cells observed in this case formed in response to cellular debris or to hemorrhage induced by cell turnover in the tumor. However, none of the multinucleated giant cells contained phagocytic debris or hemosiderin pigment. This is in contradistinction to the findings of Oyasu et al.[8] of occasional phagocytic activity of the osteoclast-like giant cells. In our case, none of the multinucleated giant cells contained detectable lysozyme. Lysozyme is a proteolytic enzyme found in reactive histiocytes and reactive histiocyte-derived multinucleated giant cells. Lysozyme may not be found in non-reactive histiocytes.[9] The absence of lysozyme in the osteoclast-like giant cells suggests that the cells are not "reactive" in the classical sense.

This case is unusual in that the metastatic lesions that prompted the patient to seek medical attention closely simulated a soft tissue malignancy—malignant giant cell tumor of soft parts. Only on autopsy was the primary squamous cell carcinoma of lung detected.

ADDENDUM

Keratin immunoperoxidase staining of sections of the primary lung tumor was performed. Only the well-differentiated squamous carcinoma component stained. The sarcomatoid component remained uniformly unstained. Negative immunoperoxidase staining of a sarcomatoid neoplasm may not reliably exclude the possibility of a spindle-cell squamous carcinoma.

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USE OF ANTIBODIES TO INTERMEDIATE FILAMENTS IN THE DIAGNOSIS OF METASTATIC AMELANOTIC MALIGNANT MELANOMA

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Immunofluorescent staining of tissue from a lung tumor detected 12 years after excision of a primary malignant melanoma of the skin was negative for prekeratin and positive for vimentin, indicating that the tumor was not epithelial in origin and excluding carcinoma from the differential diagnosis. Complementary conventional staining with hematoxylin–eosin confirmed the melanocytic origin of the tumor, indicating that it was probably an amelanotic metastasis of the original malignant melanoma. The findings in this case demonstrate the potential usefulness of immunohistochemical microscopic characterization of specific intermediate filament proteins in the diagnosis of otherwise ambiguous cases of amelanotic melanoma. HUM PATHOL 14:1006–1008, 1983.

Recent studies of the molecular composition of cytoskeletal structures have established that the subunits composing the intermediate (10-nm) filaments are distinct and specific for cells of different embryonal origins. Epithelial cells contain intermediate filaments composed of several polypeptides, called prekeratins. Mesenchymal, melanocytic, and most vascular smooth muscle cells contain filaments composed of vimentin. Desmin is the subunit of intermediate filaments in parenchymal smooth muscle, myocardium, and skeletal muscle. Glial filaments and neurofilaments are present in astrocytes and nerve cells, respectively.[1,2]

In spite of the apparent morphologic similarities between the intermediate filaments of different classes, there is little or no antigenic cross reactivity among them. These tissue-specific constituent proteins may serve as differentiation markers useful in determining the origin of various cells and tissues. Moreover, it has been shown recently that tumors arising in tissues with different embryologic origins maintain the intermediate filament subunit proteins specific for the parent tissue.[3,4] It has thus been possible to use antibodies specific for each distinct intermediate filament protein to distinguish immunohistochemically between tumors of different origins. This has proved to be especially valuable in cases of anaplastic, "non-differentiated" tumors that could not be unequivocably diagnosed by conventional histopathologic means.

An example of the usefulness of these antibodies is the diagnosis of metastatic malignant melanoma, which is often difficult when tumor cells are devoid of pigment and negative for Masson-Fontana stain, rendering the histologic findings inconclusive. This general problem assumes practical importance in the differentiation of metastases of malignant melanoma from those of anaplastic carcinoma, as exemplified in the following case:

REPORT OF A CASE

A 59-year-old man presented in 1969 with a pigmented skin lesion of the back, which was excised and diagnosed as malignant melanoma. In 1976, enlarged axillary lymph nodes were excised and found to be invaded by metastatic malignant melanoma. In November 1981, routine radiographs revealed a large tumor mass in the lower lobe of the right lung. Pneumonectomy was performed.

Several samples of tumor were fixed in formalin and embedded in paraffin, and sections were stained with hematoxylin–eosin or Masson-Fontana stain. For immunofluorescence microscopic examination, unfixed tissue blocks were frozen to −20°C, immediately sectioned at 4 to 5 μm, air-dried, and fixed in acetone at −20°C for 20 minutes. The sections were immunolabeled with the immunoglobulin G fraction of rabbit antivimentin serum or with affinity-purified antiprekeratin antibodies, according to a technique previously described.[3] Specimens from three cases of histologically characteristic malignant melanoma of the skin were processed in the same way and used as positive

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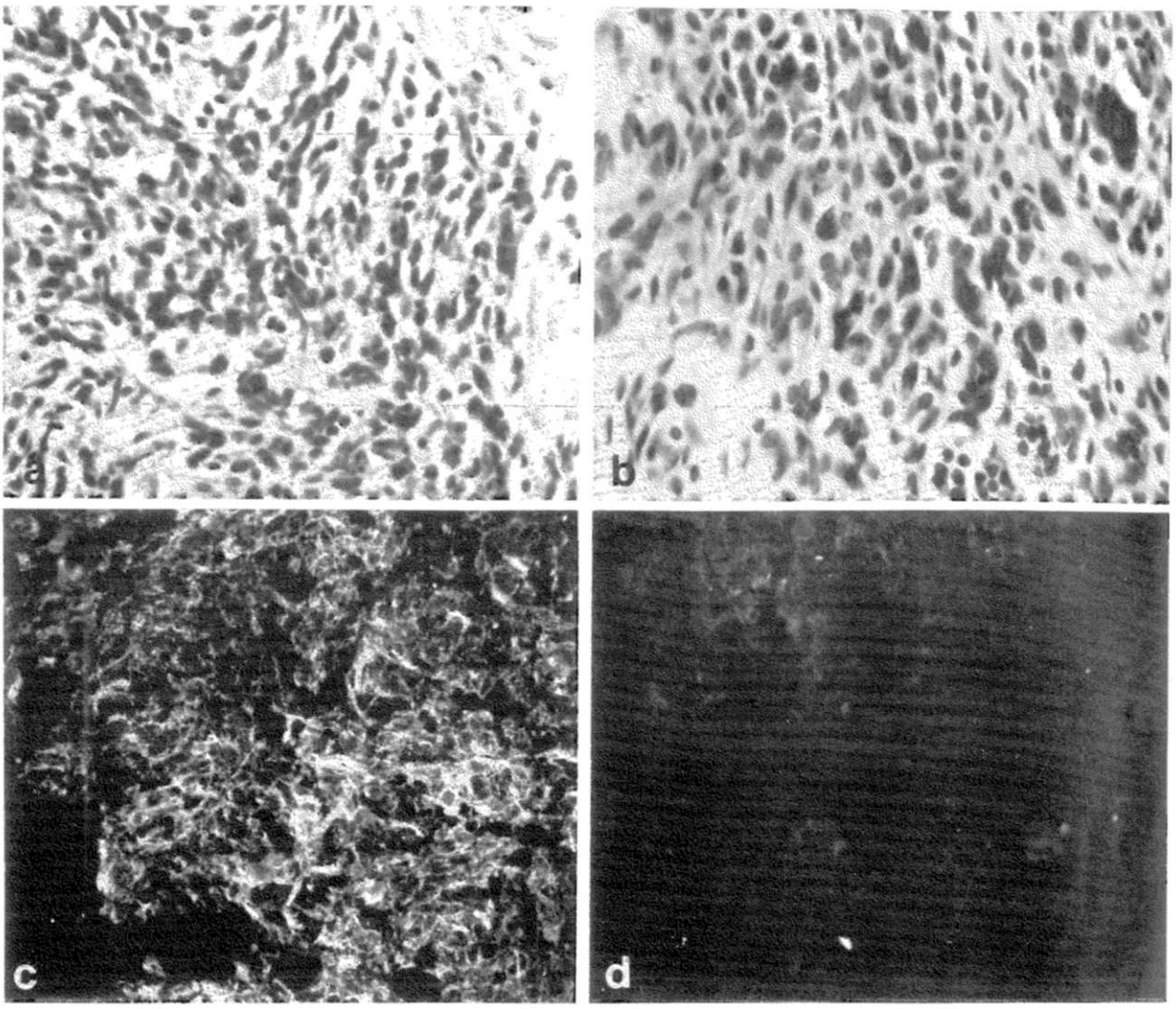

FIGURE 1. Metastatic malignant melanoma of the lung. *a* and *b*, Sections stained with hematoxylin–eosin. c, Indirect immunofluorescent labeling of frozen sections of the tumor tissue with antivimentin antibodies showing positive staining. *d*, Absence of staining on adjacent section after incubation with antiprekeratin antibodies. (*All*, ×400.)

controls. Fluorescence microscopic examination of the immunolabeled sections was performed with a Zeiss photomicroscope III.

Histologic examination revealed sheets of fusiform cells devoid of pigment and showing one to two mitotic figures per high-power field. The tumor consisted mainly of spindle-like cells. Its origin could not be clearly defined by conventional histologic examination. In one section only, two small foci that contained large cells devoid of pigment and had eosinophilic cytoplasm with one or more nuclei and prominent nucleoli were identified, which suggested malignant melanoma (fig. 1, *a* and *b*).

The tumor cells were extensively labeled with antibodies to vimentin, as shown in fig. 1*c*. Labeling with similar specificity was also obtained in the three well-defined, control cases of skin melanoma (fig. 2*a*). No specific staining with antiprekeratin antibodies or with antibodies to desmin occurred in the cells of the specimen tumor (fig. 1*d*) or of the control melanomas (fig. 2*b*). No staining with nonimmune rabbit IgG followed by rhodamine-labeled goat antirabbit IgG occurred in sections from any of the four tumors.

DISCUSSION

The lung tumor studied here was detected 12 years after the excision of a primary malignant melanoma and five symptom-free years after detection of metastases in axillary lymph nodes. Thus, a possible preoperative differential diagnosis in this case would have included, in addition to recurrent malignant melanoma, a new primary lung tumor. The histologic features of the tumor cells in most hematoxylin–eosin-stained sections were similar to those of spindle-cell carcinoma, and in only one section were there two foci of non-pigmented cells suggestive of malignant melanoma. Since Masson-Fontana staining was negative, definitive diagnosis of the tumor as either metastatic malignant melanoma or anaplastic carcinoma could not be made.

Immunofluorescent staining of the tumor tissue was negative for prekeratin, excluding a possible epithelial origin, i.e., carcinoma. The specimen tumor as well as the control malignant melanoma lesions, were positively labeled with vimentin-specific antibodies. It has been shown that the intermediate filaments of melanocytes apparently

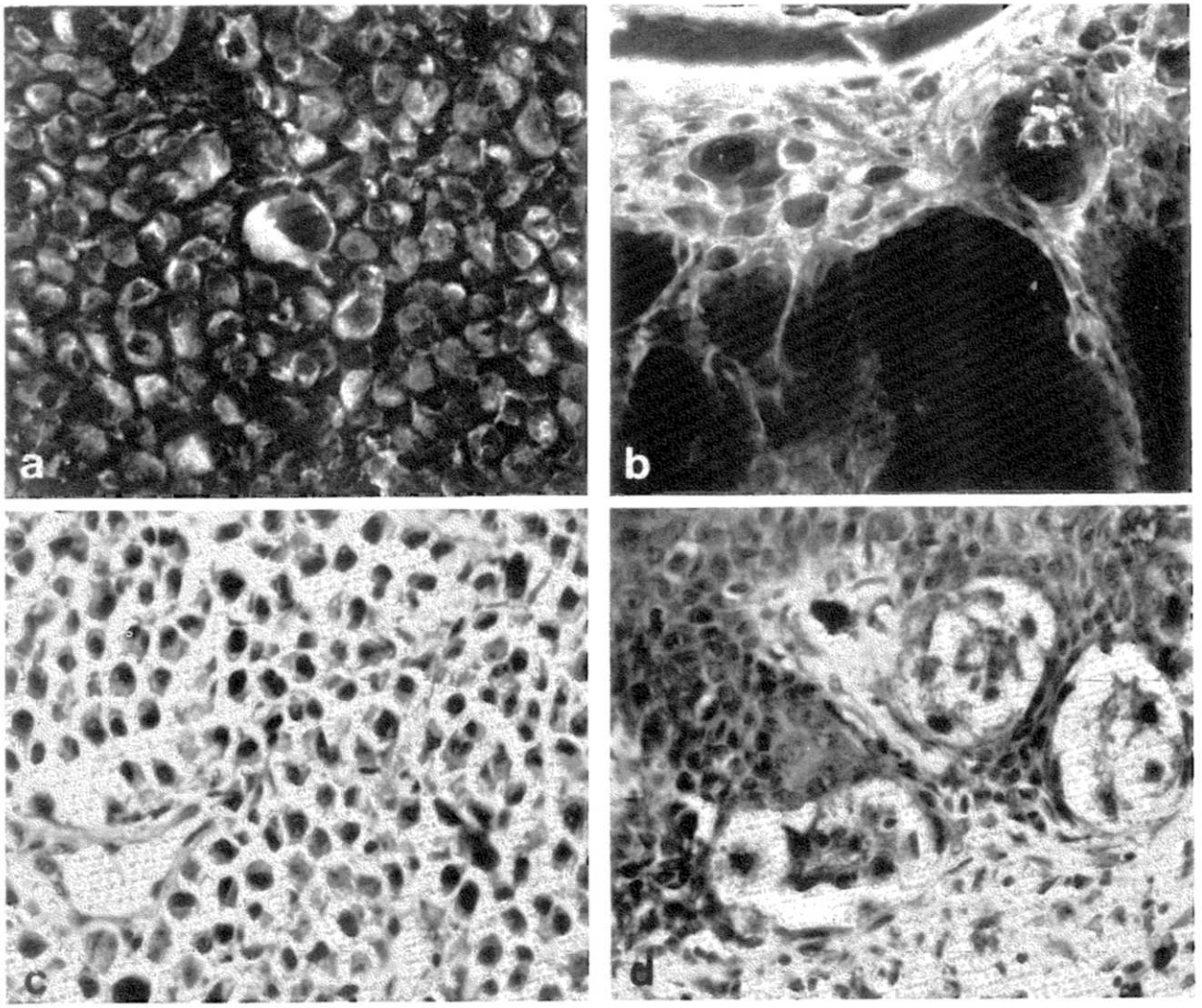

FIGURE 2. Control specimens of malignant melanomas of the skin. *a*, Positive staining of tumor cells within the deep dermis after incubation with antivimentin antibodies. *b*, In another skin melanoma, immunofluorescent labeling with antiprekeratin antibodies shows no staining in the tumor cells and strong positive labeling of the adjacent epidermis. *c* and *d*, Hematoxylin–eosin-stained sections of the same tumors shown in *a* and *b*, respectively.

contain no prekeratin[4] and are composed exclusively of vimentin[1] (M. Huszar, H. Halkin, and J. J. Bubis, unpublished observations). On the basis of the positive labeling for vimentin and the negative staining for prekeratin, we could establish only that the tumor was not epithelial in nature. This approach alone could not distinguish melanoma from several mesenchymal tumors. However, differences could be easily detected by complementary conventional hematoxylin–eosin staining. We have thus interpreted the present findings as confirmatory of the melanocytic origin of the specimen tumor. Given the patient's medical history, it appeared likely that the specimen tumor was an amelanotic metastasis of the original malignant melanoma.

Metastases in the lung are commonly found in metastatic malignant melanomas (in approximately 60 per cent of cases).[5] This phenomenon—namely, the ambiguous histologic appearance of metastasis of a malignant melanoma due to loss of pigment—is difficult to diagnose,[6,7] especially in cases in which there is no previous history of malignant melanoma. Our findings demonstrate the potential usefulness of immunohistochemical microscopic characterization of specific intermediate filament proteins in the diagnosis of ambiguous cases of amelanotic melanoma.

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