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Immunocytochemical demonstration of intermediate filament cytoskeleton proteins in human endocrine tissues and (neuro-) endocrine tumours

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Summary. The presence and distribution of intermediate filament proteins, such as cytokeratins, vimentin, neurofilament proteins and glial fibrillary acidic protein were assessed immunohistochemically in pituitary adenomas, medullary thyroid carcinomas, endocrine pancreatic tumours, gastric, intestinal and bronchial carcinoids, parathyroid adenomas, pheochromocytomas, paragangliomas and related non-neoplastic tissues. In some cases, immunohistochemical results were correlated with cytoskeletal proteins as analysed by SDS-polyacrylamide gel electrophoresis. Cytokeratin antibodies with broad range of immunoreactivity (i.e. to murine liver cytokeratin component D) reacted with epithelial cells in all non-neoplastic endocrine tissues and related neuroendocrine tumours studied, except for adrenal medulla, pheochromocytoma and paraganglioma, independently of hormone production and biological behaviour. In contrast, antibodies to epidermis-derived cytokeratins failed to stain endocrine tissues and tumours. Paranuclear cytokeratin accumulations were seen in bronchial, gastric, and intestinal carcinoids and seem to be a common feature of neuroendocrine tumours. One- and two-dimensional SDS-polyacrylamide gel electrophoresis of non-neoplastic endocrine tissues and related tumours revealed two major keratin polypeptides corresponding to cytokeratins No. 8 and 18 of the cytokeratin catalog of human cells (Moll et al. 1982). According to this cytokeratin polypeptide composition, endocrine tissues and related tumours conform to the "simple type" of epithelia. Vimentin-related immunoreactivity was restricted to stromal cells and to folliculo-stellate cells in normal pituitary gland, Schwann cells in carcinoids and satellite cells in normal adrenal medulla and in pheochromocytomas. Neurofilament protein- (70 kD)-antibodies only stained nerve fibers in normal tissues and at the periphery of carcinoid tumour cell complexes, and,

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to a variable degree, cells in nontumorous adrenal medulla, pheochromocytomas and paragangliomas. Furthermore, neurofilament reactivity was observed along with cytokeratin expression in two bronchial carcinoids.

Key words: Endocrine tissues – Endocrine tumours – Cytoskeleton – Immunohistochemistry – Gel-electrophoresis

Introduction

Intermediate filaments (IF) are cytoplasmic cytoskeletal components and can be separated into different types, i.e. cytokeratin (CK)-, vimentin-, desmin-, neuro- (NF)-, and glial filaments (GFAP) according to their chemical composition (for review see Franke et al. 1979, 1981; Osborn et al. 1980, 1982, 1983; Moll et al. 1982; Bignami et al. 1982; Cooper et al. 1985). Because of their considerable cell type specificity they may serve as histogenetic markers. Since specific antibodies to IF are available, immunohistochemical IF typing has become a valuable tool in surgical pathology, especially in cases with non-specific morphology (Denk et al. 1983, see also Osborn et al. 1983 and Cooper et al. 1985 for review and bibliography). Reports on the presence of different IF types in endocrine organs and related tumours are still based on a limited number of cases, and are occasionally conflicting.

Our investigation focused on presence and distribution of IF in various human endocrine organs, including pituitary, thyroid, parathyroid, and adrenal glands, pancreatic islets and cells of the diffuse (neuro-)endocrine system in gut and bronchial mucosa. In some cases, in which material was obtained immediately after surgery, the IF cytoskeleton was, in addition, analyzed by SDS-polyacrylamide gel electrophoresis after isolation and purification.

The aim of this paper was to study the immunoreactivity of antibodies directed to different types and subtypes of IF with non-neoplastic and neoplastic endocrine tissues and to correlate the immunohistochemical results with electrophoretic analyses of cytoskeletal proteins in selected cases.

Material and methods

Tissues. Formaldehyde (10%, phosphate-buffered)-fixed as well as unfixed specimens (summarized in table 1) were studied. Unfixed material from non-neoplastic pituitary glands, pancreas, parathyroid and adrenal glands was obtained at autopsy performed less than 6 h post mortem, other specimens were obtained at surgery. The tissues were frozen in isopentane cooled with liquid nitrogen and stored at −70° C prior to cryostat sectioning. Formaldehyde fixed tissue was embedded in paraffin and cut at 5 to 8 μm. Additional samples were fixed in freshly prepared glutaraldehyde (3%) in phosphate buffer (0.1 M pH 7.4), postfixed in osmium tetroxide (1%), and embedded in Epon 812. Thin sections were viewed in a Philips EM 400 electron-microscope at 80 KV.

Immunohistochemistry. Indirect immunofluorescence microscopy was performed on unfixed frozen sections. Air-dried cryostat sections, 5 μm thick, were submersed in acetone at −20° C for 10 min. Thereafter, they were incubated with primary antibodies for 30 min at room temperature in a moist chamber. Unbound antibodies were removed by rinsing the slides in phosphate-buffered saline (PBS, pH 7.45) and the sections were then incubated with FITC- or TRITC-

Table 1. Intermediate filament immunoreactivity of normal endocrine tissues and related tumours

	Tissues (*N*)			Antibodies to						
	total	fixed	frozen	CK/ D	CK/ 67kD	CK/ 53kD	CK/ 58kD	VIM	NF/ 70kD	GFAP
Pituitary gland	10	8	2	+	(+)	(+)	(+)	+[b]	+[a]	+[b]
Pituitary adenoma	22	22	–	+	–	–	–	–	–	–
Thyroid gland	6	4	2	+	–	–	–	–	+[a]	–
Medullary thyroid cancer (MTC)	5	3	2	+	–	–	–	–	–	–
Nontumorous pancreas										
endocrine	13	10	3	+	–	–	–	–	+[a]	–
exocrine				+	–	–	–			
Endocrine pancreatic tumour (EPT)	15	12	3	+	–	–	–	–	–	–
Gastric mucosa	27	25	2	+	–	–	–	–	+[a]	–
Gastric carcinoid	14	12	2	+	–	–	–	±	+[c]	–
Intestinal mucosa	40	35	5	+	–	–	–	–	+[a]	–
Intestinal carcinoid	38	36	2	+	–	–	–	±	+[c]	–
Lung epithelium:				+	±	±	±	–	–	–
bronchialalveolar	43	40	3	±	–	–	–	±	–	–
Bronchial carcinoid	13	10	3	+	–	–	–	±	+[c]	–
Parathyroid gland	3	2	1	+	–	–	–	–	+[a]	–
adenoma	3	1	2	+	–	–	–	–	–	–
Nontumorous adrenal-medulla				–	–	–	–	+	+	–
cortex	6	2	4	(+)	–	–	–	–	+[b]	–
Pheochromocytoma	3	1	2	–	–	–	–	±	±	–
Paraganglioma	4	1	3	–	–	–	–	+	±	–

[a] immunoreactivity restricted to nerve fibers
[b] VIM- and GFAP-reactivity restricted to folliculo-stellate cells
[c] Reactivity restricted to nerve fibers associated with tumor cells

+ positive; – negative; ± partly positive, partly negative; (+) weak reactivity

conjugated secondary antibodies for 30 min at room temperature. They were finally washed in several changes of PBS, dehydrated for 5 min in absolute ethanol and embedded in Mowiol (Hoechst, Frankfurt, FRG). The specimens were viewed and photographed using a Zeiss epillumination fluorescence microscope (Photomikroskop III, Zeiss, Oberkochen, FRG).

Peroxidase techniques (peroxidase-antiperoxidase-(PAP)-method, according to Sternberger 1979; or biotin-avidin-(ABC)-method according to Hsu et al. 1981) were used to demonstrate

Table 2. Antisera used in immunohistochemistry

Antiserum to (species)	Dilution[a]	Source references
(gp) Mouse liver cytokeratin polypeptide D (CK/D, 49 and 55 kD)	1:3,000 1: 100[b]	Denk et al. (1981)
(gp) Human epidermal prekeratin polypeptide I (67 kD)	1: 200 1: 80[b]	Denk et al. (1981)
(gp) Human epidermal prekeratin polypeptide II (58 kD)	1:1,000 1: 40[b]	Denk et al. (1981)
(gp) Human epidermal prekeratin polypeptide III (53 kD)	1:1,000 1: 60[b]	Denk et al. (1981)
(ra) Human vimentin (VIM)	1: 300 1: 15[b]	Denk et al. (1983)
(ra) Glial fibrillary acidic protein (GFAP)	undiluted	Dako (Denmark)
(m) Human neurofilament protein (70 kD; NF), monoclonal	1: 10 1: 5[b]	Sanbio (Holland)

gp Guinea pig; ra Rabbit; m Mouse

[a] PAP or ABC reaction except

[b] Indirect immunofluorescence microscopy

antigens in formaldehyde-fixed, paraffin-embedded material as described previously (Hoefler et al. 1983 and 1984c). In some instances (e.g., CK, vimentin) the dewaxed sections were pretreated with 0.6% protease (Sigma type VII, Sigma Chem. Comp., St. Louis, MO, USA, at pH 7.2) for 10 min at 37° C to enhance sensitivity (Denk et al. 1977).

Antibodies. The antibodies used are summarized in Table 2. FITC or TRITC-coupled secondary antibodies to rabbit, guinea pig, mouse, human and chicken immunoglobulins were obtained from Behring-Werke (Marburg, FRG) and Dako (Denmark). Controls included preimmune sera and non-immune sera in the first layer, coupled secondary antibodies not specific to the primary antibodies, and omission of essential steps in the PAP- or ABC-reaction.

Analysis of cytoskeletal proteins by SDS-polyacrylamide gel electrophoresis. Ten to 20 μm thick frozen sections of four endocrine pancreatic tumors, one medullary thyroid carcinoma, two parathyroid adenomas, one liver metastasis, one iliacal carcinoid and one bronchial carcinoid were microdissected (Moll et al. 1982) to remove non-tumour tissue. The remaining tissue to be analyzed was collected in medium A (96 mM NaCl, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 1.5 mM KCl,10 mM Na_2-EDTA, 0.1 mM dithiotreitol, 0.4 mM PMSF; pH 6.8), homogenized and extracted with low and high ionic strength buffers, including Triton X-100, to yield a residue enriched in IF cytoskeletal structures (Denk et al. 1979, 1981). The cytoskeleton material was analyzed by one- and two-dimensional gel electrophoresis as described by Moll et al. (1982). Furthermore, polypeptides were characterized by immunoblotting procedures as described previously (Denk et al. 1983).

Results

A detailed study of the CK distribution in human pituitary glands and adenomas has been published (Hoefler et al. 1984a). These studies revealed

a variable pattern of CK/D immunostaining in normal tissue ranging from paranuclear and focal CK accumulations to a diffuse cytoplasmic distribution. Vimentin staining was restricted to interstitial cells with arborized cytoplasm (i.e. folliculo stellate cells), some of which also reacted with GFAP antibodies (for details see Hoefler et al. 1984b). NF-reactive nerve fibers were only occasionally observed in normal pituitary glands, NF-reactive cells did not occur.

All pituitary adenomas were studied after formaldehyde fixation and paraffin embedding. A positive immunoreactivity was obtained in 17 out of 22 tumours using CK/D antibodies (Fig. 1). The negative results in 5 tumours may be due to decrease or loss of antigenicity during fixation and embedding. Antibodies to epidermal CK components, to NF protein and to GFAP failed to react with adenomas. Vimentin staining was restricted to connective tissue and vascular cells.

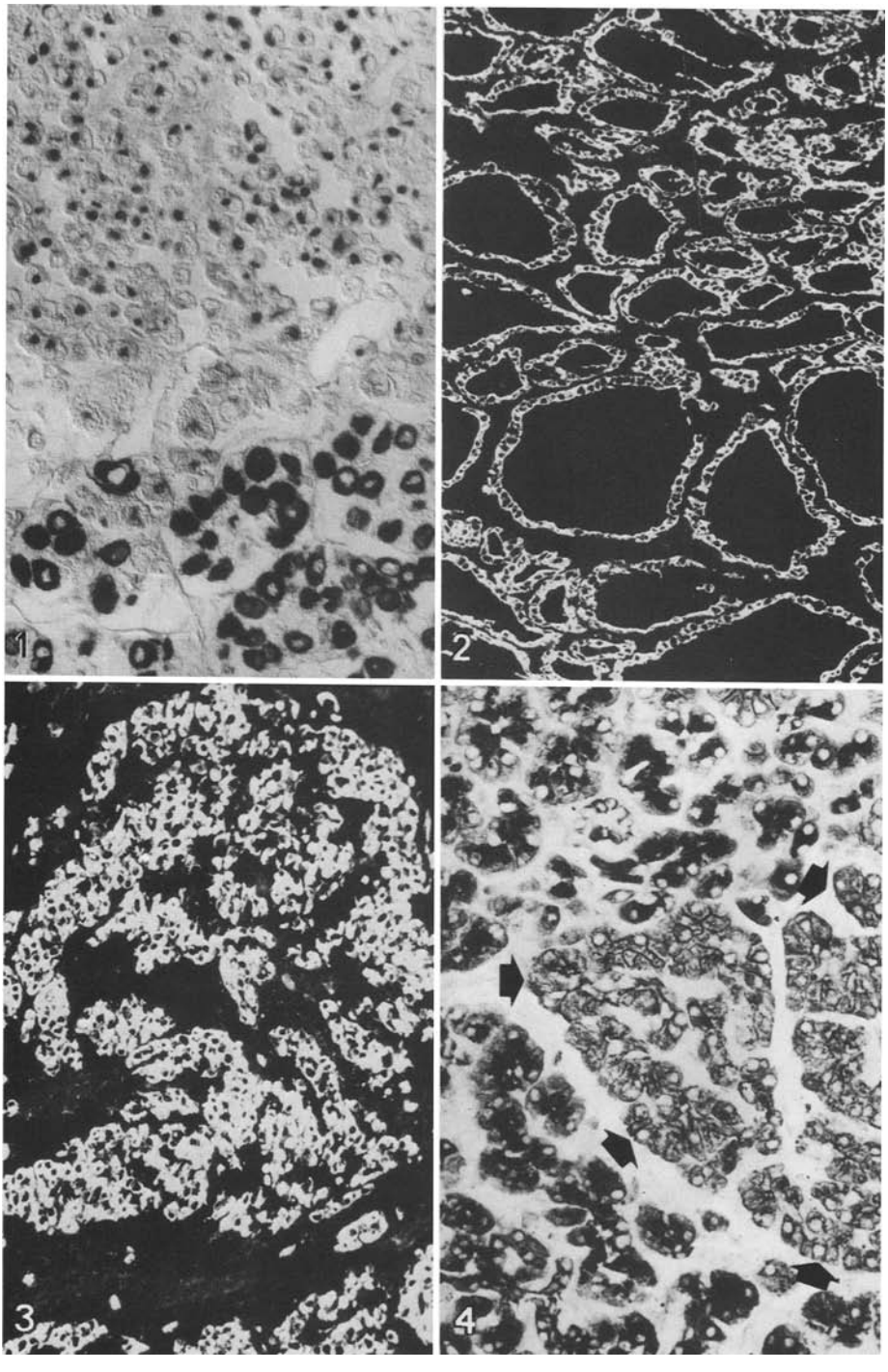
In normal thyroid gland to follicular epithelial cells and C-cells were strongly reactive with antibodies to CK/D (Fig. 2), but not with those to epidermal CK components, vimentin, and NF protein. Vimentin reactive cells as well as NF-positive nerve fibers were confined to the stroma. The immunohistochemical reactions revealed identical results in fixed and unfixed material.

All 5 medullary thyroid carcinomas studied revealed positive reactions (frozen as well as fixed specimens) with CK/D antibodies resulting in strong and evenly distributed cytoplasmic staining (Fig. 3) whereas antibodies to epidermal CKs failed to react with tumour cells. Vimentin immunoreactivity was restricted to stromal and vascular cells. NF protein-positive structures were not seen within the tumour stroma.

In normal pancreatic tissue CK/D antibodies labelled cells of the acini and the ducts conspicuously but labelled islet cells to a lesser extent. Staining was evenly distributed in the cytoplasm of all endocrine cells, differences in staining patterns depending on hormone production were not evident (Fig. 4). Antibodies to different molecular weight components of epidermal CK were not reactive. Vimentin and NF protein-related immunoreactivities were confined to stromal cells and nerve cells and axons, respectively.

All 3 unfixed endocrine pancreatic tumours were stained by CK/D antibodies (Fig. 5), but did not react with antibodies to epidermal CK. Staining by CK/D antibodies was also observed in 10 of 12 fixed tumours. The intensity of immunoreactivity was less pronounced in tumour tissue than in surrounding non-neoplastic exocrine pancreas but was roughly equal to that of normal islet cells. Cytoplasmic CK staining was evenly distributed without focal accumulation. Vimentin and NF protein-related immunoreactivities were as described in non-neoplastic pancreatic tissue. Tumour cells remained unstained.

Gastric epithelial cells of different localizations, i.e. in gastric pits, at the surface and in glands, exhibited a strong staining with antibodies to CK/D but failed to react with epidermal CK antibodies. Cells of the diffuse endocrine system showed CK reactivity, identical in distribution and intensity to other epithelial cells. Vimentin content was restricted to mesenchymal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stromal and vascular cells. NF protein was exclusively associated with neurons, epithelial cells were negative.

Tumour cells in 14 gastric carcinoids reacted with CK/D antibodies (Fig. 6). Distribution of antigenicity within the cytoplasm of the tumour cells was mostly homogeneous; only in 3 specimens was an apparently focal (paranuclear) concentration observed. Epidermal CK antibodies were non-reactive. Among the tumour cells, single vimentin-positive cells corresponding to cells of Schwann cell type which are usually associated with carcinoids were occasionally interspersed (for detailed description see Hoefler and Auboeck 1984). NF protein was demonstrable in frozen sections only and was confined to nerve fibers at the periphery of the tumour; tumour cells were NF-negative.

In the normal mucosa of the duodenum, jejunum/ileum, appendix, colon and rectum the presence and distribution patterns of IF proteins corresponded to that in gastric mucosa in that all epithelial cells were significantly stained solely with CK/D antibodies.

Without any apparent relationship to localization and biological behaviour, the primary gastrointestinal tumours ($n=38$) and their metastases ($n=2$) were strongly stained by CK/D antibodies but not by antibodies to different epidermal CK components. The intensity of CK immunoreactivity in tumour cells corresponded to that of non-neoplastic epithelial cells; cytoplasmic staining was mostly evenly distributed but in about 20% of the carcinoids a focal paranuclear accentuation was observed. Vimentin-positive single cells within the tumour cell complexes (Schwann cell type) were found in most small bowel- (8/11), in all appendix- and in 3 of 11 colonic/rectal carcinoids (for details see Hoefler and Auboeck 1984). Tumour cells were unstained with NF antibodies. NF-positive axons were present in all carcinoids in variable amounts at the periphery of the tumour cell complexes and in the surrounding stroma.

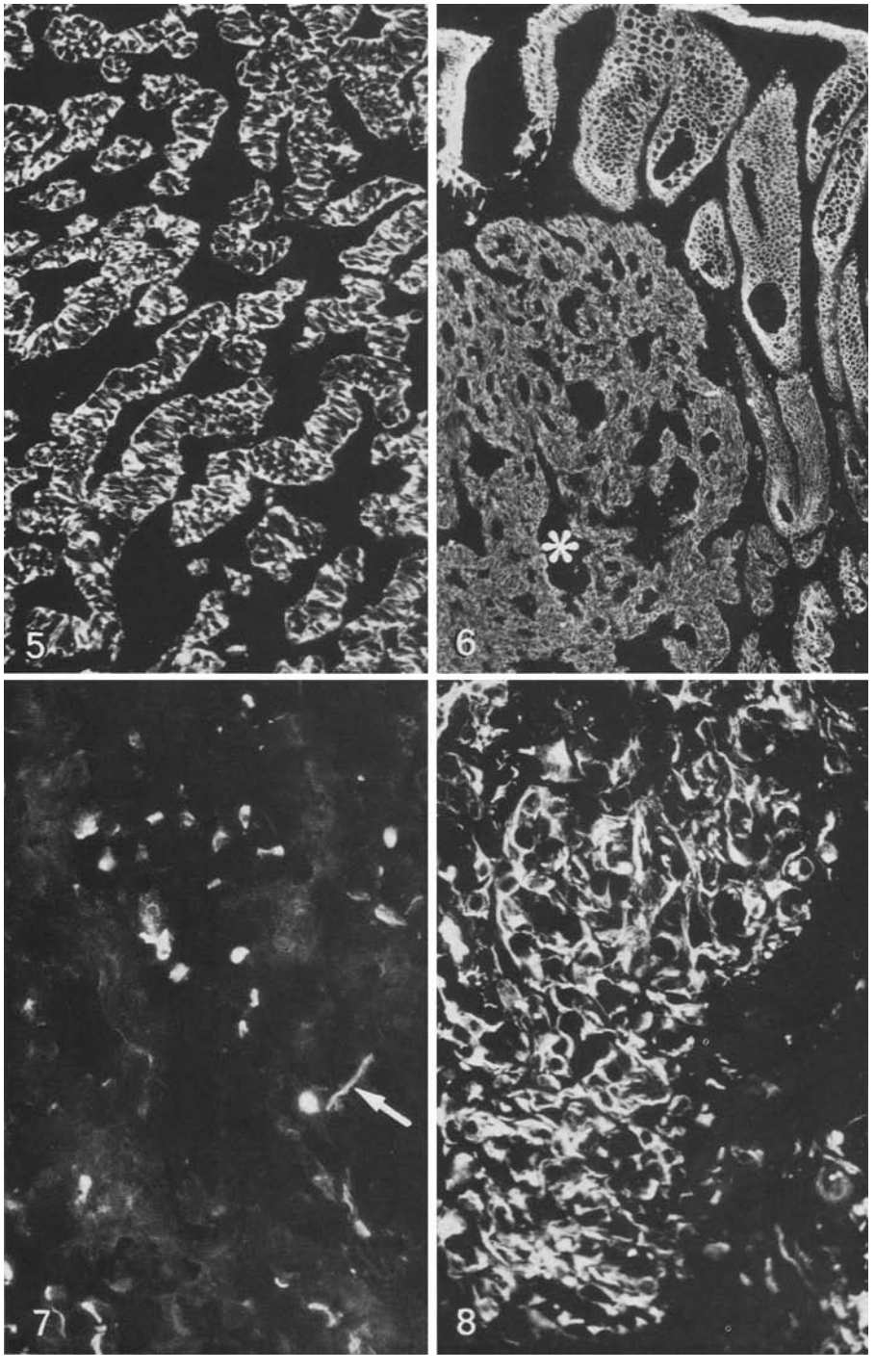
In non-neoplastic specimens independent of fixation, the epithelial cells of bronchial mucosa except the basal layer consisting of indifferent reserve cells, were strongly by CK/D antibodies. Moreover, immunoreactivity was also found in bronchiolar and alveolar epithelium as well as in bronchial

Fig. 1. Edge of a GH-producing pituitary adenoma (*top*). CK/D-immunoreactivity is concentrated as paranuclear patches. ACTH-reactive cells in non-tumorous pituitary gland (*bottom*) reveal strong and evenly distributed cytoplasmic CK staining. PAP-technique, formalin-fixed, paraffin-embedded tissue, × 1,020

Fig. 2. Non-neoplastic thyroid gland: follicular epithelium is intensely decorated by CK/D-antibodies. Indirect immunofluorescence microscopy (FITC), cryostat section, × 160

Fig. 3. Tumor cells of medullary thyroid cancer are decorated by CK/D-antibodies. The stroma remains unstained. Indirect immunofluorescence microscopy (FITC), cryostat section, × 400

Fig. 4. Normal pancreatic islet (*arrows*) and surrounding exocrine parenchyma are reactive with CK/D-antibodies. Endocrine tissue shows less intense immunoreactivity than exocrine cells. PAP-technique, formalin-fixed, paraffin-embedded tissue, × 880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glands. The basal cell layer of the bronchial epithelium and the myoepithelial cells associated with bronchial glands were selectively stained by antibodies raised against isolated epidermal CK components with molecular weights of 53 and 58 kD, whereas antibodies to the 67 kD epidermal CK polypeptide only reacted with basal cells of bronchial epithelium but not with myoepithelial cells. Alveolar epithelial cells were not decorated by any of the epidermal CK antibodies. Alveolar macrophages reacted with vimentin but not with CK antibodies as revealed by double immunofluorescence microscopy. NF-related immunoreactivity was restricted to neurons.

From 13 bronchial carcinoids included in this investigation, three tumours had already produced metastases at the time of surgery, in 3 other tumours considerable pleomorphism of cells was observed resembling the atypical carcinoids. All carcinoid cells reacted uniformly with antibodies to CK/D and lacked reactivity with antibodies to epidermal type of CK. In most tumour cells CK staining was concentrated in a paranuclear region and not uniformly distributed throughout the cytoplasm. This type of immunoreactivity corresponded to a felt-like paranuclear accumulation of IF, as revealed by electron microscooy (Fig. 9). Five of 13 tumours contained vimentin-positive (Schwann) cells interspersed between the tumour cells. NF-positive nerver fibers were present in variable numbers at the periphery of tumour cell complexes and within the stroma. The monoclonal antibody to the 70 kD NF protein did not stain tumour cells when indirect immunofluorescence microscopy was applied to unfixed frozen sections. On two frozen and unfixed carcinoids, however, a strong paranuclear patchlike NF-70 kD-immunoreactivity was obtained with the more sensitive ABC technique. The distribution of the reaction product was similar to the results obtained with CK/D antibodies.

In non-neoplastic parathyroid glands and adenomas (two chief cell adenomas, one consisting of water-clear cells) CKs were detectable (in frozen as well as in fixed material) in all epithelial cells with CK/D antibodies, but no significant staining was achieved with antibodies to the epidermal CKs. Vimentin-positivity was restricted to stromal and vascular cells. NF-positive nerves were only sparsely present in normal glands.

Fig. 5. Endocrine pancreatic tumour: the neoplastic trabeculae are intensely stained by CK/D-antibodies. Indirect immunofluorescence microscopy (FITC), cryostat section, × 400

Fig. 6. CK/D-related immunoreactivity in glandular and foveolar gastric epithelium and in carcinoid tumor cells (*asterisk*). Indirect immunofluorescence microscopy (FITC), cryostat section, × 920

Fig. 7. Phenochromocytoma with neurofilament (70 kD) immunoreactivity in some tumor cells and nerve fibers (*arrow*). Indirect immunofluorescence microscopy (TRITC), frozen section, × 400

Fig. 8. Vagal paraganglioma with vimentin reactivity in tumor cells. Indirect immunofluorescence microscopy (FITC), cryostat section, × 400

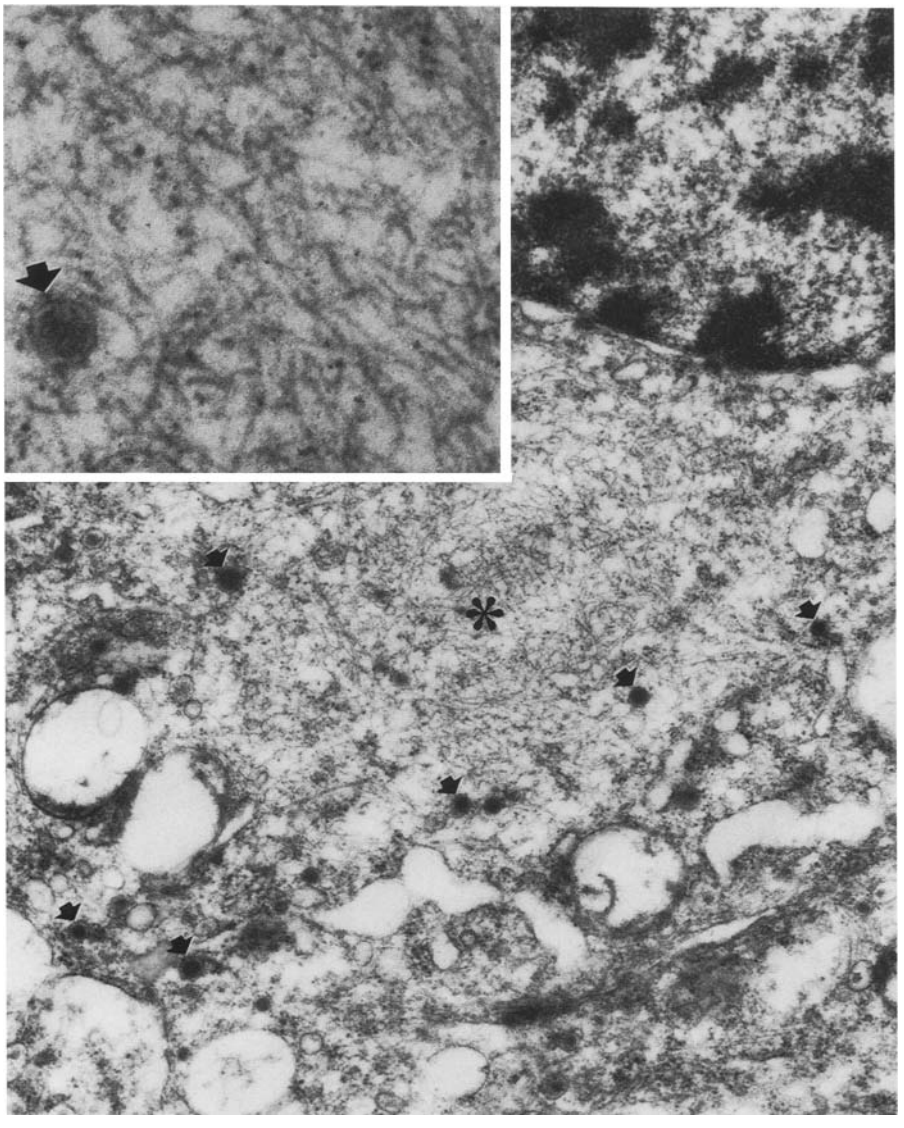

Fig. 9. Paranuclear accumulation of intermediate-sized (7–11 nm) filaments in a bronchial carcinoid (*asterisk*). Electron dense endocrine granules at the periphery are indicated by *arrows*. ×28,000. *Inset:* Higher magnification of intermediate filaments arranged in a felt-like pattern. Arrow indicates an endocrine granule. ×108,000

Cells of the non-neoplastic adrenal cortex were weakly positive with CK/D antibodies in frozen specimens, those of medulla were negative. Cells of the adrenal cortex and the medulla remained unstained with antibodies to epidermal CKs in frozen unfixed specimens. Strong NF immunoreactivity in the medulla was associated with occasional large ganglion cells with abundant cytoplasm as well as with nerve fibers. Pheochromocytes, however,

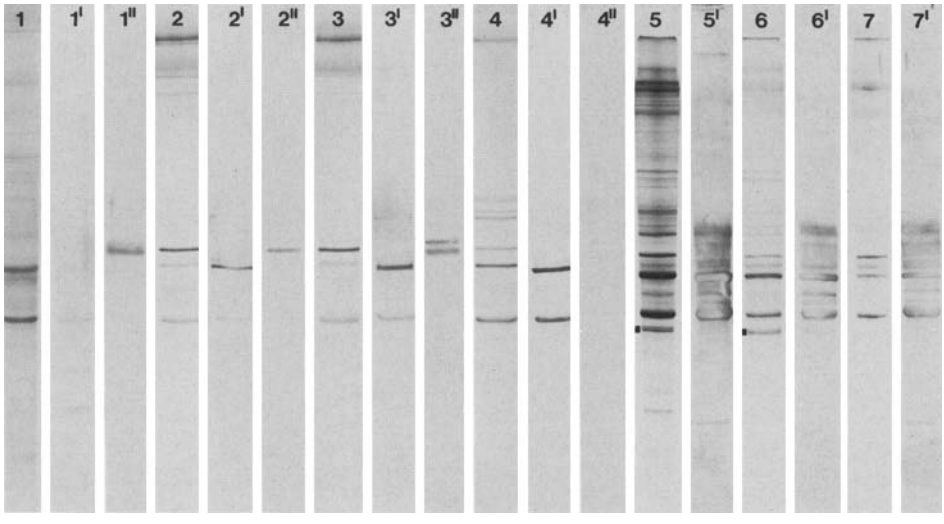

Fig. 10. SDS-polyacrylamide gel electrophoresis of cytoskeletal proteins isolated as high salt buffer-detergent resistant residues from various endocrine tumors (lane 1, medullary thyroid carcinoma; lanes 2 and 3, two different endocrine pancreatic tumours; lane 4, gastrinoma of the pancreas; lanes 5 and 6, two different parathyroid adenomas; lane 7, bronchial carcinoid). There are two major cytokeratin polypeptide components (lanes 1–4, lane 7), corresponding to cytokeratin components No. 8 and 18 (see catalog of human cytokeratins Moll et al. 1982). An additional polypeptide with M_r 40,000 is present in parathyroid adenomas (dots in lanes 5 and 6). Cytokeratin polypeptides are also identified by their reactivity with antibodies against murine liver cytokeratin component D (CK/D, lanes 1′–7′) in immunoblots. Vimentin is derived from contaminating mesenchymal cells and identified by its reactivity with vimentin antibodies (lanes 1″–4″). Double bands in lanes 1″ and 3″ represent vimentin and vimentin degradation products

reacted weakly with the NF (70 kD) antibody or were negative. Vimentin immunoreactivity was resticted to smaller cells probably resembling satellite cells. In the cortex, vimentin was associated with stromal and vascular cells.

The pheochromocytomas ($n=3$) studied were solitary, unilateral, non-metastatic tumour nodules localized in the adrenal medulla. Within tumour cells no CKs were detectable. Significant numbers of NF-reactive nerve fibers were associated with the tumour stroma (Fig. 7). Like the non-tumour adrenal medulla a minority of cells, resembling stroma and proliferated satellite cells, contained vimentin. The NF (70 kD)-reactivity of pheochromocytoma cells was of variable intensity, in most cells a weak reaction was noticed.

Two of the paragangliomas included in this investigation were associated with the carotid glomus, one with the cervical vagal nerve, and one was a jugulo-tympanic paraganglioma. All tumours were non-metastatic at the time of surgery. CK immunoreactivity was absent in unfixed as well as fixed material. Some tumour cells (cells constituting the "Zellballen" as well as the satellite cells) reacted strongly with vimentin antibodies (Fig. 8). NF reactivity was observed in nerve fibers, tumour cells revealed a weak or negative reaction.

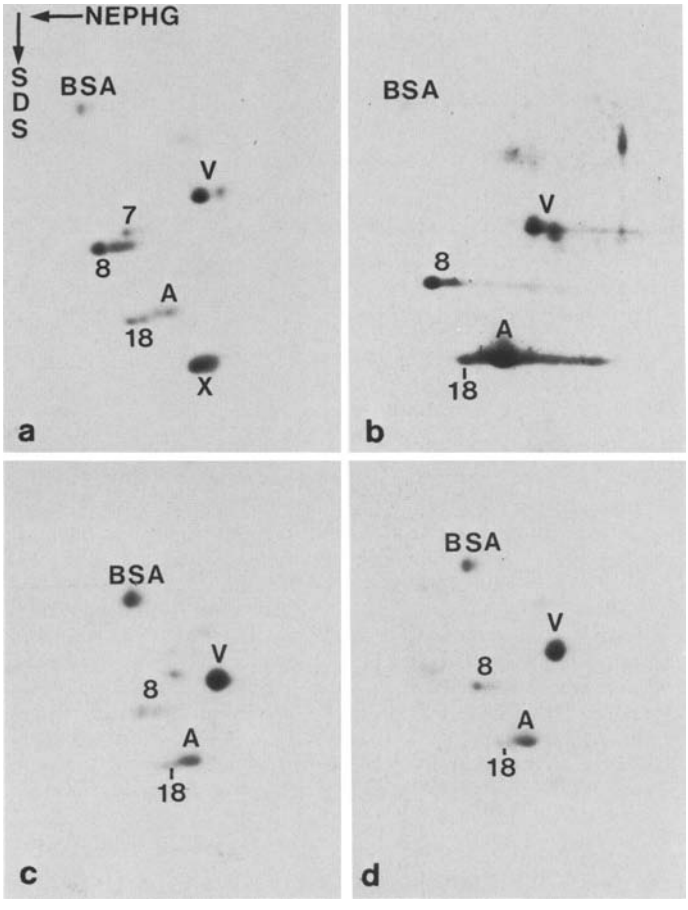

Fig. 11a–d. Two-dimensional gel electrophoresis of cytoskeletal polypeptides of parathyroid adenoma (**a**), gastrinoma (**b**) and two different endocrine pancreatic tumours (**c, d**) using non-equilibrium pH gradient electrophoresis (NEPHGE) in the first dimension (basic polypeptides are at the *left*, acidic polypeptide at the *right*). SDS indicates the direction of the second dimension electrophoresis in the presence of SDS. Major polypeptides show isoelectric points corresponding to components No. 8 (M_r 52,500; IEP 6.1) and No. 18 (M_r 45,000; IEP 5.7). In parathyroid adenoma a trace of component No. 7 is also detectable. In addition, a component with M_r of 40,000 and IEP of 5.2 is present (*X*). Vimentin (*V*) is derived from contaminating stroma cells. *BSA*, bovine serum albumin (M_r 66,500; major variant isoelectric at pH 6.35); *A*, rabbit-actin

GFAP-related immunostaining was absent in all non-tumour and tumour (neuro-)endocrine tissues studied, except for normal pituitary gland (see above). IF immunoreactivity of normal endocrine tissue and related tumours is summarized in Table 1.

One-dimensional SDS-polyacrylamide gel electrophoresis of cytoskeleton residues isolated from endocrine tumours revealed a fairly uniform pattern of constituent cytokeratin polypeptides (Fig. 10) with two major bands, one with a M_r around 53,000, corresponding to CK No. 8, and the other

with a M_r of 45,000, corresponding to CK No. 18 according to the CK catalog of human cells established by Moll et al. (1982). In two-dimensional gel electrophoresis using non-equilibrium pH gradient conditions (NEPHGE) in the first dimension, isoelectric points around 6.1 and 5.7 (major spots), respectively, were determined for these major polypeptides of several endocrine tumours (Fig. 11). Polypeptide bands at a M_r or 57,000 and slightly below were identified as vimentin and vimentin degradation products on the basis of molecular weights, isoelectric points and reactivity with vimentin antibodies in immunoblots (Fig. 10, 1′–4′), probably resulting from contaminating mesenchymal cells. The polypeptide band with M_r of 40,000 present in cytoskeletal residues from parathyroid adenomas (Fig. 10, lanes 5 and 6 in Fig. 11a) corresponds in its electrophoretic coordinates to component No. 19 described by Moll et al. (1982).

Discussion

Reports on the presence of CKs in neuroendocrine cells and related tumours, as revealed by immunohistochemistry, are numerous but conflicting. Nagle et al. (1983) were unable to detect CKs by immunohistochemistry using antibodies to human callus keratin (M_r 40–68 kD) in several endocrine tumours, including one insulin-producing pancreatic tumour, one ileocecal carcinoid, two pituitary adenomas, one paraganglioma and one parathyroid adenoma. Lack of immunoreactivity with CK antibodies was also reported with respect to an endocrine pancreatic tumour and a bronchial carcinoid by Said et al. (1983) and Schlegel et al. (1980), respectively. In several studies focused on single endocrine organs positive as well as negative reactions were reported depending on the type of CK antibodies used (Virtanen et al. 1981; von Bassewitz et al. 1982; Said et al. 1983). On the other hand, positive CK staining of several types of epithelial cells with endocrine functions, including pituitary cells, thyroid follicular cells, cells of Langerhans' islets and cells of (metastatic) gastrointestinal carcinoids was reported by Neumann et al. (1984), Ramaekers et al. (1983), Schubart and Fields (1984) and Miettinen et al. (1985a). Moll et al. (1983) demonstrated CK components Nos. 8 and 18 (for designation see Moll et al. 1982) in the normal pancreatic acinar epithelium, CK Nos. 7, 8, 18 and 19 in pancreatic duct epithelium and CK Nos. 8 and 18 in one islet cell adenomna. In a recent study by Blobel et al. (1985) CKs were demonstrated in bronchial carcinoids by immunohistochemistry and gel electrophoresis. These findings and the results of the same group (Blobel et al. (1984) concerning cells of the normal lung were confirmed by our study. Our findings of CK immunoreactivity in adrenal cortical cells are in accordance with the results published by Miettinen et al.(1985b) and Muijen et al. (1984). The latter authors, however, in contrast to our results and other papers, reported CK immunoreactivity in the adrenal medulla. Permanetter et al. (1982) reported the lack of CK-related immunostaining in medullary thyroid carcinomas in contrast with papillary carcinomas. Miettinen et al. (1984) were able to distinguish different types of thyroid carcinomas by their reactivity with antibodies

to CKs derived from epidermal and simple epithelial cells (thyroid carcinomas, except the medullary variety, were, therefore, excluded from our investigation).

The significance of tissue preparation and type of antibodies is particularly evident from this paper as well as from the work of van Muijen et al. (1984) and Kahn et al. (1983). The latter authors were able to obtain CK-specific immunostaining in carcinoids only with antibodies to low molecular weight CKs derived from human Mallory bodies. According to CK polypeptide composition, cells with a simple, i.e. with only few polypeptides expressed, and those with a rather complex set-up can be distinguished. Non-stratified, mostly secretory, epithelial cells of the digestive system and their associated glands are examples for the former, stratified epithelia of various localizations for the latter (for review and further details see Moll et al. 1982, Cooper et al. 1985). CKs associated with neuroendocrine cells and tumours, therefore, conform to the "simple type" of CKs together with those present in hepatocytes, intestinal and various gland cells and related tumours. The presence of CKs in neuroendocrine tumours and their precursor cells (Hoefler and Denk 1984) clearly allows their classification as epithelial which is, however, not in contradiction to their presumed origin from the neural crest (Pearse et al. 1980), since the expression of intermediate filaments in the adult tissues does not reflect their origin from certain germ layers.

Focal accumulation of CK filaments in paranuclear areas is an interesting feature of certain neoplastic and non-neoplastic endocrine cells. It has first been described in pituitary gland cells by Racadot et al. (1964), and these "fibrous bodies" have been more extensively studied by Horvath and Kovacs (1978). In the anterior lobe of the pituitary this peculiar type of CK staining is predominantly associated with GH producing cells. In addition, IF accumulations have been observed in bronchial and gastric (Carstens et al. 1977; Berger et al. 1984; Blobel et al. 1985, and this paper) and, less commonly, in intestinal carcinoids. Focal concentration of IF is also a significant feature of neuroendocrine (Merkel cell-) carcinomas of the skin (Hoefler et al. 1984d). Following ozon treatment of dogs, filament accumulation in parathyroid chief cells, simulating Mallory bodies at the light and electron microscopic level, has been reported by Atwal and Pemsingh (1984) (for Mallory bodies see Denk et al. 1979). The biological significance, if any, of these changes in IF arrangement is unknown. The present investigation did not provide any correlation of "fibrous bodies" to the biological behaviour of the tumours. An involvement of CK filaments in hormone gene expression has been discussed by Neumann et al. (1984) and Schubart and Fields (1984).

Within the family of CK-reactive neuroendocrine tumours, carcinoids have a special position since they consist of two different cell types: in addition to CK containing tumour cells a variable percentage (depending on localization) of carcinoids contains a cell population corresponding to Schwann cells, a population which is CK-negative but positive for vimentin.

The occurrence of Schwann cells within the tumour, however, is also a feature of paragangliomas, ganglioneuroblastomas and retinoblastomas (unpublished observation) which, in contrast to carcinoids, do not express CKs.

The reports by Altmannsberger et al. (1983), Miettinen et al. (1983) and Lehto et al. (1984 and 1985) on the presence of NF in carcinoid tumours were partly confirmed in our studies: we detected NF immunoreactivity only in two bronchial carcinoids, whereas tumours in other localizations were negative. Our results agree in principle with the studies of Blobel et al. (1985) who observed NF immunoreactivity and a polypeptide spot with electrophoretic coordinates similar to that of the 68 kD NF polypeptide in 2 out of 4 bronchial carcinoids. According to these data coexpression of CK and NF appears to occur in at least some bronchial carcinoids. We were, however, unable to confirm the results of Miettinen et al. (1985a) who reported the presence of varying numbers of NF-positive cells in islet cell tumours and in parathyroid adenomas (Miettinen et al. 1985c).

NF immunoreactivity in non-tumour adrenal medulla, human pheochromocytomas and cultured pheochromocytoma cells has been described by several authors (Osborn et al. 1982; Virtanen et al. 1981; Trojanowski and Lee 1983, 1985; Miettinen et al. 1985b). The variable NF immunoreactivity of non-tumourous pheochromocytes, pheochromocytoma cells and paragangliomas in this study is consistent with results published by Trojanowski and Lee (1985) and Miettinen et al. (1985b). We agree with Trojanowski and Lee (1985) that the inability to detect low molecular weight-NF immunohistochemically is a false negative result and does not reflect the actual absence of 70 kD NF protein. These authors speculate that the negative results may reflect the localization of 70 kD NF protein in the interior domain of intact NFs, where it may not be accessible to the NF antibody.

The immunohistochemical analysis of tumours with equivocal morphology by IF typing is a valuable adjunct in diagnosis. In general, indirect immunofluorescence microscopy on frozen sections seems to be superior to the immunoperoxidase or ABC techniques on formalin-fixed paraffin-embedded specimens. Negative results obtained with fixed material may be due to decreased antigenicity after the usual fixation and embedding procedures. Because of adverse effects of fixation and embedding on tissue antigenicity and possible cross-reactions, particularly also of monoclonal antibodies recognizing identical antigenic determinants on related and unrelated structures, cautious interpretation is mandatory. In this context, Draeger et al. (1983) reported cross-reactivity of antibodies raised against alpha-MSH and NFs. Partial sequence homologies between cytosceletal proteins and c-myc-, RSV- and adenovirus-proteins were described by Crabbe (1985). In doubtful cases, chemical analyses by gel electrophoresis and immunochemical techniques are necessary to identify the actual cytoskeletal composition and to supplement immunohistochemical data. To confirm questionable IF coexpression in single cases the demonstration of specific gene transcripts by blot- or in situ hybridisation may be necessary.

Note added in proof

In a recent paper Molle and Franke (Differentiation 30:165–175; 1985) demonstrated – in accordance with our results – the expression of CK No 8, 18 in cutaneous neuroendocrine tumors ($n=9$), bronchial carcinoids ($n=7$), medullary thyroid carcinomas ($n=2$), endocrine pancreatic tumors ($n=2$) and carcinoids of the small intestine ($n=2$) by immunohistochemistry and gel electrophoresis. Furthermore, CK No 7 and 19 were expressed in most of those tumors. In some cutaneous neuroendocrine carcinomas (4/9), bronchial carcinoids (4/7) and endocrine pancreatic tumors (1/2) 70 kD NF was detected.

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