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Choroid Plexus Tumors

An Immunocytochemical Study With Particular Reference to the Coexpression of Intermediate Filament Proteins

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Sixteen choroid plexus tumors (CPTs) have been investigated for the localization of different immunocytochemical markers of epithelial and nonepithelial nature, namely, simple epithelial-type cytokeratins, vimentin, glial fibrillary acidic protein (GFAP), a pan-epithelial antigen defined by the lu-5 monoclonal antibody (lu-5 antigen), S-100 protein, and epithelial membrane antigen (EMA). Intermediate filament proteins have been identified in paraffin sections of 14 of 16 cases (87.5%). In all these tumors, cytokeratins and vimentin were constantly coexpressed by the neoplastic cells, in a manner similar to that of the cells lining normal choroid plexus. In 7 of these 14 cases, in addition to cytokeratins and vimentin, the neoplastic cells were shown to coexpress GFAP, which is not synthesized by their normal cell counterpart. The appearance of GFAP immunoreactivity in CPTs might be related to an ependymal differentiation of the neoplastic cells, because normal ependyma and ependymomas constantly coexpress GFAP and vimentin. The simultaneous expression of three distinct intermediate filament proteins by the same neoplastic cells is an exceedingly rare phenomenon, which has never been reported by double labeling technique in neoplasms of the central nervous system. Despite the complex antigenic profile of the CPT, which includes immunoreactivity for lu-5 antigen, S-100 protein, and EMA in most of the cases, positivity for three different epithelial markers indicates that these tumors have an epithelial nature. Moreover, the immunocytochemical typing of CPT with the panel of antibodies used in the current investigation allows differentiation from other primary and metastatic central nervous system tumors. (Am J Pathol 1987, 127:519–529)

CHOROID PLEXUS tumors (CPTs) are uncommon neoplasms of the central nervous system (CNS) that arise from specialized secretory cells lining the choroid plexus. Their morphologic features cover a wide spectrum of different patterns, including papillary, cystic, acinar, mucus-secreting, oncocytic, and anaplastic variants.[1,2]

The epithelial nature of CPT has been substantiated by both electron-microscopic[3–6] and tissue-culture[6] investigations; moreover, it has been formerly confirmed by the immunocytochemical localization of the epithelial-type of intermediate filament (IF) proteins, ie, cytokeratins, in four choroid plexus papillomas investigated by Kemshead and Coakham[7] and by Coakham et al.[8]

In rather striking contrast to these findings, pointing to a definite epithelial nature of CPT, other reports provided evidence that the glial-type of IF, ie, glial fibrillary acidic protein (GFAP), was expressed in as many as 40% of these tumors.[9–12] Such a surprisingly high incidence of a nonepithelial marker in CPTs has been considered as evidence of ependymal differentiation of the neoplastic cell population.[9,11,12]

More recently, Coffin et al[13] and Miettinen et al[14] immunostained CPTs for all the five classes of IF

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proteins, providing apparently contradictory results. Indeed, though both groups of investigators showed cytokeratin immunoreactivity in almost all their cases, they reported contrasting findings as to the coexpression of other IF proteins in the same neoplasms. In fact, while Coffin et al[13] detected GFAP coexpression in 3 of 10 cases and were unable to find any vimentin immunoreactivity in CPT, Miettinen et al[14] reported coexpression of cytokeratins and vimentin in 1 of 6 cases and a simultaneous expression of cytokeratins, vimentin, and GFAP in 2 more cases. Moreover, whereas in the series of Coffin et al the GFAP coexpression was restricted to benign CPTs, Miettinen et al assessed triple expression of IF proteins also in a poorly differentiated choroid plexus carcinoma.

These investigators, however, did not try to ascertain whether the coexpression of IF proteins by CPTs was due to a heterogeneity of the neoplastic cell population or to the simultaneous expression of distinct proteins by the same neoplastic cells.

Prompted by the apparently contradictory results of the IF characterization of CPTs and by the lack of any comprehensive immunocytochemical investigation, aimed at defining the actual nature and phenotypic expression of these neoplasms, we performed an immunocytochemical study of 16 cases of CPT. In particular, we have immunostained these tumors for distinct classes of IF proteins (cytokeratins, vimentin, and GFAP), paying special attention to the recognition of a possible multiple expression of IF proteins by the same neoplastic cells.

The panel of phenotypic markers investigated also included a newly recognized cytoskeleton-associated antigen (defined by the lu-5 monoclonal antibody and expressed by all the epithelial cells and their derived tumors so far investigated),[15] the epithelial membrane antigen (EMA), and S-100 protein. This latter antigen has been shown by Nakamura et al[16] to be invariably expressed in both benign and malignant CPTs, whereas in the series of Coffin et al[13] it was confined to benign tumors.

We compared the pattern of expression of these different markers in CPTs with that of their normal cell counterpart in order to ascertain any possible phenotypic change subsequent to the neoplastic transformation, and with that of normal ependyma and ependymomas in order to give immunocytochemical support to any actual ependymal differentiation of CPTs.

Materials and Methods

Sixteen cases of surgically removed CPT have been retrieved from the files of the Surgical Pathology department of Borgo Trento Hospital in Verona. The clinical data and histopathologic diagnoses of these cases are listed in Table 1.

Ten cases of surgically removed ependymomas have also been investigated, together with normal choroid plexus and ependyma from the lateral ventricles obtained during five consecutive autopsies performed within 12 hours after death.

All the tissue samples have been fixed in 10% formalin and embedded in paraffin, according to routine histologic procedures.

Immunocytochemical Staining

From the paraffin blocks, 5-μ-thick serial sections were cut, collected on albumin-coated slides, and left to dry overnight at 37 C. In order to ascertain any possible coexpression of different markers by the same cells in adjacent sections, the serial sections from each paraffin block were consecutively numbered and then immunostained for the different antigens (simple epithelial-type cytokeratins, vimentin, GFAP, lu-5 antigen, EMA, and S-100 protein) in the same order as they are listed in Table 2, using the ABC staining procedure.[17]

Briefly, the sections were dewaxed, treated with 3% hydrogen peroxide in distilled water for inhibition of endogenous peroxidase activity, washed in 0.05M Tris-buffered saline (TBS), pH 7.6, and then sequentially incubated with 1) 1/30 dilution of normal goat or horse serum for 30 minutes, 2) specific polyclonal or monoclonal antiserum for 1 hour, 3) 1/200 dilution of biotinylated goat anti-rabbit or horse anti-mouse immunoglobulin sera for 30 minutes, and 4)

Table 1—Clinical Data on Choroid Plexus Tumors

Case	Age/Sex	Site	Histology
1	64/F	4V	CP papilloma
2	1/F	LV	CP carcinoma
3	19/F	4V	CP papilloma
4	51/M	4V	CP papilloma
5	38/M	4V	CP papilloma
6	51/M	4V	CP papilloma
7	46/M	4V	CP papilloma
8	23/F	4V	CP papilloma
9	10 mo/M	LV	CP papilloma
10	12/F	LV	CP papilloma, oncocytic
11	2 mo/F	LV	CP papilloma
12	58/M	4V	CP papilloma
13	12/F	LV	CP carcinoma, pigmented
14	26/M	4V	CP tubular adenoma
15	23/F	4V	CP papilloma
16	3/F	LV	CP carcinoma

4V, fourth ventricle; LV, lateral ventricle; CP, choroid plexus.

avidin-biotinylated peroxidase complex (ABC) for 30 minutes. The ABC solution was made 30 minutes before its use by adding 10 μl of avidin DH and 10 μl of biotinylated peroxidase to 1 ml of TBS.

Washing in TBS, three changes of 5 minutes each, was performed after steps 2, 3, and 4. Peroxidase activity was developed in the DAB medium,[18] in the dark, under gentle stirring. Finally, the sections were lightly counterstained with hematoxylin, dehydrated, and mounted in permanent medium. The immunocytochemical reactions were performed in a humidity chamber at room temperature.

For the localization of cytokeratins and lu-5 antigen, the tissue sections were treated with 0.4% pepsin (BDH) in 0.01N HCl for 7 minutes at 37 C, prior to the immunocytochemical reaction.

Control sections for specificity included staining of a breast carcinoma sample, as known positive control for cytokeratins, lu-5 antigen and EMA, whereas the endothelial lining of blood vessels and the normal nervous tissue surrounding CPTs were "built-in" positive controls for vimentin, GFAP, and S-100 protein. All these positive controls displayed strong 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 resulted unstained.

Double immunocytochemical reactions for the simultaneous localization of cytokeratins and GFAP in the same tissue sections were performed in selected cases of CPT, according to the techniques of Nakane[19] and of Tramu et al.[20]

The results of the immunoreactions were semiquantitatively evaluated with regard to the percentage of stained cells over the total number of neoplastic cells and graded in a scale ranging from − (no immunoreactive cells) to 4 + (75–100% of the cells immunostained).

Normal goat and horse sera, biotinylated antisera, and the ABC components in kit form were purchased from Vector (Burlingame, Calif). The source and the working dilutions of the specific primary antisera are given in Table 2.

Results

Normal Choroid Epithelium

Almost 100% of the normal choroid epithelial cells of the 5 cases under study showed definite immunoreactivity for both cytokeratins and vimentin, for the antigen defined by the lu-5 monoclonal antibody, and for the S-100 protein. Immunoreactivity for EMA was confined to the luminal borders of fewer (less than 25%) epithelial cells.

Cytokeratins and vimentin were evenly distributed throughout the cell cytoplasm. The two distinct classes of IF were coexpressed by the same epithelial cells, as shown by the comparative evaluation of consecutive serial sections (Figures 1 and 2), without any recognizable subcellular compartmentalization. The staining pattern of lu-5 antigen was the same as for cytokeratins and vimentin, whereas S-100 protein was localized both in the nuclei and cytoplasm of immunoreactive cells.

The reactions for the localization of GFAP constantly gave negative results.

Choroid Plexus Tumors

The immunocytochemical typing of CPT resulted in a heterogeneous staining pattern (Table 3).

Intermediate Filament Expression

With regard to the IF expression, all cases but 2 showed immunoreactivity for both cytokeratins and vimentin. The number of immunoreactive cells, however, was variable from case to case, ranging from less than 25% to almost 100% of the neoplastic cell population (Table 3). In most cases, the number of the neoplastic cells immunoreactive for cytokeratins was higher than that of vimentin-expressing cells.

Table 2—Source and Working Dilutions of Specific Antisera

Reagent	Dilution	Source	Reference
Anti-cytokeratins, 54 kd (monoclonal)	1/1000	Enzo Biochem, Inc.	21
Anti-GFAP (monoclonal)	1/100	Lab System, Inc.	50
Anti-vimentin (polyclonal)	1/50	Eurodiagnostics BV	22
Anti-cytokeratins, 50, 43 and 39 kd (monoclonal)	1/4	Becton-Dickinson	23
Anti-GFAP (polyclonal)	1/1200	Dakopatts AS	
Anti-vimentin (monoclonal)	1/5	Boehringer	32
lu-5 antibody (monoclonal)	1/100	Dr. C. Stähli Central Research Division Hoffman-La Roche	15
Anti-EMA (monoclonal)	1/25	Dakopatts AS	24
Anti-S-100 protein (polyclonal)	1/600	Dakopatts AS	

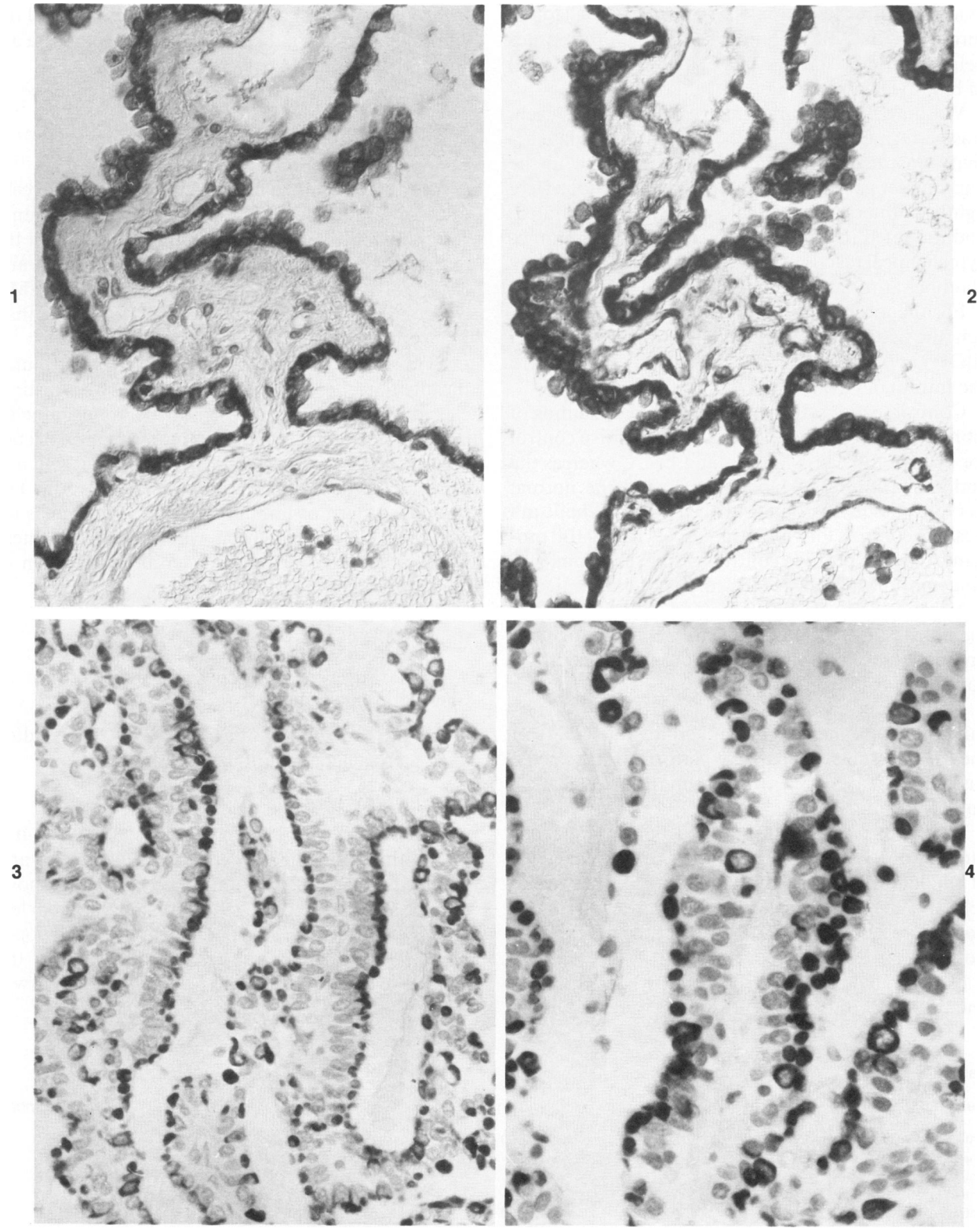


Figure 1 and 2—Immunocytochemical localization of cytokeratins (monoclonal antibody from Enzo Biochem, **Figure 1**) and vimentin (polyclonal antiserum from Eurodiagnostics, **Figure 2**) in normal choroid plexus. Choroidal cells synthesize both IF proteins, which are evenly distributed throughout the cell cytoplasm. Vimentin is also expressed by endothelial cells and some stromal cells. (Differential interference contrast optics, ×400) **Figures 3 and 4**—Immunostaining for cytokeratins (monoclonal antibody from Enzo Biochem) of a choroid plexus papilloma (Case 8). Most of the neoplastic cells are immunoreactive (**Figure 3**), and the reaction product is confined to the apical portion of the cell cytoplasm (**Figure 4**). (**Figure 3,** ×400; **Figure 4,** ×1000)

Table 3—Immunocytochemical Findings in Choroid Plexus Tumors*

Case	CK	Vim	GFAP	lu-5 ag	S-100	EMA
1	3+	2+	2+	2+	4+	1+
2	2+	2+	–	1+	3+	1+
3	4+	3+	1+	2+	3+	2+
4	2+	1+	1+	1+	4+	–
5	1+	1+	–	1+	3+	1+
6	2+	2+	1+	1+	3+	1+
7	4+	2+	–	3+	4+	–
8	4+	4+	–	4+	3+	1+
9	–	–	–	–	3+	–
10	1+	1+	–	1+	3+	–
11	–	–	–	–	3+	2+
12	3+	3+	1+	4+	4+	–
13	3+	2+	–	1+	–	1+
14	4+	3+	–	4+	4+	3+
15	4+	2+	1+	2+	4+	1+
16	3+	2+	1+	2+	3+	1+

CK, cytokeratins; Vim, vimentin; lu-5 ag, antigen defined by the lu-5 antibody.

*The results have been graded on a semiquantitative scale ranging from – (no immunoreactive cells) to 4+ (75–100% of the neoplastic cells are immunostained) The symbols 1+, 2+, and 3+ refer to the immunostaining of up to 25%, 25–50%, and 50–75% of the neoplastic cells, respectively.

In 7 of the 14 cases expressing both cytokeratins and vimentin, a focal GFAP immunoreactivity has also been demonstrated. In 6 cases, less than 25% of the cells were immunostained, whereas in the seventh case the number of immunoreactive cells comprised between 25% and 50% of the neoplastic cell population.

The results of the immunocytochemical reactions for cytokeratins, vimentin, and GFAP were not affected by the use of antisera obtained from different sources.

Two CPTs did not show immunoreactivity for any of the IF proteins tested. In the normal-appearing nervous tissue surrounding CPTs, GFAP was strictly confined to glial cells, whereas vimentin was localized in some glial cells and in endothelial cells of all the cases. Cytokeratin-immunoreactive cells were not identified.

Subcellular Compartimentalization of IF Proteins

A peculiar subcellular localization of the distinct IF proteins was assessed. Indeed, the immunoreactivity for cytokeratins was particularly strong at, or mostly confined to, the apical portion of the cytoplasm of the neoplastic cells (Figures 3 and 4). On the other hand, vimentin and GFAP appeared to be cosegregated toward the basal portion of the cell cytoplasm (Figures 5 and 6). This conspicuous subcellular compartmentalization of IF proteins was present in all the cases, including the carcinomas, being most easily recognizable in the fields with a papillary pattern of growth.

Coexpression of Distinct IF Proteins by the Same Cells

The comparative evaluation of consecutive serial sections, together with the results of the double immunocytochemical staining techniques on the same tissue sections, allowed us to ascertain the simultaneous expression of distinct IF proteins by the same neoplastic cells. Indeed, in all 14 cases in which IF proteins have been immunostained a variable number of neoplastic cells coexpressing cytokeratins and vimentin have been identified. The 7 cases in which three distinct IF classes were detectable showed, in addition to the coexpression of cytokeratins and vimentin, two other patterns of IF coexpression, ie, GFAP plus vimentin and GFAP plus vimentin and cytokeratins (Figures 7A–C). When detectable, GFAP was always associated, in the same neoplastic cells, with vimentin.

Immunostaining for lu-5 Antigen, S-100 Protein, and EMA

The antigen recognized by the lu-5 monoclonal antibody was localized in the same 14 cases immunoreactive for cytokeratins, even though the number of cells expressing the lu-5 antigen was generally lower than that of cytokeratin-immunoreactive cells. Moreover, lu-5 immunoreactivity was consistently parallel to that of cytokeratins, with regard both to the subcellular compartimentalization (Figure 8) and to the coexpression with IF proteins other than cytokeratins.

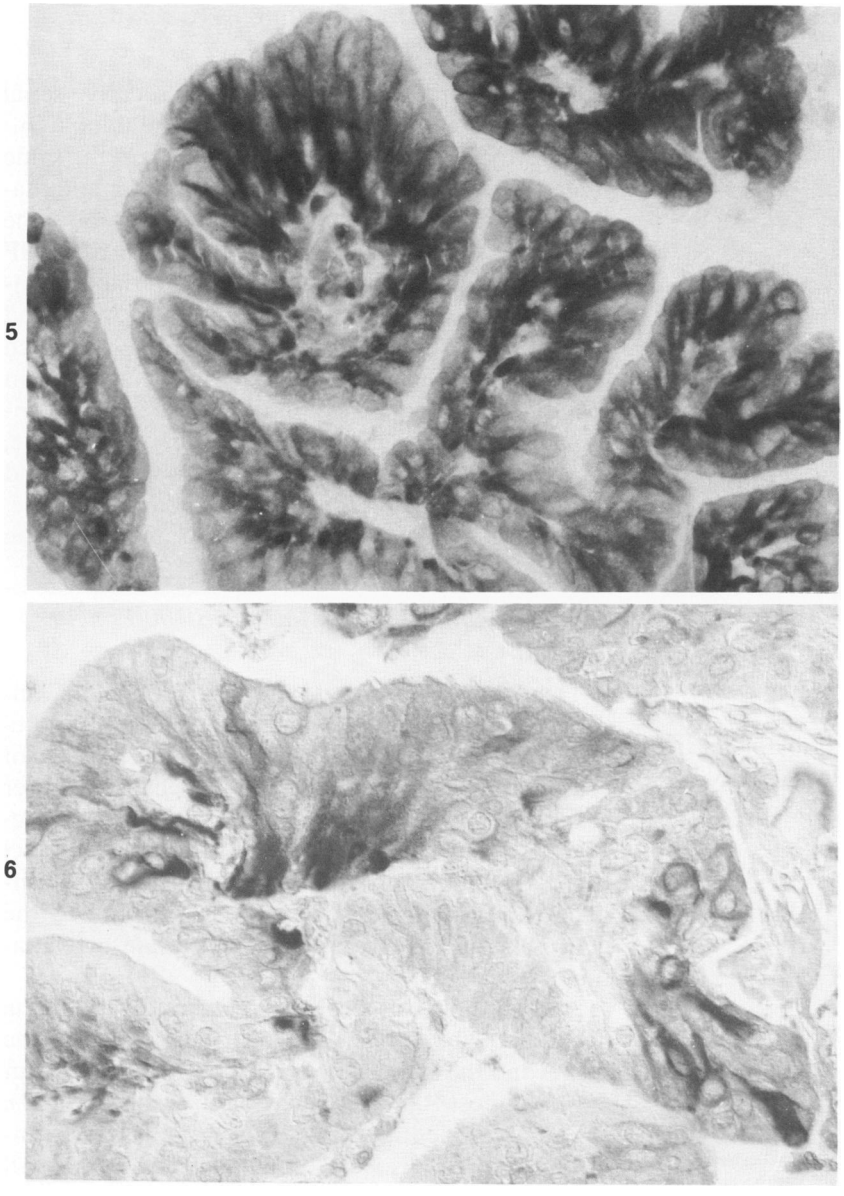
S-100 protein was localized in all the CPTs but one (Case 13). The antigen was distributed both in the nuclei and the cytoplasm of a variable number (from 50% to almost 100%) of the neoplastic cells (Figure 9).

EMA was identified in 11 out of the 16 cases. Immunoreactive cells were generally grouped in small clusters, and the reaction product was most often confined to their luminal borders (Figure 10). Immunoreactive cells represented less than 25% of the neoplastic population in 8 cases, between 25% and 50% in 2 cases, and between 50% and 75% in the remaining case.

In the normal nervous tissue surrounding CPTs, S-100 protein was localized both in the nuclei and in the cytoplasm of nervous and glial cells, whereas no cells immunoreactive for EMA or lu-5 antigen were identified.

Normal Ependyma and Ependymomas

In the 5 cases investigated, almost 100% of the normal ependymal cells were decorated by the antiserum against vimentin, whereas only a few scattered cells



Figures 5 and 6—Immunolocalization of vimentin (monoclonal antibody from Boehringer, **Figure 5**) and GFAP (polyclonal antiserum from Dakopatts, **Figure 6**). Both IF proteins are segregated toward the basal portion of the cell cytoplasm (Case 1). (×1000)

(less than 25%) were simultaneously immunoreactive for GFAP as well.

S-100 protein was localized in the nuclei and cytoplasm of most (75–100%) ependymal cells, whereas the immunoreactions for cytokeratins, lu-5 antigen, and EMA were constantly negative.

In the 10 ependymomas, the neoplastic cells showed immunoreactivity similar to that of their normal cell counterpart. Indeed, most of the tumor cells (50–100%) exhibited strong immunoreactivity for vimentin, whereas fewer of them (from less than 25% to 75%) expressed GFAP in addition to vimentin.

S-100 protein was localized in all cases, being distributed in the nuclei and cytoplasm of 50–100% of the neoplastic cells. No cells immunoreactive for cytokeratins, lu-5 antigen, or EMA were identified.

Discussion

The present immunocytochemical investigation clearly demonstrates that choroid plexus tumors are epithelial in nature, because in 15 of 16 cases (94%) it has been possible to localize in the neoplastic cell population at least one of three different, well-defined epithelial markers, namely, cytokeratins, a pan-epithelial marker defined by the lu-5 monoclonal antibody, and EMA. From our immunocytochemical study, however, it appears that CPTs actually express

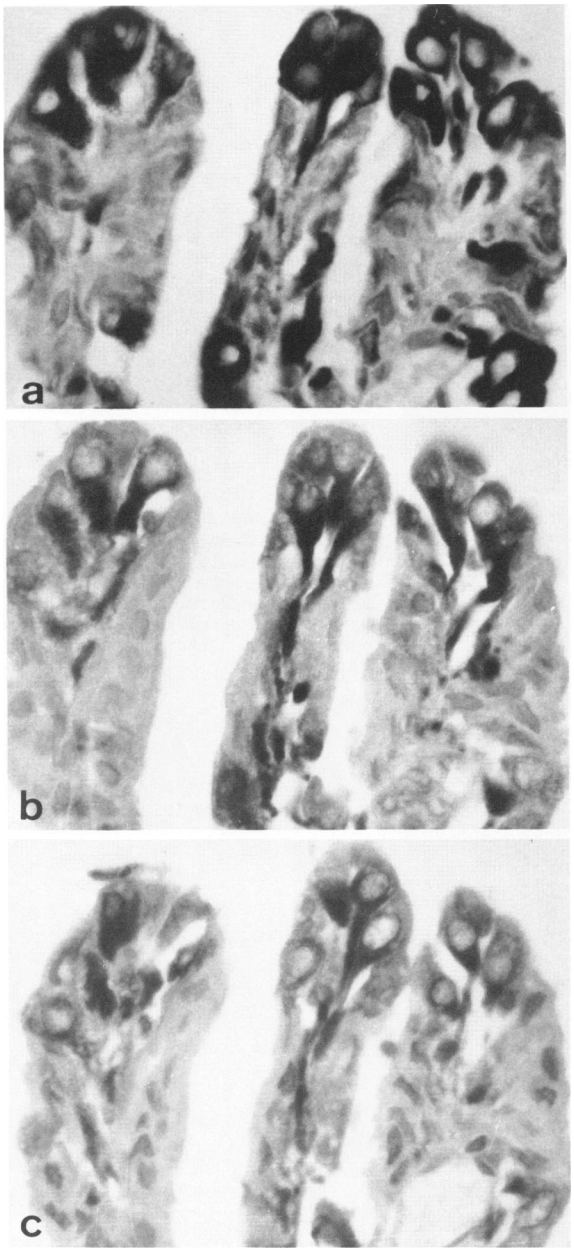


Figure 7—Colocalization of cytokeratins (monoclonal antibody from Enzo Biochem, **a**), vimentin (polyclonal antiserum from Eurodiagnostics, **b**), and GFAP (monoclonal antibody from Lab Systems, **c**) on consecutive serial sections of Case 3. (×1000)

a complex antigenic profile, which probably accounts for and allows reconciliation of the apparently contradictory results reported by previous investigators.[7-14]

The most striking feature of these neoplasms is their capability to coexpress distinct IF proteins. Indeed, we have shown that all 14 CPTs in which IF proteins were immunocytochemically detectable (87.5% of the present series) express both cytokeratins and vimentin. Although cytokeratins have already been localized in 18 of the 20 CPTs so far investigated,[7,8,13,14] vimentin immunoreactivity in the neoplastic cells has been denied[13] or detected in a few cells in 3 of 6 cases.[14] The simultaneous expression of cytokeratins and vimentin by CPTs does not seem to be a consequence of the neoplastic transformation, because the same pattern of IF expression has been observed in the 5 cases of normal choroid epithelium, which invariably co-synthesized these two classes of IF proteins.

This is at variance with the lack of immunoreactive vimentin in normal choroid epithelium of mice and rats, as reported by Schnitzer et al[25] and Pixley et al,[26] respectively. Our opposite findings, however, can be related to species-dependent differences in the pattern of IF expression. Czernobilski et al,[27] for instance, recently reported that human ovarian mesothelium contains both cytokeratins and vimentin, whereas it is decorated only by antibodies to cytokeratins in pigs and rats. Miettinen et al[14] were unable to find vimentin immunoreactivity in normal human choroid epithelium: our opposite results, however, could be related to the use of monoclonal and polyclonal antisera of a different source.

Normal choroid epithelium should therefore be added to the growing list of adult human tissues

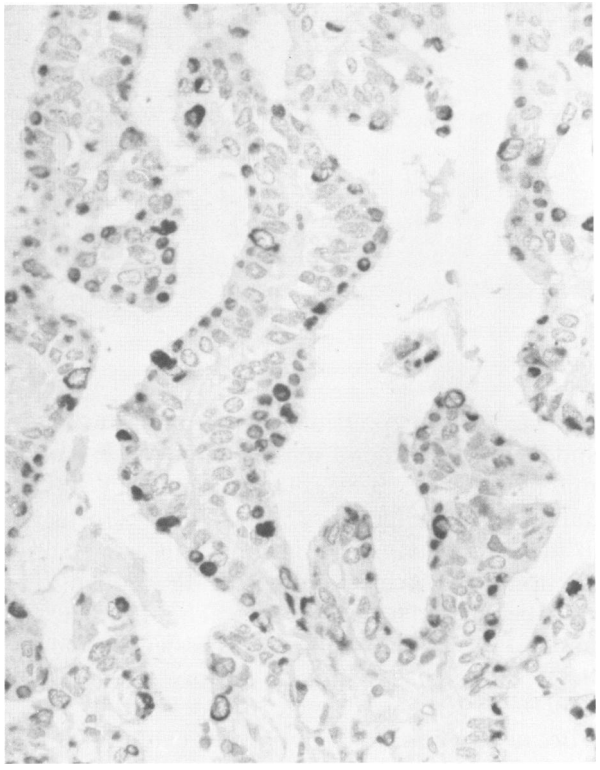
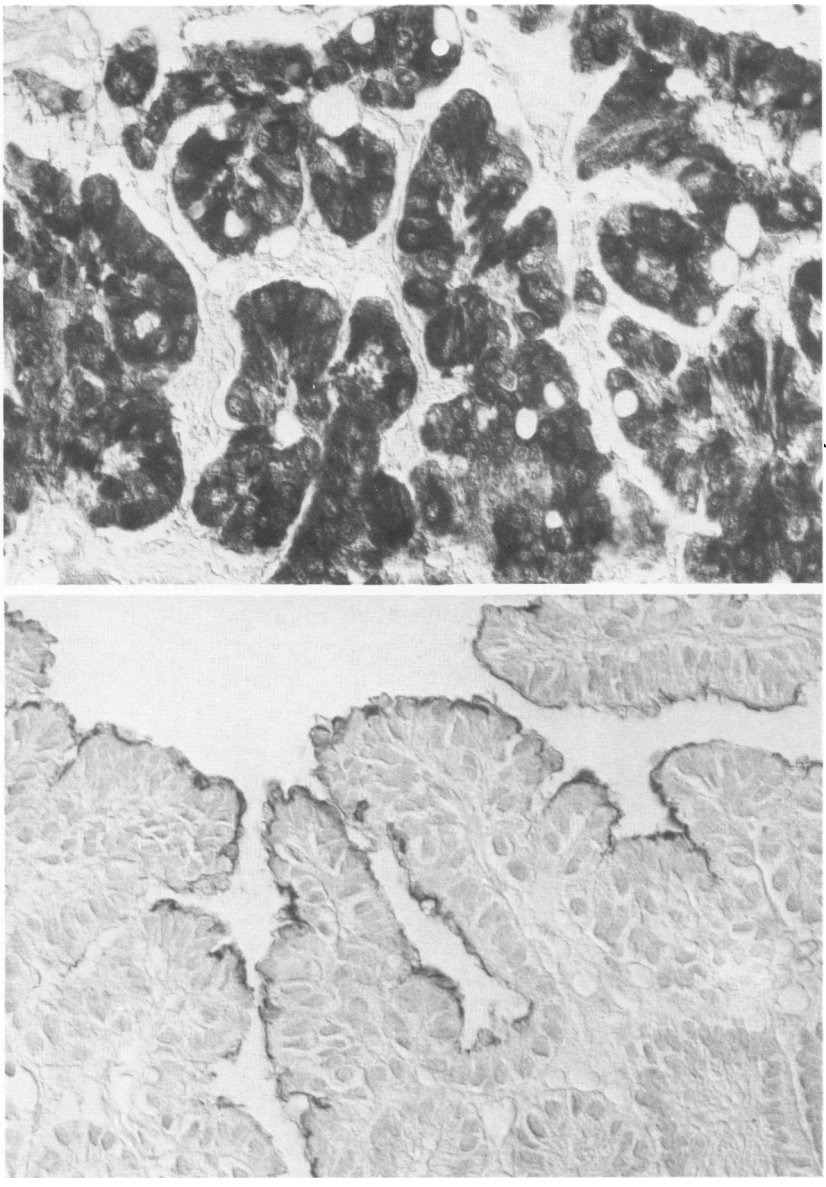


Figure 8—The antigen defined by the lu-5 monoclonal antibody shows a subcellular compartmentalization parallel to that of cytokeratins. Compare with Figure 3 (Case 8). (×400)



Figures 9 and 10—Immunolocalization of S-100 protein (Case 7, **Figure 9**) and EMA (Case 11, **Figure 10**). S-100 protein is localized both in the nuclei and cytoplasm of the neoplastic cells, whereas EMA is strictly confined to their luminal borders. (Differential interference contrast optics, ×400)

showing a dual expression of IF proteins, which includes, among others, mesothelial cells,[28] smooth muscle cells of some vessels,[29,30] some glial cells,[31,32] granulosa cells of the ovary,[27] and rete ovarii cells.[27] In all these cases, vimentin is constantly expressed in addition to the cell type-specific IF protein. This could be referred to an incomplete shift from the synthesis of vimentin—which is expressed by most of these cell lineages during embryonal development[33,34]—to the adult type of IF protein.

Because tumors arising from cells exhibiting a dual expression of IF proteins normally retain the capability to cosynthesize the same IF proteins as the normal cell counterpart,[21,28,35] the simultaneous expression of cytokeratins and vimentin by CPTs should not be regarded as an unexpected finding.

In most CPTs, however, only a portion of the neoplastic cells can be shown to express one or both IF proteins, whereas in normal conditions 100% of the choroid epithelial cells are immunoreactive for both cytokeratins and vimentin. Moreover, in 2 cases—which were the only 2 congenital choroid papillomas of our series—we have not been able to identify any IF protein. These findings can be related either to an actual reduction or suppression of IF synthesis in part of the neoplastic cell population or to changes in the IF assembly, eventually leading to the inaccessibility of some antigenic determinants. Even though forma-

lin fixation and paraffin embedding can also affect the immunoreactivity of IF proteins, in our material control structures (such as capillaries for vimentin) have been consistently positive in all cases with the antibodies used, including the 2 cases lacking expression of IF proteins by the neoplastic cells.

A second peculiar feature of CPTs is the subcellular compartimentalization of cytokeratins and vimentin, which is not apparent in the normal plexus. Because the latter, however, was obtained at autopsy, the possibility that the lack of compartmentalization is a consequence of post mortem rearrangements of IF distribution cannot be ruled out. Nevertheless, it is worthwhile stressing that IF aggregates have already been reported in tumors other than CPTs,[36-41] and that a definite subcellular compartmentalization of cytokeratins and vimentin has been ascertained also in other normal[27] and neoplastic[42] human tissues coexpressing both IF proteins. Thus, the selective segregation of the different IF proteins could well reflect still unknown different functional properties of these cytoskeletal structures in cases where they are simultaneously expressed by the same cells.

In addition to cytokeratins and vimentin, 7 of the 16 CPTs of our series (43.75%) also expressed GFAP. This finding is in keeping with previous investigations reporting GFAP immunoreactivity in a variable percentage of CPTs.[9-14] At variance with the results of Coffin et al,[13] however, we have identified GFAP immunoreactivity in one choroid plexus carcinoma. This finding, taken together with previous reports,[10,14] does not support any restriction of GFAP coexpression to benign CPTs only.

Because normal choroid epithelium lacks GFAP,[10,12,14] as we have also confirmed in the present study, the expression of this IF protein by CPTs might be strictly related to the neoplastic transformation, which—as it has been already suggested[9,10]—could elicit an ependymal differentiation of at least some of the neoplastic cells. Indeed, normal ependyma[43] and ependymomas[8,11,14,43] consistently display scattered GFAP-immunoreactive cells, which have also been demonstrated in the current investigation. The ependymal differentiation of CPTs is not surprising, because choroid and ependymal cells are embryologically closely related.[9]

GFAP was consistently coexpressed with vimentin in CPTs, and both IF proteins shared the same subcellular compartmentalization. This could well be in keeping with a previous immunoelectron-microscopic investigation on human glioma cell lines expressing both vimentin and GFAP which demonstrated that the two distinct proteins are assembled in the same IF.[44]

Moreover, in 7 cases of our series we could identify a triple expression of distinct IF proteins (cytokeratins, vimentin, and GFAP) in CPTs. Our results extend those already reported,[14] because we could demonstrate that this simultaneous expression of IF reflects an actual capability of the neoplastic cells to cosynthesize distinct IF proteins and is not due to the heterogeneity of the neoplastic cell population. This exceptional finding has never been reported in central nervous system (CNS) tumors, and the simultaneous expression of cytokeratins and GFAP by the same cell has long been considered "forbidden."[45]

Recently, however, this unique pattern of IF expression has been identified in mixed tumors of salivary glands.[46] We have also confirmed this finding in 10 mixed tumors of the salivary glands and of the skin (unpublished observations). Moreover, a triple expression of IF proteins (cytokeratins, vimentin, and neurofilaments) has been reported in medullary thyroid carcinomas.[42] It should be therefore derived from these observations that the ever-increasing number of immunocytochemical investigations on human tumors with monoclonal and polyclonal antisera specific for IF proteins makes no longer tenable a rigid restriction of a single IF protein to a given oncotype.

A major concern regarding immunocytochemical evidence for IF coexpression by the same normal or neoplastic tissues is the possibility that these data might be due to the detection of antigenic determinants common to different IF proteins.[47,48] In the absence of physicochemical and immunochemical analysis of CPT extracts, cross-reactivity cannot be completely excluded. The results of the present immunocytochemical study, however, are most unlikely to be due to the detection of common epitopes. Indeed, the selective subcellular localization of the different IF proteins and the heterogeneity in the staining pattern of different neoplastic cells in the same tumor allow us to rule out the possibility of a false colocalization of IF proteins.

As it has been already pointed out, the epithelial nature of CPTs has been further substantiated, in addition to the identification of cytokeratins, by the localization of two other epithelial markers, namely, the antigen defined by the lu-5 monoclonal antibody and EMA. As far as we know, no previous investigations on the distribution of these markers in CPTs have been reported.

The lu-5 antigen was expressed by the same CPTs immunoreactive for cytokeratins. Even though this antigen has so far not been fully characterized, it has been reported as biochemically distinct from cytokeratins.[15] Moreover, the reactivity of the lu-5 monoclonal antibody with a large series of human tumors has

been shown to be different from that of a polyclonal antiserum to cytokeratins.[15] Our results, however, point to closer relationships between the antigen defined by the lu-5 monoclonal antibody and cytokeratins (as identified by the two monoclonal antibodies used in the current study). Indeed, we could demonstrate not only that the two antigens were expressed by the same CPTs, but also that their subcellular localization was exactly the same.

Eleven of the 16 CPTs (68.7%) showed at least focal immunoreactivity for EMA, in a manner similar to that of the normal cell counterpart. EMA has been identified in most epithelia and their derived tumors.[24,49] Therefore, the expression of this antigen by CPTs is consistent with their epithelial nature.

Finally, S-100 protein was expressed by all the CPTs but 1 (a pigmented choroid plexus carcinoma), which confirmed that S-100 protein immunoreactivity is not confined to benign CPTs,[16] as has been recently claimed.[13]

In conclusion, CPTs show a complex and heterogeneous pattern of expression of different immunocytochemical markers that is not specific for any histologic subtype—among those investigated in the current study—and does not allow recognition of malignant variants. However, from a diagnostic point of view, the immunocytochemical characterization of CPTs with a panel of antibodies to IF proteins, lu-5 antigen, EMA, and S-100 protein should provide a valuable key for their differential diagnosis with respect to other primary and metastatic CNS tumors. Indeed, the localization of cytokeratins, lu-5 antigen, and EMA allows differentiation of CPTs from primary CNS tumors such as ependymomas, whereas the expression of vimentin, GFAP, and S-100 protein could be useful for distinguishing CPTs from most metastatic carcinomas.

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