Histol Histopath (1987) 2: 185-192

Histology and Histopathology

S-100 protein in human lung neuroendocrine neoplasms. Immunohistochemical study of 14 cases and review of the literature

M. Barbareschi[1], M.F. Mauri[1], M. Muscara[1], F.A. Mauri[1] and V. Lo Re[2]

[1]Department of Morbid Anatomy and Histopathology, School of Medicine, The State University of Milan, Milano, Italy;
[2]Department of Morbid Anatomy and Histopathology, City Hospital of Bollate, Milano, Italy

Summary. A group of lung neuroendocrine (NE) neoplasms are investigated in view of the possible presence of S-100 protein immunoreactivity in their cells.

The selected tumours were classified according to Gould et al. (1983a) and Mosca et al. (1985). They comprise 5 carcinoids, 3 neuroendocrine carcinomas of the well-differentiated type, or peripheral carcinoids, 5 neuroendocrine carcinomas of the intermediate cell type, or intermediate-cell, poorly differentiated carcinomas, 3 neuroendocrine carcinomas of the microcytoma type, or small cell carcinomas-SCC and a nodal metastasis of microcytoma.

All but 2 tumours were immunoreactive for neuron specific enolase (NSE).

Few S-100 immunoreactive cells were detected in 4 out of 5 carcinoids, in 1 out of 3 peripheral carcinoids, in 4 out of 5 poorly differentiated carcinomas and in the 3 microcytomas examined. No S-100 positive cells were found in the SCC's nodal metastasis.

The S-100 immunolabelled cells can be interpreted as dendritic reticulum cells migrating through the tumours.

However, in one case of typical carcinoid, abundant S-100 positive cells were detected: their stellate morphology and their intimate relation with neoplastic cells suggest that they are part of the neoplasia as a sort of satellite cell.

Key words: Lung neuroendocrine tumours — S-100 protein — Lung carcinoid — Tumourlet

Introduction

S-100 protein is an acidic, calcium binding protein, widely distributed in normal and neoplastic human tissues.

Offprint requests to: Dr. M. Barbareschi, III Cattedra di Anatomia ed Istologia Patologica, Univ. degli Studi Milano, Via Commenda 19, 20122 Milano, Italy.

With immunohistochemical methods, using polyclonal antisera, it has been detected in various neural cell types, normal and neoplastic (Cocchia and Miani, 1980; Ghandour et al., 1981; Stefansson et al., 1982; Grahm et al., 1983; Weiss et al, 1983; Barbareschi et al., 1984; Bonnin and Rubinstein, 1984; Höfler et al., 1984; Choi et al., 1985; Matsunou et al., 1985). S-100 immunoreactivity has also been described in melanocytes (Stefansson et al., 1982a), chondrocytes (Stefansson et al., 1982b), serous cells of bronchial glands, sweat glands, myoepithelial cells of salivary glands and breast (Kahn et al., 1983), interdigitating reticulum cells of lymph nodes, spleen and thymus (Takahashi et al., 1981; Khan et al., 1983; Lauriola et al., 1984 b), Langerhans cells of the skin (Cocchia et al., 1981b; Halliday et al., 1986), and adipocytes (Nakajima et al., 1982).

Immunostaining for S-100 gives positive results in tumours derived from the above mentioned cell types (Nakajima et al., 1982; Khan et al., 1983; Weiss et al., 1983) comprising the group of histiocytosis (Nakajima et al., 1982; Moscinski and Kleinschmidt-De Masters, 1985), mixed salivary gland tumours (Nakazato et al., 1982; Rozel et al., 1985), chondroid syringomas (Kahn et al., 1983), granular cell myoblastomas (Nakazato et al., 1982; Dhillon and Rode, 1983; Mukai, 1983), a variety of breast lesions (Nakajima et al., 1982; Kahn et al., 1983), chordomas (Nakajima et al., 1982; Weiss et al., 1983), and fibromatosis (Weiss et al., 1983).

New sites of human S-100 immunoreactivity have been detected with 2 different monoclonal antibodies by Vanstapel et al., (1985, 1986): they include histiocytes, striated muscle fibers and a variety of normal and neoplastic epithelial cells.

In addition to these data, S-100 labelled cells have been reported, using polyclonal antiserum, in cells strictly related to subepithelial neuroendocrine cells of human gut (Lundqvist and Wilander, 1986), in appendiceal carcinoids (Wilander et al., 1985) but not in other human gut carcinoids (Lundqvist and Wilander, 1986), and in pheochromocytomas (El Salhy et al., 1986).

S-100 immunoreactive cells have been described also in some gastrointestinal and bronchial carcinoids (Nakajima et al., 1982; El-Salhy et al., 1986). In some of the latter the labelled cells were numerous, stellate-shaped and were interpreted as Schwann or satellite cell components of the tumours.

Immunoreativity to S-100 protein has been described in various human lung tumours, but actual data seem still incomplete. Stefansson et al. (1982) report 4 SCCs devoid of any S-100 immunoreactivity. Kahn et al. (1983) describe occasional S-100 positive cells in 2 bronchioloalveolar carcinomas and in interstitial lung tissue, whereas other lung tumours are reported to be completely negative. The immunoreactive cells in interstitial lung tissue were interpreted as interdigitating reticulum cells or Langerhans cells.

Recently, Gatter et al. (1985) reported immunoreactive cells in 3 SCCs and in many of the squamous cell carcinomas and adenocarcinomas examined.

The aim of our study is to examine a series of neuroendocrine lung tumours in view of the possible S-100 positivity in their cells. This group of tumours is selected because of the supposed relationship with normal bronchial neuroendocrine cells (Becker et al., 1980; Gould et al, 1983b; D'Angati and Perzin, 1985).

For diagnostic purposes, and as part of further study on a large series of human lung neoplasms (Mosca el al. 1986), the tumours were also immunostained for neuron specific enolase (NSE), chromogranin (CG) and the 80 kd antigen of neurosecretory granules detected by the monoclonal Phe-5 antibody.

Materials and methods

The neuroendocrine lung tumours examined are classified according to Gould et al. (1985), and Mosca et al. (1985) in the following 4 groups:

- typical carcinoids or Neuroendocrine Carcinomas (NEC) of the CarciNoID type (NECNID); 5 cases studied.
- peripheral carcinoids or NEC of the WELL - Differentiated type (NECWED); 3 cases studied.
- intermediate cell poorly differentiated carcinomas or NEC of the Intermediate cell type (NECINT); 5 cases studied.
- small cell carcinomas (SCC) or NEC of the MICrocytoma type (NECMIC); 3 primitive cases and 1 nodal metastasis studied.

In our study we also examine 2 tumourlets of which one, according to the cited classification, belongs to the NECNID and the other to the NECWED group.

All specimens were surgically removed, formalin-fixed, paraffin-embedded and sectioned in 4 μm sections. For routine histology the sections were stained with hematoxylin - eosin and Alcian-blue-PAS. Preliminar investigations were also carried out by treating the specimens with a modified argyrophil Grimelius reaction (Lack and Mercer, 1977).

Immunohistochemical staining was performed using the peroxidase-antiperoxidase (PAP) method of Sternberger et al. (1970). Rabbit polyclonal antiserum to bovine brain S-100 protein was obtained from DAKO (Code No. Z311 Lot. n. 026). Rabbit polyclonal antiserum to human neuron specific enolase (NSE) was obtained from Immuno Nuclear Corp. (Cat. No. 3AO22, Lot. No. 85 44050). Rabbit polyclonal antiserum to native porcine chromogranine (CG) was obtained from Immuno Nuclear Corp (Cat. No. 63H2PT Lot. No. 8541012). Monoclonal antibody to the 80 kd antigen of neurosecretory granules was obtained from Ortho Diagnostic System S.p.A. (Cod. ENDO; ENZO BIOCHEM Cat. No. EAB-932, Lot. No. 51FC2). Sections to be stained with antiserum against NSE and CG were preincubated with a proteolytic enzyme (Protease XIV, SIGMA) to enhance the reactions.

Negative control sections were obtained by omission of the primary antibody.

Positive controls for NSE consisted in medullary cells of adrenal gland. Controls for S-100 consisted of nerve fibres of appropriately tested specimens and of intrinsic cartilage, bronchial serous glands and nerve fibres laying in proximity to the tumours being investigated.

Results

The surgical specimens were classified according to the classification of neuroendocrine neoplasms, recently proposed by Gould et al. (1983a) and Mosca et al. (1986), which is a modification of the WHO classification system.

We studied 14 macroscopically evident tumours, 2 tumourlets and one nodal metastasis (Table 1).

The 2 tumourlets examined were found in lung specimens, which were surgically removed for chronic inflammatory diseases (bronchiectasis). One was classified in the NECNID and the other in the NECWED group. Both were clearly argyrophilic with the modified Grimelius silver impregnation and stained positively with antiserum against NSE (Fig. 1) CG and the 80 kd antigen of neurosecretory granules, but, on the contrary, the tumourlets appeared clearly devoid of any S-100 positive cell.

Of the other 15 neoplasms, Grimelius silver impregnation demonstrated clear argyrophilia in the remaining 4 NECNID and 2 NECWED,in 2 out of the 5 NECINT, none in the primary SCCs and none in the metastatic one. The specimens were immunostained for detection of NSE: all but one NECINT and one SCC were positive (Fig. 2). All NECNID were also CG positive.

Immunostaining with antiserum against S-100 protein revealed three different patterns:

a) Total absence of S-100 positive cells; this being the case of 1 NECNID, 2 NECWED, 1 NECINT and of the SCC's nodal metastasis.

b) Focal and irregular presence of scarce S-100 positive cells. Most of the tumours examined presented this pattern. The S-100 reactive cells were generally irregularly shaped, with cell processes extending through tumoural elements (Fig. 3). Sometimes these cells lay in

Fig. 1. NSE immunoreactive tumourlet. Immunoperoxidase PAP-hematoxylin counterstain. x100

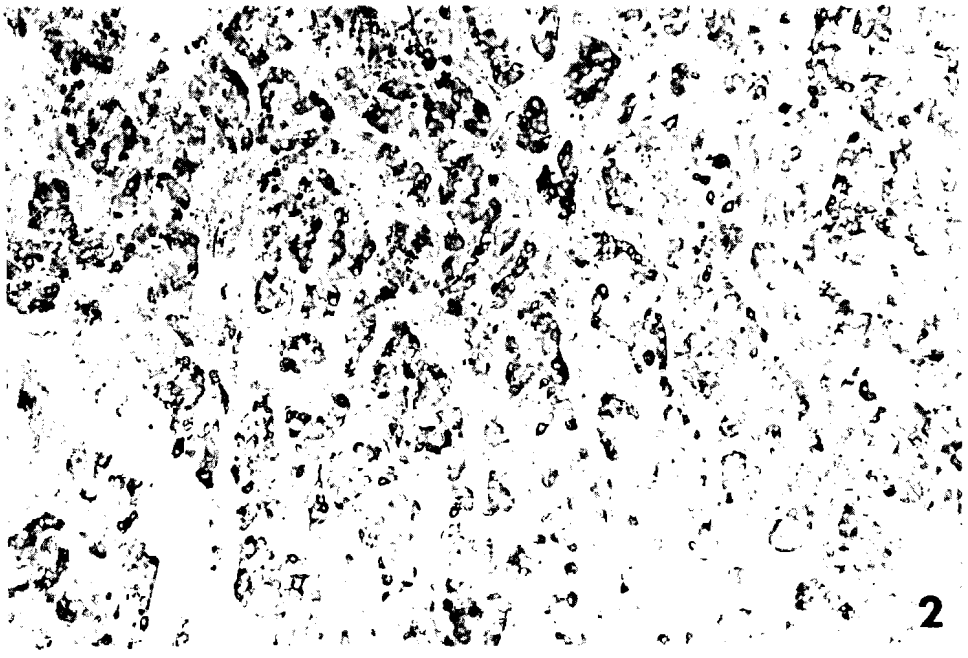

Fig. 2. NECINT with variable argyrophilia of many cells. Grimelius silver impregnation. x100

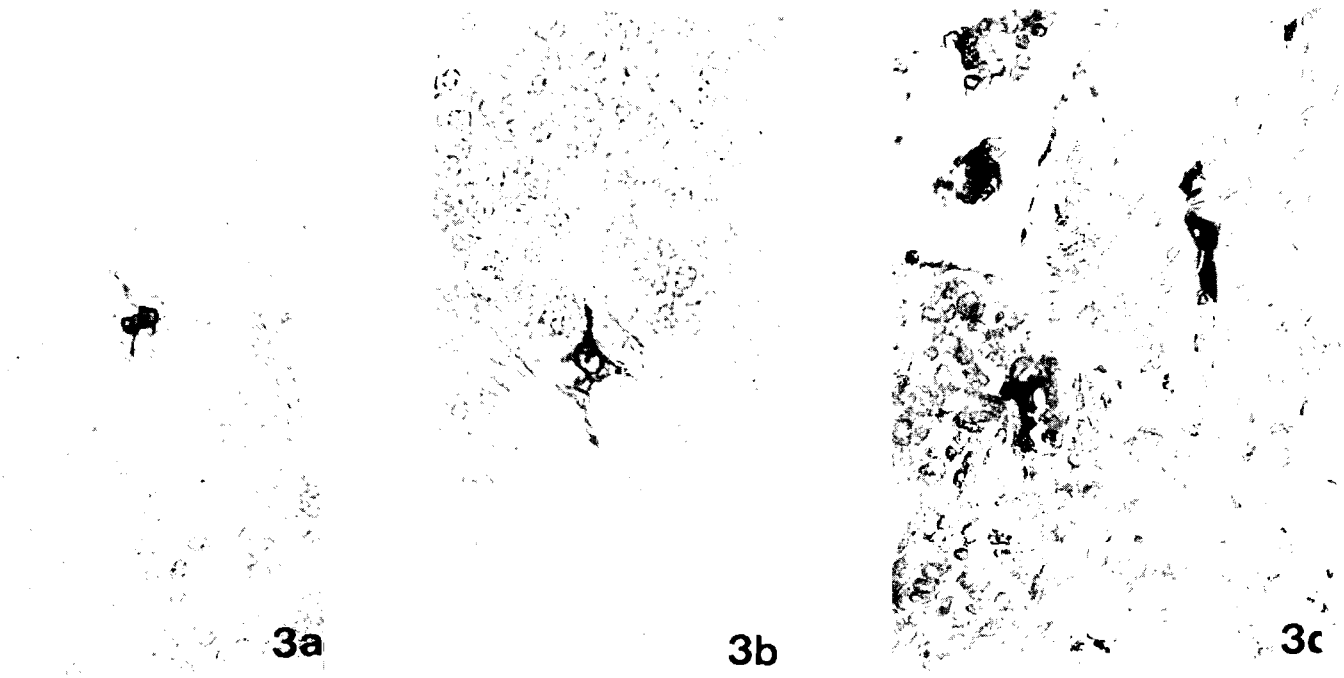

Fig. 3. S-100 immunoreactive cells in neuroendocrine lung tumours: these cells are very scarce, dendritic, stellate– or irregularly shaped. Immunoperoxidase PAP without counterstain. (a) peripheral carcinoid x250. (b) atypical carcinoid x250. (c) SCC x100

Fig. 4. Endobronchial carcinoid. (a) The tumoural growth is partly subepithelial (arrows mark epithelial layer covering the neoplasm). Hematoxylin-Eosin. x100 (b) The same tumour shows distinct NSE immunoreactivity. Immunoperoxidase PAP-Hematoxylin counterstain. x100

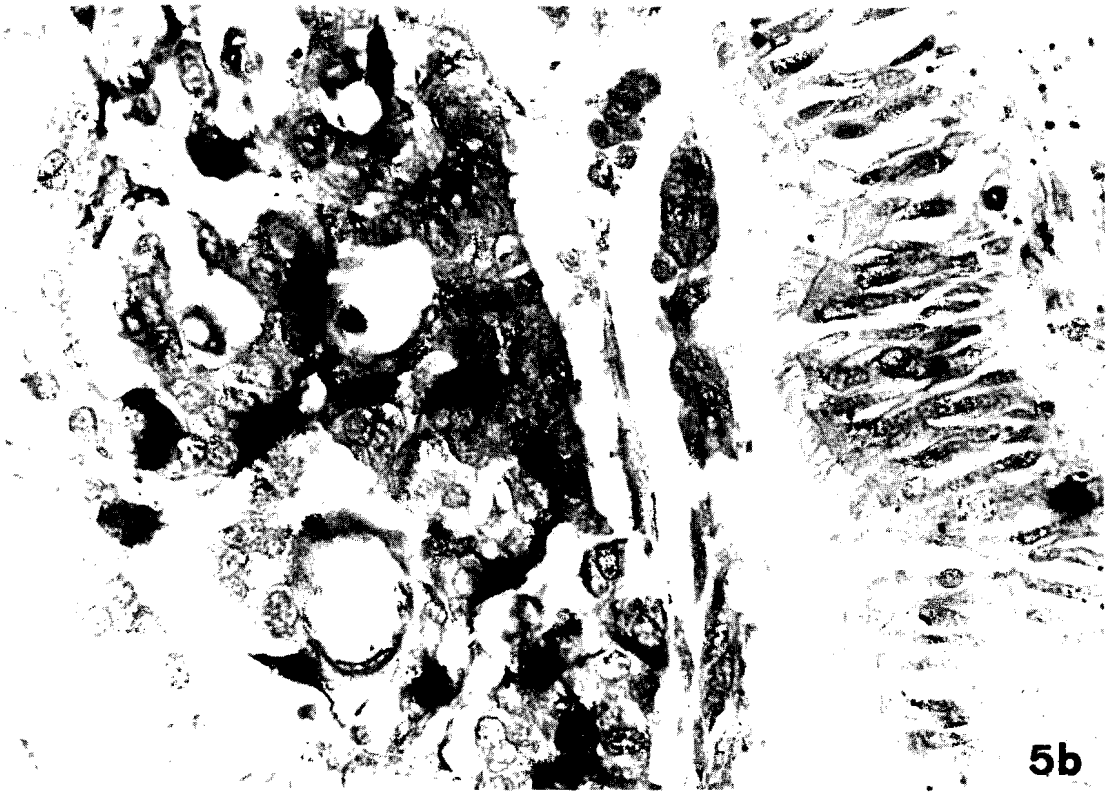

Fig. 5. Same case of Fig.4, immunostained for S-100. (a) S-100 immunoreactive cells are numerous, stellate-shaped and in intimate relation with tumoral cells. Immunoperoxidase PAP x100. (b) At higher magnification, the cell morphology appears reminiscent of other cell types with sustentacular functions. Immunoperoxidase PAP. x250

the interstitial connective tissue between neoplastic cords. Similar cells could sometimes be identified in interalveolar connective tissue of adjacent non-tumoural lung parenchyma.

c) Diffuse presence of S-100 immunoreactive cells in relation with neoplastic cells. We found only one tumour presenting this pattern. It was a centrally located carcinoid (NECNID) with a conspicuous endobronchial growth: Grimelius silver impregnation and immunostaining for NSE and CG were positive (Fig. 4). The S-100 positive cells were very abundant, intimately connected with tumoural cells, elongated or stellate-shaped, with numerous cell projections (Fig. 5).

Table 1. Examined cases and immunohistochemical findings.

TUMOUR	NUMBER	GRIMELIUS POSITIVITY	NSE POSITIVITY	S100 POSITIVITY
NECNID	5	5/5	5/5	4/5*°
NECWED	3	3/3	3/3	1/3 °
NECINT	5	2/5	4/5	4/5
NECMIC	3	0/3	2/3	3/3
Metastasis of SCC	1	0/1	N.D.	0/1

N.D.: not done.
* one of the positive tumours contained abundant S-100 reactive cells;
° the tumourlet type was negative

Discussion

The neuroendocrine tumours presented in this paper are classified according to Gould et al. (1983a) and Mosca et al. (1986). Their neuroendocrine nature has been confirmed with immunohistochemical analysis using polyclonal antiserum against NSE, which is a widely acccepted neuroendocrine marker (Said et al., 1985; Mosca et al., 1986).

The S-100 positive cells detected in our study are always, except in one case, very scarce and readily detected only in uncounterstained slides. Their morphology appears very irregular, dendritic, with elongated cell processes extending between neoplastic cells. Frequently the S-100 immunoreactive cells are also located in interstitial tissue between tumoural nests and cords.

Owing to their morphology, distribution and scarcity we belive that the S-100 positive cells are not part of the neoplastic growth. They could be interpreted as dendritic reticulum cells migrating through the tumours. Similar findings and interpretations are also described for other neoplasms, i.e. nasopharingeal indifferentiated carcinomas (Lauriola et al., 1984 a; Nomori et al., 1986), and for inflammatory lesions, i.e. cutaneous leishmaniasis (Veress and El Hassan, 1985).

In our material no correlation seems to exist between histological tumour type, presence, and quantity of S-100 immunoreactive cells.

The only exceptions are the tumourlets, which were devoid of any of such cells. This fact could perhaps be explained in view of the role of these S-100 labelled cells and the hypothetical nature of the tumourlets themselves (D'Agati and Perzin, 1985). In fact, if the detected cells are dendritic reticulum cells, they should play a role in modulation of host-tumour interactions (Lauriola et al., 1984). It could be that such interactions, which take place between host and lung tumour's, are not yet fully developed in respect to the small size of the tumourlets, which are interpretable as the very first stage of the neoplastic growth of neuroendocrine tumours (Gould et al., 1983a, b; Mosca et al., 1985).

In our study we also detected a case of bronchial central carcinoid, similar to the one described by Nakajima et al., (1982), and to the four reported by El-Salhy et al., (1986), with very abundant S-100 immunoreactive cells with peculiar stellate morphology. These cells resemble setellite cells of peripheral ganglia and adrenal medulla (Cocchia and Michetti, 1981a), folliculo-stellate cells of adenohypophysis (Nakajima et al., 1982) and the S-100 positive cells of paraganglioma (Nakaima et al., 1985) and pheochromocytomas (El-Salhy et al., 1986).

We agree that these cells are in fact part of the neoplasia, possibly a sort of satellite cell component. Similar interpretations are reported with respect to appendiceal carcinoids.

These latter tumours are believed to originate from subepithelial neuroendocrine cells (Rode et al., 1982, 1983; Lundqvist and Wilander, 1986) and to have a different histogenetic and/or differentiative pattern from other gut carcinoids. Seemingly, it could be supposed that the tumour we describe has a peculiar histogenetic and/or differentiating pattern, which sets it apart from the other lung carcinoids we examinated.

Further studies are required to establish if the presence of supposed dendritic reticulum cells in lung neuroendocrine tumours has any prognostic significance. Moreover the nature and origin of lung carcinoids with respect to their possible S-100 immunoreactivity should be further investigated.

Acknowledgements. We wish to thank Dr. J. Munguia-Barrera. Dept. of Pathology, León University, Nicaragua, for several useful comments and suggestions and Dr. B. Frigo for optimal technical assistance.

References

Barbareschi M., Floresta A.M., Mariani C., Allegranza A. and Mosca L. (1984). The nature of perineuronal cells in human gastrointestinal tract. Acta Neurol. 6, 326-327.

Becker K.L., Monaghan K.G. and Silva O.L. (1980). Immunocytochemical localization of calcitonin in Kulchitsky cells of human lung. Arch. Pathol. Lab. Med. 104, 196-198.

Bonnin J.M. and Rubinstein J.L. (1984). Immunohistochemistry of central nervous system tumours. J. Neurosurg. 60, 1121-1133.

Choi H.S.H. and Anderson P.J. (1985). Immunohistochemical diagnosis of olfactory neuroblastoma. J. Neuropath. Exp. Neurol. 44/1, 18-31.

Cocchia D. and Miani N. (1980). Immunocytochemical localization of the brain-specific S-100 protein in the pituitary gland of adult rat. J. Neurocytol. 9, 771-782.

Cocchia D. and Michetti F. (1981 a) S-100 antigen in satellite cells of adrenal medulla and the superior cervical ganglion of the rat. An immunochemical and immunocytochemical study. Cell Tissue Res.215, 103-112.

Cocchia D., Michetti F. and Donato R. (1981 b). Immunochemical and immunocytochemical localization of S-100 antigen in normal human skin. Nature 294, 85-87.

D'Agati V.D. and Perzin K.H. (1985). Carcinoid tumourlets of the lung with metastasis to a peribrochial lymph node. Cancer 55, 2472-2476.

Dhillon A.P. and Rode J. (1983). Immunohistochemical studies of S-100 protein and other neural characteristics expressed by granular cell tumour. Diagn. Histopathol. 6, 23-28.

El-Salhy M., Lundqvist M. and Wilander E. (1986). Bronchial carcinoids and pheochromocytomas. A comparative study with special emphasis on S-100 protein, Serotonin and neuron specific enolase (NSE) immunoreactivity. Acta Path. Microbiol. Scand., Sect. A. 94, 229-235.

Gatter K.C., Dunnill M.S., Pulford K.A.F., Hervet A. and Mason D.Y. (1985). Human lung tumours: a correlation of antigenic profile with histological type. Histopathol. 9, 805-823.

Ghadour M.S., Langley O.K., Labourdette G., Vincendon G. and Gombos G. (1981), Specific and artefactual cellular localization of S-100 protein: an astrocyte marker in rat cerebellum. Dev. Neurosci. 4, 66-78.

Gould V.E., Linnoila R.I., Memoli V.A. and Warren W.H. (1983a). Neuroendocrine cells and neuroendocrine neoplasms of the lung. Pathol. Ann. 18, 287-330.

Gould V.E., Linnoila R.I., Memoli V.A. and Warren W.H. (1983b). Neuroendocrine components of the bronchopulmonary tract: hyperplasias, dysplasias and neoplasms. Lab. Invest. 49, 519-537.

Graham D.I., Thomas D.G.T. and Brown I. (1983). Nervous system antigens. Histopathol. 7, 1-21.

Halliday G.M., Mc Cardle J.P., Knight B.A. and Muller H.K. (1986). New methodology for assessment of the Langerhans cell network. J. Pathol. 148, 127-134.

Höfler H., Walter G.F. and Denk H. (1984). Immunohistochemistry of folliculo-stellate cells in normal human adenohypophyses and in pituitary adenomas. Acta Neuropathol. 65, 35-40.

Kahn H.J., Marks A., Thom H. and Baumal R. (1983) Role of antibody to S-100 protein in diagnostic pathology Am. J. Clin. Pathol. 79, 341-347.

Lack E.E. and Mercer L. (1977). A modified Grimelius argyrophil technique for neurosecretory granules. Am. J. Surg. Pathol. 1, 275-277.

Lauriola L., Michetti F., Sentinelli S. and Cocchia D. (1984 a). Detection of S-100 labelled cells in nasopharyngeal carcinoma. J. Clin. Pathol. 37, 1235-1238.

Lauriola L., Michetti F., Stolfi V.M., Tallini G. and Cocchia D. (1984 b). Detection by S-100 immunolabelling of interdigitating reticulum cells in human thymomas. Virchow Arch. (Cell. Pathol.) 45, 187-195.

Lundqvist M. and Wilander E. (1986). Subepithelial neuroendocrine cells and carcinoid tumours of the human small intestine and appendix. A comparative immunohistochemical study with regard to serotonin, neuro-specific enolase and S-100 protein reactivity. J. Pathol. 148, 141-147.

Matsunou H., Shimoda T., Kakimoto S., Yamashita H., Ishikawa E. and Mukai M. (1985). Histopathologic and immunohistochemical study of malignant tumors of peripheral nerve sheath (Malignant Schwannoma). Cancer 56, 2269-2279.

Mosca L., Barbareschi M., Mauri M.F., Munguia-Barrera J., Mauri F.A., Muscara, M. and Valentini L. (1986). Neuroendocrine lung structures and tumours: immunohistochemical analysis using three neuroendocrine markers. Histopathol. (submitted for publication).

Mosca L., Ceresoli A., Anzanello E., Mauri F., Mariscotti C., Timossi R. and Mezzetti M. (1986). Neuroendocrine structures in normal and diseased human lung. Appl Pathol. (In press).

Moscinski L.C. and Kleinschmidt-Demasters B.K. (1985). Primary eosinophilic granuloma of frontal lobe. Diagnostic use of S-100 protein. Cancer 56, 284-288.

Mukai M. (1983). Immunohistochemical localization of S-100 protein and peripheral nerve myelin proteins (P2 protein, PO protein) in granular cell tumors. Am. J. Pathol. 112, 139-146.

Nakajima T., Watanabe S., Sato Y., Kameya T., Hirota T. and Shimosato Y. (1982). An immunoperoxidase study of S-100 protein distribution in normal and neoplastic tissues. Am. J. Surg. Pathol. 6, 715-727.

Nakazato Y., Ishizeki J., Takahashi K. and Yamaguchi H. (1982). Immunohistochemical localization of S-100 protein in granular cell myoblastoma. Cancer 49, 1624-1628.

Nomori H., Watanabe S., Nakajima T., Shimosato Y. and Kameya T. (1986). Histiocytes in nasopharyngeal carcinoma in relation to prognosis. Cancer 57, 100-105.

Rode J., Dhillon A.P. and Papadaki L. (1983). Serotonin-immunoreactive-cells in the lamina propria plexus of the appendix. Hum. Pathol. 14, 464-469.

Rode J., Dhillon A.P., Papadaki L. and Criffiths D. (1982). Neurosecretory cells of the lamina propria of the appendix and their possible relationship to carcinoids. Histopathol. 6, 69-79.

Rozell B., Stenman G., Hansson H.A., Dahl D., Hansson G.K. and Mark J. (1985). Intermediate filaments in cultured human pleomorphic adenomas. An immunohistochemical study. Acta Pathol. Microbiol. Immunol. Scand. Sect. A/93, 335-343.

Said J.W., Vimadal S., Nash G., Shintaku P., Heusser R.C., Sassoon A.F. and Lloyd R.V. (1985). Immunoreactive neuron-specific enolase, bombesin and chromogranin as markers for neuroendocrine lung tumours. Human Pathol. 16, 236-240.

Stefansson K., Wollmann R. and Jerkovic M. (1982 a) S-100 protein in soft tissue tumour derived from Schwann cells and melanocytes. Am. J. Pathol. 106, 261-268.

Stefansson K., Wollmann R., Modre B.W. and Arnason B.G.W. (1982 b). S-100 protein in human chondrocytes. Nature 295, 63-64.

Sternberger L.A., Hardy P.H., Cuculis J.J. and Meyer H.G. (1970). The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J. Histochem. Cytochem. 18/5, 315-333.

Takahashi K., Yamaguchi H., Ishizechi J., Nakajima T. and Nakazato J. (1981). Immunohistochemical and immunoelectron microscopic localization of S-100 protein in the interdigitating reticulum cells of human limph node. Virchows Arch. (Cell Pathol.) 37, 125-135.

Vanstapel M.J., Gatter K.C., de Wolf-Peeters C., Mason D.Y. and Desmet V.D. (1986). New sites of human S-100 immunoreactivity detected with monoclonal antibodies. Am. J. Clin. Pathol. 85, 160-168.

Vanstapel M.J., Peeters B., Cordell J., Heyns W., de Wolfepeeters C., Desmet V. and Mason D. (1985). Methods in laboratory investigation. Production of monoclonal antibodies directed

against antigenic determinants common to the α and β-chain of bovine brain S-100 protein. Lab. Invest. 52, 232-238.

Veress B. and El Hassan A.M. (1985). Immunohistochemical demonstration of S-100 protein antigen-containing cells in chronic cutaneous leishmaniasis. Acta Path. Microbiol. Immunol. Scand. Sect. A/93, 331-334.

Weiss S.W., Langloss J.M. and Enzinger F.M. (1983). Value of S-100 protein in the diagnosis of soft tissue tumors with particular reference to benign and malignant Schwann cell tumors. Lab. Invest. 49, 299-308.

W.H.O. (1982): Histological typing of lung tumours. II Ed. Am. J. Clin Pathol. 77, 123-136.

Wilander E., Lundqvist M. and Movin T. (1985). S-100 protein in carcinoid tumours of appendix. Acta Neuropathol. 66, 306-310.

Accepted December 12, 1986