American Journal of Pathology, Vol. 133, No. 2, November 1988

Glial Fibrillary Acidic Protein Immunoreactivity in Human Respiratory Tract Cartilages and Pulmonary Chondromatous Hamartomas

GIUSEPPE VIALE, MD, CLAUDIO DOGLIONI, MD, PATRIZIA DELL'ORTO, BScD, GIANFRANCO ZANETTI, MD, PAOLO IUZZOLINO, MD, LAMBERTO BONTEMPINI, MD, and GUIDO COGGI, MD

From the Second Department of Pathology, University of Milan, School of Medicine, Milano, and the Departments of Surgical Pathology, Ospedale Borgo Trento, Verona, and Ospedale S. Maria del Prato, Feltre, Italy

Immunocytochemical investigation was performed on a large series of adult, neonatal, and fetal respiratory tract cartilages to ascertain their immunoreactivity for glial fibrillary acidic protein (GFAP) and vimentin. Two polyclonal and six different monoclonal antibodies were used to document the presence of GFAP-immunoreactive chondrocytes in all the fetal and neonatal cartilages as well as in the adult epiglottis, arythenoids, and lobar, segmental, and subsegmental bronchi. The number of chondrocytes showing GFAP immunoreactivity decreased from fetal life to adulthood. Simultaneous immunoreactivity for GFAP and vimentin has also been ascertained in chondrocytes and in perichondrial stellate or elongated cells of the 25 chondromatous hamartomas investigated. These findings document yet another "inappropriate" pattern of intermediate filament immunoreactivity in normal and neoplastic human cells, and contradict the widely held supposition that the expression of GFAP is restricted to cells of glial origin. (Am J Pathol 1988, 133:363–373)

GLIAL FIBRILLARY acidic protein (GFAP) is the major protein constituent of the glial filaments expressed in various cells of the neuroglia,[1–10] in interstitial cells of the pineal gland,[11–14] and in stellate cells of the pituitary gland.[15–17] In some of these cells, GFAP is coexpressed with vimentin, and previous immunoelectron microscopic observations have documented that these two proteins are assembled in the same filament.[18,19]

Because the expression of GFAP is maintained by these cells even after neoplastic transformation, its immunocytochemical localization is a useful tool for the histopathologic diagnosis of central nervous system (CNS) tumors.[8,20–26] Outside the CNS, GFAP has been localized in various cells of the peripheral nervous system,[27–36] and even in cells not associated with the nervous tissue.[37–40]

A peculiar aspect of the GFAP expression in these extra-CNS sites is represented by the fact that it is strikingly species-dependent[32,36,37,40] and significantly affected by the use of polyclonal or monoclonal antibodies (MAb) of different sources. Indeed, the stellate perisinusoidal cells of the liver[39] and the peripheral nervous system-associated cells of rats[31] are immunostained by rabbit polyclonal and some monoclonal antisera to GFAP, but not by other MAbs to the same protein.[36] This has suggested an apparent lack of complete antigenic identity between the CNS and the peripheral GFA polypeptides.[31]

In humans, GFAP expression outside the CNS has been consistently documented with both polyclonal and monoclonal antibodies only in individual nerve sheath cells,[35,36,40] in a subpopulation of epithelial

Supported in part by Ministero della Pubblica Istruzione, Roma, Italy.

Accepted for publication July 6, 1988.

Address reprint requests to Giuseppe Viale, MD, 2nd Department of Pathology, University of Milan, Via Di Rudini', 8. 20142 Milano, Italy.

Table 1—Source and Working Dilution of Specific Antisera

Reagent	Dilution	Source
Anti-GFAP (polyclonal)	1:2000	Dakopatts AS
Anti-GFAP (polyclonal)	1:5	Ortho
Anti-GFAP (1B4-2E1-4A11 MAb)	1:3	Biomedical Technologies[52]
Anti-GFAP (GA5 MAb)	1:5	Boehringer[53]
Anti-GFAP (12-24 MAb)	1:1000	Prof. W.W. Franke* [36]
Anti-GFAP (6F2 MAb)	1:10	Sanbio
Anti-GFAP (G1 MAb)	1:1000	Amersham[54]
Anti-GFAP (MAb)	1:200	Lab System[55]
Anti-vimentin (V9 MAb)	1:5	Boehringer[56]
Anti-cytokeratins (lu-5 MAb)	1:100	Dr. C. Stähli† [57,58]

* MAb commercially available from Progen.
† Central Research Division, Hoffmann-La Roche.

cells (most likely myoepithelial in nature) of the parotid gland,[36,42] and in some neoplastic cells of pleomorphic adenomas,[36,41–43] nerve sheath tumors,[44–47] and malignant mixed mullerian tumors.[48]

In 1984, Kepes and colleagues,[38] in the course of a study on the role of astrocytes in the formation of cartilage in gliomas, incidentally reported immunoreactivity for GFAP in some chondrocytes of the human epiglottis using a rabbit polyclonal antiserum. Because normal human cartilages from articular surfaces, the ribs, the tracheal rings, and the bronchial wall did not show any GFAP immunoreactivity, the authors suggested that the expression of GFAP in the epiglottic cartilage could be related to its possible origin from the neural crest. In a subsequent immunocytochemical investigation, Budka[40] confirmed expression of GFAP in two human epiglottis, using both monoclonal and polyclonal antibodies to this IF protein. This study, however, did not include other respiratory tract cartilages.

Prompted by these incidental observations, the authors performed an extensive investigation on normal cartilages of the human respiratory tract to ascertain the actual capability of their chondrocytes to express GFAP, alone or in association with vimentin, which is the intermediate filament (IF) protein peculiar of human chondrocytes.[49]

Because of the reported discrepancies on GFAP immunolocalization with the use of different polyclonal and monoclonal antibodies, two anti-GFAP rabbit polyclonal antisera and a panel of six well-characterized mouse MAbs to this protein were used. Moreover, consecutive serial sections were immunostained for the localization of different IF proteins to document any possible coexpression of GFAP with vimentin or cytokeratins.

Because immunoreactivity for GFAP was demonstrated in the chondrocytes of different cartilages of the human respiratory tract in adults, the investigation was extended to neonatal and fetal respiratory tract cartilages as well as to 25 pulmonary chondromatous hamartomas to verify if GFAP-immunoreactivity appears early during the fetal development of these cartilages and if it is also maintained in neoplastic chondrocytes.

Materials and Methods

Tissues

A total of 243 specimens of the different cartilages of the human respiratory tract, from the epiglottis to the subsegmental bronchi, were included in the current study, together with 25 pulmonary chondromatous hamartomas.

Healthy adult cartilages were collected from surgical specimens—ten laringectomies (eight men and two women) and ten pneumonectomies (five men and five women) performed for invasive carcinomas—and from ten consecutive autopsies (five men and five women) performed within 12 hours after death. Neonatal cartilages were obtained at autopsy from two full-term stillborn male fetuses and three immature (small for date) newborn infants (two boys and one girl) who died of pulmonary distress.

Fetal cartilages were collected from five fetuses (12-, 14-, 16-, 19-, and 22-week-old, respectively) after spontaneous or therapeutic aborption.

The 25 pulmonary chondromatous hamartomas were retrieved from the files of the three institutions participating in this study, and had all been surgically removed.

All the samples of healthy adult and neonatal cartilages, as well as of the cartilages of the 19- and 22-week-old fetuses, were subdivided in three parts, one of which was snap-frozen in liquid nitrogen, while the remaining two were fixed in 10% formalin and in methacarn, respectively, and embedded in paraffin. For the cartilages of the 12-, 14-, and 16-week-old fetuses and the pulmonary hamartomas, only specimens fixed in 10% formalin and embedded in paraffin were available.

When needed, fixed samples were decalcified with a solution containing 8% formic acid and 5% HCl in distilled water for 16 hours.

Immunocytochemical Staining

Five-micron serial sections were cut from the blocks of paraffin-embedded material, collected on albumin-coated slides, left to dry overnight at 37 C, and then immunostained for the localization of GFAP, vimentin, and cytokeratins, using the specific polyclonal and monoclonal antibodies listed in Table 1.

The immunocytochemical reactions were performed in a humidity chamber at room temperature (unless otherwise specified) according to the avidin-biotin-peroxidase complex (ABC) method[50] as detailed previously.[51]

Normal goat and horse sera, biotinylated antibodies, and the ABC components in kit form were purchased from Vector (Burlingame, CA). The source and the working dilution of the specific primary antisera are given in Table 1.

For the localization of cytokeratins, the tissue sections of formalin-fixed samples were treated with 0.4% pepsin (BDH) in 0.01 N HCl for 7 minutes at 37 C before the immunocytochemical reaction. Preliminary experiments demonstrated that proteolytic digestion with pepsin, pronase, or trypsin failed to significantly enhance, or even decreased (with the GA5 and 6F2 MAbs) GFAP immunoreactivity in formalin-fixed material, and it was therefore omitted in subsequent immunoreactions.

Serial sections (5–7 μ thick) of the frozen samples were cut on a cryostate, mounted on albumin-coated slides, air-dried at room temperature for 12 hours, fixed in chloroform:acetone (1:1) for 5 minutes at 4 C, and stored at −70 C. Immediately before staining, the tissue sections were thawed, refixed in chloroform: acetone for 5 minutes, washed in PBS for 20 minutes, and then immunostained in the same manner as for paraffin-embedded material, avoiding endogenous peroxidase inhibition.

Selected cases of frozen samples containing peripheral bronchi from two fetuses, two neonates, and two adults also were immunostained for GFAP with the panel of monoclonal and polyclonal antibodies according to an indirect immunofluorescence staining technique. After incubation with the specific anti-GFAP antibodies, the tissue sections were incubated with a 1:20 dilution of FITC-conjugated swine anti-rabbit immunoglobulin or rabbit anti-mouse immunoglobulin sera (both from Dakopatts, Denmark) for 30 minutes at room temperature. After washing, the slides were mounted with a water-based medium.

Control sections for specificity included staining of 10 samples of human cerebellum obtained at autopsy and frozen or fixed in a similar manner as for the respiratory tract samples as known positive controls for GFAP, whereas the endothelial lining of blood vessels and the respiratory epithelium, respectively, provided built-in positive controls for vimentin and cytokeratins. All these controls displayed strong and specific immunostaining for the corresponding antigens.

Negative controls were obtained with the replacement of the specific antisera with the immunoglobulin fraction of nonimmune rabbit or mouse sera, and constantly were unstained.

Results

The results of the present immunocytochemical investigation were not affected by the sex of the patients from which the samples were obtained, or by the pretreatment of the tissue samples with the decalcifying solution. The immunolocalization of the different IF proteins was partially affected by the staining of frozen or fixed tissue samples and, with regard to the localization of GFAP, by the use of different monoclonal or polyclonal antibodies. Indeed, the comparative evaluation of the results obtained on frozen and on fixed sections of the same tissue samples allowed the authors to ascertain that, irrespective of the antisera employed, the number of GFAP-immunoreactive cells in methacarn- and formalin-fixed tissues was reduced to 75 and 60%, respectively, of the cells that were immunostained by the corresponding anti-GFAP antibodies in the frozen sections of the same cartilages or of the same control samples of human cerebellum. Similar findings were also observed in the immunolocalization of cytokeratins and vimentin.

As far as the use of different antibodies to GFAP is concerned, a higher number of cells was immunostained by the two polyclonal antisera to GFAP than by the six MAbs, both in frozen and in fixed samples from the respiratory tract and the control cerebellum. In particular, the 12–24 and 6F2 MAbs stained from 25–50% (in formalin-fixed tissues) to 50–75% (in frozen or methacarn-fixed samples) of the cells immunoreactive to the polyclonal antisera, while the remaining MAbs immunostained from 75–100% of these cells in frozen and formalin- or methacarn-fixed sections.

Normal Cartilages

In all the samples examined, the chondrocytes of the epiglottic cartilages were immunoreactive for both GFAP and vimentin. The number of GFAP-immunoreactive chondrocytes, as evaluated on sections immunostained with polyclonal antisera, was different in fetal, neonatal, and adult cartilages, however. (Table 2) Indeed, in the epiglottis of the 19- and 22-week-old fetuses all the cells of the chondroblastic mesenchyme were strongly immunolabeled, as were the cells of the neonatal cartilage. In the adults, GFAP immunoreactivity was a consistent feature of the peripheral chondrocytes and of several perichondrial spindle or stellate cells, whereas it was detectable only in some (from 10–30%) of the chondrocytes in the central ar-

Table 2—Immunoreactivity for GFAP in Normal Respiratory Tract Cartilages

	Adult	Neonatal	Fetal
Epiglottis	+(N = 20)	+(N = 2)	+(N = 2)
Arythenoid	+(N = 20)	+(N = 5)	ND
Cricoid	−(N = 20)	(+)(N = 5)	(+)(N = 2)
Thyroid	−(N = 20)	(+)(N = 5)	(+)(N = 2)
Tracheal rings	−(N = 10)	(+)(N = 5)	(+)(N = 5)*
Main bronchi	−(N = 20)	(+)(N = 5)	(+)(N = 5)*
Lobar, segmental, and subsegmental bronchi	+(N = 60)	+(N = 15)	+(N = 12)†

−, no immunostained chondrocytes; (+), immunostained cells only in frozen tissue sections; +, immunostained cells both in frozen and in fixed and paraffin-embedded sections; ND, not determined.

* Frozen samples were available only for the 19- and 22-week-old fetuses.

† Lobar, segmental, and subsegmental bronchi were not identified in the 12-week-old fetus.

eas of the cartilage (Figure 1). This peculiar distribution of GFAP-positive cells also was observed in the frozen sections of the same cartilages.

The elastic moiety of adult and neonatal arythenoids showed a pattern of GFAP immunostaining similar to that of the corresponding epiglottic cartilages, whereas in the hyaline portions only occasional peripheral chondrocytes were immunolabeled. Arythenoids were not identified in the fetuses examined.

The chondrocytes of the thyroid and cricoid cartilages, the tracheal rings, and the main bronchi were consistently devoid of GFAP immunoreactivity in all the fixed and paraffin-embedded specimens studied (Figure 2). When frozen tissues were immunostained, however, some (less than 10%) of the peripheral chondrocytes were shown to contain GFAP in the fetal and neonatal, but no adult, cartilages.

In lobar and segmental bronchi, a variable number of GFAP-immunoreactive chondrocytes was identified in all the adult, neonatal, and fetal cartilages. In both paraffin-embedded and frozen samples, however, only a minority of the cells (never exceeding 10% of the chondrocytes in the adult and 30% in the neonatal and fetal samples) were immunolabeled. The positive cells were confined mostly to the periphery of the cartilages.

In the adult subsegmental bronchi, the number of GFAP-immunoreactive chondrocytes apparently increased with the reduction of the bronchial lumen, varying from 20% of the chondrocytes to almost 50% in the smaller bronchi (Figure 3). Labeled chondrocytes were still localized preferentially in the peripheral portions of the cartilages. In neonatal and fetal samples, however, all the chondrocytes of the peripheral bronchi showed strong immunostaining for GFAP (Figures 4 and 5a). Identical results of GFAP immunolocalization in peripheral bronchi were obtained in immunofluorescence experiments (Figure 4, inset).

GFAP was not localized in cells other than chondrocytes and perichondrial cells, except for nerves that were immunolabeled by the two polyclonal antisera to GFAP and by all but 6F2 MAb. The polyclonal antisera immunostained both nerve sheath cells and axons, whereas the MAbs decorated only occasional nerve sheath cells, in agreement with previous observations.[36]

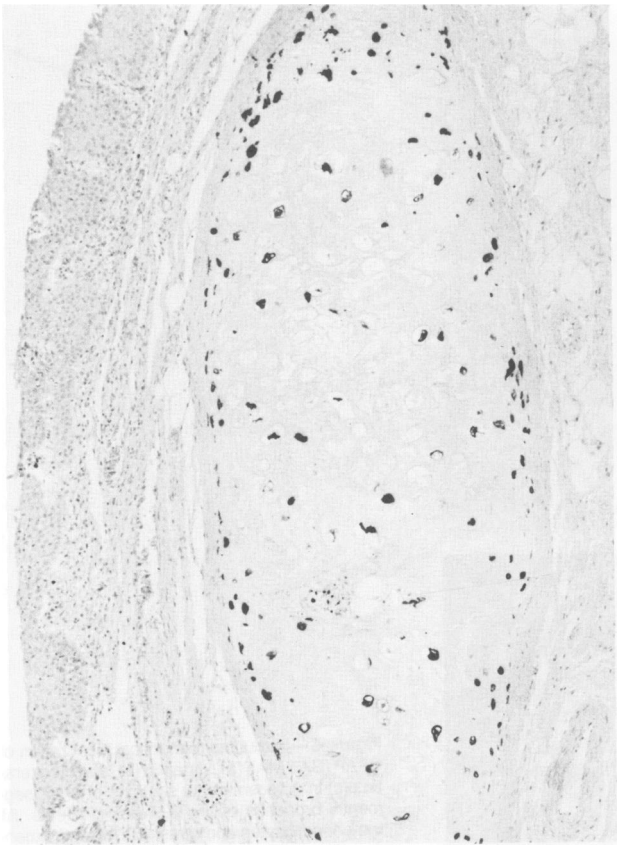
Vimentin was expressed by all the chondrocytes of the different cartilages. The comparative evaluation of serial tissue sections immunostained for GFAP and vimentin allowed the authors to ascertain that all the GFAP-immunoreactive chondrocytes also expressed vimentin (Figures 5a and b). No cytokeratin-immunoreactive chondrocytes were identified in the samples studied (Figure 5c). Indeed, this IF protein was expressed exclusively by the pneumocytes and the bronchial epithelium. Moreover, in all the fetal and neonatal samples of the latter epithelium, vimentin was consistently expressed in addition to cytokeratins (Figures 5b and c).

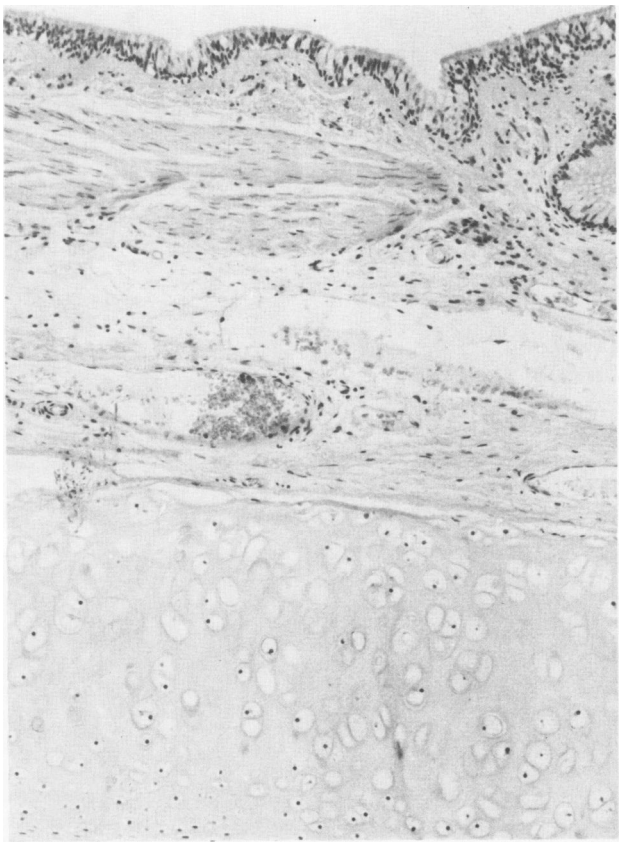
Pulmonary Chondromatous Hamartomas

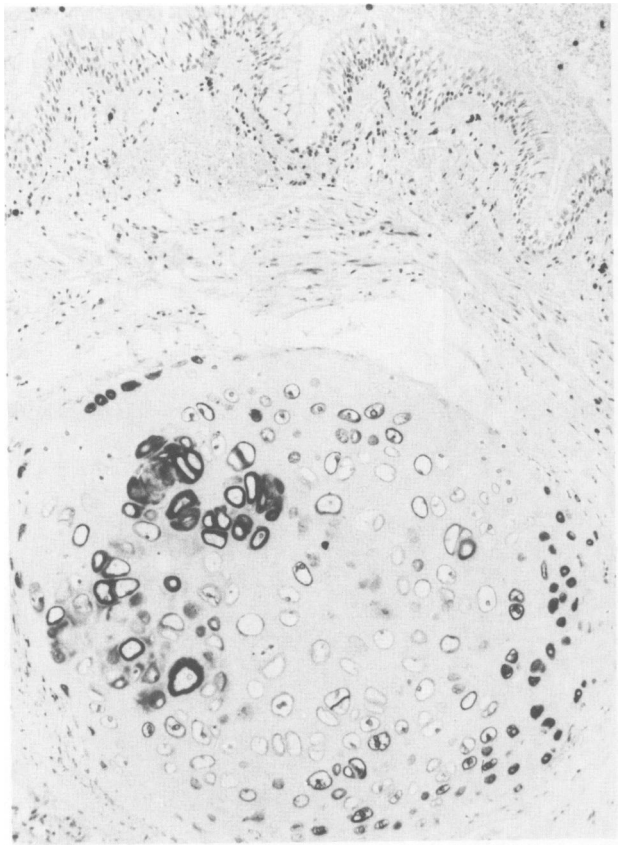
In the 25 pulmonary hamartomas, a variable percentage (from 25–50%) of cells in the chondroid areas showed GFAP immunoreactivity. The immunolabeled cells were confined mostly to the periphery of the chondroid foci and showed features of stellate or elongated spindle cells (Figure 6a). The number of mature chondrocytes that showed GFAP expression decreased from the periphery to the central portions of the cartilagineous islands, where only occasional cells were immunolabeled.

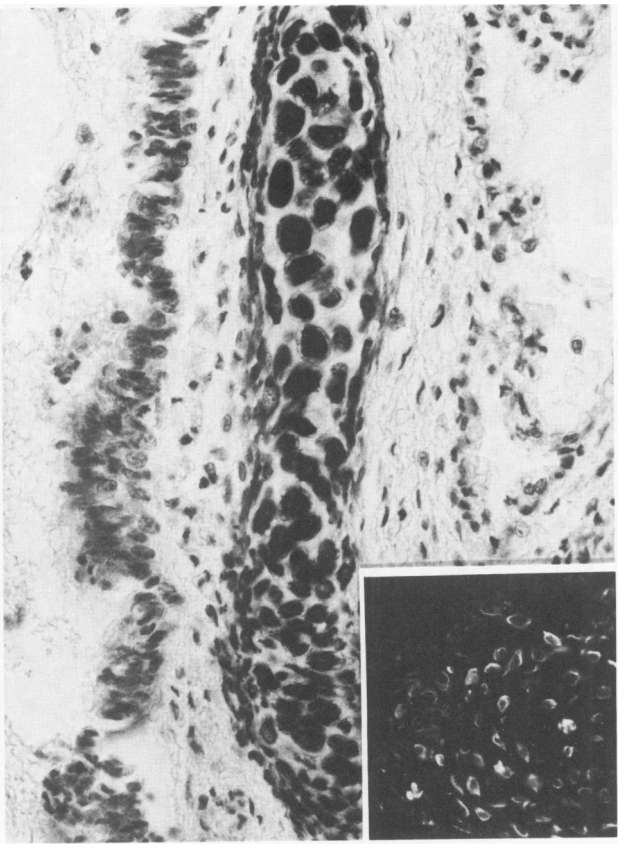
Vimentin was expressed constantly by all the cells of the chondroid foci, including those immunoreactive for GFAP (Figure 6b), whereas cytokeratin immunoreactivity was strictly confined to the epithelial component of the hamartomas (Figure 6c).

Figure 1—Immunocytochemical localization of GFAP in chondrocytes of adult human epiglottis. Immunoreactive cells are mainly located at the periphery of the cartilage. (Formalin fixation, polyclonal antiserum from Dakopatts, ×250) **Figure 2**—The cartilages of adult main bronchi do not show GFAP-immunoreactive chondrocytes. (Formalin fixation, polyclonal antiserum from Dakopatts, ×250) **Figure 3**—Several GFAP-immunoreactive chondrocytes are identifiable in the adult subsegmental bronchi. (Methacarn fixation, G1 MAb, ×250) **Figure 4**—All the chondrocytes of neonatal subsegmental bronchi display strong immunoreactivity for GFAP. (Methacarn fixation, G1 MAb, ×400) **Inset**—GFAP-immunoreactivity in the same cartilages after immunofluorescence staining of frozen tissue sections. (G1 MAb, ×400)









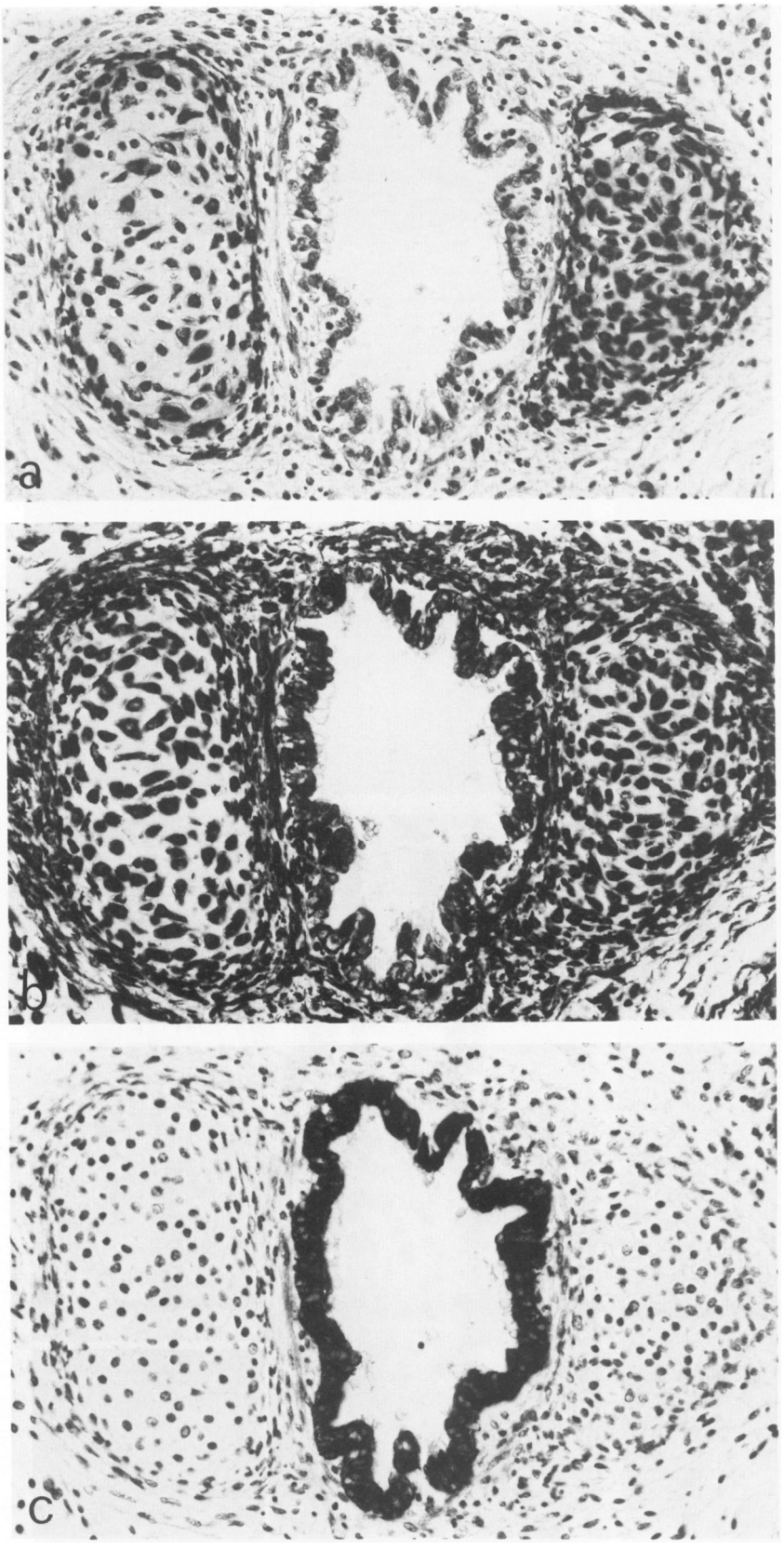


Figure 5—Immunocytochemical localization of GFAP (GA5 MAb (**a**), vimentin (**b**), and cytokeratins (**c**) in adjacent serial sections of a subsegmental bronchus of the 22-week-old fetus. All the chondrocytes coexpress GFAP and vimentin, whereas most epithelial cells coexpress cytokeratins and vimentin. (Methacarn fixation, ×400)

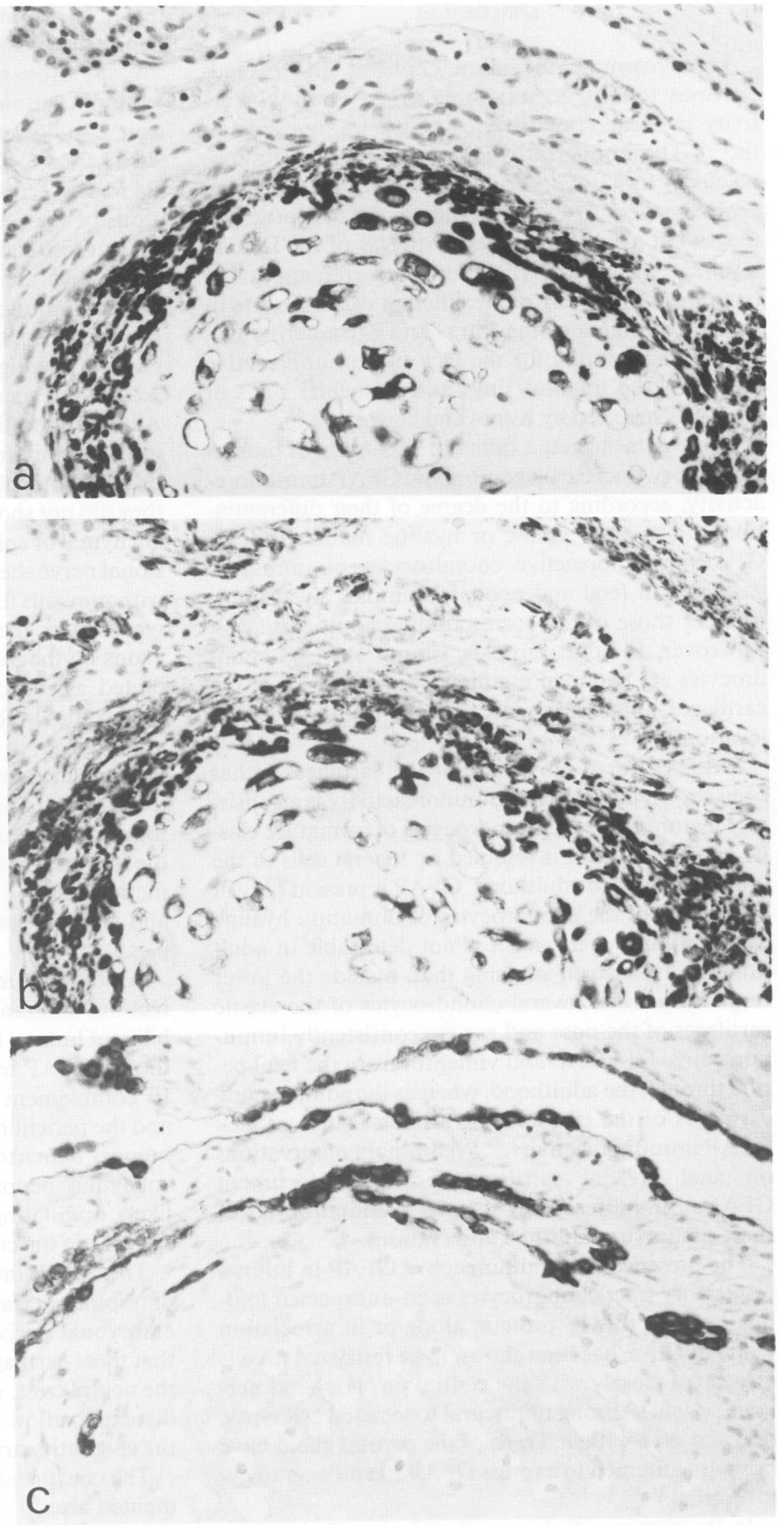


Figure 6—Immunocytochemical localization of GFAP (GA5 monoclonal antibody (**a**), vimentin (**b**), and cytokeratins (**c**) in adjacent serial sections of a chondromatous hamartoma of the lung. Peripheral chondrocytes and perichondrial cells coexpress GFAP and vimentin, whereas epithelial cells are decorated only by the anti-cytokeratins antibody. (Formalin fixation, ×400)

Discussion

The present investigation confirms and extends previous reports documenting GFAP-immunoreactivity in some chondrocytes of the human epiglottis.[38,40] The authors have demonstrated that immunoreactivity for GFAP, in addition to that for vimentin, is observed consistently not only in the epiglottic cartilages but also in the other cartilages of the human respiratory tract. This IF pattern, however, appears to be expressed variably in the different cartilages and in the different ages of the intra- and extrauterine life, possibly accounting for the lack of immunoreactive GFAP in the tracheal rings and bronchial walls of adults, as reported by Kepes and coworkers.[38]

These data suggest a different capability of human respiratory tract cartilages to show GFAP immunoreactivity, according to the degree of their differentiation and to their elastic or hyaline nature. Indeed, GFAP-immunoreactive chondrocytes of immature cartilages in fetal and neonatal samples always outnumber those of the corresponding adult cartilages. Moreover, in adult samples, immunolabeled chondrocytes are confined mainly to the periphery of the cartilages, and are only occasionally found in central locations.

With regard to the nature of the cartilages, it has been shown that GFAP immunoreactivity is a consistent feature of all the chondrocytes of immature elastic cartilages and it is retained by several cells of the same cartilages in adulthood. GFAP is present only in a minority of the chondrocytes of immature hyaline cartilages, however, and it is not detectable in adult samples. It is worth stressing that, outside the lower respiratory tract, several chondrocytes of the elastic cartilages of the nose and ear are consistently immunoreactive for GFAP and vimentin from the fetal period through the adulthood, whereas the adult hyaline cartilages of the ribs and the articular surfaces lack GFAP immunoreactivity.[38] Preliminary observations on fetal skeletal cartilages, however, document GFAP-immunoreactivity in several chondrocytes of these tissues (unpublished observation).

The presence of immunoreactive GFAP in human respiratory tract chondrocytes is an unexpected finding, because this IF protein, alone or in association with vimentin, has been shown to be restricted to cells associated closely with the central or peripheral nervous system. Among nonneural-associated cell types, only the myoepithelial cells of the parotid gland have been documented to express GFAP in addition to cytokeratins.[36,42]

In the absence of physico-chemical analysis of human cartilage extracts, the authors have performed an extensive immunocytochemical investigation to exclude the possibility that the results could be affected by any cross-reactivity of anti-GFAP antisera with epitopes pertaining to other IF proteins.[59] For this reason, two polyclonal and six different, well-characterized MAbs to GFAP were used. The GA5 and the 12-24 MAbs recognize epitopes localized in different portions of the GFAP molecule;[36,53] the 1B4-2E1-4A11 mixture is made of three MAbs of different specificities,[52] and the 6F2 MAb is specific for an epitope whose immunoreactivity, at variance with that of the epitopes recognized by the other MAbs used in the current investigation, is greatly reduced by trypsin digestion but remains unaffected by pronase digestion. All the antibodies resulted in a definite and consistent immunostaining of chondrocytes as well as of glial cells in control sections of human cerebellum, but they did not show any cross-reactivity with other mesenchymal or epithelial cell types. The staining of occasional nerve sheath cells by five of the six MAbs agrees with previous findings documenting GFAP immunoreactivity in these cells,[35,36,40] whereas the staining of axons by the two polyclonal antisera has been interpreted as reflecting the presence of contaminating specificities in these preparations.[36]

Moreover, to exclude any conformational or chemical modification of IF proteins induced by the fixatives and eventually leading to the appearance of epitopes immunoreactive to the anti-GFAP antibodies, the authors investigated both fixed (in formalin and methacarn) and frozen samples of the same cartilages and no false-positive staining was seen in fixed samples.

All these immunocytochemical data provide indirect but substantial confirmation of the actual capability of human respiratory tract chondrocytes to synthesize GFAP in addition to vimentin. This peculiar IF complement is maintained by the chondrocytes and the perichondrial stellate and spindle cells of pulmonary hamartomas, which are considered true mesenchymal neoplasms of the bronchial wall, most likely originating from undifferentiated multipotential cells in the connective tissue.[60,61]

The GFAP-immunoreactivity of the chondrocytes of respiratory tract cartilages cannot be related to their embryonal derivation because there is no evidence that these cartilages share a common derivation from the neural crest, which has been suggested[38] as a possible explanation for the GFAP immunoreactivity in the epiglottic cartilage.

The coexpression of GFAP and vimentin, if documented also in other human cartilages and their derived tumors outside the respiratory tract, might have relevant implications in investigations on cell differ-

entiation and tumor histogenesis, with particular reference to the mixed tumors of the salivary glands and of the skin. Indeed, the presence of neoplastic cells expressing GFAP and vimentin (with or without cytokeratins) both in pleomorphic adenomas of the salivary glands[36,40–43] and in mixed tumors (chondroid syringomas) of the skin (manuscript in preparation) could simply reflect a chondroid differentiation of the tumor cells instead of implying their derivation from normal parent cells with the same pattern of IF expression, which have been so far only occasionally identified in the parotid glands[36,62] but never documented in other salivary glands and in the skin adnexa.[36] A similar condition has been described for choroid plexus tumors, which coexpress GFAP in addition to cytokeratins and vimentin, whereas normal choroid epithelial cells express cytokeratins and vimentin only.[51] The appearance of GFAP in these tumors has been related to their differentiation toward ependymal cells, which normally cosynthesize vimentin and GFAP.[63]

As an ancillary finding of the current study, coexpression of cytokeratins and vimentin in the fetal and neonatal, but not adult, bronchial epithelium also has been demonstrated. This epithelium should therefore be added to other human epithelia showing transient expression of vimentin, in addition to cytokeratins, during development and differentiation, for example, the tubular epithelium of the kidney[64,65] and the surface epithelium of the tongue.[66]

In conclusion, this investigation further emphasizes the unexpected complexity of IF expression in several human cell types, documenting yet another example of "inappropriate" synthesis of IF proteins by respiratory tract cartilages and pulmonary hamartomas, and contradicting the widely held supposition that the expression of GFAP is restricted to cells of glial origin.

References

1. Eng LF, Vanderhaeghen JJ, Bignami A, Gerstl B: An acidic protein isolated from fibrous astrocytes. Brain Res 1971, 28:351–354
2. Bignami A, Eng LF, Dahl D, Uyeda CT: Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain Res 1972, 43:429–435
3. Eng LF, De Armond SJ: Immunochemistry of the glial fibrillary acidic protein, Progress in Neuropathology. Vol 5. Edited by HM Zimmerman. New York, Raven Press, 1983, pp 19–39
4. Dahl D, Bignami A: Intermediate filaments in nervous tissue, Cell Tissue Motility. Vol 6. Edited by JW Shay. New York, Plenum Press, 1985, pp 75–96
5. De Armond SJ, Eng LF, Rubinstein LJ: The application of glial fibrillary acidic (GFA) protein immunohistochemistry in neurooncology. Pathol Res Pract 1980, 168:374–394
6. Yen S-H, Fields KL: Antibodies to neurofilament, glial filament, and fibroblast intermediate filament proteins bind to different cell types of the nervous system. J Cell Biol 1981, 88:115–126
7. Schnitzer J, Franke WW, Schachner M: Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. J Cell Biol 1981, 90:435–447
8. Deck JHN, Eng LF, Bigbee J, Woodcock SM: The role of glial fibrillary acidic protein in the diagnosis of central nervous system tumors. Acta Neuropathol (Berl) 1978, 42:183–190
9. Roessmann U, Velasco ME, Syndley SD, Gambetti P: Glial fibrillary acidic protein (GFAP) in ependymal cells during development: An immunocytochemical study. Brain Res 1980, 200:13–21
10. Shaw G, Osborn M, Weber K: An immunofluorescence microscopical study of the neurofilament triplet proteins, vimentin and glial fibrillary acidic protein within the adult rat brain. Eur J Cell Biol 1981, 26:68–82
11. Moller M, Ingild A, Bock E: Immunohistochemical demonstration of S-100 protein and GFA protein in interstitial cells of rat pineal gland. Brain Res 1978, 140: 1–13
12. Lowenthal A, Flament-Durand J, Karcher D, Noppe M, Brion JP: Glial cells identified by anti-a-albumin (anti-GFA) in human pineal gland. J Neurochem 1982, 38:863–865
13. Paposozomenos SCh: Glial fibrillary acidic (GFA) protein-containing cells in the human pineal gland. J Neuropathol Exp Neurol 1983, 42:391–408
14. Higley HR, McNully JA, Rowder G: Glial fibrillary acidic protein and S-100 protein in pineal supportive cells: An electron microscopic study. Brain Res 1984, 304:117–120
15. Suess U, Pliska V: Identification of the pituicytes as astroglial cells by indirect immunofluorescence-staining for the glial fibrillary acidic protein. Brain Res 1981, 221:27–33
16. Velasco ME, Roessmann U, Gambetti P: The presence of glial fibrillary acidic protein in the human pituitary gland. J Neuropathol Exp Neurol 1982, 41:150–163
17. Hoefler H, Walter GF, Denk H: Immunohistochemistry of folliculostellate cells in normal human adenohypophyses and in pituitary adenomas. Acta Neuropathol (Berl) 1984, 65:35–40
18. Sharp G, Osborn M, Weber K: Occurrence of two different intermediate filament proteins in the same filament in situ within a human glioma cell line. Exp Cell Res 1982, 141:385–395
19. Wang E, Caincross JG, Liem RKH: Identification of glial filament protein and vimentin in the same intermediate filament system in human glioma cells. Proc Natl Acad Sci USA 1984, 81:2102–2106
20. Uyeda CT, Eng LF, Bignami A: Immunological study of the glial fibrillary acidic protein. Brain Res 1972, 37: 81–89
21. Bignami A, Dahl D: Astrocyte-specific proteins and neuroglial differentiation: An immunofluorescence study with antibodies to the glial fibrillary acidic protein. J Comp Neurol 1974, 153:27–38
22. Deck JH, Eng LF, Bigbee J: A preliminary study of glioma morphology using the peroxidase-antiperoxidase

method for the GFA protein (Abstract). J Neuropathol Exp Neurol 1976, 35:362
23. Eng LF, Rubinstein LJ: Contribution of immunohistochemistry to diagnostic problems of human cerebral tumors. J Histochem Cytochem 1978, 26:513–522
24. Velasco ME, Dahl D, Roessmann U, Gambetti P: Immunohistochemical localization of glial fibrillary acidic protein in human glial neoplasms. Cancer 1980, 45: 484–494
25. Bonnin JM, Rubinstein LJ: Immunohistochemistry of central nervous system tumors: Its contribution to neurosurgical diagnosis. J Neurosurg 1984, 60:1121–1133
26. Perentes E, Rubinstein LJ: Recent applications of immunoperoxidase histochemistry in human neuro-oncology: An update. Arch Pathol Lab Med 1987, 111: 796–812
27. Jessen KR, Mirsky R: Glial cells in the enteric nervous system contain glial fibrillary acidic protein. Nature 1980, 286:736–737
28. Barber PC, Lindsay RM: Schwann cells of the olfactory nerves contain glial fibrillary acidic protein and resemble astrocytes. Neuroscience 1982, 7:3077–3090
29. Dahl D, Chi NH, Miles LE, Nguyen BT, Bignami A: Glial fibrillary acidic (GFA) protein in Schwann cells: Fact or artifact? J Histochem Cytochem 1982, 30:912–918
30. Jessen KR, Mirsky R: Astrocyte-like glia in the peripheral nervous system: An immunohistochemical study of enteric glia. J Neurosci 1983, 3:2206–2218
31. Jessen KR, Thorpe R, Mirsky R: Molecular identity, distribution and heterogeneity of glial fibrillary acidic protein: An immunoblotting and immunohistochemical study of Schwann cells, satellite cells, enteric glia and astrocytes. J Neurocytol 1984, 13:187–200
32. Eng LF, Smith ME, de Vellis J, Skoff RP: Recent studies of the glial fibrillary acidic protein, Intermediate Filaments. Edited by E Wang, D Fishman, RKH Lien, T-T Sun. Ann NY Acad Sci 1985, 455:525–537
33. Jessen KR, Mirsky R: Glial fibrillary acidic polypeptides in peripheral glia: Molecular weight, heterogeneity and distribution. J Neuroimmunol 1985, 8:377–393
34. Yen S-H, Fields KL: A protein related to glial filaments in Schwann cells, Intermediate Filaments. Edited by E Wang, D Fishman, RKH Lien, T-T Sun. Ann New York Acad Sci 1985, 455:538–551
35. Fields KL, Yen S-H: A subset of Schwann cells in peripheral nerves contain a 50-kilodalton protein antigenically related to astrocyte intermediate filaments. J Neuroimmunol 1985, 8:311–330
36. Achtstatter T, Moll R, Anderson A, Kuhn C, Pitz S, Schwechheimer K, Franke WW: Expression of glial filament protein (GFP) in nerve sheaths and non-neural cells re-examined using monoclonal antibodies, with special emphasis on the co-expression of GFP and cytokeratins in epithelial cells of human salivary gland and pleomorphic adenomas. Differentiation 1986, 31:206–227
37. Hatfield JS, Skoff RP, Maisel H, Eng EL: Glial fibrillary acidic protein is localized in the lens epithelium. J Cell Biol 1984, 98:1895–1898
38. Kepes JJ, Rubinstein LJ, Chiang H: The role of astrocytes in the formation of cartilage in gliomas: An immunohistochemical study of four cases. Am J Pathol 1984, 117:471–483
39. Gard AL, White FP, Dutton GR: Extra-neural glial fibrillary acidic protein (GFAP) immunoreactivity in perisinusoidal stellate cells of the rat liver. J Neuroimmunol 1985, 8:359–375
40. Budka H: Non-glial specificities of immunocytochemistry for the glial fibrillary acidic protein (GFAP): Triple expression of GFAP, vimentin and cytokeratins in papillary meningioma and metastasizing renal carcinoma. Acta Neuropathol (Berl) 1986, 72:43–54
41. Nakazato Y, Ishizeki J, Takahashi K, Yamaguchi H, Kamel T, Mori T: Localization of S-100 protein and glial fibrillary acidic protein-related antigen in pleomorphic adenoma of the salivary glands. Lab Invest 1982, 46:621–626
42. Nakazato Y, Ishida Y, Takahasi K, Suzuki K: Immunohistochemical distribution of S-100 protein and glial fibrillary acidic protein in normal and neoplastic salivary glands. Virchows Arch [A] 1985, 405:299–310
43. Rozell B, Stenman G, Hansson H-A, Dahl D, Hansson GK, Mark J: Intermediate filaments in cultured human pleomorphic adenomas. Acta Pathol Microbiol Immunol Scand (A) 1985, 93:335–343
44. Tascos NA, Parr J, Gonatas NK: Immunocytochemical study of the glial fibrillary acidic protein in human neoplasms of the central nervous system. Hum Pathol 1982, 13:454–458
45. Memoli VA, Brown EF, Gould VE: Glial fibrillary acidic protein (GFAP) immunoreactivity in peripheral nerve sheath tumors. Ultrastruct Pathol 1984, 7:269–275
46. Gould VE, Moll R, Moll I, Lee I, Schwechheimer K, Franke WW: The intermediate filament complement of the spectrum of nerve sheath neoplasms. Lab Invest 1986, 55:463–474
47. Reifenberger G, Szymas J, Wechsler W: Differential expression of glial- and neuronal-associated antigens in human tumors of the central and peripheral nervous system. Acta Neuropathol (Berl) 1987, 74:105–123
48. Liao SY, Choi BH: Expression of glial fibrillary acidic protein by neoplastic cells of mullerian origin. Virchows Arch [B] 1986, 52:185–193
49. Miettinen M, Lehto V-P, Dahl D, Virtanen I: Differential diagnosis of chordoma, chondroid, and ependymal tumors as aided by anti-intermediate filament antibodies. Am J Pathol 1983, 112:160–169
50. Hsu S-M, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
51. Doglioni C, Dell'Orto P, Coggi G, Iuzzolino P, Bontempini L, Viale G: Choroid plexus tumors: An immunocytochemical study with particular reference to the coexpression of intermediate filament proteins. Am J Pathol 1987, 127:519–529
52. McLendon RE, Burger PC, Pegram CN, Eng LF, Bigner DD: The immunohistochemical application of three anti-GFAP monoclonal antibodies to formalin-fixed, paraffin-embedded, normal and neoplastic brain tissues. J Neuropathol Exp Neurol 1986, 45:692–703
53. Debus E, Weber K, Osborn M: Monoclonal antibodies specific for glial fibrillary acidic (GFA) protein and for each neurofilament triplet polypeptides. Differentiation 1983, 25:193–203

54. Gheuens J, de Schutter E, Noppe M, Lowenthal A: Identification of several forms of the glial fibrillary acidic protein, or a-albumin, by a specific monoclonal antibody. J Neurochem 1984, 43:964–970
55. Virtanen I, Miettinen M, Letho V-P, Kariniemi A-L, Paasivuo R: Diagnostic application of monoclonal antibodies to intermediate filaments. Ann NY Acad Sci 1985, 455:635–648
56. Osborn M, Debus E, Weber K: Monoclonal antibody specific for vimentin. Eur J Cell Biol 1984, 34:137–143
57. von Overbeck J, Stähli C, Gudat F, Carmann H, Lautenshlager C, Durmuller U, Takacs B, Miggiano V, Staehelin Th, Heitz PhU: Immunohistochemical characterization of an anti-epithelial monoclonal antibody (MAb lu-5). Virchows Arch [A] 1985, 407:1–12
58. Franke WW, Winter S, von Overbeck J, Gudat F, Heitz PU, Stähli C: Identification of the conserved, conformation-dependent cytokeratin epitope recognized by monoclonal antibody (lu-5). Virchows Arch [A] 1987, 411:137–147
59. Pruss RM, Mirsky R, Raff MC, Thorpe R, Dowding AJ, Anderton BH: All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody. Cell 1981, 27:419–428
60. Bateson EM: So-called hamartoma of the lung: A true neoplasm of fibrous connective tissue of the bronchi. Cancer 1973, 31:1458–1467
61. Perez-Atayde A, Seiler MW: Pulmonary hamartoma: An ultrastructural study. Cancer 1984, 53:485–492
62. Born IA, Schechheimer K, Maier H, Otto HF: Cytokeratin expression in normal salivary glands and in cystadenolymphomas demonstrated by monoclonal antibodies against selective cytokeratin polypeptides. Virchows Arch [A] 1987, 411:583–589
63. Rubinstein LJ, Brucher J-M: Focal ependymal differentiation in choroid plexus papillomas: An immunoperoxidase study. Acta Neuropathol (Berl) 1981, 53: 29–33
64. Holthöfer H, Miettinen A, Passivuo R, Lehto VP, Linder E, Altthan O, Virtanen I: Cellular origin and differentiation of renal carcinomas: A fluorescence microscopic study with kidney-specific antibodies, antiintermediate filament antibodies and lectins. Lab Invest 1983, 49:317–326
65. Holthöfer H, Miettinen A, Lehto VP, Lehtonen E, Virtanen I: Expression of vimentin and cytokeratin types of intermediate filament proteins in developing and adult human kidneys. Lab Invest 1984, 50:552–559
66. Van Muijen GNP, Ruiter DJ, Warnaar SO: Coexpression of intermediate filament polypeptides in human fetal and adult tissues. Lab Invest 1987, 57:359–369

Acknowledgment

The authors thank Mr. Tiziano Pellencin, Mr. Luciano Schenal, and Mr. Alfonso Scopel for their competent technical assistance. Special thanks are also due to Professor W. W. Franke for the gift of the 12-24 monoclonal antibody.