Virchows Archiv A Pathol Anat (1990) 417:97–103

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistochemical study of ependymal neoplasms: histological subtypes and glial and epithelial characteristics

Yoichi Kaneko[1,2], Iwao Takeshita[2], Toshio Matsushima[2], Toru Iwaki[1], Takatoshi Tashima[2], and Masashi Fukui[2]

Departments of [1] Neuropathology and [2] Neurosurgery, Neurological Institute, Faculty of Medicine, Kyushu University 60 Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan

Received August 30, 1989 / Received after revision February 7, 1990 / Accepted February 14, 1990

Summary. An immunohistochemical study on ependymal tumours was performed in order to determine what relationships exist between histological subtypes and epithelial or glial characteristics. Thirty-eight ependymal tumours were examined with antibodies to cytokeratin (CK), epithelial membrane antigen (EMA), transthyretin (TTR) and glial fibrillary acidic protein (GFAP) using the avidin-biotin-complex technique. They included 23 ependymomas, 13 anaplastic ependymomas, and 2 myxopapillary ependymomas. Only 3 of the 23 ependymomas were positive with EMA but 19 reacted with GFAP. None of them were positive with CK. Six of the 13 anaplastic ependymomas were positive with EMA, 3 with CK and 10 with GFAP. Five of the 6 anaplastic ependymomas which had epithelial marker proteins were either negative or weakly positive for GFAP. The present study demonstrates that most benign ependymomas exhibit GFAP positivity while the anaplastic ones tend to suppress their glial nature in favour of epithelial differentiation. However, ependymal tumours showed few characteristics of choroid plexus cells; only one of the examined cases was positive for TTR.

Key words: Cytokeratin – Ependymal tumour – Epithelial membrane antigen – Glial fibrillary acidic protein – Immunohistochemical study – Transthyretin

Introduction

Ependymal tumours have been extensively studied by anti-glial fibrillary acidic protein (GFAP) immunostaining; many authors have shown that most of them have GFAP positivity.

Epithelial membrane antigen (EMA) and cytokeratin (CK) have been widely accepted as epithelial differentiation antigens in both normal and neoplastic tissues (Frierson et al. 1986; Mills et al. 1987; Moll et al. 1982; Sloane and Ormerod 1981). In the central nervous system (CNS), both epithelial marker proteins were demonstrated in the choroid plexus cell but not in the ependymal cell (Cruz-Sanchez et al. 1988; Doglioni et al. 1987; Kasper et al. 1986; Sloane and Ormerod 1981). In neoplasms, they were expressed not only in choroid plexus tumours (Doglioni et al. 1987; Mannoji and Becker 1988; Matsushima et al. 1988; Miettinen et al. 1986) but also in meningiomas (Holden et al. 1987; Schnitt and Vogel 1986). Ependymal neoplasms have also showed positivity in a few studies (Cruz-Sanchez et al. 1988; Mannoji and Becker 1988). Transthyretin (TTR, prealbumin) is a retinol and thyroxin binding protein which is produced in the normal and neoplastic choroid plexus (Aleshire et al. 1983; Matsushima et al. 1988; Stauder et al. 1986) but ependymomas have not been studied for TTR expression.

An immunohistochemical examination of the 38 ependymal tumours was performed in order to search for a correlation between the histological subtypes and the epithelial or glial characteristics using anti-EMA, CK, TTR and GFAP antibodies.

Materials and methods

Surgical specimens were obtained from 38 ependymal tumours: 23 ependymomas, 13 anaplastic ependymomas and 2 myxopapillary ependymomas. A tumour composed predominantly of uniform ependymal cells forming rosettes, tubules, clefts and papillations as epithelial properties, and perivascular pseudorosettes was diagnosed as ependymoma. Although epithelial-like structures are less common than perivascular pseudorosettes, when present they are diagnostic. The ependymomas were well differentiated and showed no anaplastic features. The anaplastic ependymomas were highly cellular with necroses and frequent mitoses. The presence of pleomorphism, endothelial proliferation and multinucleation suggested the anaplastic ependymoma. The clinical manifestations and histopathological diagnoses of the cases are summarized in Table 1. Ependymomas showed a tendency to be located infratentorially, while the anaplastic ones were more often supratentorial.

Immunohistochemical analysis was performed with anti-EMA

Offprint requests to: Y. Kaneko

Table 1. Clinical and immunohistochemical findings of ependymal tumours

Case	Age/sex	Site	EMA	CK	TTR	GFAP
Ependymomas						
1	23/F	4th v	+[a]	−	−	+
2 (1)	2/M	4th v	+[a]	−	−	+
(2)	4		+[a]	−	−	+
(3)	6	−	+[a]	−	+	+
3	6/M	4th v	+[a]	−	−	+
4	53/F	Cbll-4th v	−	−	−	(+)
5	21/F	4th v	−	−	−	+
6	8/M	4th v-C_2	−	−	−	+
7	3/M	4th v-C_2	−	−	−	+
8	10/F	Lat v-P-O-T	−	−	−	+
9	24/M	4th v	−	−	−	+
10	27/M	Lat v	−	−	−	−
11	2/F	P-T	−	−	−	−
12	10/F	4th v	−	−	−	+
13	44/F	T_{6-9}	−	−	−	+
14	46/F	4th v	−	−	−	−
15	11/F	4th v	−	−	−	+
16	8/M	4th v	−	−	−	(+)
17	1/F	4th v	−	−	−	+
18	18/F	4th v	−	−	−	+
19	0/M	4th v	−	−	−	+
20	44/M	C_{5-6}	−	−	−	+
21	62/M	C_{2-5}	−	−	−	+
22	43/F	C_1	−	−	−	+
23	57/F	T_{7-10}	−	−	−	+
Anaplastic ependymomas						
24	7/F	Fr	+[b]	(+)	−	(+)
25	14/M	Lat v-P	+[a]	−	−	−
26	0/M	4th v	+[b]	+	−	(+)
27	13/M	Fr-P	+[a]	−	−	−
28 (1)	20/F	Fr	+[b]	(+)	−	(+)
(2)	22		+[b]	+	−	−
29	8/F	4th v	+[a]	−	−	+
30	6/M	Fr-P-T	−	−	−	+
31	13/F	P-T-O	−	−	−	−
32	18/F	3rd v-C2	−	−	−	+
33	15/M	3rd–4th v	−	−	−	+
34	16/M	Fr-P	−	−	−	+
35	5/M	4th v	−	−	−	+
36	3/M	Fr	−	−	−	+
Myxopapillary ependymomas						
37	16/M	Cauda equina	−	−	−	+
38	36/M	Cauda equina	−	−	−	+

M, Male; F, female; Fr, frontal; T, temporal, P, parietal; O, occipital; Cbll, cerebellar; C, cervical; T, thoracic; Lat, lateral; v, ventricle

+[a] The EMA positivities were mainly confined to the luminal surface of the epithelial like structures as linear deposits

[b] Plasma membrane of the tumour cells in glial region were stained with anti-EMA

(+) A few positive cells

monoclonal antibody (DAKO, Copenhagen, Denmark, 1:100), anti-CK monoclonal antibody (Becton-Dickinson Mountain View CA USA, 25 μg/ml), anti-TTR (Kitamoto et al. 1985; 1:1000) and anti-GFAP (Mannoji et al. 1981; 1:1000) using the avidin-biotin complex (ABC) method. Streptavidin and biotin reagents were obtained from Biogenix Laboratories (Dublin CA USA). The specimens were fixed in 10% formalin, embedded in paraffin and sectioned at 6 μm serially. The sections were stained with haematoxylin and eosin. Deparaffinized sections were incubated in 0.3% H_2O_2 in methanol to inhibit the endogenous peroxidase activity for 30 min. The sections were subsequently incubated in primary antibody diluted in 0.05 M Tris-HCl pH 7.6 with 10% normal goat serum added at 4° C overnight, biotin-labelled goat anti-rabbit or mouse for 30 min at room temperature, and streptavidin-biotinylated peroxidase complex for 30 min at room temperature. Each step was followed by three 10 min washes in 0.05 M Tris-HCl pH 7.6. The peroxidase reaction was developed with 0.01% 3,3′-diaminobenzidine (Nakarai Chemicals Kyoto Japan) and 0.003% H_2O_2 in 0.05 M Tris-HCl. The sections were counterstained lightly with haematoxylin. When anti-CK was used, the sections were pretreated with 0.5% pepsin in 0.01 N HCl for 45 min at 37° C before blocking of endogenous peroxidase activity.

Results

Three of the 23 ependymomas showed positivity with anti-EMA immunostain. In these cases, the immunoreactive products were present as linear deposits on the luminal surface of the epithelial-like structures such as ependymal rosettes and tubules, ependymal epithelia and papillary structures (Fig. 1A). In 1 of the 3 positive cases, anti-EMA also showed a granular cytoplasmic positivity. However, all of the 23 ependymomas were negative to CK.

Anti-TTR reacted to one case of ependymoma (case 2), in which three operations had been performed. TTR positivity was observed in the ependymal epithelium of the specimen obtained at the third operation (Fig. 1B), where immunoreactive products were visualized as intracytoplasmic granular deposits (Fig. 1C). TTR-positive cells were also stained with anti-EMA (Fig. 1D) but not with anti-CK.

Nineteen of the 23 cases showed positivity for GFAP. Immunoreactive products with anti-GFAP were mainly present in the cell processes of so-called perivascular pseudorosettes extending to the vascular wall. Tumour cells forming ependymal true rosettes were occasionally positive. The cell bodies of the interposed tumour cells were stained with anti-GFAP to various degrees.

Six of the 13 cases of anaplastic ependymomas demonstrated positivity for EMA. Anti-EMA stained anaplastic ependymomas in two ways: (1) immunoreactive products were mainly confined to the luminal surface of the epithelial like structures (cases 25, 27 and 29) or (2) the linear deposits (containing some granular deposits) at the peripheral aspects of the tumour cell (which presumably represented the cell surface) were observed in the cellular part of the tumour (cases 24, 26 and 28). All of 3 cases of the former group showed CK negativity, whereas 3 cases of the latter showed CK positivity. Immunostaining with anti-TTR revealed no positive cells in any of the anaplastic ependymomas. In 10 cases the tumours were stained with anti-GFAP. However, 5 of the 6 anaplastic ependymomas which had epithelial differentiation antigen were negative or weakly positive for GFAP.

In case 27, a considerable number of ependymal rosettes and tubules were present (Fig. 2A), and anti-EMA gave reaction to their luminal surfaces and microcysts as linear deposits (Fig. 2B). Intracytoplasmic granular deposits were occasionally seen. In this case, tumour cells were stained with neither anti-GFAP nor anti-CK.

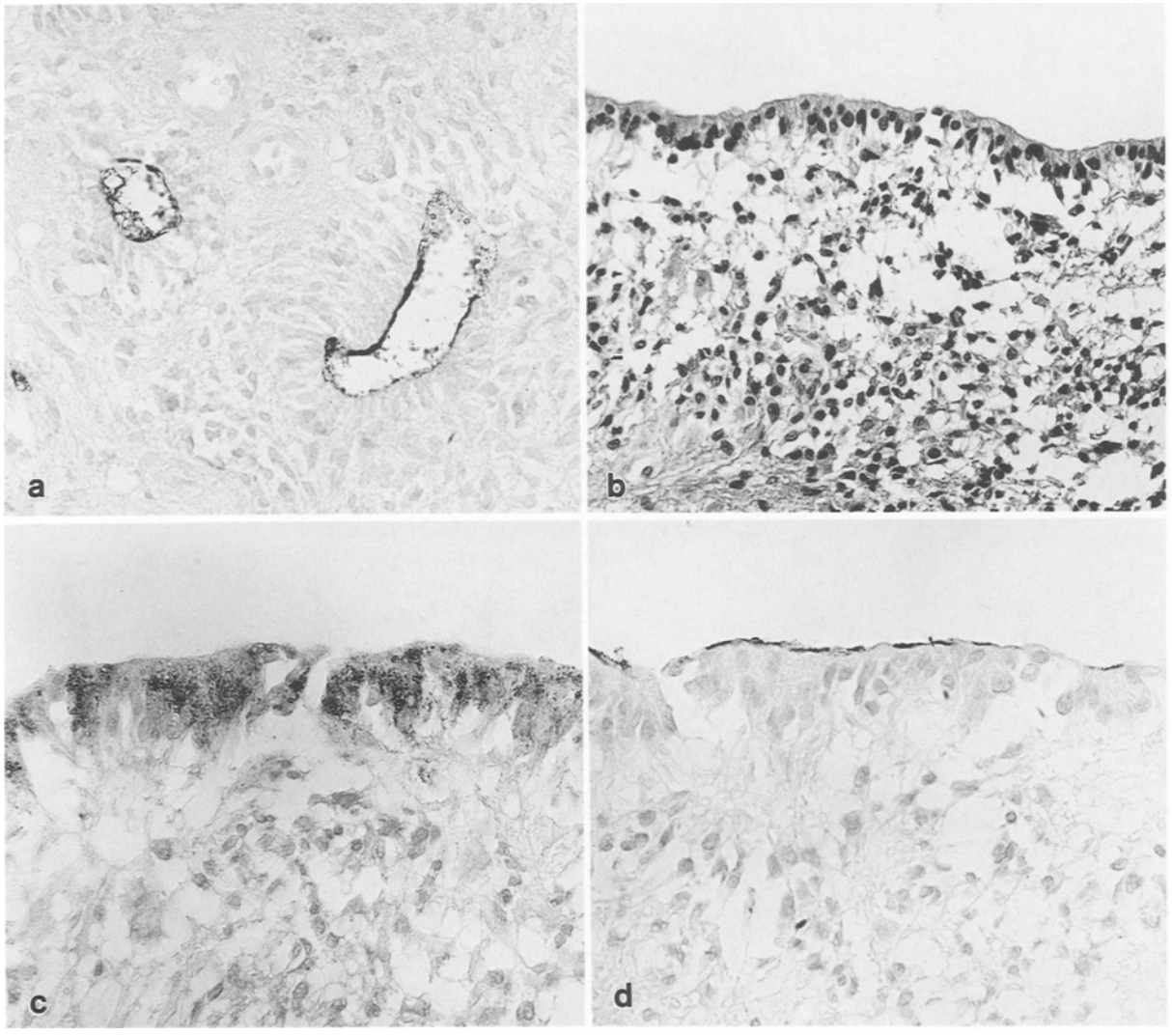

Fig. 1 A–D. Case 2. Ependymal rosette and tubule (**A**) and ependymal epithelium (**B–D**) in the sections obtained at the third operation. **A** Anti-EMA reacts with the luminal surface of the ependymal rosette and tubule, ×400. **B** H & E, ×200. **C** Immunoreactive products are visualized as intracytoplasmic granular deposits (anti-TTR stain), ×400. **D** Anti-EMA reacts with the apical portion of the ependymal epithelium as linear deposits, ×400

In case 26, the tumour cells were poorly differentiated and proliferated in papillary and sheet-like patterns (Fig. 3A). Ependymal rosettes were seen sporadically. Anti-EMA stained the plasma membrane of the tumour cells diffusely (Fig. 3B). The anti-CK reacted with the cytoplasmic components of the tumour cells (Fig. 3C). GFAP positive cells were focally seen (Fig. 3D). We did not observe the co-existence of GFAP and the epithelial marker proteins in the same neoplastic cells at serial sections.

Two operations were performed in case 28. Perivascular pseudorosettes and epithelial-like structures were not clearly seen in the specimen from the first operation. However, perivascular pseudorosettes and ependymal epithelia were evident in the specimen from the second operation (Fig. 4A) and ependymal rosettes also appeared in part. EMA positive cells were seen diffusely in both specimens (Fig. 4B, C). Anti-EMA reacted with the cell membrane, and with the cytoplasm as granular deposits. In contrast with the EMA positive cells, CK positive cells were very rare in the specimen from the first operation, but a fair number of cells became positive to CK in the specimen from the second operation (Fig. 4D). Immunoreactive products were observed in the cytoplasm and cell processes extending to the vascular walls. GFAP-positive cells were focally seen in the section from the first operation but were not seen in the section from the second operation.

The 2 cases of myxopapillary ependymoma showed positivity to GFAP but not to EMA, CK or TTR.

Discussion

There is an intimate relationship between the development of the choroid plexus and that of the ependyma,

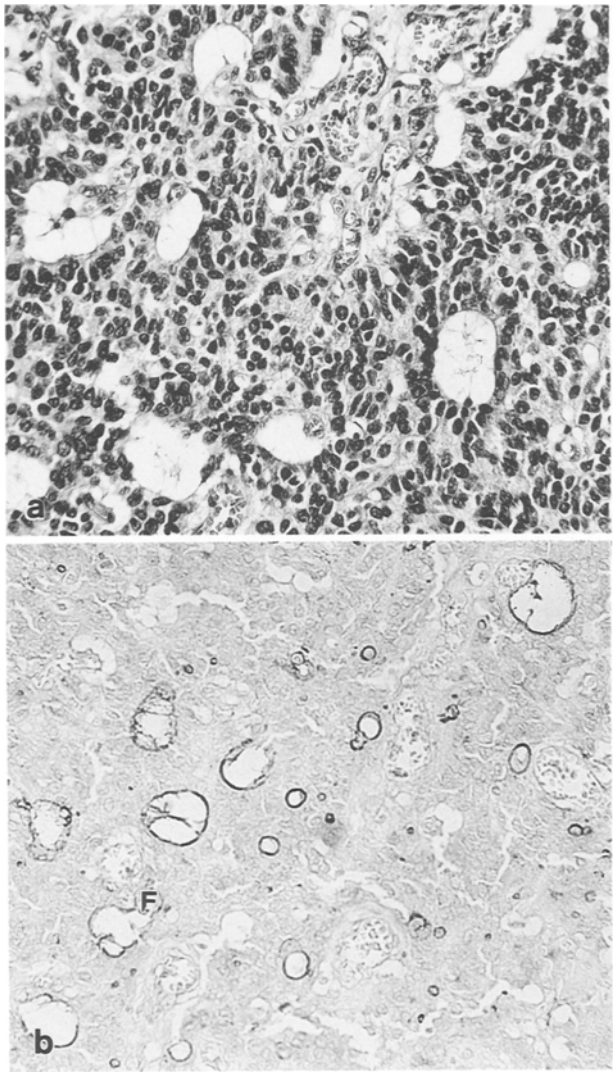

Fig. 2A–D. Case 27. **A** Ependymal rosettes and tubules are abundant. H & E, ×200, **B** Luminal surface of the ependymal rosettes and tubules were stained with anti-EMA. Microcysts were also stained, ×200

but the mature cells of both show different characteristics. The epithelial nature of choroid plexus cells has been demonstrated by electron microscopical and immunohistochemical studies (Dohrmann and Bucy 1970; Kasper et al. 1986). In contrast to the choroid plexus cell, the ependymal cell is considered to glial cell even if the cell shows some epithelial feature. TTR can be identified in the choroid plexus cell after 6 weeks of gestation (Jacobsen et al. 1982), but the ependymal cell does not express TTR (Aleshire et al. 1983).

The normal choroid plexus does not have GFAP, but choroid plexus tumours often express GFAP as a glial marker (Doglioni et al. 1987; Miettinen et al. 1986; Rubinstein and Brucher 1981; Teratuto et al. 1983). This has been explained as ependymal differentiation because ependymal cells are positive for GFAP transiently in the embryonic stage (Roessmann et al. 1980) and often in the pathological state (Miettinen et al. 1983; Tajika et al. 1988; Velasco et al. 1980). We postulated that the correlation between the choroid plexus cell and ependymal cell might be more intimate when they undergo neoplastic transformation. For that reason, ependymal tumours were studied with antibodies against epithelial differentiation antigens and TTR.

Table 2. Immunohistochemical features of ependymal tumours

Tumour group	No. of cases	No. of positive cases for			
		EMA	CK	TTR	GFAP
Ependymomas	23	3(+[a] 3, +[b] 0)	0	1	19
Anaplastic ependymomas	13	6(+[a] 3, +[b] 3)	3	0	10
Myxopapillary ependymomas	2	0	0	0	0

For footnotes, see Table 1

Ependymal neoplasms have been examined using EMA and CK antibodies, and many authors have failed to find either EMA or CK positivity in them (Doglioni et al. 1987; Miettinen et al. 1983, 1986). However, Mannoji and Becker (1988) recently reported 5 cases of CK-positive ependymal tumours, and Cruz-Sanchez et al. (1988) found EMA-positive ependymoma cases. These observations indicate that some ependymal neoplasms display epithelial differentiation.

The present investigation on 38 ependymal tumours revealed that 9 were positive with EMA, 3 with CK, and 1 with TTR. EMA and CK expression was strongly related to the histological subtypes. EMA and CK were more often observed in the anaplastic ependymomas than in the benign ones. It might be generally considered that benign ependymomas differentiate more closely in the direction of normal ependymal epithelia than do anaplastic tumours. It was reported that benign ependymomas were more frequently stained with anti-GFAP than anaplastic ones (Tajika et al. 1988). In our study, anaplastic ependymomas with either none or a few GFAP-positive cells were likely to express EMA and CK. In the cases of poor differentiation, some ependymal neoplasms might have suppressed GFAP expression and shown an epithelial nature. However, their characteristics with epithelial differentiation were different from those of normal and neoplastic choroid plexus cells because they did not show TTR positivity.

In electron microscopic studies, it was pointed out that ependymoblastoma cells closely resembled the cells lining the normal, embryonic neural tube (Hirano et al. 1973). GFAP, EMA and CK-positive tumour cells were observed in case 26. This fact suggests that some tumour cells have the capacity to differentiate in both directions. Thus, undifferentiated ependymoma cells might roughly correspond to the early embryonic ependymal cells which have not yet developed into choroid plexus cells. Mannoji and Becker (1988) postulated the presence of a transitional cell between the choroid plexus cell and the ependymal cell, and reported two cases of papillary

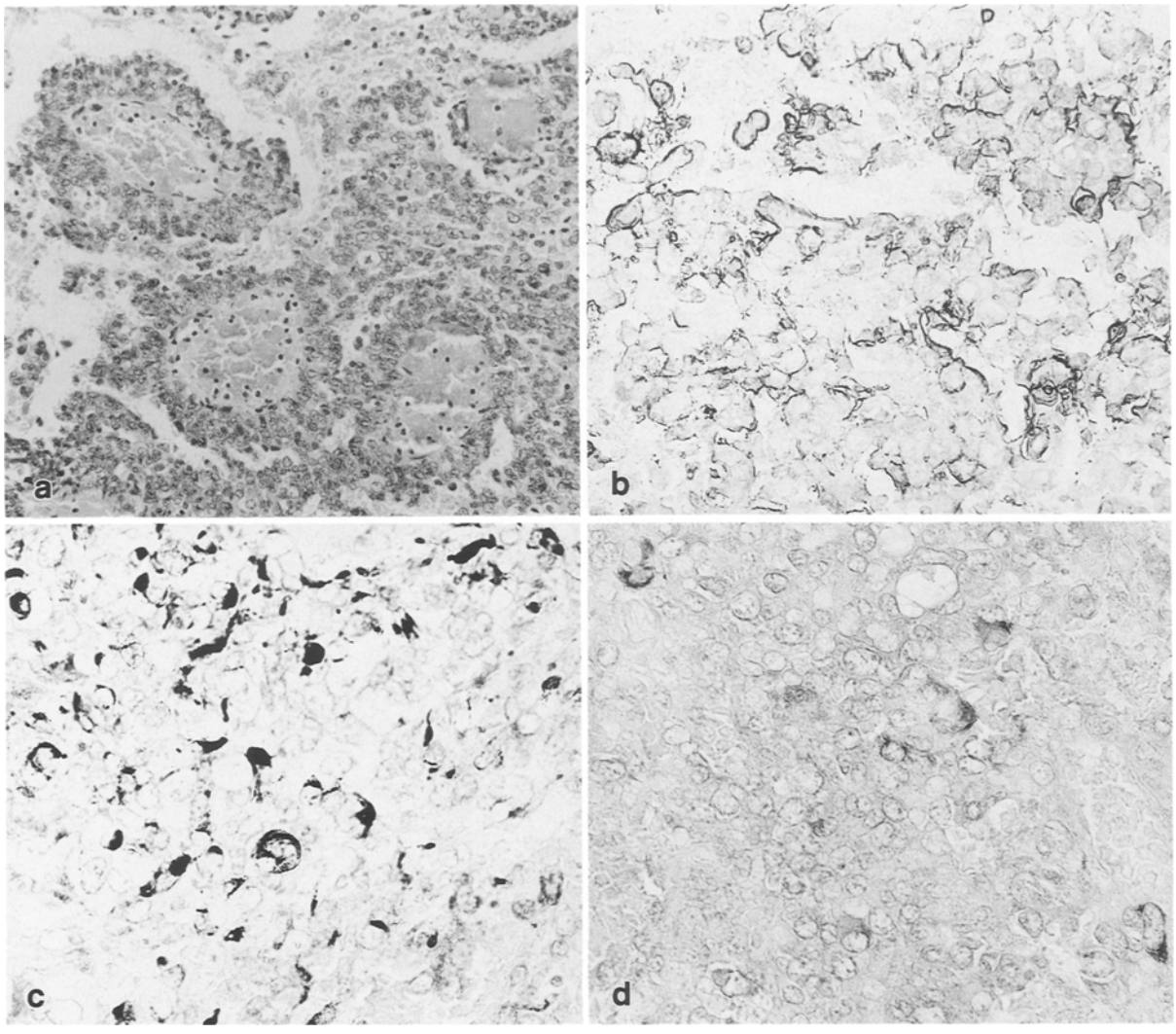

Fig. 3A–D. Case 26. **A** Highly cellular tumour with papillary structures. Tumour cells have round or oval nuclei and a scanty cytoplasm. H & E, ×150. **B** The plasma membrane of many tumour cells was immunolabelled with anti-EMA, ×400. **C** Anti-CK reacts with the cytoplasmic components of a considerable number of tumour cells, ×400. **D** GFAP-positive cells are occasionally seen, ×400

ependymoma corresponding to them. However, in our cases, no transitional cells showing glial and epithelial differentiation were found.

The immunostaining patterns of anti-EMA antibody were divided into the two patterns, as described above. Three cases in which anti-EMA reacted to the plasma membrane of tumour cells in cellular region were also positive to CK. In case 28, EMA-positive cells were abundant in the sections from two operations. CK-positive cells markedly increased in number in the specimen of the second operation, whereas GFAP-positive cells decreased. In this case, epithelial differentiation seemed to have progressed at an interval of 2 years between the two operations.

It was interesting to observe that epithelial-like structures stained with anti-EMA did not exhibit any immunoreactivity to CK. The discrepancy of immunoreactivity with anti-EMA and CK is often observed in undifferentiated carcinomas (Frierson et al. 1986; Mills et al. 1987). It is possible that these cases did not differentiate enough to express CK. Anti-EMA reacts not only to epithelial tumours but also to some mesenchymal-type tumours with an epithelioid differentiation (Pinkus and Kurtin 1985; Sloane et al. 1983). Therefore, it is supposed that EMA might be expressed more extensively than CK as an epithelial marker protein.

One of the 38 ependymomas was positive to TTR. Seitz et al. (1987) examined non-ependymal gliomas using anti-TTR. TTR positivity was found in the interstitium of tumour tissue but not in tumour cells. Matsushima et al. (1988) reported that only benign choroid plexus papillomas showed TTR positivity. This is the first paper to report on the TTR expression in an ependymal tumour.

In conclusion, most benign ependymomas do not exhibit antigenicity to epithelial marker proteins but do to glial ones. Some anaplastic ependymomas may ex-

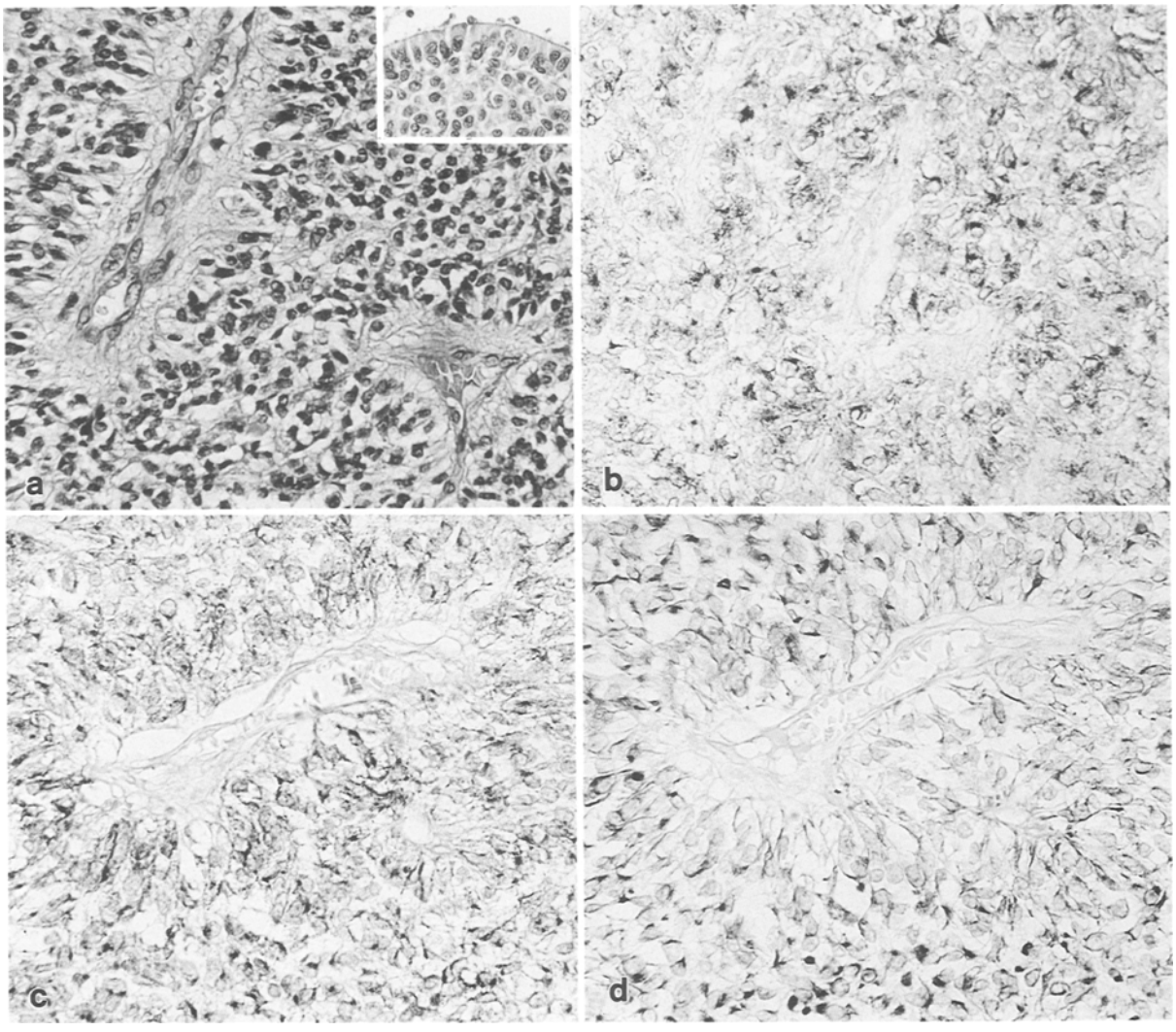

Fig. 4A–D. Case 28. The specimens (**B**) were obtained from the first operation while others (**A, C, D**) were from the second operation. **A** Tumour cells are poorly differentiated with perivascular pseudorosette formation. Ependymal epithelium is shown in the inset. H & E, ×270. **B** EMA-positive cells are seen diffusely. Perivascular pseudorosettes are not apparent, ×370. **C** Anti-EMA reacted with many tumour cells like the section from the first operation, ×370. **D** Anti-CK reacts with the cytoplasm of the tumour cells. Cell processes extending to the vascular wall were also stained, ×370

press epithelial marker proteins but at the same time suppress their glial characteristics.

Acknowledgements. We thank the kindness of Miss K. Hatanaka in assisting our laboratory works and B. Quinn for comments on the manuscript.

References

Aleshire SL, Bradley CA, Richardson LD, Parl FF (1983) Localization of human prealbumin in choroid plexus epithelium. J Histochem Cytochem 31:608–612

Cruz-Sanchez FF, Rossi ML, Esiri MM, Reading M (1988) Epithelial membrane antigen expression in ependymomas. Neuropathol Appl Neurobiol 14:197–205

Doglioni C, Dell'orto P, Coggi G, Iuzzolino P, Bontempini L, Viale G (1987) Choroid plexus tumors. An immunohistochemical study with particular reference to the coexpression of intermediate filament proteins. Am J Pathol 127:519–529

Dohrmann GJ, Bucy PC (1970) Human choroid plexus: a light and electron microscopic study. J Neurosurg 33:506–516

Frierson HF Jr, Mills SE, Fechner RE, Taxy JB, Levine PA (1986) Sinonasal undifferentiated carcinoma. An aggressive neoplasm derived from schneiderian epithelium and distinct from olfactory neuroblastoma. Am J Surg Pathol 10:771–779

Hirano A, Ghatak NR, Zimmerman HM (1973) The fine structure of ependymoblastoma. J Neuropathol Exp Neurol 32:144–152

Holden J, Dolman CL, Churg A (1987) Immunohistochemistry of meningiomas including the angioblastic type. J Neuropathol Exp Neurol 46:50–56

Jacobsen M, Jacobsen GK, Clausen PP, Saunders NR, Mollgard K (1982) Intracellular plasma proteins in human fetal choroid plexus during development. II. The distribution of prealbumin, albumin, alpha-fetoprotein, transferrin, IgG, IgA, IgM, and alpha-1-antitrypsin. Dev Brain Res 3:251–262

Kasper M, Karsten U, Stosiek P (1986) Detection of cytokeratin(s) in epithelium of human Plexus choroideus by monoclonal antibodies. Acta Histochem (Jena) 78:101–103

Kitamoto T, Tateishi J, Hikita K, Nagara H, Takeshita I (1985)

A new method to classify amyloid fibril proteins. Acta Neuropathol (Berl) 67:272–278
Mannoji H, Becker LE (1988) Ependymal and choroid plexus tumors: cytokeratin and GFAP expression. Cancer 61:1377–1385
Mannoji H, Takeshita I, Fukui M, Ohta M, Kitamura K (1981) Glial fibrillary acidic protein in medulloblastoma. Acta Neuropathol (Berl) 55:63–69
Matsushima T, Inoue T, Takeshita I, Fukui M, Iwaki T, Kitamoto T (1988) Choroid plexus papillomas: an immunohistochemical study with particular reference to the coexpression of prealbumin. Neurosurgery 23:384–389
Miettinen M, Lehto VP, Dahl D, Virtanen I (1983) Differential diagnosis of chordoma, chondroid, and ependymal tumors as aided by anti-intermediate filament antibodies. Am J Pathol 112:160–169
Miettinen M, Clark R, Virtanen I (1986) Intermediate filament proteins in choroid plexus and ependyma and their tumors. Am J Pathol 123:231–240
Mills SE, Wolfe JT III, Weiss MA, Swanson PE, Wick MR, Fowler JE, Young RH (1987) Small cell undiferentiated carcinoma of the urinary bladder. A light-microscopic, immunocytochemical, and ultrastructural study of 12 cases. Am J Surg Pathol 11:606–617
Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24
Pinkus GS, Kurtin PJ (1985) Epithelial membrane antigen – a diagnostic discriminant in surgical pathology. Immunohistochemical profile in epithelial, mesenchymal, and hematopoietic neoplasms using paraffin sections and monoclonal antibodies. Hum Pathol 16:929–940
Roessmann U, Velasco ME, Sindely SD, Gambetti P (1981) Glial fibrillary acidic protein (GFAP) in ependymal cells during development. An immunohistochemical study. Brain Res 200:13–21
Rubinstein LJ, Brucher J-M (1981): Focal ependymal differentiation in choroid plexus papillomas. An immunohistochemical study. Acta Neuropathol (Berl) 53:29–33
Schnitt SJ, Vogel H (1986) Meningiomas. Diagnostic value of immunoperoxidase staining for epithelial membrane antigen. Am J Surg Pathol 10:640–649
Seitz RJ, Wechsler W (1987) Immunohistochemical demonstration of serum proteins in human cerebral gliomas. Acta Neuropathol (Berl) 73:145–152
Sloane JP, Ormerod MG (1981) Distribution of epithelial membrane antigen in normal and neoplastic tissues and its value in diagnostic tumor pathology. Cancer 47:1786–1795
Sloane JP, Hughes F, Ormerod MG (1983) An assessment of the value of epithelial membrane antigen and other epithelial markers in solving diagnostic problems in tumour histopathology. Histochem J 15:645–654
Stauder AJ, Dickson PW, Aldred AR, Schreiber G, Mendelsohn FAO, Hudson P (1986) Synthesis of transthyretin (pre-albumin) mRNA in choroid plexus epithelial cells, localized by in situ hybridization in rat brain. J Histochem Cytochem 34:949–952
Tajika Y, Kubo O, Himuro H, Inoue N, Tajika T, Toyama T, Sakairi M, Kitamura K (1988) Immunohistochemical study of ependymoma. Brain Tumor Pathol 5:13–18
Taratuto AL, Molina H, Monges J (1983) Choroid plexus tumors in infancy and childhood. Focal ependymal differentiation: an immunohistochemical study. Acta Neuropathol (Berl) 59:304–308
Velasco ME, Dahl D, Roessmann U, Gambetti P (1980) Immunohistochemical localization of glial fibrillary acidic protein in human glial neoplasms. Cancer 45:484–494