Distinctive Immunofluorescent Labeling of Epithelial and Mesenchymal Elements of Carcinosarcoma with Antibodies Specific for Different Intermediate Filaments

MONICA HUSZAR, MD,* EMIL HERCZEG, MD,† YAIR LIEBERMAN, MD,† AND BENJAMIN GEIGER PhD*

A carcinosarcoma of the lung, as well as the paratracheal lymph nodes from the same patient, were subjected to immunofluorescent labeling with antibodies to tissue-specific intermediate-filament subunits, including desmin, vimentin, and prekeratin. Within the tumor mass two distinct populations of malignant cells were found: prekeratin-positive cells, corresponding to the carcinomatous component of the tumor, and vimentin-positive cells, corresponding to the sarcomatous elements. Tumor cells were also detected in lymph node metastases in which only the prekeratin-containing carcinoma cells were found. In view of the strict specificity of antivimentin and anti-prekeratin for cells of mesenchymal or epithelial origin, respectively, it is proposed that the two components of the carcinosarcoma are derived from distinct cell types and are not morphologic variants of the same tumor. HUM PATHOL 15:532–538, 1984.

Carcinosarcoma is a rare type of mixed tumor, containing malignant cells of both epithelial and mesenchymal appearance.[1–6] Either or both of the two elements of the carcinosarcoma can metastasize to lymph nodes or other organs, maintaining either the carcinomatous or the sarcomatous form. Among the few cases described in the literature to date (only 33 cases had been reported until recently[6]), considerable morphologic variability was reported. Thus, in some cases squamous carcinoma was identified, while in others adenocarcinoma or large cell unidentified carcinoma was found. The sarcoma cells had the typical appearance of fibrosarcoma, chondrosarcoma, or osteosarcoma[6].

What are the origins of the two cellular components of the carcinosarcoma? Is the carcinosarcoma a carcinoma with metaplastic changes, or are there two distinct and independent tumors that intermingle and grow side by side?

To distinguish between these two possibilities, we used antibodies specific for subunits of different intermediate filaments, i.e., desmin, vimentin, and prekeratin. Experience of the last few years indicated that most cells contain only one type of intermediate filament. Thus, epithelial cells contain prekeratin, and mesenchymal cells contain vimentin. Desmin is the subunit of intermediate filaments of myocytes, and glial fibrillary protein and neurofilament subunits are found in glial and many neuronal cells, respectively.[7–10] An important and useful observation was that tumoral cells contain the same intermediate filament components as their nontumoral parental cells. These findings have recently been used widely in tumor diagnosis, especially in cases of anaplastic tumors in which the conventional histologic findings could not lead to conclusive diagnoses.[9–15]

In the present study we labeled a carcinosarcoma of the lung for desmin, vimentin, and prekeratin. We found that the carcinomatous elements of the tumor contained prekeratin and were not stained with antivimentin or antidesmin, and that the sarcomatous cells were positively labeled for vimentin exclusively. In view of the strict specificity of prekeratin for epithelial cells and of vimentin for mesenchymal cells, it was concluded that the two elements found in this tumor were of distinct cellular origins.

MATERIALS AND METHODS

Histologic Techniques

Sections of paraffin-embedded tissue stained with hematoxylin–eosin were prepared according to standard procedures.[16] For immunofluorescent labeling, blocks of freshly excised tumor tissue were either frozen in isopentane cooled with liquid nitrogen or fixed in absolute ethanol for three days and embedded in paraffin. For frozen sectioning, tissue blocks were trimmed, and 5-μm sections were cut at −20°C in Frigocut 2000 (Jung-Reichert, Federal Republic of Germany). The sections were air-dried for several hours and then fixed in acetone at −20°C for 20 minutes, rinsed with phosphate-buffered saline solution (PBS), and immunolabeled. From the paraffin-embedded tissue, 5-μm sections were cut with a microtome, deparaffinized in xylene and alcohol series (100 per cent, 90 per cent, and 80 per cent), and washed with PBS.

Immunochemical Reagents

Antibodies to electrophoretically purified vimentin from baby hamster kidney (BHK) cells were prepared either in guinea pigs or in rabbits and used as whole serum or as the DEAE cellulose-purified IgG fraction. The antibodies to bovine epidermal keratins used were either rabbit antibodies, affinity-purified on Sepharose-bound prekeratin,[17] or recently developed mouse monoclonal antibodies P_G

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Address correspondence and reprint requests to Dr. Geiger.

8.13.[18] Antibodies to chick desmin were prepared as described previously.[19] The specificity of these antibodies was verified by immunoblotting analysis and by intensive immunohistochemical survey of human and bovine tissue. They all exhibited exclusive reactivity with the intermediate filament subunits used as immunogens. Goat antibodies to rabbit or to mouse IgG (affinity-purified) were prepared as described and conjugated to rhodamine–lissamine sulfonyl chloride.[20,21]

Immunofluorescent Labeling

Sections on glass slides were incubated with 50 to 70 μl of the antibodies at room temperature in a humidified chamber for 45 minutes. Excess reagent was removed by three ten-minute washings with PBS, and the sections were incubated with rhodamine–labeled goat antirabbit IgG or goat antimouse IgG. The PBS-rinsed sections were mounted in Elvanol and examined in a photomicroscope equipped with filter sets for selective fluorescein and rhodamine fluorescence using oil immersion objective Plan-Neofhar 25/0.8.

REPORT OF A CASE

A 52-year-old man was admitted to the Sheba Medical Center in January 1983. Two months prior to admission he experienced coughing, hemoptysis, and weight loss, and later he had marked dyspnea. The patient's previous medical history was essentially uneventful.

Chest radiographs revealed a solid mass occupying most of the right lower lobe, obscuring the contour of the diaphragm. Results of bronchoscopic examination were negative, but cytologic analysis of the patient's sputum revealed cells suggestive of malignant tumor with an appearance of squamous cell carcinoma. A right lower lobectomy (12 × 9 × 9 cm) was performed, and paratracheal and mediastinal lymph nodes were removed for pathologic examination. The costal surface of the pleura contained a thick, irregular patch about 7 cm in diameter. A transverse

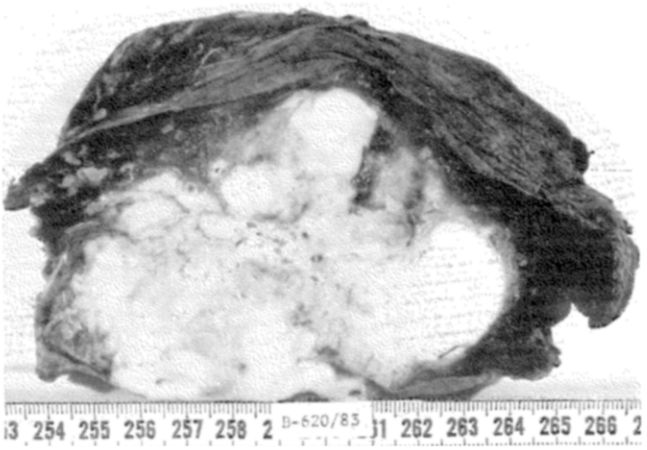

FIGURE 1. Transverse section through the right lower lobe of the lung showing a soft, yellowish tumor mass with cysts, occupying almost the entire lobe.

section through the lobe (fig. 1) revealed a yellowish mass with soft areas as well as cysts occupying almost the entire lobe. No pathologic lesions were seen in the main bronchus.

RESULTS

Histologic Findings

Hematoxylin–eosin-stained sections from different regions of the tumor revealed two distinct cellular patterns: 1) fascicles of atypical spindle cells; and 2) solid islands of polygonal, poorly differentiated squamous epithelium (defined by its overall squamoid pattern). In some areas the two cell types were segregated into clearly distinct regions (fig. 2*A*), while in others the two intermingled, with no distinct border between them (fig. 2*B*). The sarcomatous cells often formed storiform patterns (fig. 3*A*) and were pleomorphic, with numberous atypical mitoses (fig. 3*B*) and multinucleated giant cells (fig. 3*C*). The carcinomatous cells showed marked dysplasia, with few mitotic figures. Intercellular bridges were not de-

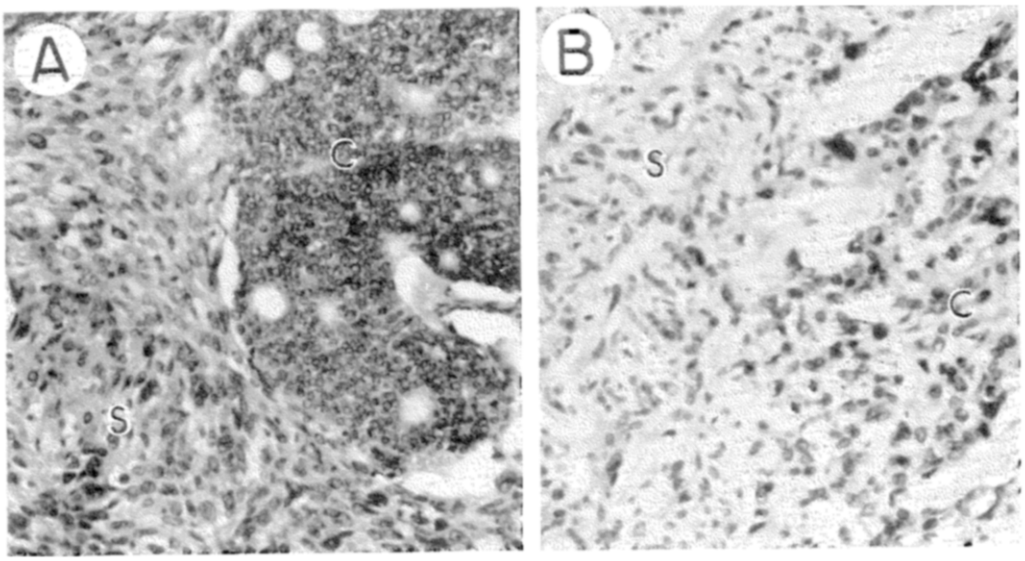

FIGURE 2. Distribution of carcinomatous (C) and sarcomatous (S) foci in the exised tumor: The two types of cells either segregate into clearly distinct areas (*A*) or intermingle, leaving no distinct border between them (*B*). (Hematoxylin–eosin stain. ×100.)

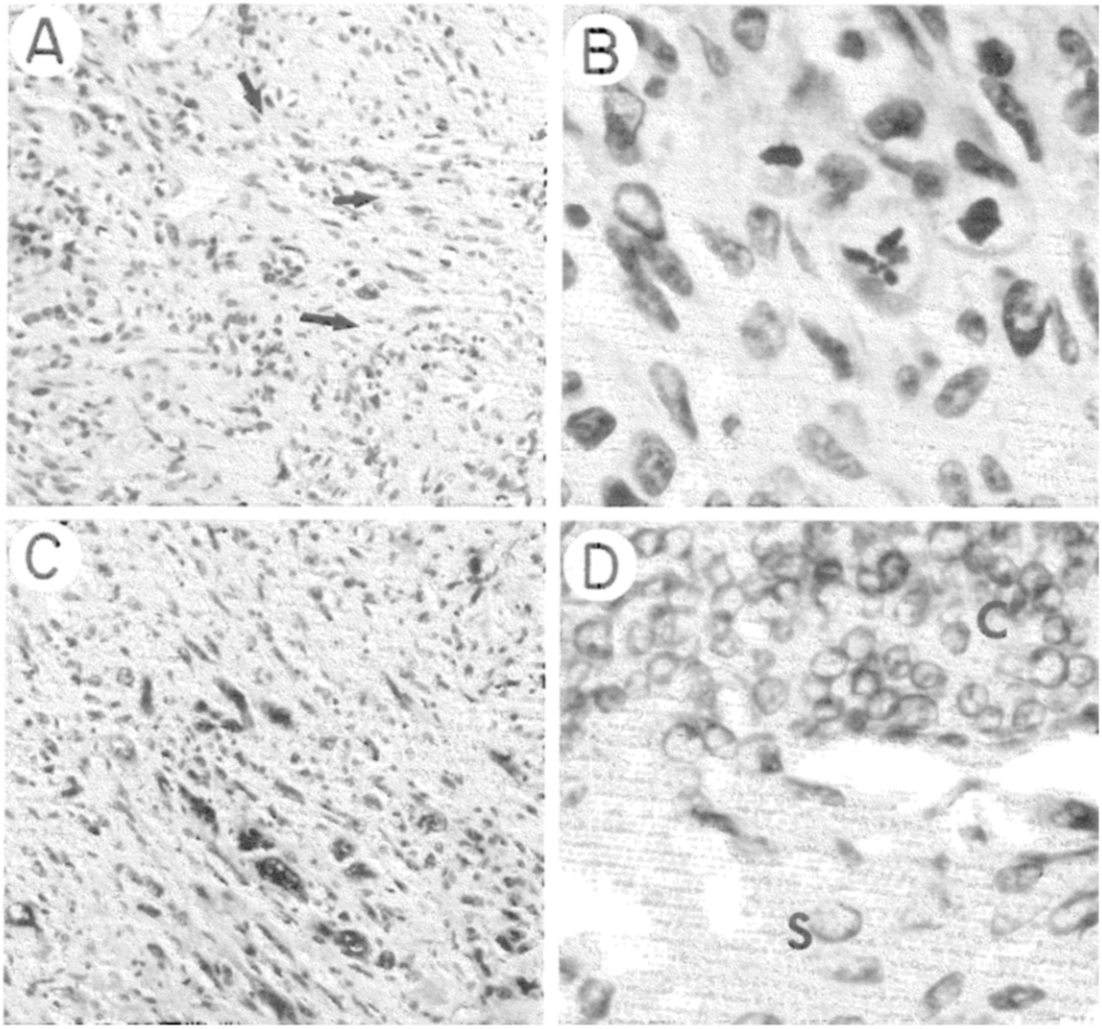

FIGURE 3. Typical cellular patterns found in a carcinosarcoma, including storiform patterns (*A, arrows*), atypical mitoses (*B*), and giant cells (*C*), all found in the sarcomatous portion of the tumor. The carcinoma cells show marked dysplasia, and intercellular bridges cannot be detected (*D*). C, carcinomatous orea; S, sarcomatous area. (Hematoxylin–eosin stain. *A* and *C*, ×100; *B* and *D*, ×380.)

tected by light microscopy, and keratinization was not noticed (fig. 3*D*). On the basis of these observations we diagnosed the tumor as a carcinosarcoma with a poorly differentiated squamous epithelial component and a malignant fibrous histiocytoma component.

Immunofluorescent Labeling of the Carcinosarcoma with Antibodies to Tissue-specific Intermediate Filaments

Sections of both frozen and alcohol-fixed blocks of the carcinosarcoma were stained with antibodies to desmin, vimentin, and prekeratin and then with rhodamine-labeled secondary antibody. Antibodies to prekeratin labeled specifically the carcinomatous cells of the tumor. These cells appeared either as solid, coherent islands of cells (fig. 4*A*) or as single or small groups of cells embedded in the sarcomatous stroma (fig. 4*B*). The positive staining for prekeratin was cytoplasmic, often with fibrillary patterns, and was demonstrated with monoclonal antibodies P_G 8.13 and with regular rabbit antibodies. Neither surrounding sarcoma cells nor elements of the connective tissue showed significant labeling (areas labeled "S" in figs. 4*A* and *B*). Vimentin-positive cells were found in the sarcomatous portion of the tumor exclusively. These were typically spindle-shaped cells as seen in longitudinal (fig. 4*C*) and transverse (fig. 4*D*) sections. The labeling was intense and strictly cytoplasmic. The intercellular matrix between the cells was not labeled with either antivimentin or anti-prekeratin. The carcinomatous islands of the tumor (labeled "C" in fig. 4*E*) were not labeled for vimentin. Immunofluorescent labeling of the same region with antibodies to desmin (fig. 4*F*) or with normal rabbit immunoglobulin (not shown) did not result in specific staining.

Lymph Node Metastases of Lung Carcinosarcoma

At the time of surgery, one paratracheal and one mediastinal lymph node were removed and fixed with either formaldehyde or ethanol. Histologic ex-

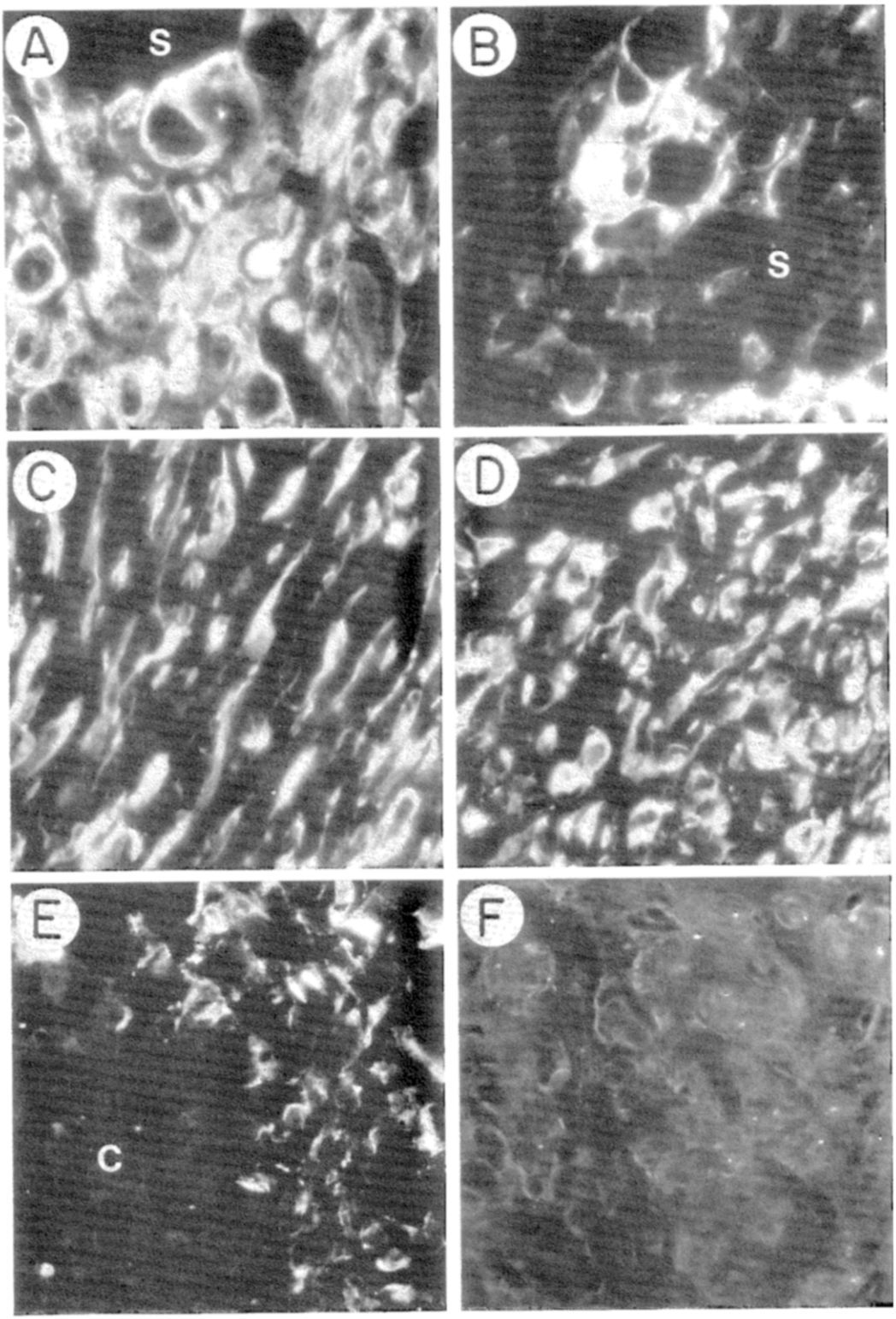

FIGURE 4. Indirect immunofluorescent labeling of the carcinosarcoma for intermediate filament subunits; prekeratin (*A* and *B*), vimentin (*C, D,* and *E*) and desmin (*F*). The carcinomatous cells, including single cells or small islands, are strongly labeled for prekeratin, while the sarcomatous cells (S) are essentially not labeled. The sarcomatous cells shown in longitudinal (*C*) and transverse (*D*) sections are positively labeled for vimentin, while the intercellular connective tissue or neighboring carcinoma islands (C) are negative. No specific labeling is obtained with desmin antibodies (*F*, time of exposure double that used in figs. 4*A* to *E*). (×380.)

amination of hematoxylin–eosin-stained sections revealed neoplastic cells in the paratracheal lymph node only. The metastatic lesion morphologically resembled poorly differentiated squamous cell carcinoma and was surrounded by necrotic areas (figs. 5*A* and *B*). The overall morphology of the cells was similar to that of the carcinomatous component of the primary tumor. Extensive screening of the lymph nodes did not reveal metastatic lesions of the sarcoma cells.

Immunofluorescent labeling of sections from the same lymph node showed distinct masses of prekeratin-positive cells surrounded by residual lymphatic tissue and necrotic areas (fig. 5*C*). These epithelial cells were widely distributed, occupying most of the

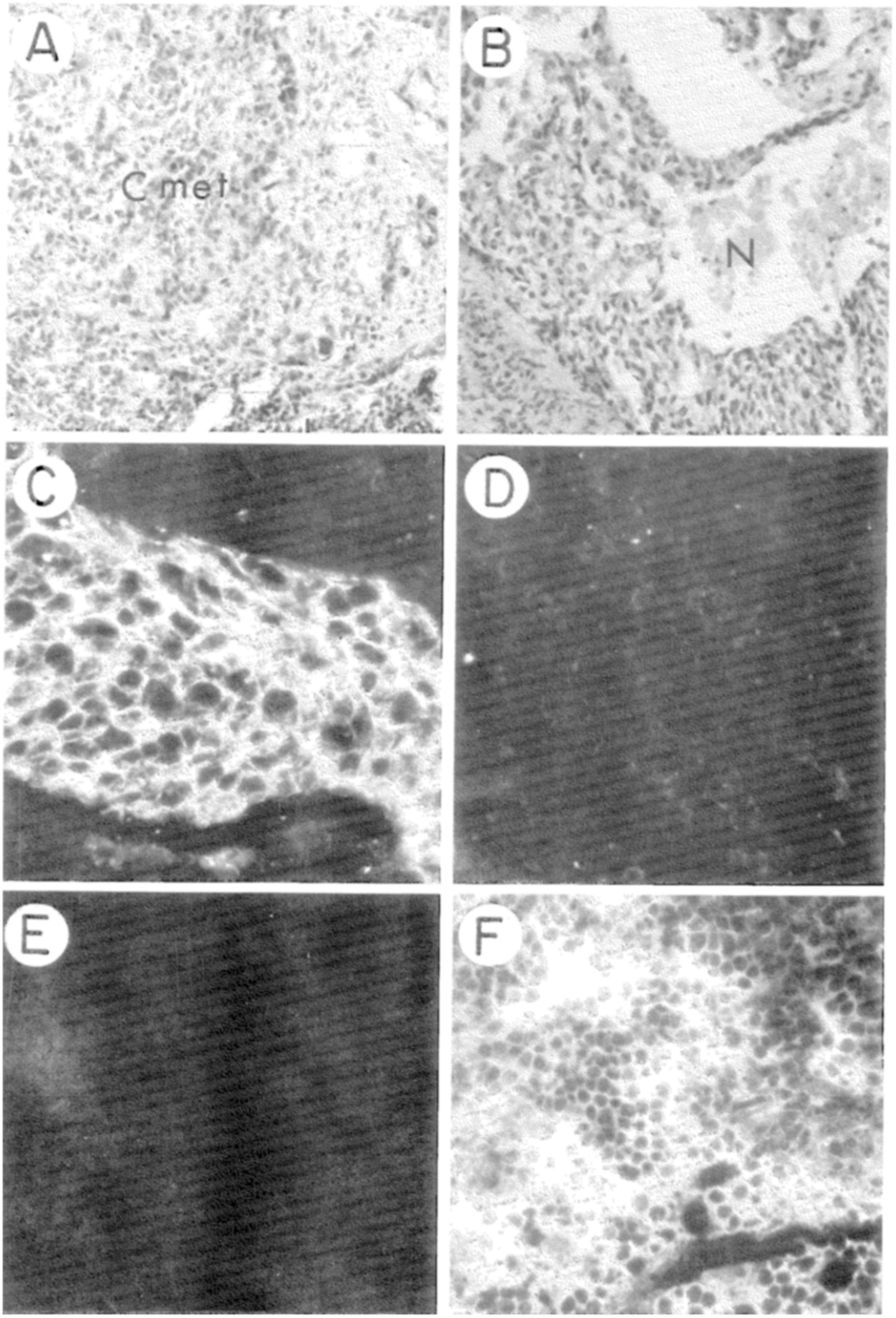

FIGURE 5. *A* and *B*, lymph node metastasis (paratracheal lymph node) showing carcinomatous cells (Cmet) with only residual lymphatic tissue (L) or necrotic areas (N). *C* to *F*, indirect immunofluorescence of the same lymph node (*C* and *D*) or of a normal lymph node, without metastatic deposits (*E* and *F*) for intermediate filament components. The sections were labeled for prekeratin (*C* and *E*) or vimentin (*D* and *F*). Notice the specific labeling of the carcinoma cells for prekeratin and the absence of vimentin from those areas. Normal lymph nodes on the other hand, contain no prekeratin-positive elements (*E*), and the normal lymphatic tissue is clearly vimentin–positive (*F*). (×380.)

lymph node (residual lymphatic tissue, shown in fig. 4*A*). The metastatic cells within the lymph node were not labeled with vimentin-specific antibodies (fig. 5*D*). It should be emphasized that lymph nodes that do not contain metastatic deposits show no labeling with prekeratin-specific antibodies (fig. 5*E*), while the lymphatic tissue is extensively labeled with antivimentin (fig. 5*F*).

DISCUSSION

The development of a malignant tumor is usually believed to result from the proliferation of a single

clone of neoplastic cells. It is, however, a common observation that cells derived from the same tumor often display considerable morphologic heterogeneity.[22] An extreme case in which morphologically distinct tumor cells develop within the same tumor is the relatively rare tumor defined as carcinosarcoma. As mentioned above, it consists of two morphologically distinct cellular elements: carcinoma cells and sarcoma cells. The interrelations between these two cell types are not clear. Most of the information available to date concerning this type of tumor is based on standard histologic examinations of relatively few specimens (about 33 until recently) performed during the last two decades.[6]

Several alternative explanations have been offered for the coexistence of the two types of cells within the same tumor. One possibility is that sarcoma-like cells are derived from the carcinoma due to metaplastic changes. If such an explanation were correct it would imply that the "sarcomatous" elements of the tumor could, in fact, be considered as carcinoma with spindle cell metaplasia. Alternatively, the two cellular elements of the carcinosarcoma may have distinct histologic origins (epithelial and mesenchymal). In this case it may be assumed that the two tumors develop in parallel or sequentially and proliferate at the same site.

To distinguish between these possibilities we used immunofluorescent labeling of the tumor with antibodies to tissue-specific intermediate filaments. It has been clearly shown that all epithelial cells contain intermediate filaments of the prekeratin class exclusively. This includes carcinoma cells, as well as several of their anaplastic derivatives.[9–15] Moreover, it has been shown for several types of carcinomas that the composition of the prekeratin polypeptides of the tissue of origin is largely retained in the neoplastic cells.[23] Mesenchymal tumors, including various types of sarcomas, lymphomas, and melanomas, contain vimentin exclusively.[8–10,24] This finding enabled us, and many other laboratories, to use these antibodies for tumor diagnosis in cases in which the conventional histologic findings were not conclusive. Using such an approach, we were recently able to distinguish between spindle cell variants of squamous cell carcinoma (prekeratin-positive) and sarcoma cells (vimentin-positive; Huszar et al., unpublished data) and between malignant metastatic melanoma and anaplastic carcinoma.[15]

The results of the present study, i.e., the exclusive presence of prekeratin in the carcinomatous elements of the carcinosarcoma and the presence of vimentin in the sarcomatous cells, strongly suggest that the two cell types of this tumor have distinct cellular origins and are thus not derived from each other. We cannot exclude the possibilty that the expression of prekeratin was lost and the expression of vimentin switched on during a putative metaplastic process. However, in view of the accumulating data concerning intermediate filament diversity, this possibility seems unlikely.

In addition to its diagnostic value, the method used in this study, immunofluorescent labeling for prekeratin and other intermediate filament constituents, allowed the detection of individual carcinoma cells or small foci that were embedded in the surrounding sarcomatous matrix or connective tissue (fig. 4*B*). By standard histologic staining procedures such distributions of tumor cells would scarcely be detected. This property may be of great general advantage for the visualization of sparsely spread carcinomatous tumor cells within surrounding connective tissue, lymph nodes, etc.

It should also be pointed out that the two elements of the tumor investigated in the present study showed distinct and independent metastatic activities; only the carcinoma portion could be detected in the lymph node examined. Previous reports of the metastatic spread of carcinosarcomas indicated that both cellular elements could be metastatic, though their interrelations in the metastatic lesions have not been described in detail.

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REFERENCES

1. Bergmann M, Ackerman LV, and Kemler RL: Carinosarcoma of the lung. Review of the literature and report of two cases treated by pneumectomy. Cancer 4:919, 1951
2. Prive L, Tellem M, Meranze DR, et al: Carcinosarcoma of the lung. Arch Pathol 72:351,1961
3. Jenkins BJ: Carcinosarcoma of the lung. Report of a case and review of literature. J Thorac Cardiovasc Surg 55:657, 1968
4. Stackhouse EM, Harrison, EG Jr, Ellis FH Jr: Primary mixed malignancies of lung: carcinosarcoma and blastoma. J Thorac Cardiovasc Surg 57:385, 169
5. Razzuk MA, Urschel MC, Race GJ, et al: Carcinosarcoma of the lung. Report of two cases and review of the literature. J Thorac Cardiovasc Surg 61:541, 1971
6. Carter D, Eggleston JC: Tumors of the lower respiratory tract, fascicle 17, second series. Atlas, of Tumor Pathology. Armed Forces Institute of Pathology, Washington DC, 1980, p 208
7. Lazarides E: Intermediate filaments as mechanical integrators of cellular space. Nature 283:249, 1980
8. Franke WW, Schmid E, Shiller DL, et al: Differentiation-related patterns of expression of proteins of intermediate-sized filaments in tissues and cultured cells. Cold Spring Harbor Symp Quant Biol 46:431, 1981
9. Osborn M, Weber K: Intermediate filaments: cell type specific markers in differentiation and pathology. Cell 31:303, 1982
10. Damjanov I: Antibodies to intermediate filaments and histogenesis. Lab Invest 47:215, 1982
11. Gabbiani G, Kapanci Y, Barrazone P, et al: Immunochemical identification of intermediate-sized filaments in human neoplastic cells: a diagnostic aid for the surgical pathologist. Am J Pathol 104:206, 1981
12. Battifora H, Sun T-T, Bahu RM, et al: The use of antikeratin antiserum as a diagnostic tool: thymoma versus lymphoma. HUM PATHOL 11:635, 1980
13. Bannasch P, Zerban H, Schmid E, et al: Liver tumors distinguished by immunofluorescence microscopy with antibodies to proteins of intermediate-sized filaments. Proc Natl Acad Sci USA 77:4948, 1980
14. Schlegel R, Banks-Schlegel S, McLeod JA, et al: Immunoperoxidase localization of keratin in human neoplasms. Am J Pathol 110:41, 1980

15. Huszar M, Halkin H, Herczeg E, et al: Use of antibodies to intermediate filaments in the diagnosis of metastatic amelanotic malignant melanoma. HUM PATHOL 14:1006, 1983
16. Lillic RD: Histopathologic Technics and Practical Histochemistry. New York, Blakiston 1976, p 45
17. Horwitz B, Kupfer H, Eshhar Z, et al: Reorganization of arrays of prekeratin filaments during mitosis immunofluorescence microscopy with multiclonal and monoclonal prekeratin antibodies. Exp Cell Res 134:281, 1981
18. Gigi O, Geiger B, Eshhar Z, et al: Detection of a cytokeratin determinant common to diverse epithelial cells by a broadly cross-reacting monoclonal antibody. The EMBO J 11:1429, 1982
19. Geiger B, Singer SJ: Association of microtubules and intermediate filaments in chicken gizzard cells as detected by double immunofluorescence. Proc Nat Acad Sci USA 77:4769, 1980
20. Geiger B, Singer SJ: The participation of -actinin in the capping of cell membrane components. Cell 16:213, 1979
21. Brandtzaeg P: Conjugates of immunoglobulin G with different fluorochromes. I. Characterization by anionic-exchange chromatography. Scand J Immunol 2:273, 1973
22. Ashley DJB: Evans' Histological Appearances of Tumours 3rd edition. New York, Churchill Livingston, 1978, p 638
23. Moll R, Franke WW, Schiller DL, et al: The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11, 1982
24. Miettinen M, Lehto V-P, Badley, et al: Expression of intermediate filaments in soft-tissue sarcomas. Int J Cancer 30: 541, 1982