Intermediate Filament Proteins in Choroid Plexus and Ependyma and Their Tumors

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The intermediate filament protein types of normal choroid plexus and ependymal tissue and their putative tumors were investigated. In normal human choroid plexus tissue, but not in ependyma, keratin could be demonstrated immunohistochemically. By immunoblotting, keratins 8, 18, and 19 were found, but glial fibrillary acidic protein (GFAP) was absent. In mouse and rat, choroid plexus epithelium and ependymal lining cells were keratin-positive. In addition, many ependymal cells were vimentin-positive. Keratin was immunohistochemically found in three of four choroid plexus papillomas, two of two choroid plexus carcinomas, and the lining cells of three neuroepithelial cysts. GFAP-positive cells were present in some choroid plexus tumors. In contrast, none of the eight ependymomas contained keratin, but all were strongly positive for GFAP. The results show that choroid plexus lining cells and choroid plexus tumors have true epithelial characteristics in their cytoskeleton, in contrast to ependymomas, which do not show keratin positivity but show glial filaments, as would be seen in astrocytic tumors. (Am J Pathol 1986, 123:231–240)

TYPING of the cytoskeletal intermediate filament proteins has become a powerful tool in the analysis of cell differentiation both in normal and in neoplastic tissues.[1–6] In general terms which also pertain to malignant neoplasms, epithelial cells contain keratin(s); mesenchymal nonmuscular cells, vimentin; muscle cells, desmin; neural cells, neurofilaments; and glial cells, glial fibrillary acidic protein (GFAP).[1–6]

In the central nervous system, tumors that show true neural differentiation contain neurofilaments,[7] most gliomas contain GFAP,[8–11] and meningiomas contain vimentin.[12] The only keratin-positive primary neoplasms of the central nervous system reported so far seem to be the choriod plexus tumors.[13,14] Choroid plexus cells have the morphologic features of epithelial cells, and hence one would expect that tumors featuring these cells would express keratin, conforming with their epithelial nature.

We undertook the present study to verify whether choroid plexus tumors indeed expressed keratins. Because there were no data on the intermediate filament content of normal choroid plexus cells, we also examined normal choroid plexus tissues by immunofluorescence microscopy and immunoblotting. Furthermore, we compared the choroid plexus epithelium with the ependymal lining cells, as well as ependymomas, to obtain some insight into the differences and/or similarities between these cells. Human material, which was never optimally preserved for immunochemical studies, was supplemented with animal tissue. We show that in mouse and rat, the choroid plexus lining cells, as well as the ependymal cells, express keratin. In humans, keratin was demonstrated in choroid plexus cells and corresponding tumors, whereas GFAP was demonstrated in ependymomas and focally in some choroid plexus tumors.

Materials and Methods

Tissue Material

Frozen sections from choroid plexus tissue and ependymal linings of the posterior horns of the lateral ventricles from adult humans (n = 7) were obtained from autopsies performed less than 24 hours after death. For comparative purposes, sagittal sections from cerebral

Supported by the Finnish Medical Research Council.
Accepted for publication November 27, 1985.
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hemispheres of adult Wistar rats (n = 6) and Swiss Webster mice (n = 7), including choroid plexus and ependymal linings of the lateral, third, and fourth ventricles were also studied. We also studied frozen sections of fetal mouse brains of 15–18 gestational days and the brains from newborn 1-, 4-, 7-, and 14-day-old mice. The following human tumors were studied: neuroepithelial cysts of the third ventricle (n = 3), choroid plexus papillomas of lateral ventricles (n = 4), choroid plexus carcinomas (n = 2), and ependymomas mainly from the fourth ventricle region (n = 8). The clinical data of the tumor patients is summarized in Table 1. No tumors were found elsewhere in the follow-up of patients with choroid plexus carcinomas. All tumor material had been originally fixed in formalin and routinely embedded in paraffin.

Antibodies

PKK1, a monoclonal antibody to keratin cytokeratin, described elsewhere[15–17] reacts with keratins of molecular weights 52, 45, and 40 kd (ie, keratins 8, 18, and 19, according to the numbering system of Moll et al.[18] Rabbit antiserum to vimentin,[19] monoclonal antibodies to vimentin,[20] and monoclonal antibodies to GFAP[16,21] have been fully characterized elsewhere.

Three monoclonal antibodies to neurofilaments were used: NF1, which reacts with the 200-kd subunit protein and NF2, which reacts with the 200-kd and the 70-kd subunit protein.[16] In some cases, a monoclonal antibody, NF3 (Virtanen et al, in preparation), reacting with the 200-kd and the 150-kd neurofilament subunit protein was used. Monoclonal antibodies PKK1, NF1, and anti-GFAP and the rabbit antiserum to vimentin react with formalin-fixed and paraffin-embedded tissues. Monoclonal antibodies to vimentin, NF2, and NF3 antibodies do not react with paraffin-embedded tissues and were used only on frozen sections.

Immunostaining

The staining was performed as described previously.[22] Frozen sections were fixed in methanol or acetone (−20 C for 10 minutes). After deparaffinization, the paraffin sections of formalin-fixed material were subjected to proteolytic treatment with 0.4% pepsin in 0.01 N HCl for 30 minutes to 2 hours at 37 C in order to enhance antigen exposure.[6,23] The primary antibodies were used at a concentration of 25–50 μg/ml and after careful washing were followed by fluorescence isothiocyanate-conjugated (FITC) goat anti-mouse IgG or goat anti-rabbit IgG (Cappel Laboratories, Cochranville, Pa) and coverslipped with phosphate-buffered glycerol (pH 7.4).

Immunoblotting Experiments

Choroid plexus tissues from two autopsies performed less than 24 hours after death were studied. The tissue was mechanically dissociated into a single-cell suspension in 0.15 M phosphate-buffered saline (pH 7.4). The suspension was centrifuged, and the supernatant was discarded. The cells in the pellet were homogenized with a glass homogenizer in 50 mM Tris-buffer the presence of chelating agents (EDTA, EGTA), protease inhibitors (antipain, pepstatin, and phenylmethylsulfonylfluoride) and 0.1% triton X-100. The homogenate was centrifuged and the pellet was treated with the cytoskeleton extraction buffer containing 1% SDS and 50 μM dithiotreitol in 25 mM Tris buffer (pH 7.5) at 100 C for 5 minutes. After the extraction buffer treatment, the homogenate was centrifuged, and the supernatant was used for polyacrylamide gel electrophoresis in 10% slab gels in the presence of SDS according to Laemmli et al.[24] The separated polypeptides were electrophoretically transferred to nitrocellulose membranes according to Towbin et al,[25] and immunostained with monoclonal antikeratin antibodies AE1, AE3, Chrome 1 and anti-GFAP. AE1 reacts with keratins of 40, 48, 50, 54, 55, and 56.5 kd (keratins of the acidic subfamily corresponding the numbers 19, 16, 14–15, 13, and 12, respectively)[26,27]; AE3 reacts with all keratins of the basic subfamily (52 to 67 kd) corresponding the numbers 8, 7, 6, 5, 4, 3, and 1–2, respectively[26,27]; Chrome 1 reacts with keratin of 45 kd from the acidic subfamily (keratin 18).[28] After the primary antibodies, horseradish peroxidase-coupled goat anti-mouse IgG antisera were applied, and the color was developed with 0.06% diaminobenzidine in the presence of 0.03% hydrogen peroxide in 0.05 M Tris buffer (pH 7.7). Samples of known keratin polypeptide content were electrophoresed in parallel with the choroid plexus tissue extracts for proper identification of the keratins.

Results

The immunohistochemical data on the human, rat, and mouse choroid plexus and ependyma and the corresponding human tumors are summarized in Tables 1 and 2.

Normal Human Choroid Plexus and Ependymal Lining Cells

The lining cells of the normal choroid plexus reacted with the PKK1 antibody to keratin. The basal portions of the cytoplasm of the lining cells showed the strongest reactivity (Figure 1a). The stromal, but not the epithelial, cells were positive for vimentin (Figure 1b). No

Table 1—Clinical Data and Intermediate Filament Immunostaining Results of Neuroepithelial, Choroid Plexus, and Ependymal Tumors

Case	Age	Sex	Diagnosis	Keratin	Vimentin	GFAP
1	28 years	Male	Neuroepithelial cyst of the third ventricle	+	−	−
2	29 years	Male	Neuroepithelial cyst of the third ventricle	+	−	−
3	46 years	Male	Neuroepithelial cyst of the third ventricle	+	−	−
4	37 years	Female	Papilloma of plexus choroideus	+	+	+
5	33 years	Male	Papilloma of plexus choroideus	+	−	−
6	25 months	Male	Papilloma of plexus choroideus	+	+	−
7	5 months	Male	Papilloma of plexus choroideus	−	−	−
8	10 months	Male	Carcinoma of plexus choroideus (poorly differentiated)	+	+	+
9	37 years	Female	Carcinoma of plexus choroideus (well differentiated adeno-carcinoma)	+	−	−
10	16 months	Male	Ependymoma of the cerebellum	−	+	+
11	21 months	Male	Ependymoma of the fourth ventricle region	−	+	+
12	4 years	Male	Ependymoma	−	+	+
13	9 years	Female	Ependymoma, Parieto-occipital right cerebral hemisphere, in ventricle	−	+	+
14	10 years	Male	Ependymoma fourth ventricle and vermis	−	+	+
15	11 years	Female	Ependymoma of fourth ventricle region	−	+	+
16	28 years	Female	Ependymoma, fourth ventricle filling tumor	−	+	+
17	46 years	Female	Ependymoma of fourth ventricle region	−	+	+

The results refer to the tumor cells, and vimentin positivity present in the stromal elements has been excluded.

Table 2—Immunostaining Results for Intermediate Filament Proteins in Adult Human and Developing and Adult Mouse and Rat Choroid Plexus and Ependymal Lining Cells

	Choroid plexus lining cells			Ependymal lining cells		
	Keratin	Vimentin	GFAP	Keratin	Vimentin	GFAP
Human, adult	+	−	−	−	−	−
Mouse, 16th intrauterine day	−	+	−	−	+	−
Mouse, 18th intrauterine day	(+)	+	−	(+)	+	−
Mouse, 1st postnatal day	(+)	(+)	−	(+)	+	−
Mouse, 4th postnatal day	+	−	−	+	+	−
Mouse, 7th postnatal day	+	−	−	+	+	−
Mouse, 14th postnatal day	+	−	−	+	+	−
Mouse/rat adult	+	−	−	+	+	−

+, most cells positive; (+), a minority of cells positive.

GFAP or neurofilament-positive cells could be detected in the normal choroid plexus. Human ependymal lining cells of the lateral ventricles showed no distinct reactivity with any of the intermediate filament protein antibodies used in this study, except some neurofilament-positive nerve fibers between the ependymal lining cells. Attempts to unmask the possible masked antigenic determinants as shown by Franke et al[29] also gave negative results. The neuropil underneath the ependyma reacted with antibodies to GFAP and vimentin but was unreactive with antibodies to neurofilaments except for some scattered axons (data not shown). In immunoblotting experiments, the choroid plexus tissues from two autopsies consistently showed keratins

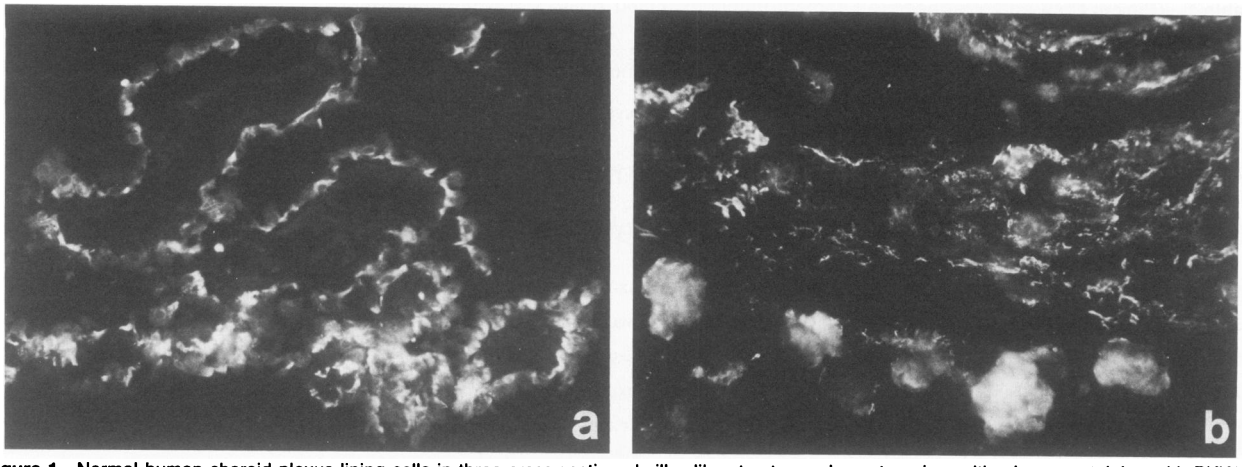


Figure 1—Normal human choroid plexus lining cells in three cross-sectioned villus-like structures show strongly positive immunostaining with PKK1 monoclonal antibodies to keratin (**a**). Monoclonal antibodies to vimentin react with stromal but not epithelial cells of choroid plexus in three longitudinally sectioned villuslike structures. Note also the fluorescence in psammoma bodies (**b**). (Immunofluorescence, ×200)

of 40, 45, and 52 kd (keratins 19, 18, and 8, respectively) (Figures 2 and 3). No glial fibrillary acidic protein could be detected (Figure 4).

Animal Tissues

In adult rat (Figure 5a) and mouse tissue both the choroid plexus lining cells and the cuboidal ependymal cell layer lining the lateral third and fourth ventricles were strongly positive for keratin and were negative for neurofilaments. GFAP positivity appeared in cells with cytoplasmic processes just underneath the ependymal lining but not within it (Figure 5b). These cells probably represented the so-called tanacyte type of ependymal cells. Vimentin positivity could be found in a major portion of the ependymal lining cells; but in choroid plexus, positivity was seen only in the stroma and not in the lining cells (Figure 5c and d). Fetal mice showed only vimentin, and not keratin, positivity in the ependymal lining and the developing choroid plexus until the 18th day, when isolated keratin-positive ependymal

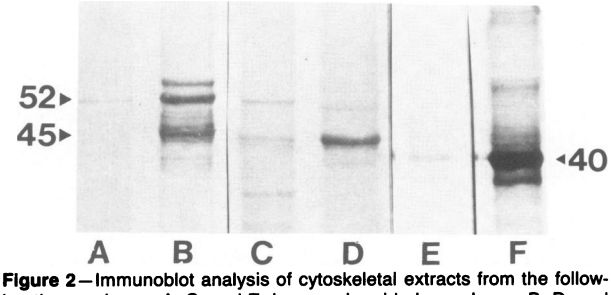


Figure 2—Immunoblot analysis of cytoskeletal extracts from the following tissues. Lanes A, C, and E, human choroid plexus; Lanes B, D, and F, human term placenta, as controls. Lanes A and B were immunostained with monoclonal antibody AE-3. Lane A contains one immunoreactive band, corresponding to keratin 8 (52 kd). Lane B contains two immunoreactive bands corresponding to keratins 7 (54 kd) and 8 (52 kd). Lanes C and D were immunostained with monoclonal antibody Chrome-1. A 45-kd immunoreactive band corresponding to keratin 18 can be seen in both lanes. Lanes E and F were immunostained with monoclonal antibody AE-1. A 40-kd immunoreactive band corresponding to keratin 19 can be seen in both lanes.

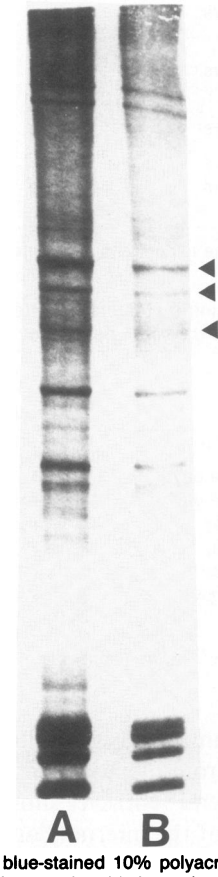


Figure 3—Coomassie blue-stained 10% polyacrylamide gel containing cytoskeletal extracts of human choroid plexus: Lane A, heavily loaded with sample, as shown in Figures 2 and 3; Lanes B, less heavily loaded with sample, shown for clarity. *Arrowheads* indicate the position of the keratin polypeptides.

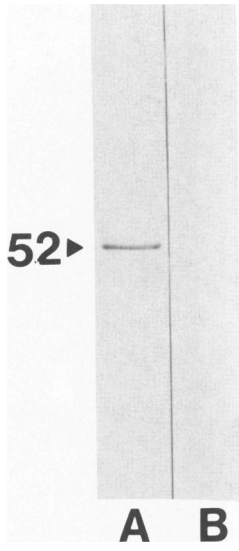


Figure 4—Immunoblot analysis of cytoskeletal extracts of mouse brain (Lane A) and human choroid plexus (Lane B). This blot was reacted with a monoclonal antibody immunoreactive with GFAP. A single 52-kd immunoreactive band is detected in Lane A; no immunoreactive band is seen in Lane B.

and choroid plexus lining cells could be detected (Table 2, Figure 6a). At the 18th gestational day and in newborn mice all ependymal and choroid plexus cells still reacted with the antibody to vimentin (Figure 6b), but the staining intensity and number of positive cells was diminishing in choroid plexus lining cells during the development. Some GFAP-positive cells were found lining the ventricles of late prenatal and young mice (Table 2, Figure 6c). These cells probably did not belong to the ordinary ependymal lining cells and may reflect the incompleteness of the developing ependymal cell layer.

Neuroepithelial Cysts

All three neuroepithelial cysts (colloid cysts of the third ventricle) showed keratin-positivity in the epithelial-like lining cells. (Figure 7). The underlying stromal cells were vimentin-positive. GFAP or neurofilament positivity was seen only in the included fragments of brain tissue.

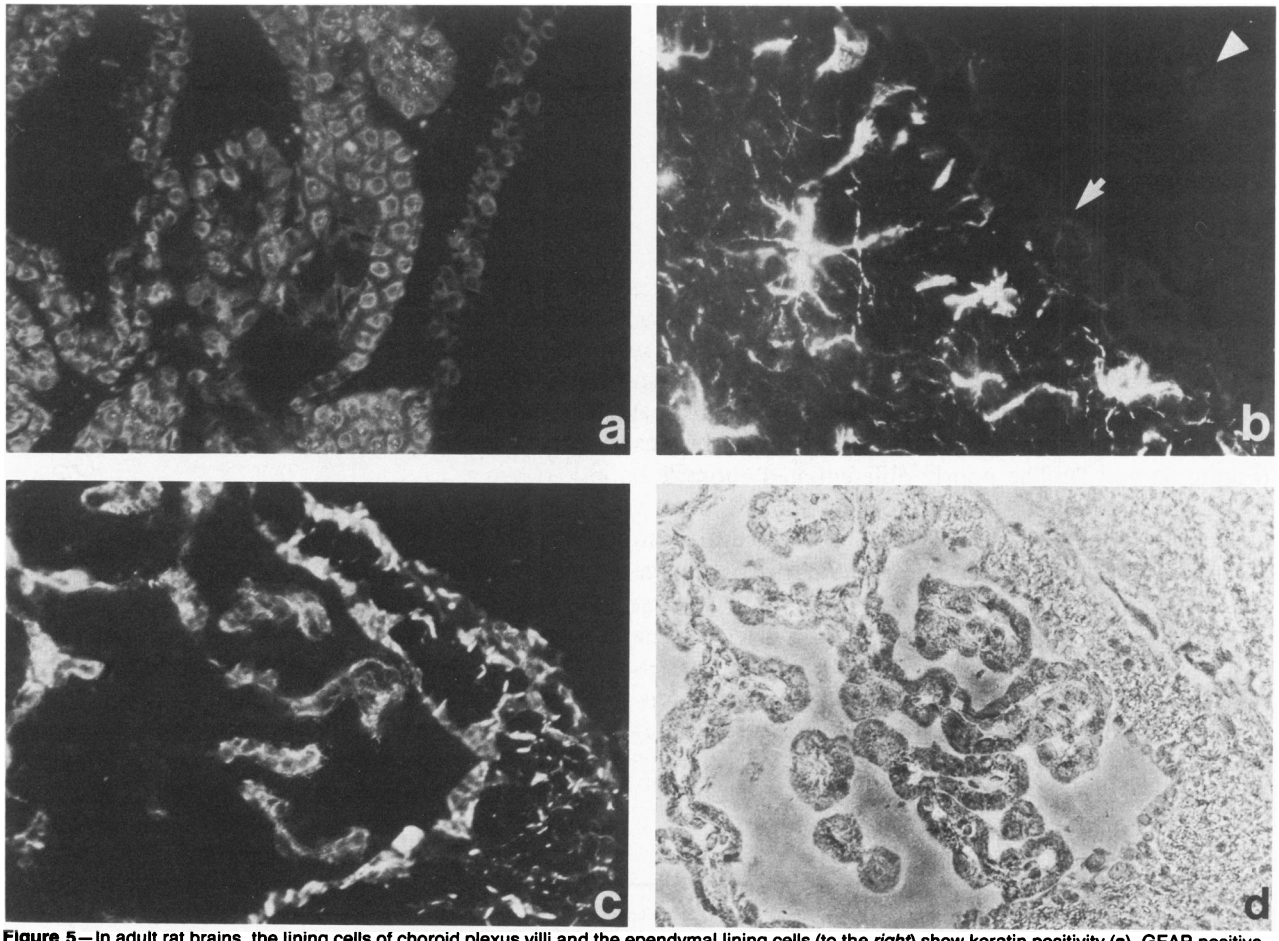


Figure 5—In adult rat brains, the lining cells of choroid plexus villi and the ependymal lining cells (to the *right*) show keratin positivity (**a**). GFAP-positive cells are found subependymally and within the brain substance but not in the ependymal lining (*arrow*). The choroid plexus structures are negative (*arrowhead*) (**b**). Vimentin positivity is seen in the stromal, but not epithelial, elements of the choroid plexus, in the ependydmal lining cells and many cells underneath the ependyma (**c**). **d**—Phase-contrast micrograph of the same area as vimentin-immunostaining. **a–c**, immunofluorescence; **a**, **c**, and **d**, ×150; **b**, ×250)

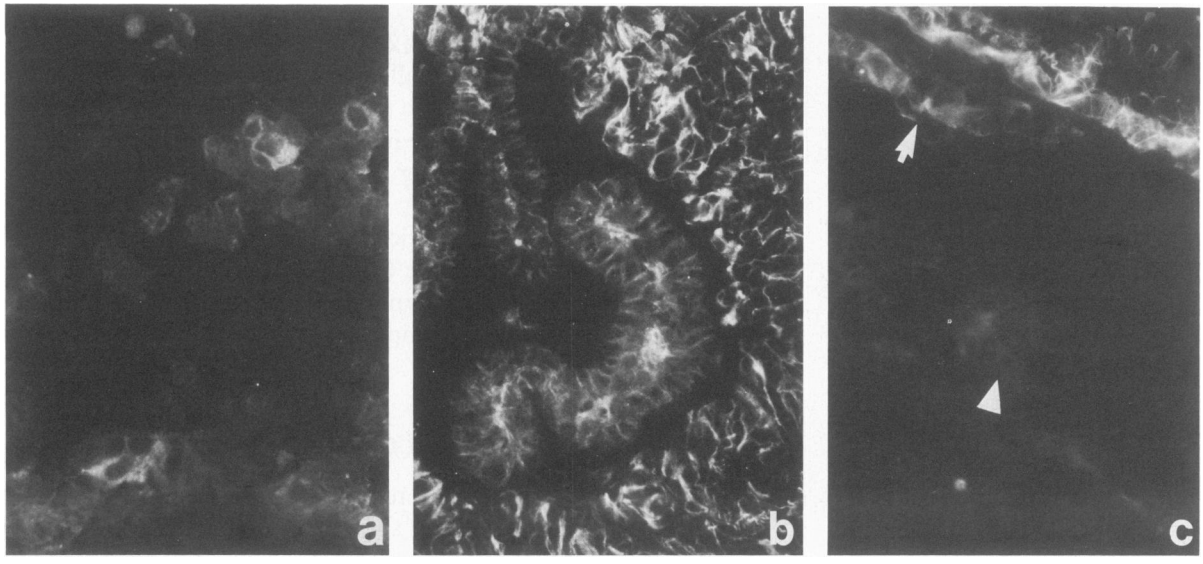


Figure 6—In developing mouse brain on the 18th gestational day, the first keratin-positive cells appear in the ependymal lining (*bottom*) and choroid plexus (*top right*), whereas most of the cells are still negative for keratin (**a**). Vimentin positivity is seen among all the brain cells, including ependymal lining cells, and also in the choroid plexus in newborn mice (**b**). GFAP positivity is seen in some cells (*arrow*) close to the ependymal lining in newborn mice, whereas choroid plexus tissues are negative (*arrowhead*). (Immunofluorescence; **a**, ×300; **b**, ×150; **c**, 200)

Choroid Plexus Tumors

In three of four choroid plexus papillomas, keratin-positivity appeared in the tumor cells lining the papillae (Figure 8a and b). A certain number of tumor cells were also GFAP-positive, and a few tumor cells were vimentin-positive, like the stromal and vascular components. In choroid plexus carcinomas, keratin was present in all tumor cells in an adenocarcinoma-like neoplasm (Figure 8c), and occasional groups of GFAP-positive stromal cells were present (Figure 8d). The majority of tumor cells were keratin-positive in a poorly differentiated choroid plexus carcinoma with solid areas but still papillomatous elements elsewhere in the tumor (Figure 8e and f). The poorly differentiated choroid plexus carcinoma also showed GFAP (Figure 8g) and vimentin-positive tumor cells (Figure 8h). No neurofilament positivity was found in choroid plexus tumor cells.

Ependymomas

All the cerebellar ependymomas of the fourth ventricle region and cerebral ependymomas showed GFAP positivity in the majority of tumor cells. The pattern of staining varied and included either prominent fibrillar GFAP positivity in typical ependymomas with perivascular rosettes (Figures 9a and b) or was in form of perinuclear cytoplasmic staining in a papillary ependymoma (Figures 9c and d). No keratin-positive cells were detected in any of the ependymomas studied. Varying numbers of vimentin-positive cells were seen in all ependymomas, and no neurofilament-containing tumor cells were found.

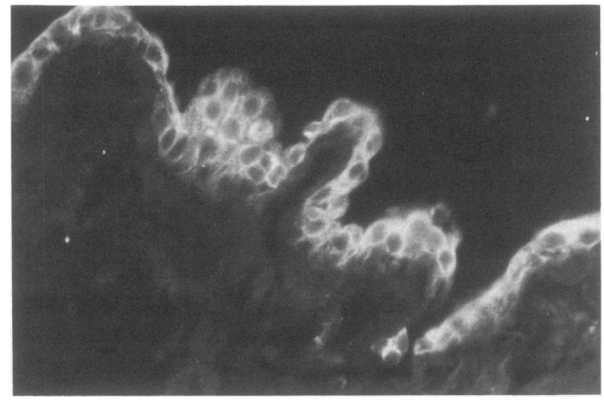
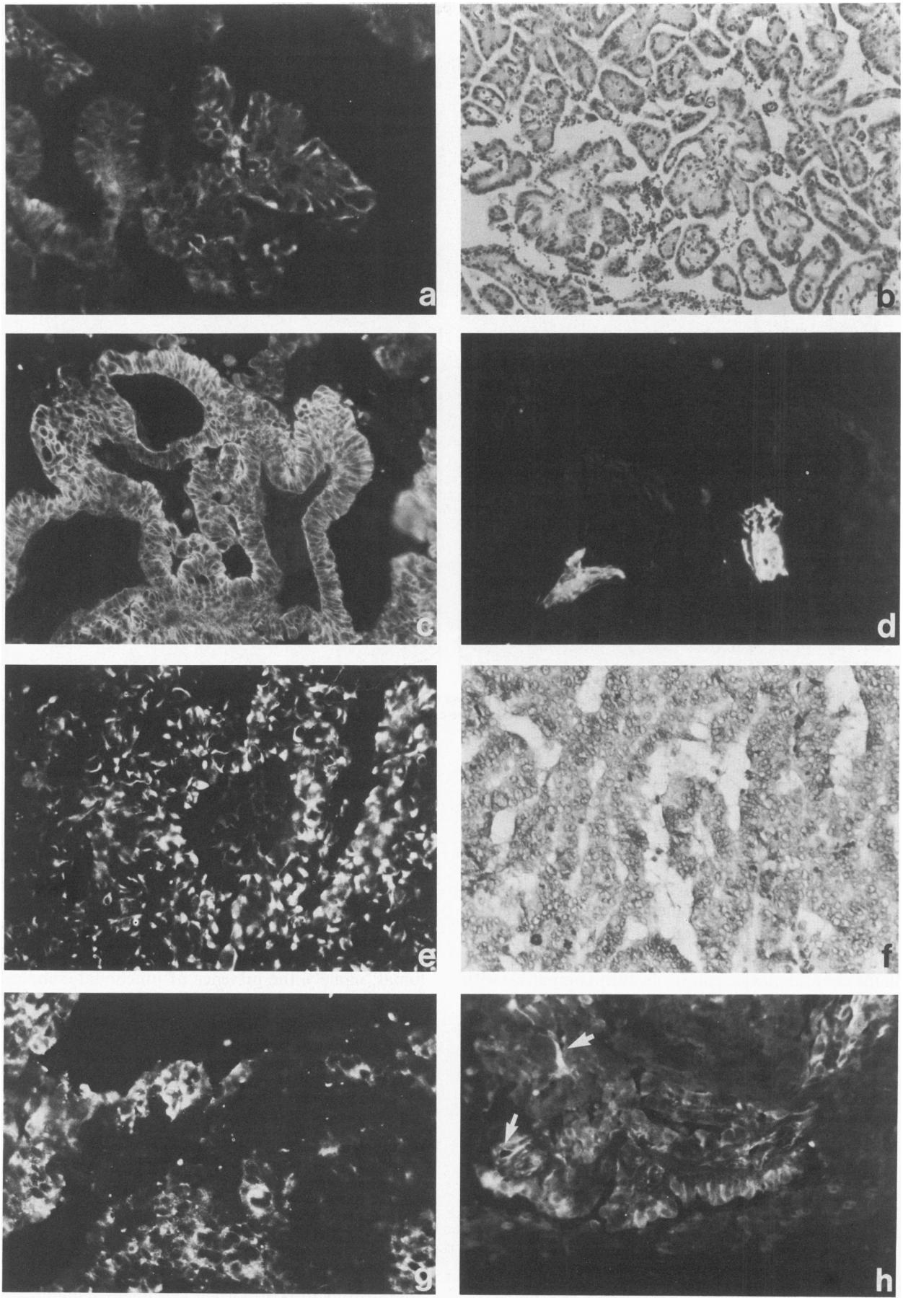


Figure 7—The lining cells of a neuroepithelial cyst are uniformly keratin-positive, and the stroma is negative. (Immunofluorescence, ×350)

Figure 8—Choroid plexus papilloma shows keratin positivity in most of the lining cells (**a**). A hematoxylin and eosin staining of the same tumor is shown (**b**). A choroid plexus carcinoma featuring moderately differentiated adenocarcinoma shows keratin positivity in the epithelial-like lining cells (**c**). The same tumor shows some GFAP-positive stromal cells in the same area, while the epithelial elements are negative (**d**). A choroid plexus carcinoma with solid and trabecular areas shows keratin positivity in most tumor cells (**e**). A hematoxylin and eosin staining of the tumor is shown in (**f**). Many tumor cells show GFAP positivity in the same choroid plexus carcinoma (**g**). Vimentin is present in some tumor cells. Note also vimentin positivity in the vascular endothelial cells (*arrows*) (**h**). (Immunofluorescence; **a**, **c**, **d**, **f–h**, ×250; **b**, ×100; **e**, ×150)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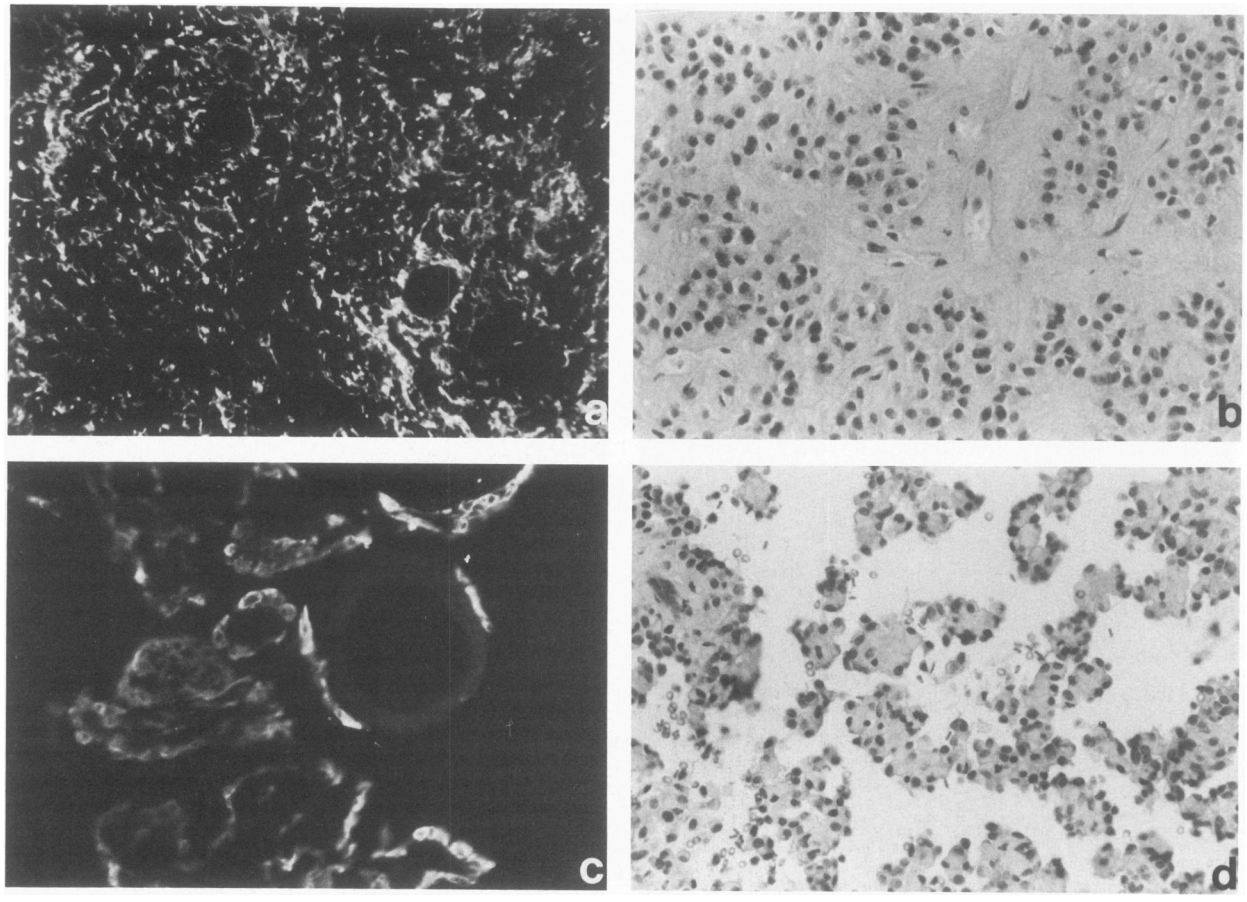

Figure 9—A solid ependymoma with perivascular rosettes (**a** and **b**) shows strong GFAP positivity in most tumor cells. A papillary ependymoma also shows GFAP positivity in the lining cells of papillary structures (**c** and **d**). (**a** and **c**, immunofluorescence; **b** and **d**, hematoxylin and eosin, **a** × 150; **b–d**, × 200)

Discussion

In this study, we examined the intermediate filament protein expression in choroid plexus and ependymal cells and their putative tumors.

The results show that human choroid plexus lining cells contain keratins as studied with both immunofluorescence staining and immunoblotting technique. Keratin positivity indicates true epithelial differentiation in the choroid plexus lining cells, fully compatible with the morphologic epithelial-like appearance of these cells. The choroid plexus lining cells appear to contain simple epithelial type keratins 8, 18, and 19 in the numbering system of Moll et al.[18] In this respect these cells resemble other simple nonstratified epithelial cells, for instance, gastrointestinal epithelial cells.[18] The keratin positivity of choroid plexus lining cells was also confirmed in rat and mouse brain samples. The epithelial cytoskeleton in choroid plexus lining cells is in line with the ultrastructural appearance of these cells, which rest on a basement membrane and show desmosome-like junctions and arrays of apical microvilli, resembling those present for instance in proximal tubules of the kidney.[30]

Choroid plexus tumors and neuroepithelial cysts showed keratin-positive tumor cells and thus have true epithelial characteristics. Keratin positivity has recently been reported in choroid plexus papillomas and carcinomas,[13,14] but has been thought to be in contrast with the situation in choroid plexus tissues,[14] which we now show to contain keratins. Ultrastructural observations also suggest epithelial features in choroid plexus papillomas, namely, the presence of junctional complexes and basal laminae underneath the cells.[31] The focal GFAP positivity observed in some choroid plexus tumors can be regarded as glial differentiation feature. Focal GFAP positivity in choroid plexus papillomas was also noted by Rubinstein et al.[32] The presence of keratin in choroid plexus tumors indicates that keratin-positive primary brain tumors exist and are not necessarily metastases.

Ependymal lining cells in adult rats and mice contained keratin and vimentin immunoreactivity, whereas in humans we were not able to demonstrate any immunofluorescence immunoreactivity. Whether this is due to species differences or the less than optimal nature of human postmortem specimens remains to be

determined. In adult human ependyma, however, GFAP negativity is in line with previous findings.[32,33] The keratin positivity of rat ependyma is compatible with its ultrastructural appearance similar to epithelial linings.[34] Thus, rat ependymal cells contain zonula occludens and zonula adherens type of junctions and perinuclear whorls of intermediate filaments suggestive of tonofilamentlike organization.[34] Our results on embryos are in line with previous data showing that rat embryos have vimentin positivity throughout the neural tube[35] from Day 12 on. Previous data also indicate that vimentin is present in adult rat and mice ependyma.[36,37] The development of keratin-positive cells to neuroepithelium probably is a similar switch in the intermediate filament expression, as is, for example, the formation of keratin-positive renal tubules from their vimentin-positive precursors.[38] Transient presence of GFAP-positive cells lining the ventricles in embryonal and early postnatal mice might reflect the incompleteness of the ependymal lining, and it seems probable that these cells do not, in fact, belong to the ordinary ependymal cell population, but to glial cells which have their processes to reach the ventricles at some developmental stages. However, GFAP positivity in developing ependymal cells has been described in humans.[33]

Cerebellar ependymomas contained GFAP-positive cells, as shown before by several groups,[8,9,39] but did not contain keratins and therefore sharply contrast with choroid plexus tumors, a feature of potential differential diagnostic significance. By their content of GFAP and vimentin, ependymomas show features parallel to early developmental human ependymal cells to which ependymomas have been histogenetically linked; ependymomas have been considered to represent tumors featuring primitive ependymoglial tissue.[40] In light of ultrastructural features, namely, the presence of cilia and junctional complexes,[41] and their patterns of intermediate filaments expression, the relationship of ependymomas to primitive ependymoglia seems indeed possible.

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