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Simultaneous Standard Light Microscopy and Immunohistology on Bronchoscopically Procured Lung Cancer Specimens

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Abstract—*A method is described which enables the routine assessment of* in situ *phenotypic differences in lung cancer specimens. In this method, bronchoscopically procured biopsies are mildly fixated and split into two parts. One part is processed according to standard histological procedures, ensuring optimal morphology for normal pathological evaluation of the case. The other part is used for monoclonal antibody based immunohistology.*

The reactivity of a panel of monoclonal antibodies directed against different lung cancer and human leucocyte associated antigens has been assessed. It is shown that antigens that are normally destroyed during routine fixation and embedding technology are still reactive in the procedure.

It is concluded that the procedure enables a study of monoclonal antibody defined phenotypic features, without interfering with the normal histopathological evaluation of the case.

INTRODUCTION

MOST monoclonal antibodies isolated until now react with antigens detectable in cryostat sections but are destroyed during the tissue fixation and embedding procedures normally used in pathology. Generally, bronchoscopically procured formalin fixed and paraffin embedded biopsies are used for the histological diagnosis of lung cancer. Snap-freezing of a bronchoscopically procured specimen may interfere with proper light microscopic evaluation of the case [1]. In this report a procedure is described which combines monoclonal antibody based immunohistological staining of small specimens with a simultaneously conducted embedding technology to produce high quality normal histology.

MATERIALS AND METHODS

Bronchoscopy procedure

Rigid bronchoscopy was performed under general anesthesia. The bronchoscope (R. Wolf, Knittlinger, F.R.G.) had a distal cuff to facilitate artificial respiration during general anesthesia. Flexible bronchoscopy was performed under local anesthesia with an Olympus BF-P10. Biopsies were taken under visual control and placed in Nakane's fixative [2]. The diameter of the biopsies ranged from 1.5 to 2.5 mm.

Fixative and handling of the biopsies

Nakane's fixative [2] was prepared as follows: Firstly, a lysine–phosphate buffer was made by adding as much 0.1 M dibasic sodium phosphate to 50 ml 0.1 M lysine–HCl until the pH reached 7.4. Then 0.025 M sodium phosphate buffer with an osmolarity of 280 mOsm was added to fill up the volume to 100 ml. Subsequently the fixative can be prepared shortly before use by combining three parts of the lysine–phosphate buffer with 1 part paraformaldehyde solution (4% w/v according to Robertson *et al.* [3]), after which 2.14 mg Na-meta-periodate/ml was added.

The specimens were placed in the fixative for 3 h. Then, the specimen was divided into two parts by cutting with a razor blade. One part was rinsed and kept in the sodium phosphate buffer to wash out the fixative for at least 12 h. This tissue part was snap-frozen subsequently in OCT compound (Miles Laboratories, Inc., U.S.A.) in liquid freon and kept at −80°C until use. Cryostat sectioning and immunostaining of the specimen were performed

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Table 1. Reactivity of monoclonal antibodies with bronchoscopically procured lung cancer specimens. Both SCLC and non-SCLC express the epithelial markers recognized by MOC-31 and RGE-53. The expression of neuroendocrine differentiation antigens is largely restricted to the SCLC group

	Neuroendocrine differentiation antigens						Epithelial differentiation antigens		
	MOC-1	MOC-21	MOC-32	MOC-51	MOC-52	MNF	MOC-31	RGE-53	RKSE-60
SCLC	56/61	43/61	38/61	19/60	53/61	3/55	61/61	44/60	0/59
non-SCLC	2/18	0/18	0/18	1/18	0/18	0/18	17/18	11/18	3/18

according to described methods [4]. A control reaction, in which the primary antibody was omitted, was carried out in all staining procedures to ensure that the observed reactions are specific. The other part was further fixed in formalin and embedded in plastic for light microscopy.

Light microscopy

Routine hydroxy-ethylmethacrylate embedding [5] (JB-4 embedding, Polysciences Inc., Warrington, PA, U.S.A.) was performed to obtain optimal light microscopic histology. The diagnosis SCLC or non-SCLC was set according to WHO criteria [6].

Monoclonal antibodies tested after Nakane fixation

We have produced a number of monoclonal antibodies (MOC-1, MOC-21, MOC-31, MOC-32, MOC-51 and MOC-52) using SCLC-cell lines derived antigens [1, 7]. Using a large series of frozen and paraffin embedded normal and malignant tissues, these antibodies have been screened in both our laboratory and by the participants of the first international workshop on monoclonal antibodies directed against SCLC [8]. MOC-1, MOC-21, MOC-32 and MOC-52 were assigned to SCLC-Cluster 1, comprising a group of monoclonal antibodies reactive with normal and malignant neuroendocrine tissues including SCLC.

MOC-31 was assigned to SCLC-Cluster 2, a group of monoclonal antibodies reactive with a subset of normal and malignant epithelial tissues, including both SCLC and non-SCLC.

The tissue reactivity of the MOC series has been published before [1]. MOC-51, which has not been clustered yet, also recognizes a neuroendocrine differentiation antigen [1]. The epitopes recognized by MOC-1, MOC-51 and MOC-52 are destroyed during formalin fixation and subsequently performed paraffin or plastic embedding.

In the present study also monoclonal antibodies RGE 53 and RKSE 60, directed against cytokeratin 18 and 10 respectively, and MNF, directed against the 210 and 68 kDa components of neurofilaments [9–11], have been included. In addition, a series of monoclonal antibodies reactive with human leucocyte associated antigens [leu 2^a, leu 3^a, leu 4, leu 5 (Beckton Dickinson), directed against CD 8, CD 4, CD 3, CD 2 respectively] was used. These antigens, recognized by RGE 53, RKSE 60, and the leu series, are destroyed by formalin fixation and subsequent plastic or paraffin embedding.

RESULTS

Immunohistochemistry was performed on 96 bronchoscopically procured specimens. From these, 79 specimens contained tumor in the part which was used for immunohistochemistry.

All antigens recognized by the MOC series, by the leu series and by the monoclonal antibodies RGE 53, RKSE 60 and MNF remained detectable after fixation in the Nakane fixative.

A heterogeneity of expression of neuroendocrine differentiation antigens can be appreciated in the SCLC group, whereas the assessed epithelial antigens are heterogeneously expressed in the non-SCLC group (Table 1). In two non-SCLC specimens neuroendocrine differentiation features (reactivity with MOC-1 and, in one case, with MOC-51) could be demonstrated. Figures 1 and 2 show examples of monoclonal antibody based immunohistological staining in one part, and normal histology in the other part of a bronchoscopically procured biopsy.

DISCUSSION

From a clinical point of view, the most important differential diagnosis in lung cancer is that between SCLC and non-SCLC. Immunohistology of a biopsy with monoclonal antibodies directed against pan-SCLC associated antigen (such as MOC-1, MOC-52 [8]) may add to the establishment of a proper diagnosis, especially in cases where only small biopsies with poor morphology are available. The procedure described in this paper does not interfere with the normal histopathological evaluation of a case, and therefore enables the routine assessment of the immunohistology of a biopsy for such purposes.

A further consideration to use immunohistology in addition to normal histology stems from the fact that the detection of specific antigenic features in a biopsy may enable the further refinement of a diagnosis. In the case of lung cancer this possibility can be illustrated as follows. Further subtyping of

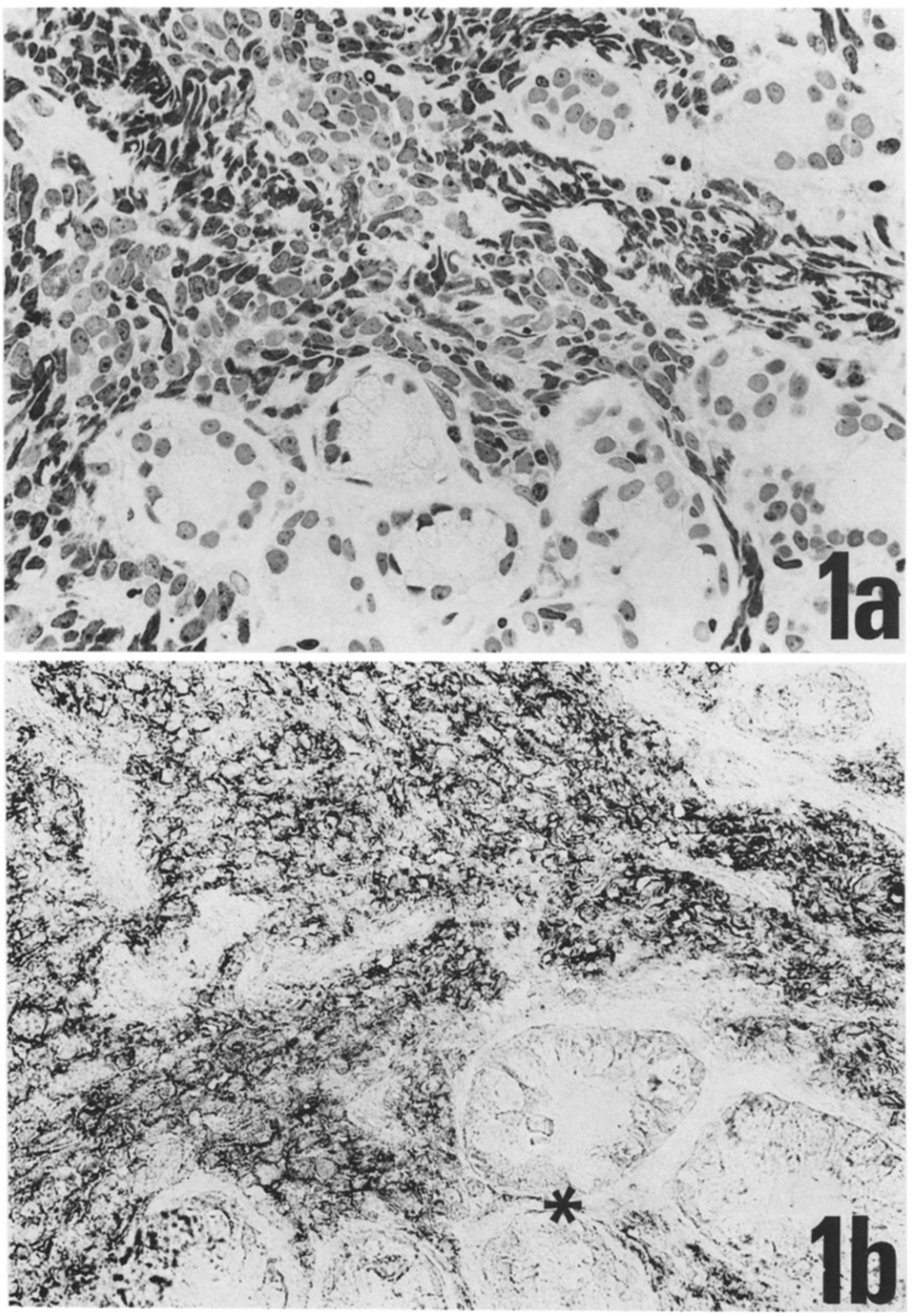


Fig. 1. Bronchoscopically taken SCLC specimen after fixation in Nakane's fixative. One part is further processed by formalin fixation, hydroxy-ethylmethacrylate embedding and hematoxylin–eosin staining. In the section a submucous bronchial gland surrounded by SCLC can be seen. Tumor fields with non-crushed and crushed morphology are present (a). Another part of the same specimen has been used for immunoperoxidase staining with MOC-1. It can be appreciated that the submucous bronchial gland (indicated by an asterix) does not stain with MOC-1. In the submucosa infiltrating SCLC cells reactive with MOC-1 can be seen (b). Magnification: 400 ×.

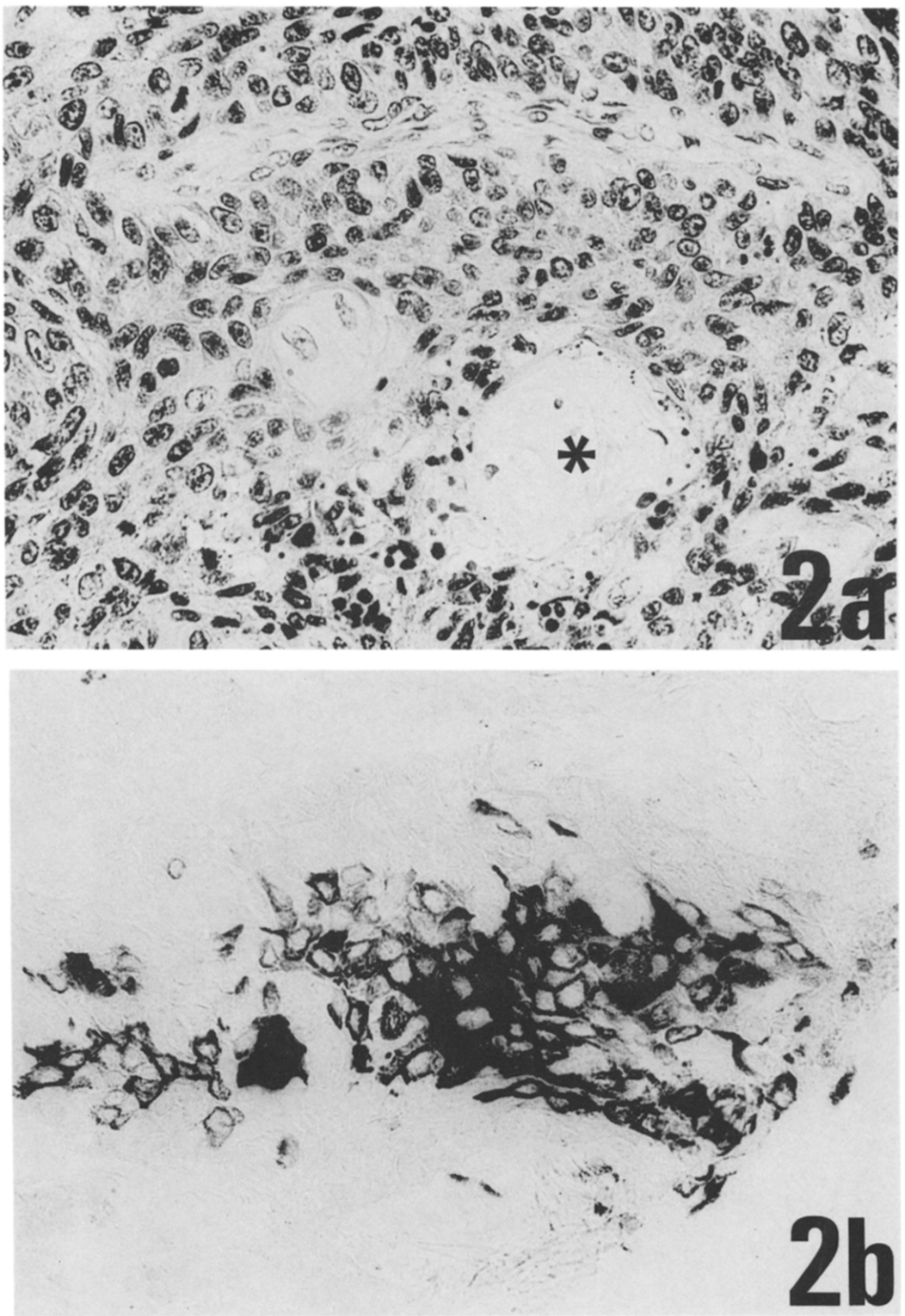
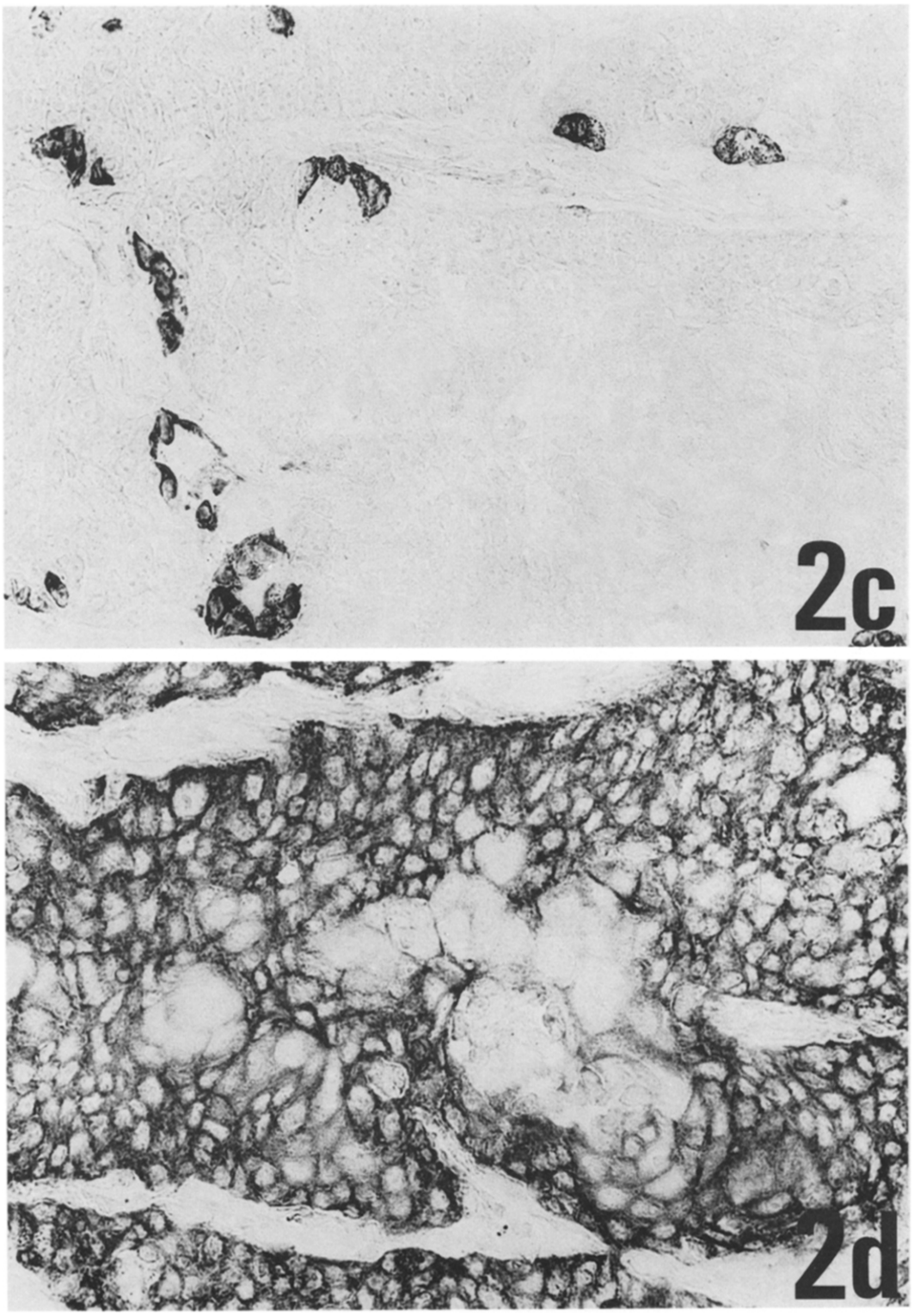


Fig. 2. Squamous cell carcinoma specimen after fixation in Nakane's fixative. A part of the specimen is further processed by formalin fixation, hydroxy-ethylmethacrylate embedding and HE staining. Keratinizing areas can be appreciated in the section (asterix) (a). Part of the same specimen is shown after cryostat sectioning and immunoperoxidase staining. Keratinizing areas of the tumour react with RKSE 60 (b). Isolated non-keratinizing cells react with RGE 53 (c). All tumor cells, except for the highly keratinized areas show reactivity with MOC-31 (d). Magnification: 400 ×.



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both SCLC and non-SCLC on the ground of normal histopathological parameters does not provide additional information concerning clinically important parameters such as the tumors' response to chemotherapy or patients' survival. Only the presence of admixtures of large cells in a SCLC biopsy may be taken as an indication for worse prognosis [12].

In vitro studies, using established lung tumor derived cell lines, have indicated that further subtyping of both SCLC and non-SCLC on non-morphological parameters may be feasible. In *in vitro* SCLC it has been shown that the specific lack of some neuroendocrine features, such as the presence of gastrin releasing factor (GRF) and DOPA-decarboxylase, predicts a decreased sensitivity of the tumor cells to radiotherapy [13]. In agreement with this suggestion we have shown recently that, also *in vivo*, a diminished expression of specific neuroendocrine antigens in SCLC tumor appears to correlate with a decreased reactivity to subsequently applied polychemotherapy [14]. On the other hand, in *in vitro* non-SCLC, the presence of neuroendocrine markers may mark a subgroup showing increased drug sensitivity as compared to normal, neuroendocrine marker negative non-SCLC [15]. There are no *in vivo* data to sustain such a conclusion, yet.

In conclusion, immunohistology appears to be a powerful tool which, in combination with the use of specifically selected monoclonal antibodies, may be helpful in some cases to establish a normal pathological diagnosis, and, in addition, may extend the possibilities to set a more differentiated pathological diagnosis. A prerequisite for such an application is, however, that the immunohistological staining procedure can be done in a routine fashion, under conditions in which the reactivity of the antigens is not affected by fixation. In addition, the tissue morphology should be optimally preserved to enable optimal normal histopathological evaluation. The procedure described in this paper meets these conditions and can be done on all diagnostic specimens. The work-up is illustrated in our study with bronchoscopically taken biopsies. All lung tumor biopsies taken in this way during 1985 and 1986 have been processed according to the procedure and have been successfully used for both setting the pathological diagnosis and immunophenotyping the tumor cells. Immunohistological findings in biopsy specimens are not only useful for pathological purposes. Careful correlation of these data with the clinical course of a patient during intra-individual follow-up may also add to our understanding of the biological changes within a tumor in the course of time.

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