American Journal of Pathology, Vol. 132, No. 2, August 1988

Immunocytochemical Patterns of Islet Cell Tumors as Defined by the Monoclonal Antibody HISL-19

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A series of 51 islet cell tumors removed from 28 patients was investigated immunohistochemically with the monoclonal antibody HISL-19. The antibody was produced after immunization of BALB/c mice with human islet cells and was found to react with a wide range of neuroendocrine and neural cells. All tumors presented positive immunoreaction showing various combinations of 2 basic patterns. The first pattern reflected the immunostaining of the secretory granules of the tumor cells. This "granular" staining was predominantly associated with benign neoplasms and with the tumoral production of glucagon and pancreatic polypeptide (PP), while it was absent or inconsistent in most insulin-secreting tumors. The second pattern consisted of focal immunoreactive aggregates located in a peri- (and, in polarized cells, supra-) nuclear position. This "cluster-type" staining showed a good morphologic and topographic correspondence with the Golgi apparatus of the cells of the same tumors, as shown by electron microscopy. The latter pattern was well represented in all types of islet cell tumors except those producing PP. Moreover, it was more apparent in less differentiated tumors in which the granular pattern was often absent or inconsistent. Cluster-type (but not granular) immunoreactivity was frequently found in some nonendocrine, nontumoral pancreatic structures, particularly in the epithelium of small ducts. However, the immunoreactive aggregates of nonendocrine cells were distinctly less prominent than those of endocrine cells. On the basis of a comparison with other immunohistochemical markers for neuroendocrine cells, it is concluded that the HISL-19 monoclonal antibody presents specific staining characteristics useful for the cytologic analysis of islet cell tumors. (Am J Pathol 1988, 132:249–257)

THE MONOCLONAL ANTIBODY (MAb) HISL-19 was produced after immunization of BALB/c mice with human islet cells isolated from cadaveric pancreatic specimens.[1] This MAb reacts with islet cells of human, bovine, and porcine origin but not with those of rodents or of the angler fish. In addition, it reacts with endocrine cells of the gut and lung, all cell types of the anterior pituitary, the parafollicular C cells of the thyroid, the adrenal medullary cells, and central and peripheral neurons.[1,2]

Immunoblotting experiments with tumoral islet cell tissue and with isolated bovine islets showed that the antigenic epitope is present in 4 islet cell proteins with molecular weights in the region of 120, 69, 67, and 56 kd.[1,2] Although these proteins may correspond to different molecules sharing crossreactive antigenic determinants, it seems more likely that they represent related products resulting from sequential posttranslational cleavage and processing of a common precursor protein.[1,2] More recently, it has been shown that the 67 kd (35/32 dimer) protein detected by MAb HISL-19 shares many biochemical and molecular key features with the chromogranin proteins.[3] As demonstrated by one-dimensional and two-dimensional gel electrophoresis, immunoblotting, monoclonal antibody HISL-19 immunoaffinity chromatography, and immunoelectron microscopy, it is a water soluble, acidic protein stored (after passing the Golgi apparatus) within secretory granules of peptide hormone producing cells, and it is released in detectable amounts into the serum of patients bearing neuroendocrine carcinomas. Differences of the HISL-19 pro-

Supported in part by grants from the Italian Ministry of Public Education and the Italian Association for Cancer Research (A.I.R.C.).

Accepted for publication March 31, 1988.

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tein and chromogranins A, B, and C are indicated by their different tissue distribution, the discrepancy of their apparent molecular weights in SDS gels and isoelectric points, and by the lack of cross-reactivity of their specific antibodies.[3]

The spectrum of immunoreactive cells, covering both a wide range of neuroendocrine cells and different subsets of neural cells, suggests that the HISL-19 MAb defines a new family of neuroendocrine molecules.[1] The observation, however, that low but detectable amounts of immunoreacting material are also present in a small number of nonendocrine nonneural cells, such as those forming the epithelia of the gallbladder, the bronchial mucosa, and the small ducts of salivary glands,[2] indicates that the proteins, or at least one of them, may also occur in some exocrine epithelial tissue, although in a markedly lower concentration.

A previous paper by one author[2] has shown that the HISL-19 MAb has potential applications in diagnostic histopathology because it appears as an almost universal marker for neoplasms originating from most neuroendocrine tissues. Islet cell tumors were included in the series of neoplasms investigated and their reactivity to HISL-19 MAb was already described. The aim of the present study is to extend the analysis to a different, larger series of pancreatic endocrine neoplasms. In particular, an attempt is made to assess whether there is a relationship between the pattern of HISL-19 reactivity of tumor cells and: 1) the type of hormone produced by the tumors; 2) the benign or malignant nature of the neoplasms; and 3) the degree of differentiation of tumor cells. In addition, a parallel investigation performed in the same series of tumors with a number of others neuroendocrine markers recently proposed[4] offered the opportunity of a comparative evaluation of the respective immunoreactive patterns.

Materials and Methods

Materials

Fifty-one pancreatic endocrine tumors collected from 28 patients were investigated. The neoplasms were classified according to the symptom-causing hormone and/or the majority islet cell population shown by immunocytochemistry. Tumors discovered as incidental findings at autopsy or during operation and showing only occasional hormone-containing cells in immunohistochemistry but otherwise clearly recognizable as islet cell tumor on the basis of the histologic characteristics and of the immunoreactivity to neuroendocrine markers (such as chromogranin A, neurone-specific enolase or protein gene product 9.5) were classified as nonfunctioning tumors. Tumor tissues were fixed in Bouin's fluid in all cases except 4, in which 10% formalin was used. After dehydration the specimens were routinely embedded in paraffin. Serial sections were then cut at 5 μ and stained with hematoxylin and eosin (H&E), the Grimelius[5] silver method, and polyclonal antisera against insulin, glucagon, somatostatin, pancreatic polypeptide (PP), gastrin, and vasoactive intestinal peptide (VIP) for histopathologic and hormonal characterization. The immunoreactions were visualized by either immunofluorescence or immunoperoxidase techniques. Details on the immunohistochemical techniques and sources of antisera are given elsewhere.[6,7] Tissue specimens processed for conventional electron microscopy as previously detailed[8,9] were available for 22 tumors.

Preparation of MAb HISL-19

The MAb HISL-19 was produced as described in detail previously.[1] Briefly, female BALB/c mice were immunized with human islet cells isolated from cadaveric human pancreatic specimens. Spleen cells from the immunized mice were fused with the nonsecretor murine myeloma cell line P3x63, 653 using the standard polyethylene glycol technique.[10,11] The resulting hybrids were initially screened for reactivity with human islets using the indirect immunofluorescence technique on cryostat sections of human pancreas. Positive colonies were cloned several times, expanded, and monoclonal antibody ascites was raised in mice. The characterization of the proteins recognized by the MAb HISL-19 was obtained with the Western immunoblotting technique.[1,2]

Immunohistochemical Procedure

The immunoreactivity of MAb HISL-19 was evaluated using an indirect immunoperoxidase technique. Sections were incubated with MAb HISL-19 (dilution 1/4000 of ascites fluid) and subsequently incubated with peroxidase conjugated rabbit anti-mouse Ig antibody (Dakopatts, Copenhagen, Denmark; dilution 1/100) and then with peroxidase conjugated swine anti-rabbit Ig antibody (Dakopatts, dilution 1/100). Peroxidase activity was detected by the 3,3'-diaminobenzidine tetrahydrochloride (DAB) reaction and the sections were counterstained with hematoxylin.

Results

Table 1 reports the distribution of the 51 tumors investigated in this study along with the relevant results after immunostaining with the HISL-19 MAb. All neoplasms had a positive immunoreaction show-

Table 1—Results of Staining with the MAb HISL-19 in a Series of 51 Pancreatic Endocrine Tumors: Distribution of the Immunohistochemical Patterns and Occurrence of Immunoreactive Material in the Extratumoral Exocrine Tissue

Type of tumor	No.	Immunohistochemical pattern*		Extratumoral tissue†	
		Granular‡	Cluster-type	Ducts	Acini
		Benign tumors			
Insulinoma	10	4 (5)	8	8 (9)	8 (9)
Glucagonoma	12	12	10	3 (4)	3 (4)
PP-oma	15	15	2	2 (3)	2 (3)
Nonfunctioning	1	1	1	– (1)	1 (1)
TOTAL	38	32 (5)	21	13 (17)	14 (17)
		Malignant tumors			
Insulinoma	4	1 (1)	4	1 (1)	NE
Glucagonoma	3	2 (1)	3	NE	NE
Gastrinoma	2	1 (1)	2	NE	NE
VIP-oma	1	–	1	NE	NE
PP-oma	1	– (1)	–	NE	NE
Nonfunctioning	2	2	2	NE	NE
TOTAL	13	6 (4)	12	1 (1)	

* See text for details.

† Numbers in parentheses refer to the cases in which extratumoral tissue was available for the study. NE = not examined.

‡ Numbers in parentheses refer to tumors in which only occasional discrete cells showed granular type immunoreactivity.

ing various combinations of 2 basic patterns. One pattern, thereafter designed as granular, was characterized by a fine granular cytoplasmic staining. This staining reproduced the intracellular distribution of secretory granules with frequent polarization and accumulation at the capillary pole of the tumor cells (Figure 1). The other pattern, thereafter designed as cluster-type, was characterized by focal and often large perinuclear aggregates of coarse material (Figure 2). These clusters, sometimes presenting a crescentlike or a ringlike shape (Figure 2), in favorable conditions of cell structure and sectioning were shown to be localized in the supranuclear region, ie, opposite to that containing the basally accumulated secretory granules (Figure 3). The combination of the 2 patterns of immunoreaction resulted in a readily identifiable staining of all tumors except one PP-producing carcinoma that harbored rare granular stained cells only.

The granular pattern of HISL-19 immunoreactivity was often found to coexist with the cluster pattern (Figure 3), although the latter tended to be masked when the tumor cells were filled with secretory granules. The granular pattern was found to present the

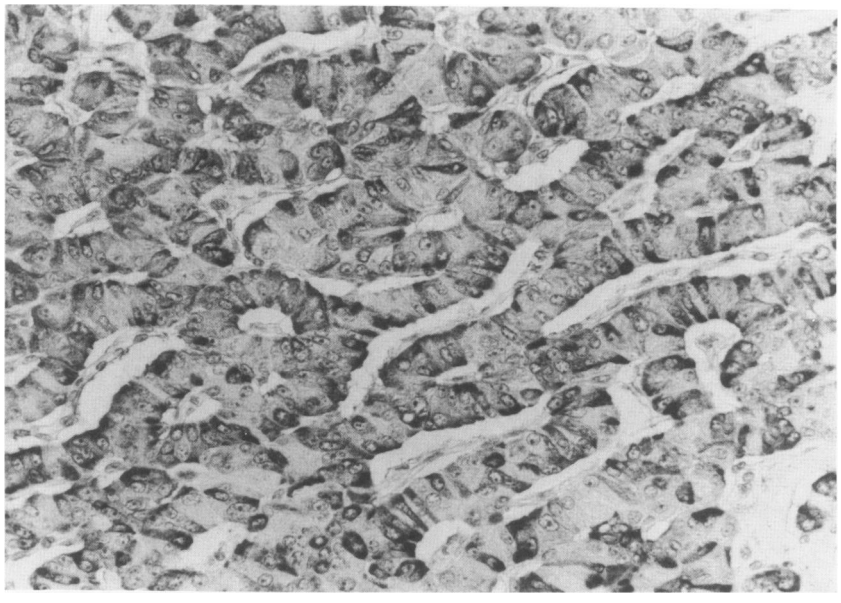

Figure 1—Granular pattern of immunoreactivity to HISL-19 monoclonal antibody in a benign PP-oma from a patient with multiple endocrine neoplasia type I (MEN-I). Several tumor cells show polarization of the immunoreactive material. No cluster-type immunoreactivity is detectable. (Indirect immunoperoxidase technique with hematoxylin counterstaining, ×275)

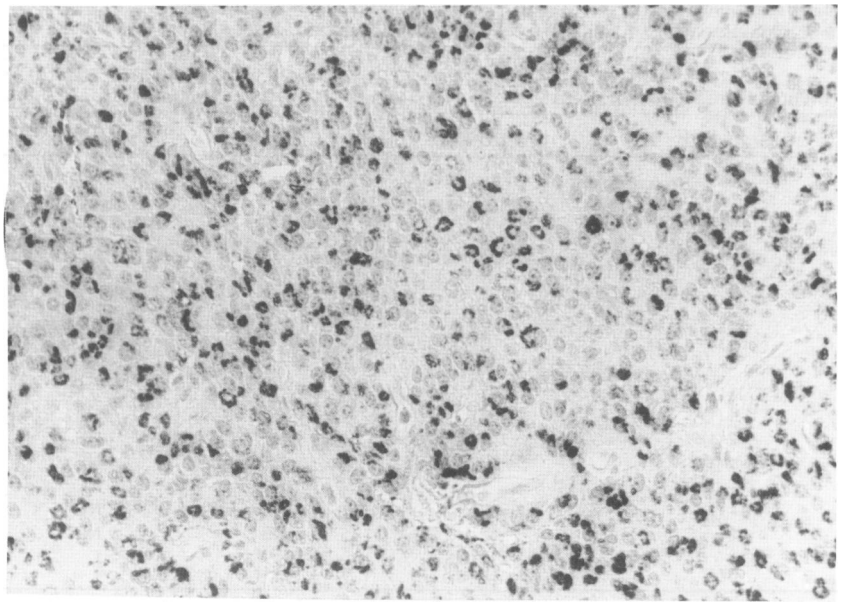

Figure 2—Cluster-type pattern of HISL-19 immunoreactivity with absence of granular staining in an insulin-secreting adenoma. The paranuclear position and the crescent or ringlike shapes of the immunostained aggregates are consistent with the Golgi apparatus of tumor cells. (Same technique as in Figure 1, ×275)

most diffuse expression when the tumors exhibited a gyriform-ribbonlike arrangement (Figure 1).

A granular staining of the majority of tumor cells occurred in virtually all benign glucagonomas and PP-omas (Figure 1). The correspondence of this type of immunoreaction with glucagon and PP immunoreactivity was better appreciated in those tumors in which an association of areas rich and areas poor in these hormones was present (Figure 4). In contrast, a diffuse granular staining for the HISL-19 MAb was found in only 4 of 10 benign insulinomas. The only feature discriminating these 4 neoplasms from the others was the predominance of the gyriform-ribbonlike histologic structure. In 5 of the remaining benign insulin-secreting tumors the granular reaction was confined to isolated cells or to small discrete cell clusters (Figure 5), which in consecutive sections were not necessarily associated with insulin immunoreactivity but revealed either absent or variable hormone content. On occasion discrete clusters of positive cells were found to correspond to nonneoplastic islets trapped by the tumor.

Granular HISL-19 immunostaining of most tumor cells was less frequent in malignant tumors, being present in 6 of 13 cases. Of interest is 1 of these 6 tumors, an insulin-secreting carcinoma showing 2 major cell populations, 1 composed of insulin producing cells and the other composed of argyrophil cells, as

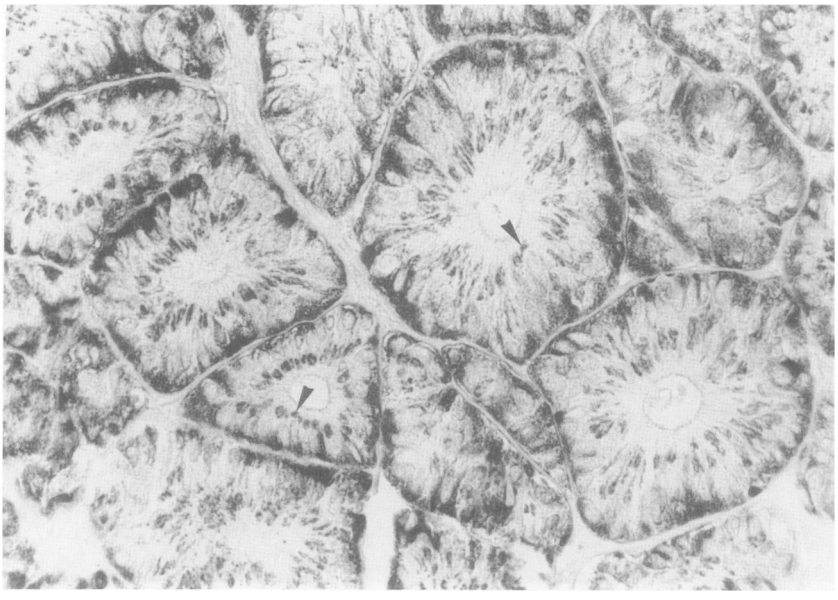

Figure 3—Coexistence of granular and cluster-like patterns of HISL-19 immunoreactivity in insulin-producing cells of a metastatizing tumor. The glandlike arrangement of tumor cells emphasizes the basal accumulation of granular immunostained material and the supranuclear localization of the immunoreactive focal aggregates related to the Golgi complex (arrowheads). (Same technique as in Figure 1, ×255)

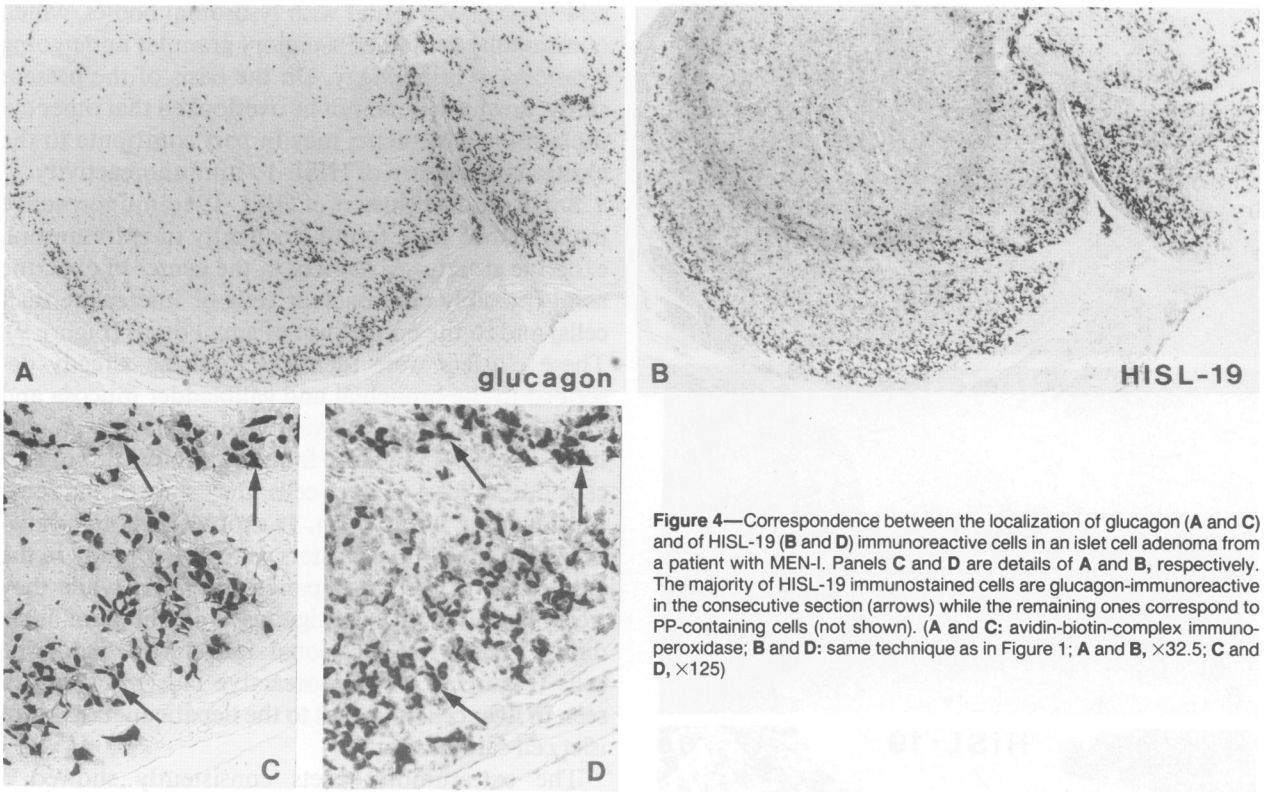

Figure 4—Correspondence between the localization of glucagon (**A** and **C**) and of HISL-19 (**B** and **D**) immunoreactive cells in an islet cell adenoma from a patient with MEN-I. Panels **C** and **D** are details of **A** and **B**, respectively. The majority of HISL-19 immunostained cells are glucagon-immunoreactive in the consecutive section (arrows) while the remaining ones correspond to PP-containing cells (not shown). (**A** and **C**: avidin-biotin-complex immunoperoxidase; **B** and **D**: same technique as in Figure 1; **A** and **B**, ×32.5; **C** and **D**, ×125)

described in detail previously.[12] In this tumor the granular pattern was shown extensively by the insulin cells while it was inconspicuous in the argyrophil cells (Figure 6). Tumor tissue removed from the same patient 8 years later revealed complete degranulation of tumor cells after immunostaining for either insulin or HISL-19. The granular pattern also was lacking in another insulin-secreting carcinoma, composed mostly of argyrophil cells.

The cluster-type pattern of HISL-19 immunoreactivity was clearly recognizable in most tumors, without apparent relations with the type of hormone produced by the neoplastic cells. A notable exception was represented by PP-producing tumors, the majority of which showed sparse or no cluster-type immunoreaction. This pattern was the only type of immunoreaction seen in most malignant tumors. Such a feature reflected a lesser degree of tumor cell differentiation, as exemplified by the previously mentioned case of the tumor re-examined after 8 years, in which the clear-cut variation over time from a predominant granular pattern to a lonely cluster-type pattern was

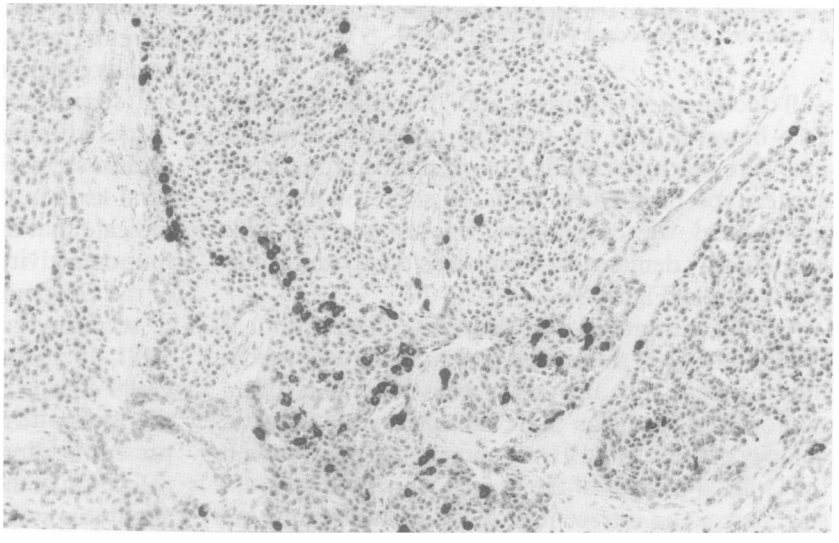

Figure 5—Isolated cells showing granular HISL-19 immunostaining in a benign tumor exclusively composed of insulin-containing cells. The hormonal content of the discrete immunoreactive cells could not be identified. (Same technique as in Figure 1, ×110)

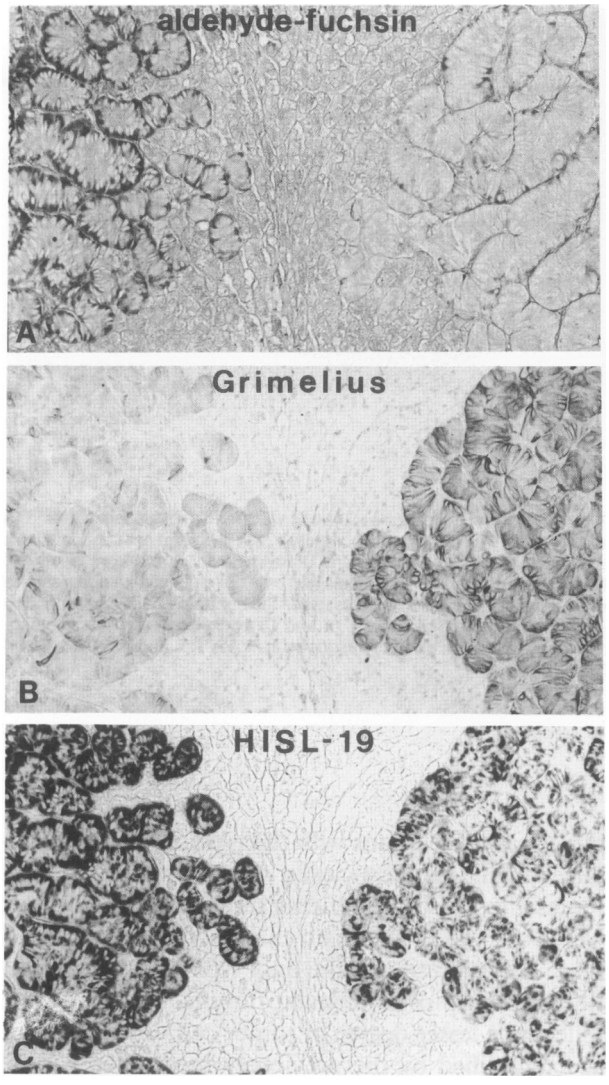

Figure 6—Liver metastases of an islet cell carcinoma composed of insulin-containing (**A**) and argyrophil (**B**) cell with segregation of the two cell populations to different tumor clusters. While the cluster pattern of HISL-19 immunostaining (**C**) is present in both cell types, the granular pattern is prominent in insulin cells (on the left) and absent or inconspicuous in argyrophil cells (on the right). (**A**: aldehyde-fuchsin stain for beta granules; **B**: consecutive serial section to **A** stained with Grimelius' silver method; **C**: same section as **B** decolorized and immunostained for HISL-19, ×130)

well documented (Figures 6 and 7). It must be noted also, however, that the majority of benign insulinomas were characterized by a marked predominance of cluster-type immunoreaction. In some of these tumors, areas with prominent immunoreactive cells were sharply demarcated from areas composed of unreactive cells (Figure 8).

Although immuno-ultrastructural localization of HISL-19 MAb in this material was prevented by technical reasons,[3] the analysis of conventional electron micrographs from the same tumors revealed a close analogy of the HISL-19 immunoreactive clusters with the Golgi apparatus of the neoplastic cells in terms of size, shape, and intracellular location. In contrast, no relationship was found with lysosomal bodies, which occasionally contained secretory granules undergoing a process of crinophagy. On the basis of the present study, however, it cannot be overlooked that other cytoplasmic constituents may in part contribute to the cluster-type pattern of HISL-19 immunoreactivity.

Small, dotlike clusters of HISL-19 immunoreactive material also were found frequently in extratumoral exocrine structures, namely in the center of exocrine acini (possibly centroacinar cells or intercalate duct cells) and in the epithelium of small ducts (Figure 9). These clusters were identical to those already described in the bronchial and gallbladder mucosa and in the small ducts of salivary glands.[2] They were distinctly smaller than those found within the tumor islet cells but, at least in duct cells, they showed the same supranuclear localization. It should be noted, however, that the dotlike clusters were present only in the epithelium of the small pancreatic ducts while they were absent in the contiguous epithelium of large ducts, except for occasional intraductal endocrine cells (Figure 9). Immunoreactive clusters were not seen in liver cells adjacent to the hepatic metastases of islet cell carcinomas.

The extratumoral islets consistently showed a marked granular staining with the HISL-19 MAb. The reaction was clearly more intense in the peripherally located glucagon-containing cells.

Discussion

It has been shown that immunostaining of islet cell tumors with the MAb HISL-19 results in 2 patterns of immunoreaction, which for the intracellular location and the structure of the immunoreactive material can reasonably be assigned to different compartments of the tumor cells. The first pattern, showing fine granular material often accumulated in the basal cytoplasm of tumor cells, obviously reflects the cell content of secretory granules. The second pattern is characterized by irregular supranuclear clusters of coarse material sometimes having a crescentlike or a ringlike shape. Although technical reasons prevented a direct immunohistochemical localization at the ultrastructural level in this material, several lines of evidence indicate that the cluster-type pattern of HISL-19 immunoreactivity mostly corresponds to the structures of the Golgi apparatus of the tumor cells. In addition to secretory granules, a selective localization of the HISL-19 protein to the cisternae and vesicles of the Golgi complex was demonstrated in bovine pancreatic islets with immuno-electron microscopy using the pre-embedding procedure.[3] In the same study, the localization of the HISL-19 reactive protein in the Golgi apparatus was also demonstrated in the cells of the

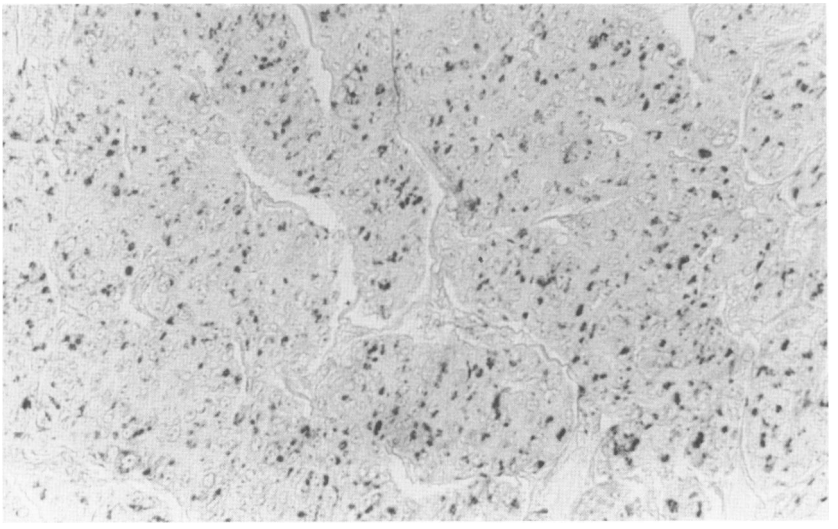

Figure 7—Extensive cluster-type and absent granular-type HISL-19 immunostaining in tumor tissue of the same patient as in Figure 6 removed 8 years later. Neither insulin immunoreactivity nor argyrophil staining persisted in tumor cells examined in consecutive sections. (Same technique as in Figure 1, ×275)

adrenal medulla where, however, chromaffin granules were unreactive. Moreover, the present investigation showed a close analogy between the optical appearance of HISL-19 immunoreactive clusters and the ultrastructure of the Golgi complexes of the same tumor in terms of size, shape, and intracellular location. A minor contribution of other cytoplasmic constituents to the cluster-type immunoreactivity cannot be excluded, however.

The staining characteristics of the HISL-19 MAb show some interesting differences from those of the most common neuroendocrine markers proposed in recent years. These markers can be conveniently subdivided into 2 categories[4]: 1) those reacting with antigens stored in the cell secretory granules, such as antibodies against chromogranin A,[13] PHE5,[14] and prealbumin[15]; and 2) those reacting with antigens diffused in the cell cytoplasm, such as antibodies against neuron-specific enolase[16] and PGP 9.5.[17] Studies with chromogranin A[18] have shown that the staining of neuroendocrine granular markers essentially corresponds to that found after the Grimelius[5] silver impregnation.

The granular pattern of immunoreaction obtained with HISL-19 MAb largely overlaps the pattern presented by neuroendocrine markers against granular antigens, as it could be verified by staining of adjacent sections of the tumors included in the present study.[4] In this regard it is pertinent to recall that the HISL-19 protein(s) are not related with chromogranins.[3] As a marker for the secretory granules contained in the tumor cells, the granular pattern of HISL-19 immuno-

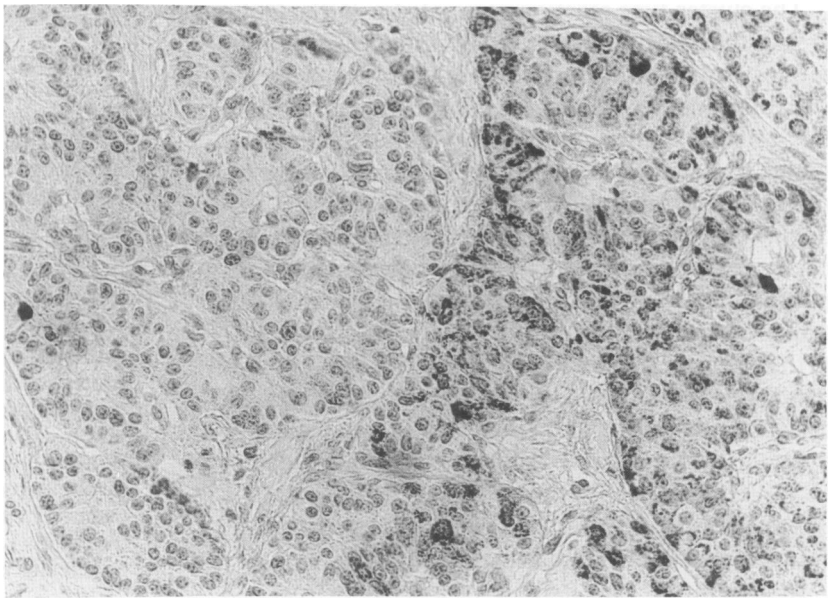

Figure 8—Insulin-secreting adenoma with coexistence of well-defined areas showing both types of HISL-19 immunostaining (on the right) and of areas virtually unreactive (on the left). (Same technique as in Figure 1, ×275)

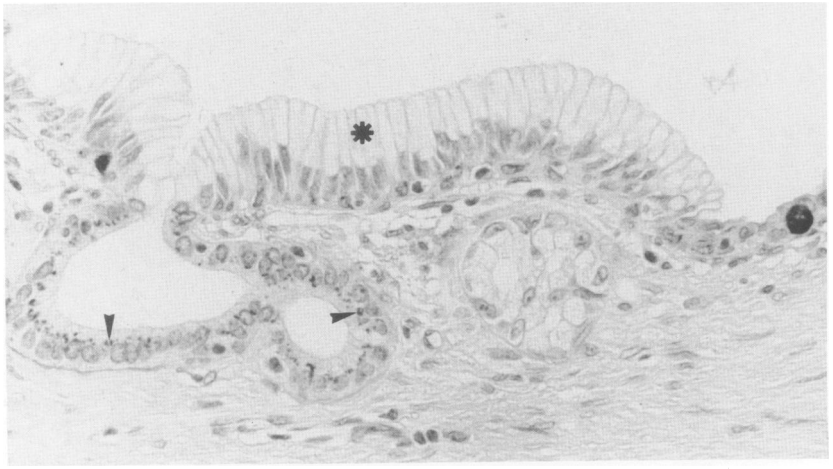

Figure 9—Small dotlike structures (arrowheads) showing HISL-19 immunoreactivity are present in the supranuclear area of the epithelial cells of a small duct found in the peritumoral tissue. Contiguous columnar cells (*) of a large duct do not present immunoreactive reaction. Strong granular staining is shown by 2 endocrine cells intercalated with the ductal epithelial cells. (Same technique as in Figure 1, ×365)

reactivity has the same advantages and limitations of other neuroendocrine granular markers.[4] Indeed, it appears to be specific for neuroendocrine tissues but it is strictly dependent on the granule content of the cells. Thus, its use as a diagnostic tool in the study of metastatic tissues of unknown origin is limited by the frequent cell degranulation of malignant endocrine tumors. Moreover, in the specific case of islet cell tumors it is more effective in granulated neoplastic cells containing glucagon or PP than in those containing insulin. The present results also indicate that the HISL-19 protein(s) are either absent or below the threshold of immunohistochemical detection in the secretory granules of most insulin-producing tumors, either benign or malignant. In addition, granular HISL-19 immunostaining also was inconsistent in the argyrophil cells of insulin-secreting tumors, a type of granulated cells whose secretory product has not yet been identified.[19]

The cluster-type reaction associated with the Golgi area of the islet tumor cells is a peculiar feature of immunostaining with the HISL-19 MAb not observed with other neuroendocrine markers. In contrast with the granular pattern, the cluster-type reaction was found to occur consistently in insulin-secreting tumors while it was absent or negligible in most PP-producing tumors. Although the cluster-type pattern also occurs in some nonendocrine tissue and related neoplasms,[2] the large and irregular size of the HISL-19 stained aggregates in islet cell tumors, which is related to the often conspicuous development of the Golgi apparatus in these peptide synthesizing and processing cells, may be an useful marker for the recognition of the tumor origin. Due to the demonstration of several HISL-19 proteins with different molecular weight,[1] it is possible that the proteins identified in the Golgi complex and in the granules are different.

In conclusion, the staining of a series of islet cell neoplasms with the MAb HISL-19 has revealed 2 patterns of immunoreaction: 1) a granular pattern, mostly associated with well-differentiated tumors and with tumors producing glucagon and PP; and 2) a cluster, Golgi-related pattern, which was well represented in all types of tumors except those producing PP and was independent from the benign or malignant nature of the tumors. These distinctive staining characteristics, in particular the latter one, allow the HISL-19 MAb to provide more information useful for the cytologic analysis of the endocrine tumors of the pancreas than other neuroendocrine markers currently available. It is also suggested that islet cell tumors, with their frequent large collections of islet cells showing peculiar cytologic conditions, may represent an useful tool for the study of the intracellular localization of antigens defined by monoclonal antibodies.

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