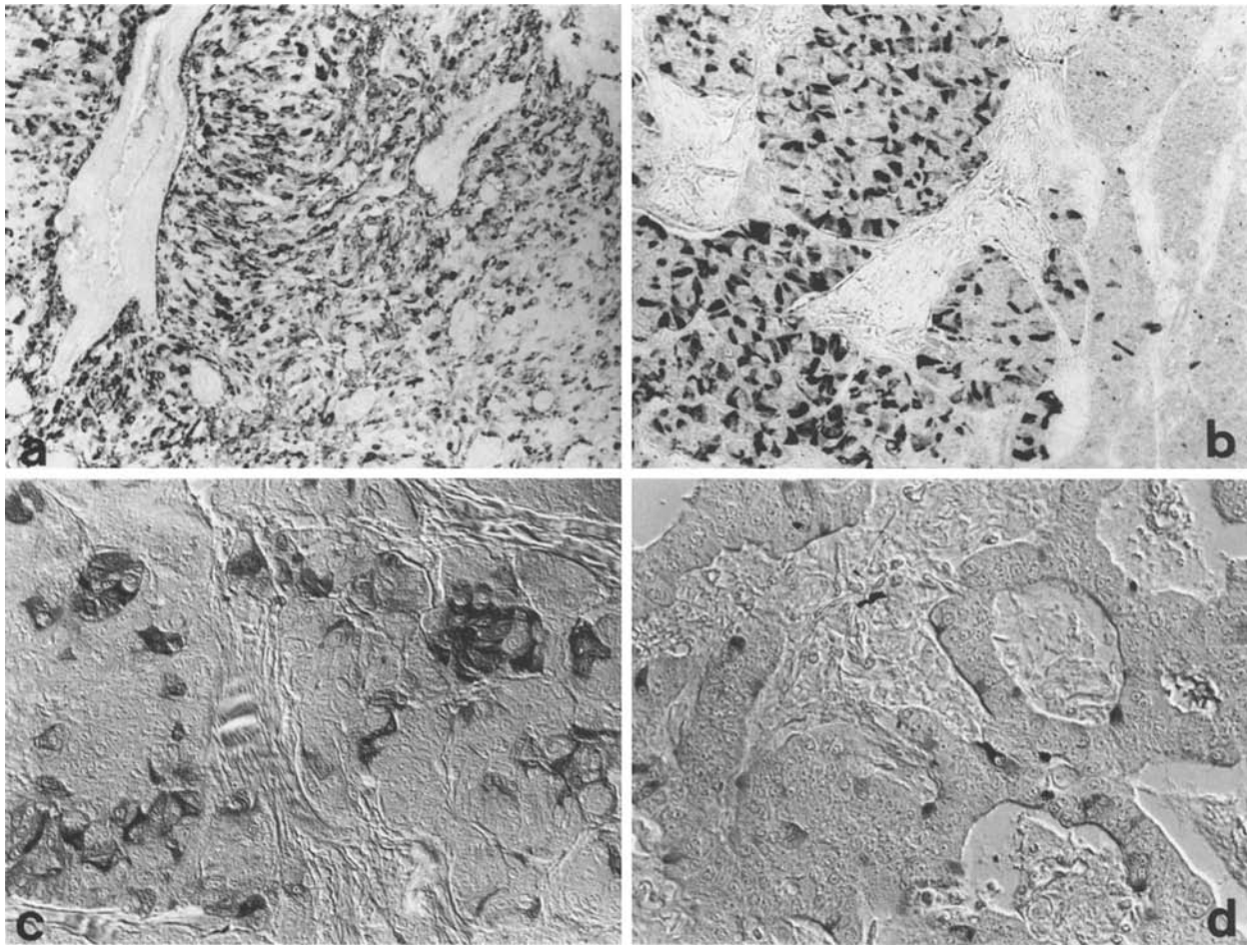


Fig. 7a–d. Expression of alpha subunit of human chorionic gonadotropin in malignant (**a, c**) and benign (**b, d**) tumors. Immunoreactive cells of carcinomas are diffusely distributed in a gastrinoma **a** or mostly clustered in an insulinoma **c**. Those of two benign insulinomas (**b, d**) shows a frequency comparable to that of carcinomas in **b** and a scattered distribution in **d** (**a** and **b**: immunogold-silver staining, ×100; **c** and **d**: avidin-biotin-complex peroxidase interference contrast optics, ×235)

granules of tumour cells and often presenting a polarized staining consistent with the intracellular granule distribution.

The first group of markers includes NSE and PGP 9.5. In agreement with previous observations (Lloyd et al. 1984b; Rode et al. 1985; Nash and Said 1986; Chejfec et al. 1987) these soluble cytoplasmic proteins are expressed by all tumours regardless of their hormone production or clinical behaviour.

The specificity of NSE and PGP 9.5 as markers for neuroendocrine tumours is limited by their expression by a range of non-neural, non-neuroendocrine neoplasms (Vinores et al. 1984; Leader et al. 1986; Gould 1987; Rode et al. 1985). Moreover, these markers present a varying and, sometimes, marked nuclear staining occuring both in tumour and in normal islet cells. Nuclear labelling after immunostaining for the soluble cytoplasmic proteins NSE and PGP 9.5 has already been reported (Haan et al. 1982; Rode et al. 1985; Leader et al. 1986; Thompson and Day 1988). Its significance remains unclear but an influence of tissue fixation (Haan et al. 1982) seems unlikely since in our study no nuclear staining is observed with the other neuroendocrine markers used.

The group of markers for granular antigens includes CgA, PHE5, Pa and, in part, HISL-19. With these markers a heavy, consistent reaction, virtually identical in appearance to that shown by antisera against specific hormones, is seen in benign glucagonomas and PP-omas. In contrast, the majority of benign insulinomas and all malignant tumours show a less intense staining, often restricted to sparse cells. In these cases Pa and the granular staining pattern of HISL-19 are less frequently expressed than PHE5 and CgA. Our results indicate that in malignant tumours the degree

of immunoreaction for granular markers is inversely related to the degree of cell differentiation.

The specificity of granular antigens as markers for neuroendocrine tumours is high (Gould 1987). However, the occurence of sparse endocrine cells immunoreactive for CgA in non endocrine carcinomas of the pancreas (Kay et al. 1985) requires caution in the interpretation of scattered cells immunoreactive for neuroendocrine granular markers as a proof for an endocrine origin of tumours not otherwise identified.

The relation between the various antigens colocated in the granules of normal and neoplastic islet cells remains unknown. Immunological and structural differences seem to indicate a heterogeneous population of proteins co-stored with the specific hormone within the granules. However, it cannot be excluded that these substances may, at least in part, represent the cleavage products of a single large precursor. The absence or low concentration of granular markers in the insulin-producing B cells is intriguing. Beta-granin, a 21 kD protein stored in and secreted from beta granules, has been identified and found to be immunologically related to CgA (Hutton et al. 1985). However, the concentration of this small protein in most tumour tissue is probably lower than the threshold of the sensitivity of the method, thus explaining the frequent inconsistency of immunostaining for CgA of insulin producing neoplasms.

Our subdivision of neuroendocrine markers into two groups has practical implications. Indeed for diagnostic purpose no significant advantages can be expected by the association of markers of the same group while immunoprobes of different groups may offer complementary, useful information. The identification of immunoprobes for other cytoplasmic components may be useful for avoiding the limitations presented by the two classes of markers mentioned above. In this regard it is noteworthy the property of HISL-19 of labeling also the Golgi complex of tumour cells. This characteristic allows this *mab* to show positive immunoreaction in all cases including those in which the low content of tumour cell secretory granules is responsible for a negative result of granular markers. However, immunostaining of Golgi complexes by HISL-19 is occasionally found in some non endocrine tumours (Krisch et al. 1986). Synaptophysin, a 38 kD protein located to intracellular small vesicles independent from secretory granules and distributed in the Golgi region or throughout the cytoplasm (Navone et al. 1986), may represent another promising candidate (Chejfec et al. 1987; Gould 1987).

The distribution of the immunoreactivity for the alpha-subunit of glycoprotein hormones (α-HCG) was unrelated with that of other markers investigated in the present study. The subunit was largely expressed in 56.2% of malignant tumours, a figure slightly lower but essentially in accordance with a previous study (Heitz et al. 1983). Moreover, we found α-HCG-immunoreactive cells (although usually with a lower frequency than in carcinomas) in four tumours which for their histological and clinical features could be confidently classified as benign. All these cases were associated with the insulinoma syndrome. Although in accordance with data from one group (Lloyd et al. 1984a), our results in benign tumours are at variance with those of the large study of Heitz et al. (1983) and do not support the assumption that the expression of α-HCG by pancreatic endocrine tumours is a specific marker of malignancy.

The cytological composition of the "benign" tumour showing the higher α-HCG immunoreactivity, with a mixed composition of insulin and PP cells and a minor somatostatin cell population, is unusual in our experience and may be of relevance to the ectopic expression of the α-subunit in this case. Additional explanations for the discrepancy between the results of this and the previous study (Heitz et al. 1983) may include: 1) our α-HCG immunoreactive tumours, despite their benign appearance, actually represented potentially malignant neoplasms cured before the onset of a metastatic spread; 2) the immunohistochemical procedures employed in the present investigation for visualization of the immune reaction (IGSS and ABC) are more sensitive that the PAP technique used by Heitz et al. (1983); and 3) the antisera used in this and the previous studies recognized different epitopes of the α-subunit or of an immunologically related molecule. Further studies are indeed warranted to clarify the issue but it may be suggested that differences in the expression of the α-subunit of glycoprotein hormones between benign and malignant islet cell tumours may be quantitative rather than qualitative. Our results are not necessarily in contrast with the absence of increased circulating levels of α-HCG in patients with benign islet cell tumours shown by Kahn et al. (1977) since the lower tumour concentrations of immunoreactive material and the high degree of tumour cell differentiation may prevent inappropriate release of the subunit.

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