Expression of a Neural Type of Intermediate Filament as a Distinguishing Feature Between Oat Cell Carcinoma and Other Lung Cancers

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Expression of intermediate filaments was studied immunohistologically in oat-cell (6 cases), epidermoid (9 cases), adeno- (7 cases), large-cell anaplastic (3 cases), and bronchioalveolar carcinoma (3 cases), of lung. Affinity-purified antibodies against epithelial (anti-keratin), neural (anti-neurofilament), muscle (anti-desmin) and mesenchymal (anti-vimentin) intermediate filaments were used. In indirect immunofluorescence microscopy of oat-cell carcinomas a positive cytoplasmic fluorescence was seen only with antibodies against neural intermediate filaments, neurofilaments, while no staining of the tumor cells was observed with antibodies against other types of intermediate filaments. On the other hand, all the epidermoid, adeno, anaplastic, and bronchioalveolar carcinomas showed constantly a strong reaction with anti-keratin antibodies in a varying number of cells but no decoration with anti-neurofilament antibodies. The results show that expression of neural intermediate filaments is a major distinguishing feature between oat-cell carcinomas and other lung cancers and suggest that anti-neurofilament antibodies can be used as a diagnostic aid in the surgical pathologic study of pulmonary neoplasms. (Am J Pathol 1983, 110:113–118)

OAT CELL CARCINOMA, comprising about 10% of lung cancers, is a distinct tumor type with characteristic histologic and cytological features.[1,2] Ultrastructurally identifiable neuroendocrine granules and histochemically and chemically detectable serotonin, histaminase, L-dopa decarboxylase, and calcitonin in the tumor cells have been well documented and taken as an evidence of a neuroendocrine origin of these tumors.[1,3–5] Recently, the presence of a neuropeptide, bombesin, has been reported in oat-cell carcinomas.[6] In this study we show, using monospecific antibodies and immunofluorescence microscopy, another major distinguishing feature between oat-cell carcinoma and other lung cancers; the expression of neural intermediate filaments in oat-cell carcinoma in contrast to the expression of epithelial intermediate filaments in other types of lung cancers (epidermoid, adeno-, large-cell anaplastic, and bronchioalveolar carcinomas). This finding offers the possibility of using anti-neurofilament antibodies as a differential diagnostic aid in the surgical pathologic study of lung cancers. We also briefly discuss the significance of this finding with regard to the histopathogenesis of oat-cell carcinoma.

Materials and Methods

Twenty-eight cases of histologically verified lung carcinomas, including 9 cases of epidermoid, 7 cases of adeno-, 3 cases of bronchioalveolar, 3 cases of large anaplastic, and 6 cases of small-cell carcinomas were studied. For diagnostic light-microscopic examination, surgically removed samples of the tumors were fixed in formalin and embedded in paraffin in a routine manner, sectioned, and stained with hematoxylin and eosin (H&E). For immunofluorescence

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Table 1—Pulmonary Carcinomas Tested for Intermediate Filaments in Indirect Immunofluorescence Microscopy

Histologic type	Total number of cases	Number of cases expressing different intermediate filaments			
		Keratin	Neurofilaments	Vimentin	Desmin
Epidermoid carcinoma	9	9	0	0	0
Adenocarcinoma	7	7	0	0	0
Bronchialveolar carcinoma	3	3	0	0	0
Anaplastic (large-cell) carcinoma	3	3	0	0	0
Oat-cell carcinoma	6	0	6	0	0

microscopic examination, the sections were deparaffinized and treated with 0.4% pepsin (Merck AG, Darmstadt, FRG) in 0.01 N HCl for 2 hours.[7] Fresh- and deep-frozen tumor material was also available for about half the cases in each group, and for indirect immunofluorescence microscopy (IIF), either touch imprints or frozen sections were made, which were then fixed in methanol at −20 C for 10 minutes. To visualize intermediate filaments, we reacted the sections first with rabbit anti-vimentin, anti-desmin, anti-keratin, or anti-neurofilament antibodies and then exposed them to fluorescein-isothiocyanate(FITC)-conjugated goat anti-rabbit IgG (Cappel Laboratories, Cochraneville, Pa). For control purposes, the specimens were also exposed to normal rabbit serum, followed by the FITC-conjugated goat anti-rabbit IgG. The specificity of the staining reaction was also controlled by the use of antibodies preabsorbed with the corresponding antigens: vimentin isolated electrophoretically from

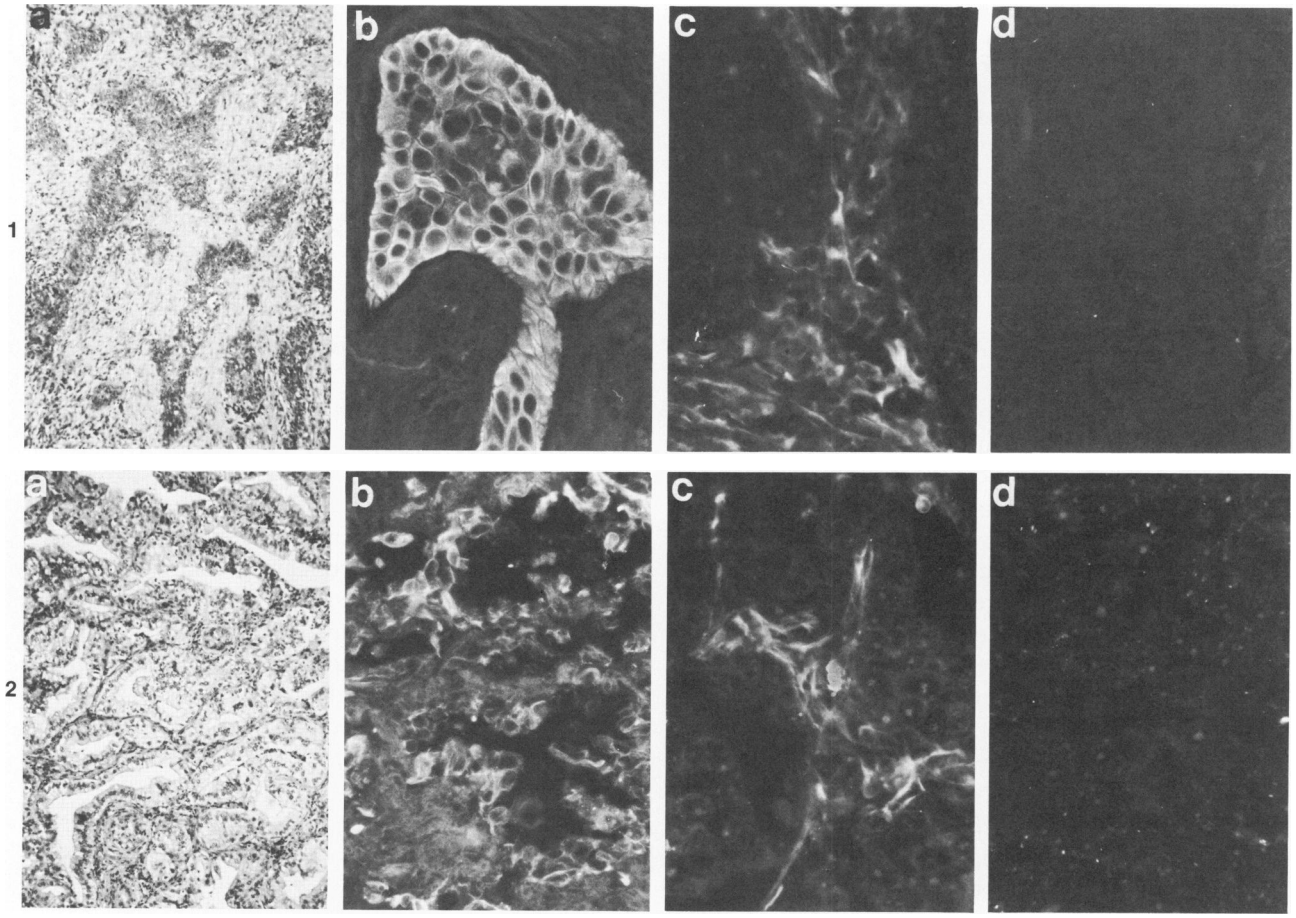

Figure 1—Epidermoid carcinoma of the lung. H&E staining (**a**). IIF staining with anti-keratin shows distinct decoration of the carcinoma cells but no staining of the stroma (**b**), while anti-vimentin antibodies decorate stromal cells but not tumor cells (**c**). No reaction with anti-neurofilament antibodies is seen (**d**). **Figure 2**—Adenocarcinoma of the lung. H&E staining (**a**). IIF staining with anti-keratin shows a positive reaction with the carcinoma cells (**b**), while anti-vimentin antibodies decorate only scattered mesenchymal cells (**c**). No staining with anti-neurofilament antibodies is seen (**d**).

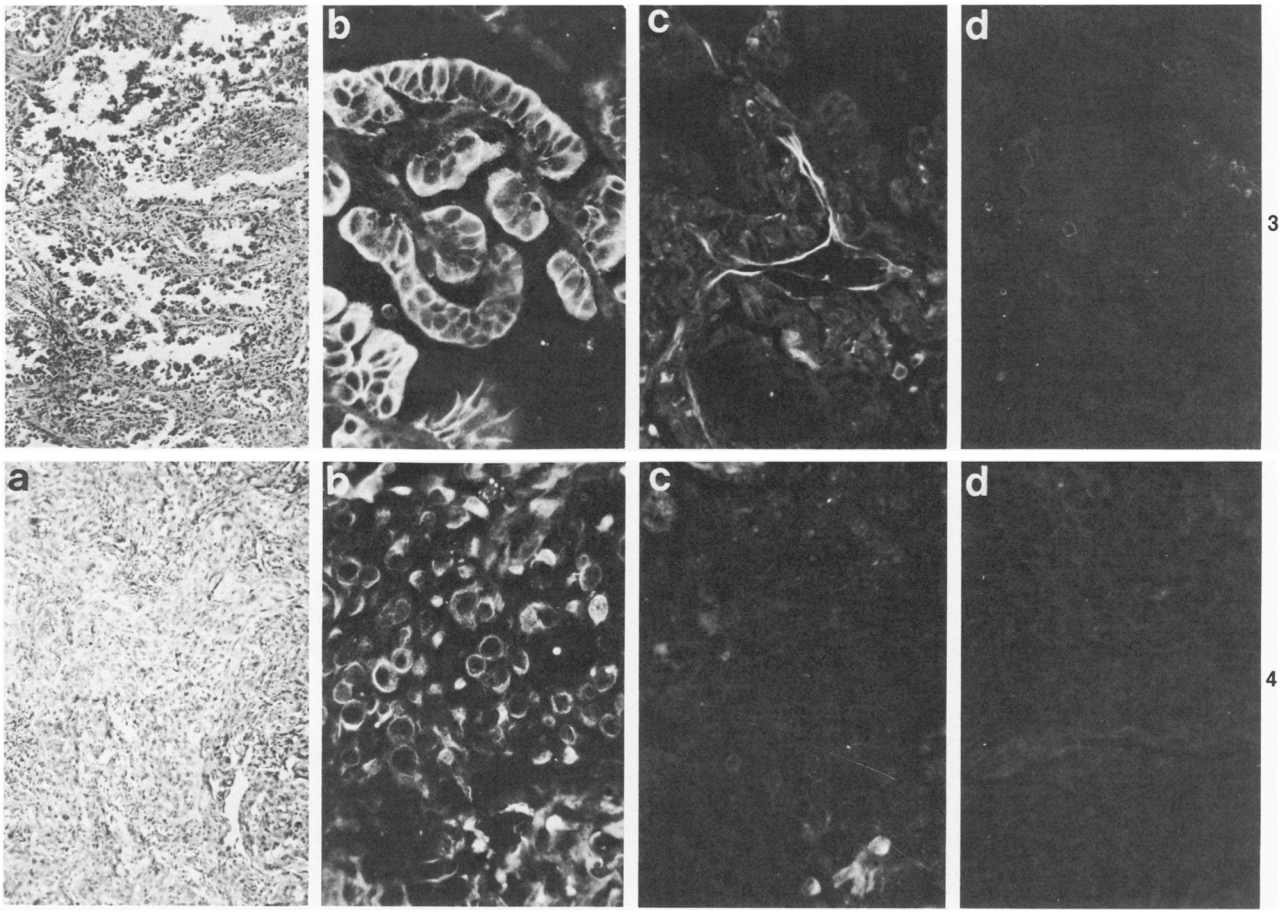

Figure 3— Bronchioalveolar carcinoma of the lung. H&E staining (**a**). IIF staining with anti-keratin shows a strong reaction with the carcinoma cells (**b**), while anti-vimentin decorates only stromal cells (**c**). No staining with anti-neurofilament antibodies can be seen (**d**). **Figure 4**— Large-cell anaplastic carcinoma. H&E staining (**a**). IIF staining with anti-keratin shows a positive reaction with carcinoma cells (**b**). Anti-vimentin decorates only some entrapped mesenchymal cells (**c**). No staining is seen with anti-neurofilament antibodies (**d**).

cultured human fibroblasts,[8] keratin isolated from human plantar callus,[8] desmin isolated from chicken gizzard,[9] or neurofilament-rich material isolated from human brain.[10] The specimens were studied in a Zeiss Universal microscope equipped with an epi-illuminator III RS and filters for FITC-fluorescence. The production and specificity of anti-vimentin,[8] anti-keratin,[8] anti-desmin,[9] and anti-neurofilament[11] antibodies have been presented elsewhere. Anti-desmin antibodies were a kind gift from Dr. R. A. Badley, Unilever Research, Sharnbrook, England.

Results

Histologic typing and immunofluorescence findings are summarized in Table 1. The histologic typing was done independently by three pathologists following the classification presented by Rosai.[1] Typical examples of the histologic pictures of the carcinomas studied are given in Figures 1–5.

Immunofluorescence microscopy of all 9 cases of epidermoid carcinomas showed a bright decoration with anti-keratin antibodies (Figure 1b), while anti-vimentin antibodies stained only scattered mesenchymal cells and vascular endothelium (Figure 1c). No staining with anti-neurofilament (Figure 1d) or anti-desmin antibodies was seen in epidermoid carcinomas. A similar staining profile was seen in adeno- and bronchioalveolar carcinomas. The tumor cells reacted only with anti-keratin (Figures 2b and 3b), while stromal cells showed reactivity with anti-imentin antibodies (Figures 2c and 3c). No staining was seen with anti-neurofilament antibodies (Figure 2d and 3d). In large-cell anaplastic carcinomas a large proportion of the neoplastic cells did not show reactivity toward any of the antiserums used. However, in all cases anti-keratin–reactive cells were found (Figure 4b). No staining of the tumor cells with anti-vimentin (Figure 4c), anti-neurofilament (Figure 4d), or anti-desmin (data not shown) anti-

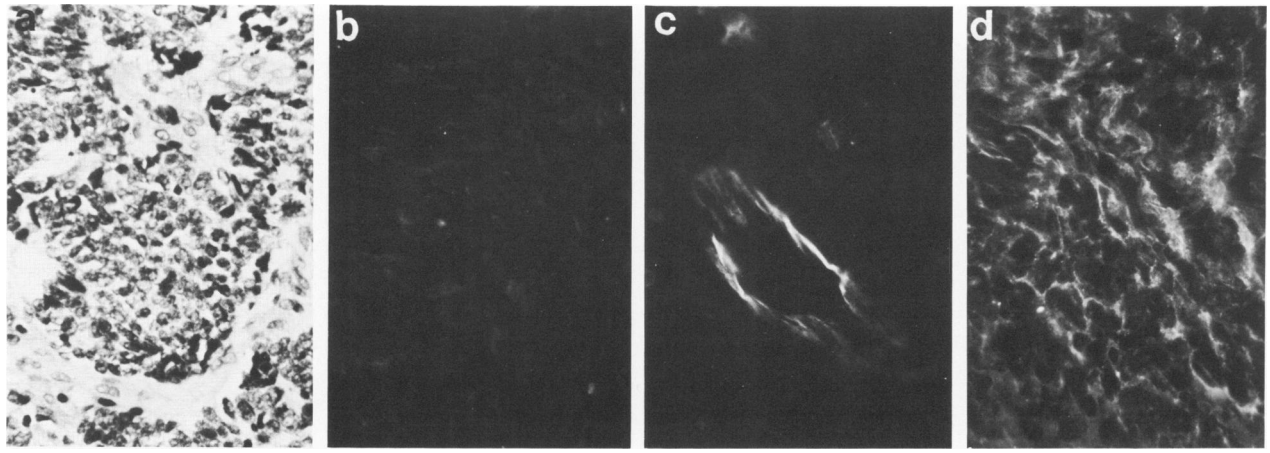

Figure 5—Oat-cell carcinoma of the lung. H&E staining (**a**). No staining of the carcinoma cells can be seen with anti-keratin (**b**) or antivimentin antibodies (**c**). Anti-vimentin decorates, however, endothelium of entrapped vessels. Anti-neurofilament antibodies, on the other hand, show a strong reaction with the carcinoma cells (**d**).

bodies were seen. The staining reactions could be inhibited in all cases by preabsorption of the antiserums with their corresponding antigens. No fluorescence was seen in any of the tumors after exposure to normal rabbit serum (not shown).

In contrast to other carcinomas studied, oat-cell carcinoma cells showed a strong reactivity with anti-neurofilament antibodies (Figure 5d) but not with anti-keratin or anti-vimentin antibodies (Figures 5b and 5c). Anti-vimentin antibodies decorated, however, scattered mesenchymal cells and endothelium of the vessels entrapped among the tumor cells (Figure 5c). The anti-neurofilament staining reaction in sections derived from paraffin sections was weaker than in cryostat sections of deep-frozen material. A distinct decoration of cytoplasmic filaments with anti-neurofilament antibodies with a lack of reactivity with anti-keratin and anti-vimentin antibodies was also seen in touch imprint preparations of the tumors (Figure 6).

Discussion

Intermediate filaments (IMF) are polymeric cytoplasmic structures that form part of an elaborate filamentous network collectively called the cytoskeleton.[12,13] Although morphologically similar, five biochemically and immunologically distinct subclasses of IMFs can be distinguished: vimentin-containing IMFs in mesenchymal cells, keratin-containing IMFs in epithelial cells, desmin-containing IMFs in muscle cells, glial-fibrillary-acidic-protein-containing IMF's

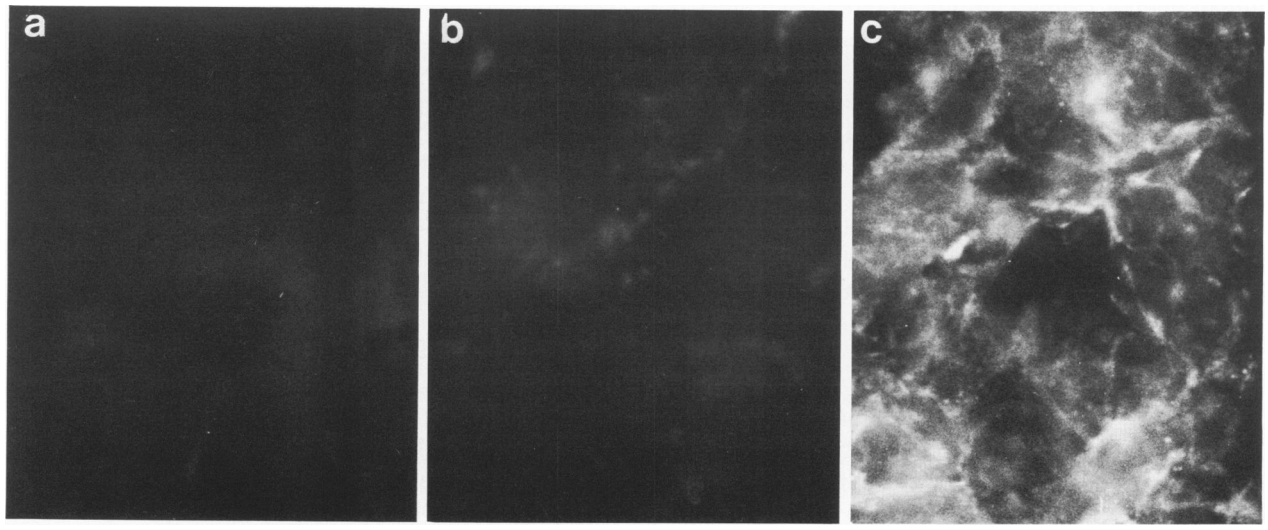

Figure 6—Touch imprint preparations of oat-cell carcinoma of the lung. Anti-keratin (**a**) and anti-vimentin (**b**) antibodies show no reaction with carcinoma cells, while a strong reaction can be seen with anti-neurofilament antibodies (**c**).

in glial cells, and neurofilaments in neural cells.[8,13,14] Different types of intermediate filaments show a high degree of tissue specificity *in vivo* and have therefore been successfully used as tissue markers in both embryologic and histopathologic studies.[15,16] Anti-vimentin and anti-keratin antibodies, for instance, have been applied as an aid in the differential diagnosis of lymphomas and carcinomas[17-19]; and we have also used anti-desmin antibodies to identify poorly differentiated rhabdomyosarcomas.[20]

In this study we have shown that oat-cell carcinomas of lung express only neural intermediate filaments, in contrast to epidermoid, adeno-, bronchial-veolar, and also large-cell anaplastic carcinomas, which express only epithelial intermediate filaments (cytokeratin). This finding is in accordance with recent results of Gusterson et al,[21] who have demonstrated keratin positivity in most epidermoid carcinomas and keratin negativity in most oat-cell carcinomas. In their study adenocarcinomas and anaplastic carcinomas showed a higher heterogeneity in keratin expression when compared with our results. This difference may be due to the well-known variability in the ability of anti-keratin antibodies to recognize different epithelia.[22,23] Moreover, Bergh et al have recently described two oat-cell-carcinoma-derived cell lines that do not express keratin.[24] In neither of these studies were anti-neurofilament antibodies used.

Neurofilaments could be detected in all cases studied, although a part of the malignant cells did not show any reaction with either anti-neurofilament or any other type of anti-intermediate filament antibodies. The reason for this lack of reactivity may be related to the processing of the specimens; formaldehyde fixation and paraffin embedding may have destroyed partially the antigenic sites of the filaments.[15] It is also possible that in poorly differentiated cells with sparse cytoplasm the quantity of the IMF protein is under the detection level of the immunofluorescence technique. However, the use of cryostat sections and cytologic smears from the fresh tumor tissue enabled better visualization of the cytoplasmic filaments in poorly differentiated cells.[15] With these technical precautions in mind, anti-neurofilament antibodies can be used as a useful differential diagnostic aid in distinguishing oat-cell carcinomas from other pulmonary carcinomas that express keratin filaments and also from most mesenchymal tumors, which express vimentin or desmin.[25]

Neurofilaments, present in all neurons of the central and the peripheral nervous system, form part of the neuronal cytoskeleton.[26] They are suitable as differentiation markers because, during embryologic development they seem to be present exclusively in neural crest and neuroectodermal cells from the earliest detection of these structures.[27-29] On the other hand, keratin has been generally regarded as a reliable indicator of epithelial cells.[14-16] Hence, the expression of neurofilaments and the lack of keratin in oat-cell carcinomas has also important bearing in the discussion of the origin of these carcinomas, strengthening the view that oat-cell carcinomas are derived from specialized neural cells of the bronchial tree.[6,30,31] This view is in agreement with our previous finding that bronchial carcinoids, which share common endocrine, biochemical, and morphological characteristics with oat-cell carcinomas, also seem to express only neural intermediate filaments.[32]

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