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# Coexistence of cytokeratin, vimentin and neurofilament protein in human choroid plexus

## An immunohistochemical study of intermediate filaments in neuroepithelial tissues

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**Summary.** The expression of intermediate filament proteins in human brain ependyma and choroid plexus epithelium has been studied by immunohistochemistry using a panel of monoclonal antibodies directed against all five classes of intermediate filaments. Ependymal cells express GFAP and vimentin filaments, whereas plexus epithelium simultaneously contains neurofilaments, cytokeratins and vimentin, a phenomenon not previously observed in normal cells in vivo. By means of specific antibodies we were able to establish that cytokeratins 8 and 18 but not 19 are present in plexus epithelium.

**Key words:** Cytokeratin – Vimentin – Neurofilament – Human choroid plexus

## Introduction

In a previous communication (Kasper et al. 1986) we reported on the coexistence of cytokeratins and vimentin in the normal epithelium of choroid plexus. This unexpected result prompted us to carry out a more comprehensive investigation of the expression of intermediate filament proteins (IFP) in these brain structures. The present paper describes the results obtained with monoclonal antibodies (mabs) against neurofilaments, glial fibrillary acidic protein (GFAP) and desmin, in addition to an enlarged panel of anti cytokeratin and anti vimentin mabs. We demonstrate for the first time the coexistence of three classes of IFP in normal cells.

## Materials and methods

*Tissues.* Normal human brain was obtained from autopsy 3 to 12 h post mortem. Tissue samples containing choroid plexus and ependyma were prepared from 6 adults (aged 61, 73, 74, 75, 80, 91) and from one fetus (20th week of pregnancy). They were embedded in a commercial mounting medium (Jung, Heidelberg) and stored in liquid nitrogen. Cryostat sections (4 μm) were cut by means of a Frigocut 2700 microtome (Reichert, Vienna).

*Cytological samples.* Smears from choroid plexus and ependyma were prepared on slides and air dried. The immunocytochemical procedure was carried out immediately.

*Antisera.* The monoclonal antibodies used in this study were as follows (see Table 1 for their detailed specificity). A45-B/B3 and A53-B/A2 are our own anti cytokeratin antibodies (Karsten et al. 1983, 1985). LE (Lane 1982) and TROMA (Brulet et al. 1980) are other anti cytokeratin mabs obtained from Dr. E.B. Lane, Imperial Cancer Research Fund, London, and Dr. A. Wobus, Central Institute of Genetics, Gatersleben, respectively. V101 is an antivimentin mab (weakly cross-reacting with desmin) donated by Dr. Viklicky, Institute of Molecular Genetics, Prague). The other mabs used are commercially available from Labsystems Oy, Boehringer Mannheim and Sternberger Meyer Immunochemicals (SMI 34). The last monoclonal antibody to the neurofilament 200 kD subunit is refered to by Sternberger and Sternberger (1983) as 07-5. The mouse polyclonal serum has been characterized by Perry et al. on immunostrips (unpublished results). Polyacrylamide gels containing the proteins of interest are sliced at 70 μm and then immunostained.

*Immunoperoxidase staining.* Unfixed frozen sections or smears were air dried. Endogenous peroxidase was inactivated by dipping the slides in absolute methanol containing 0.3% $H_2O_2$ for 30 min. After incubation with the first mab for 30 min at room temperature, the slides were washed with phosphate buffered saline (PBS) in three subsequent washing steps. The slides were then reacted with peroxidase-conjugated goat anti mouse immunoglobulin (obtained from H. Typlt, Karl Marx University, Leipzig), diluted 1:100, and washed five times again. Peroxidase activity was revealed using 3'3-diaminobenzidine as a substrate. Sections were counterstained with haematoxylin and mounted in Canada balsam. Controls were performed by replacing the primary antiserum with a) an irrelevant hybridoma supernatant and b) PBS.

*Bodian's Silver Method.* For staining of nerve fibers and nerve endings we used a modified Bodian's silver method as described (Gambetti et al. 1981). Sections (5 μm) of paraffin-embedded tissue were taken.

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**Table 1.** Distribution of intermediate filaments in human choroid plexus epithelium and ependyma

| Denomination of antibody | Specificity | Reactivity with Plexus epithelium | Ependyma |
|---|---|---|---|
| A45-B/B3 | Cytokeratin (broad spectrum) | ++ | – |
| LE 34 | Cytokeratin (broad spectrum) | ++ | – |
| TROMA 1 | Cytokeratin 8 (52 kD) | + | – |
| CK2[a] | Cytokeratin 18 (45 kD) | ++ | – |
| LE 61 | Cytokeratin 18 | ++ | – |
| A53-B/A2 | Cytokeratin 19 (40 kD) | – | – |
| TROMA 2 | Cytokeratin | – | – |
| NR4[a] | Neurofilament protein (68 kD) | – | – |
| – | Neurofilament protein (200 kD)[b] | ++ | – |
| 07-5 (SMI 34) | Neurofilament protein (200 kD) | ++ | – |
| – | All three neurofilament subunits[c] | ++ | – |
| G-A-5[a] | GFAP | – | ++ |
| – | GFAP[b] | – | ++ |
| V9[a] | Vimentin | ++ | ++ |
| V101 | Vimentin (+ Desmin) | + | ++ |
| DE-B-5[a] | Desmin | – | – |

[a] = mab from Boehringer Mannheim
[b] = mab from Labsystems Oy
[c] = polyclonal antibody (mouse antiserum)

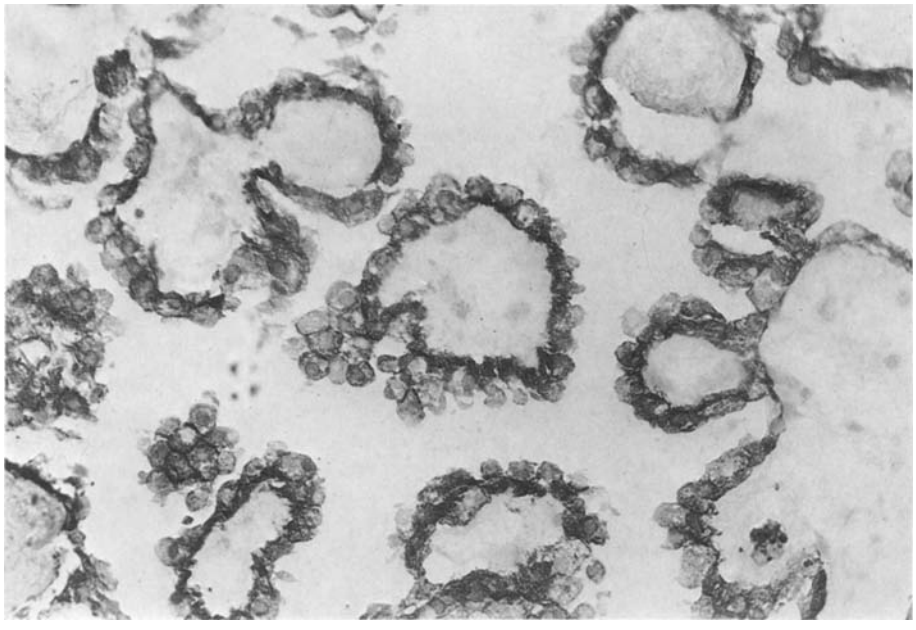

**Fig. 1.** Human choroid plexus epithelial cells stained with monoclonal anti cytokeratin antibody. A45-B/B3 Indirect immunoperoxidase technique. 280×

## Results

In this study frozen sections and cell smear preparations of choroid plexus epithelium were reacted with a panel of monoclonal antibodies against all five classes of IFPs. In our earlier communication (Kasper et al. 1986) we found both the ependyma and plexus positive for vimentin, whereas plexus epithelium also contained IFP of the cytokeratin type. These results have now been confirmed using different anti vimentin and anti cytokeratin mabs (Table 1, Figs. 1 and 3). Furthermore, the application of mabs specific for individual cytokeratin proteins allows us to state that at least cytokeratins no. 8 and 18 but not 19 are expressed in plexus epithelium. The paired expression of cytokeratins 8 and 18 is a common feature probably related to the cytokeratin filament architecture (Cooper 1985). The presence of other cytokeratins cannot be excluded. The availability of mabs against neurofilaments and GFAP in this study revealed the presence of 200 kD neurofilaments in plexus epi-

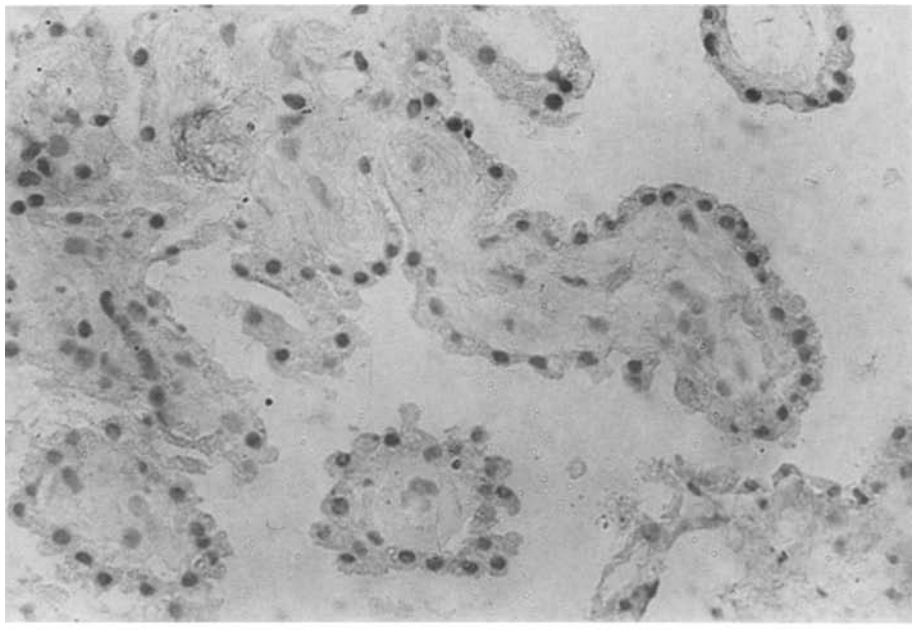

**Fig. 2.** Human choroid plexus reacted with monoclonal anti GFAP antibody: no staining, 280×

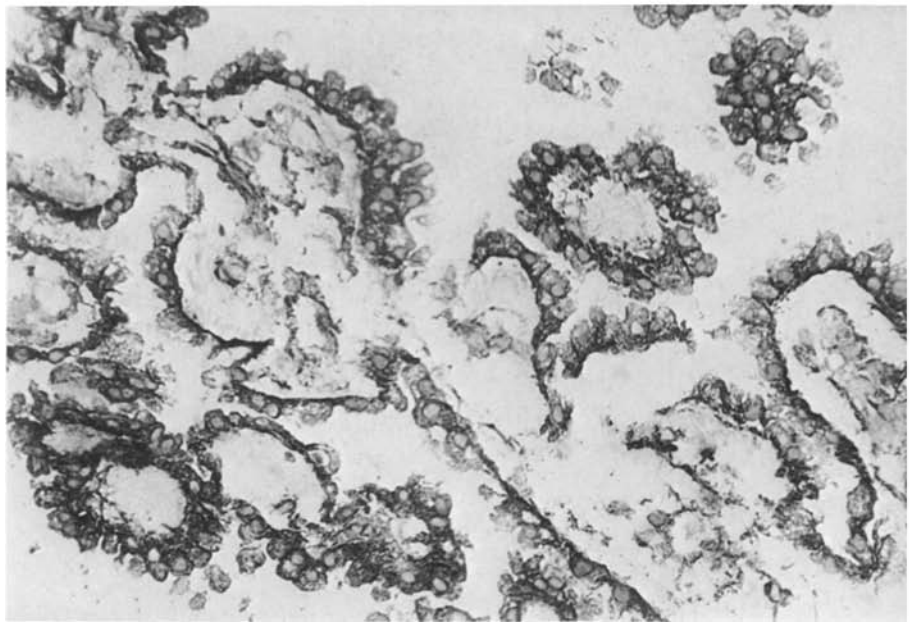

**Fig. 3.** Human choroid plexus stained with monoclonal anti vimentin antibody V9. Positive reaction in plexus epithelium, in small blood vessel walls, and partially in the plexus stroma. 280×

thelium in addition to IFP of the cytokeratin and vimentin type (Fig. 4). GFAP was not present in plexus epithelium (Fig. 2). Ependyma, on the other hand, was strongly positive for GFAP and vimentin, but negative for neurofilament protein. Desmin was absent in ependyma and plexus as was expected.

## Discussion

The coexpression of two types of IFP in some tissues, one of which being vimentin, has already been described (see Gown and Gabbiani 1984). Shaw et al. (1981) examined the distribution of vimentin, neurofilaments and GFAP in the adult rat brain. Their findings are comparable to our results obtained with human material but the authors did not include antisera against cytokeratin and desmin in their study. With the demonstration of the presence of cytokeratins in plexus epithelium in addition to 200 kD neurofilament protein and vimentin, this is the first report of a tissue in which three classes of IFP are simultaneously expressed.

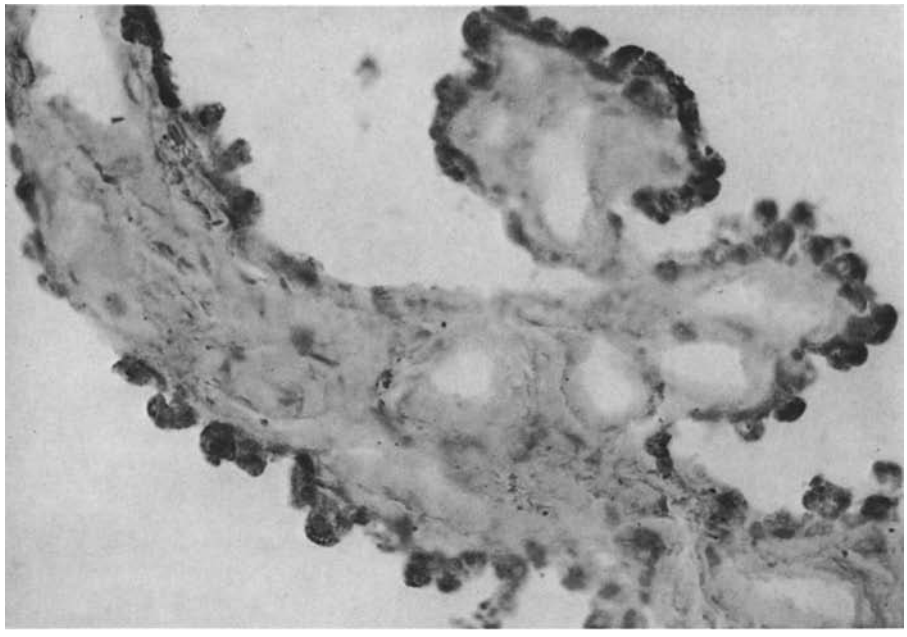

**Fig. 4.** Human choroid plexus reacted with monoclonal anti neurofilament antibody. Positive reaction in epithelial cells, 280 ×

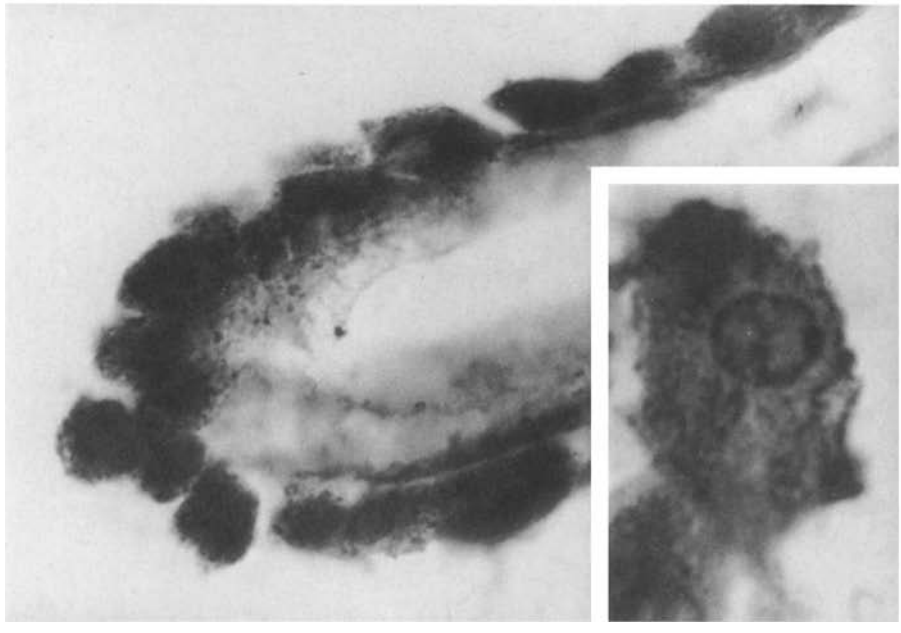

**Fig. 5.** Staining of plexus epithelial cells with Bodian's silver method. ×280; inset ×1,120

We consider the coexistence of all three types of IFP to be true coexpression (i.e. coded for by the plexus cells' own genome), but cannot exclude the possibility that cytokeratins are taken up from the blood by plexus cells in connection with their role in fluid production during the ultrafiltration and processing of blood plasma. Minimal amounts of cytoskeletal proteins may reach the circulation as a consequence of tissue damage or degradation where they seem to provoke autoantibodies (Dellagi et al. 1982). In this connection it is, however, interesting to note that we were able to demonstrate cytokeratin (together with vimentin and neurofilaments) by the 20$^{th}$ week of fetal development well before the onset of liquor production (24$^{th}$ week of pregnancy, Oksche 1984).

Using Bodian's silver stain we could see a reaction with filamentous structures in plexus epithelial cells (Fig. 5). The decoration of filaments with Bodian's silver reinforces the findings of immuno-

staining with anti neurofilament antibodies. Ependyma and plexus epithelium are of neuroectodermal origin. Their common precursors seem to be able to develop into two directions: first, ependyma for which GFAP is a specific marker; and second, plexus epithelium which is characterized by neurofilaments (plus cytokeratins). This developmental process parallels the differentiation of glial cells (GFAP positive) and neurones (containing neurofilaments). Considering this, the striking feature of IFP expression is that it is determined mainly by functional differentiation and less by the germ layer of origin (Fujita et al. 1981; Gown and Gabbiani 1984). The lack of information about the function(s) of the different IFPs is, however, a disadvantage in such considerations. Choroid plexus epithelial cells possess multiple filament structures, some of which appear to increase with age (Oksche 1984). It would be of interest to correlate vimentin, neurofilament, and cytokeratin proteins with these different structures in immunoelectron microscopic studies. Such studies could answer the question whether one of these IFP types found could represent a cell constituent no longer functioning but remaining in the cell as a residual body. This would lead one to consider the triple conjunction of IFP's as an intermediate state during changes in differentiation pathways. The patterns of intermediate filament proteins in neuroectodermal tumours are rarely known. In a recent paper (Vandevelde et al. 1985), one out of 9 ependymomas was GFAP positive, whereas all of 4 choroid plexus papillomas were negative.

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