Colloid Cysts of the Third Ventricle:  
Immunohistochemical Evidence for Nonneuroepithelial Differentiation

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The histogenesis of colloid cysts (CCs) of the third ventricle has been a subject of controversy. We examined, using immunohistochemical techniques, four CCs for the presence of cytokeratins (CKs), glutathione S-transferase isoenzymes (GST-\(\tau\), GST-\(\mu\)), and glial fibrillary acidic protein. Antibodies to both low molecular weight CKs (anti-CK8) and to a mixture of CKs (AE1/AE3) were used. For comparison, normal fetal and adult choroid plexus, ependyma, and nasal mucosa were also examined. The epithelium lining all four CCs showed positive immunostaining for the CKs and GST-\(\tau\) but not for GST-\(\mu\) or glial fibrillary acidic protein. Fetal and adult nasal mucosa showed a pattern of immunohistochemical staining almost identical to that of CCs. In contrast, fetal and adult choroid plexus tissue showed positive immunostaining for GST-\(\tau\) and low molecular weight CKs but not for the CK mixture (AE1/AE3). Fetal and adult ependyma were negative for both CKs and GST-\(\tau\). These results suggest that CCs differentiate along nonneural lines distinct from the neuroepithelial differentiation of the choroid plexus and ependyma. Hum Pathol 23:811-816. Copyright © 1992 by W.B. Saunders Company

To determine whether immunohistochemical staining characteristics could be used to assist in the identification of the direction of differentiation of CCs, we compared four cases of CCs of the third ventricle to normal human adult and fetal choroid plexus, ependyma, and nasal epithelium. Immunohistochemical stains for broad-spectrum and low molecular weight cytokeratins (CKs), glutathione S-transferase-\(\tau\) (GST-\(\tau\)), glutathione S-transferase-\(\mu\) (GST-\(\mu\)), and glial fibrillary acidic protein (GFAP) were used. Our results suggest that CCs differentiate along nonneural lines similar to the epithelium of the trachea and sphenoid sinus.

MATERIALS AND METHODS

Tissue

Four patients underwent surgical resection of CCs of the third ventricle between 1980 and 1989 at The Johns Hopkins Hospital. Resected tissue was fixed in 10% phosphate-buffered formalin and embedded in paraffin. In two of the four cases, normal choroid plexus tissue was attached to the CC. For purposes of comparison, normal choroid plexus and normal ependyma were obtained from tissue resected from patients with intractable epilepsy. Nasal mucosa was obtained from transsphenoidal hypophysectomies. Human choroid plexus, ependyma, and nasal mucosa were obtained from two fetuses aborted at the ninth and 17th week of gestation. Positive control tissues for GST-\(\tau\), GST-\(\mu\), and GFAP included human placenta, human liver, and normal human cerebral cortex, respectively. All tissues were sectioned at a thickness of 5 \(\mu\)m.

Antibodies

A monoclonal antibody to broad-spectrum CKs (AE1/AE3) was obtained from Hybridtech Inc (San Diego, CA). This mixture of antibodies contains the anti-CR monoclonal antibody AE1, which recognizes the 50- and 56.5-kd CKs (CKs 10 and 14), and AE3, which recognizes the 58-, 65.5-, and 68-kd CKs (CKs 1, 2, and 5). A monoclonal antibody to low molecular weight CK was obtained from ENZO Biotech Inc (New York, NY). This antibody recognizes the 52.5-kd CK (CK 8) (manufacturer’s data). Rabbit anti-cow GFAP antibody was obtained from DAKO Co (Carpinteria, CA).

Rabbit antiserum to human GST-\(\tau\) and affinity-purified rabbit antibody to human GST-\(\mu\) were obtained from Medlabs (Dublin, Ireland). The specificities of these antibodies were confirmed on immunoblotting following sodium dodecyl sulfate polyacrylamide gel electrophoresis in which purified GST-\(\tau\) and GST-\(\mu\) are run (MEDLABS, Dublin, Ireland).

Immunohistochemistry

Deparaffinized sections were pretreated with 0.03% \(\text{H}_2\text{O}_2\) in methanol for 30 minutes at room temperature to block
endogenous tissue peroxidase activity. Sections for CK staining were also predigested with pronase (Calbiochem, San Diego, CA) 1 mg/mL in 0.05 mol/L tris-buffered saline supplemented with 0.23% EDTA for 30 minutes at 37°C. Pronase activity was arrested by immersing the sections in 95% ethyl alcohol for 5 minutes. To block nonspecific antibody binding, sections stained for GST-μ, GST-π, and GFAP were incubated with normal goat serum (1:10) for 30 minutes at room temperature. For CK staining, sections were incubated with normal horse serum (1:20) for 30 minutes at room temperature. Sections were then incubated with the following primary antibodies at the dilutions indicated: AE1/AE3 (1:2,000 dilution), anti-CK 8 (1:2,000 dilution), GFAP (1:500 dilution), GST-π (1:1,000 dilution), or GST-μ (1:100 dilution), all for 12 hours at 4°C. Normal rabbit serum and normal mouse immunoglobulin G (IgG) were used as negative controls. Sections were incubated with the secondary antibody; biotinylated anti-mouse IgG horse immunoglobulin (Vector Laboratories Inc, Burlingame, CA) for CK or biotinylated anti-rabbit IgG goat Ig (Vector Laboratories Inc) for 30 minutes at room temperature. Sections for CK staining were incubated with peroxidase-conjugated streptavidin (1:1,000 dilution) (Jackson ImmunoResearch Laboratories Inc, West Grove, PA); all other sections were incubated with avidin-biotin-peroxidase complex (Vector Laboratories Inc) for 30 minutes at room temperature. Sections were immersed in aminoethyl carbazole (Biomeda Corp, Foster City, CA) for 10 minutes or in diaminobenzidine solution (Sigma, St Louis, MO) for 5 minutes and then counterstained with hematoxylin.

RESULTS

Colloid Cysts

Hematoxylin-eosin–stained sections of the four CCs of the third ventricle demonstrated that the epithelial lining of the CCs presented here was fundamentally the same epithelial lining (ie, ciliated and nonciliated cuboidal to columnar epithelium) as is seen in the nasal mucosa. The percentage of ciliated cells varied among cases and in two of the four cases, normal colloid plexus tissue was attached to the CC (Fig 1).

Immunohistochemical staining of the four CCs revealed intense staining for broad-spectrum CKs in all four cases (Fig 1). The staining was localized to the cytoplasm of ciliated and nonciliated columnar to cuboidal epithelia. A similar staining pattern was observed for the low molecular weight CK (Fig 2), but the intensity of staining was less. All four cases also stained for GST-π (Fig 3). Patchy positive staining was observed in both the cytoplasm and nucleus of ciliated and nonciliated columnar to cuboidal epithelia. In contrast, positive staining for GST-μ and GFAP was not observed.

Normal Tissues

Fetal and adult choroid plexus tissues were also examined. In these tissues staining for broad-spectrum CKs was not identified, while positive staining for low molecular weight CK was observed in the cytoplasm of both fetal and adult choroidal epithelia. Positive staining for GST-π was found mainly in the cytoplasm of fetal and adult choroidal epithelia, but occasional nuclear staining was identified in adult choroidal epithelia. Positive staining for GST-μ or GFAP was not detected in these tissues.

DISCUSSION

The histogenesis of CCs of the third ventricle is controversial. Paraphysis, neuroepithelium, and endoderm all have been reported as possible sites of origin based on the morphologic similarity of the cyst epithelial lining to these structures. This confusion over the "cell of origin" of CCs is mirrored in the nomenclature applied to these lesions. For example, CCs have been referred to as ependymal cysts, choroidal epithelial cysts, neuroepithelial cysts, and epithelial cysts.

The majority of the initial investigations into the histogenesis of CCs were based on detailed light microscopic observations. In 1955, Kappers suggested that most CCs of the third ventricle were derived from either the diencephalic vesicle (ie, ependyma) or the paraphysis, a rudimentary organ in the human fetus that disappears by 3.5 months of gestation. Ten years later, however, Shuangshoti et al suggested that there was no morphologic difference between diencephalic vesicle and paraphysis, and proposed that CCs of the third ventricle are derived from the neuroepithelium common to both the paraphysis and the diencephalic vesicle. Based on their observations, Shuangshoti et al suggested that the term "colloid cyst" be replaced by the term "neuroepithelial cyst."

The introduction of electron microscopy has added to the understanding of the direction of differentiation of CCs. In 1974, Hirano and Ghatak examined three CCs of the third ventricle and noted that the fine structure of the epithelial cells lining these cysts differed from that of normal choroidal epithelium and ependyma. The cysts they examined were lined by two types of cells, a lighter ciliated cell and a darker nonciliated cell. These cells rested on a basement membrane, and the nonciliated cells had microvilli with a constant diameter and coated substance on their luminal surfaces. Hirano and Ghatak noted the ultrastructural similarities between the epithelium lining CCs of the third ventricle and the tracheal epithelium and, based on these observations, speculated that CCs of the third ventricle might arise from midline endodermal diverticula during embryogenesis. In support of this idea, epithelial cysts in the fourth ventricle and spinal cord with morphologic appearances similar to those of CCs of the third ventricle have been reported, while ependyma usually lacks a basement membrane and choroidal epithelia have club-shaped microvilli.

The purpose of this study was to examine, using immunohistochemical techniques, the direction of dif-
differentiation of CCs of the third ventricle. Antibodies to CKs, to GSTs, and to GFAP were used. The anti-CK antibodies used in this study included an antibody to low molecular weight CKs and a mixture of antibodies against CKs (AE1/AE3). The mixture of the anti-CK antibodies AE1 and AE3 recognizes the CKs 1, 2, 5, 10, and 14, which are widely distributed in various kinds of epithelium. It is interesting to note that CKs (AE1/AE3) have not been detected in the choroidal epithelia or in the ependyma, although it has been reported that low molecular weight CKs (CK 8, 18, and 19) are present in human choroidal epithelia as well as in CCs of the third ventricle. We found the epithelial lining of CCs of the third ventricle to be strongly positive for the mixture of CKs (AE1/AE3). Although the intensity of staining was less, a similar pattern of staining was observed when antibodies to the low molecular weight CKs were used. This positive staining for low molecular weight CKs suggests that the epithelium lining CCs of the third ventricle is distinct from choroidal epithelium.

The second group of antibodies used in this study was to the GSTs. Glutathione S-transferases are ubiquitous intracellular enzymes that participate in the metabolism and detoxification of a wide range of electrophilic compounds by conjugation with glutathione. The GST isoenzymes are grouped by their isoelectric points into three classes: basic, GST-α-ε; near-neutral, GST-μ; and acidic, GST-π. We chose to examine the patterns of expression of GST-π and GST-μ in this study because they are potentially useful markers of differentiation in

FIGURE 1. (Top) Immunohistochemical stain for CK (AE1/AE3) demonstrating that the epithelium of the CC (arrow) is strongly positive for CK while the normal choroid plexus (arrowhead) attached to the cyst wall is negative. (CK AE1/AE3; magnification x50.) (Bottom) High-power view of the top panel showing intense staining for CK in the columnar to cuboidal epithelium of the CC. (CK AE1/AE3; magnification x400.)
the brain. Glutathione S-transferase-μ and GST-π have been identified in normal human brain.\textsuperscript{18-20} GST-π has been found in normal choroid plexus, and the expression of GST-π has been found to increase in various premalignant\textsuperscript{21,22} and malignant neoplasms.\textsuperscript{21,33-47} We found that GST-π was not expressed in human ependyma, although it was demonstrated in choroidal epithelia and nasal mucosa. The epithelium lining CCs of the third ventricle stained positively for GST-π.

The third group of antibodies used in this study were against GFAP. Glial fibrillary acidic protein was not expressed in the epithelial lining of the four CCs in our cases, although ependyma should express GFAP at certain developmental stages.\textsuperscript{28-30} In toto these immunohistochemical results indicate that the epithelium lining CCs of the third ventricle is similar to nasal mucosa and unlike ependyma and choroidal epithelia (ie, neuroepithelium). These immunohistochemical results are not entirely surprising. Kondziolka and Bilbao\textsuperscript{31} reported that CCs of the third ventricle are distinct from choroid plexus based on the staining of CCs with pan-epithelial and pan-CK antibodies and the absence of staining with these same antibodies in the choroid plexus. Our finding that both CCs and choroid plexus stain positively for the low molecular weight CKs, while only CCs stain for the broad-spectrum CKs, suggests that it is the high molecular weight CKs that distinguish CCs from the choroid plexus.
Of interest, the fine structure of the epithelial lining of CC also is very similar to that of symptomatic Rathke's cleft cysts and to the mucosa of the sphenoid sinus. The epithelial lining of the CCs presented here also showed immunohistologic as well as morphologic similarities to nasal mucosa. For example, intracellular mucin has been found in a substantial number of CCs but is usually not present in choroid plexus epithelium. It is interesting to note that Rathke's cleft and nasal mucosa are considered to be derived from similar origin (i.e., stomodeum).

While the results of the immunohistochemical studies presented here suggest that CCs of the third ventricle differentiate along lines similar to those of the nasal mucosa, two of the four cysts we examined were closely associated with histologically normal choroid plexus tissue. This finding emphasizes the important distinction between differentiation and site of origin of tumors. Although there is a general correspondence between morphologic characteristics and organ of origin, striking exceptions have been reported. For example, hepatocellular carcinomas of the pancreas and stomach have been reported.

Our studies help define the direction of differentiation of CCs. This direction of differentiation is along
TABLE 1. Immunohistochemical Results for Colloid Cysts, Neuroepithelium, and Nasal Mucosa

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<th>Age (yr)/Sex</th>
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Abbreviations: CK, cytokeratin; LMW, low molecular weight; GST, glutathione S-transferase; GFAP, glial fibrillary acidic protein; NP, not performed.
* Fetal age in gestational weeks.

REFERENCES