Desmoplastic supratentorial neuroepithelial tumors of infancy with divergent differentiation potential ("desmoplastic infantile gangliogliomas")

Report on 11 cases of a distinctive embryonal tumor with favorable prognosis

Scott R. VandenBerg, M.D., Ph.D., Estelle E. May, M.D., Lucien J. Rubinstein, M.D., Mary M. Herman, M.D., E. Perentes, M.D., S. A. Vinueses, Ph.D., V. Peter Collins, M.D., and T. S. Park, M.D.

Division of Neuropathology (Department of Pathology) and Department of Neurological Surgery, University of Virginia School of Medicine, Charlottesville, Virginia, and Department of Tumor Pathology, Karolinska Hospital, Stockholm, Sweden

Eleven cases of supratentorial neuroepithelial tumor presenting in infancy are reported. The tumors were characterized by their voluminous size, their intense desmoplasia, and the frequent presence of divergent astrocytic and ganglionic differentiation as demonstrated by special neurohistological and immunohisto- and immunocytochemical techniques. All the tumors presented in subjects below the age of 18 months, usually within the first 4 months of life. They most often involved the frontal and parietal regions and were composed predominantly of a dense desmoplastic tissue superficially resembling a moderately cellular fibroma. The fibroblastic elements were admixed with variable numbers of pleomorphic neuroepithelial cells. Divergent astrocytic and neuronal differentiation was demonstrable in nine of the 11 tumors. All showed astrocytic differentiation. The study of one example by electron microscopy, immunocytochemistry, and tissue culture disclosed that the astrocytic tumor cells were partly invested by a pericytoplasmic basal lamina. Successful total or near-total surgical resection has been followed by a favorable postoperative course extending in some cases over many years of tumor-free survival. The name "desmoplastic infantile ganglioglioma" is proposed for this apparently distinct clinicopathological entity, whose massive size is indicative of a pre- or perinatal origin. Its identification can be achieved by careful histological analysis and is of obvious prognostic significance.

Key Words • embryonal neuroepithelial tumor • desmoplasia • ganglioglioma • cell differentiation • immunocytochemistry • brain tumor
Desmoplastic infantile gangliogliomas

examined by us (Cases 8 and 9). The other nine cases were referred to one of us (L.J.R.) from other centers. Four (Cases 1, 3, 9, and 10) were previously included in a series of 35 primary cerebral neuroblastomas, where they were listed as examples of an intensely desmoplastic variant of cerebral neuroblastoma presenting clinically in infancy (Cases 32 to 35).9

In all but one case (Case 5), multiple paraffin blocks were available to allow extensive sampling of different parts of the tumor. Wet formalin-fixed tissue was available in five cases (Cases 1, 3, 4, 8, and 9) for silver impregnation of frozen sections to demonstrate axonal and other neurofibrillary material (see below). In one case (Case 8), fresh unfixed tissue obtained at surgery was also available for tissue culture studies and for electron microscopic processing (both described below).

Histological Techniques

In addition to routine hematoxylin and eosin, special stains on paraffin sections included iron-hematoxylin and van Gieson stain, Mallory's phosphotungstic acid-hematoxylin, Gordon and Sweets' silver method for reticulin, and Nissl (cresyl violet or toluidine blue) stain, and a variant of the Bielschowsky silver impregnation for neurites, as previously described.9 The latter was also applied to frozen-section material of all cases in which wet tissue was available.

Tumor Culture Studies

Tissue for tumor culture was obtained from Case 8 during a third craniotomy for removal of tumor from the right frontoparietal region when the patient was aged 11 months. It was immediately placed into sterile Waymouth's MB752/1 medium supplemented with 15% fetal calf serum.* Part of the tissue was processed for standard light and electron microscopy. The remainder of the tumor was transferred to Waymouth's medium MB752/1 which had been supplemented with insulin (0.09 U/ml), 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and 15% (v/v) fetal calf serum.† The latter was composed of 50% unfiltered serum and 50% filtered serum.‡ The tumor was trimmed into about 0.5-cm pieces within 90 minutes and was explanted within 4 hours after surgical removal onto acid-cleaned uncoated glass coverslips and organ culture dishes,§ the latter using Gelfoam as the supporting matrix, and fixed as previously described.18 Approximately half of the cultures were grown in complete Waymouth's medium (as above), and the remaining cultures were grown in a 50:50 mixture of reconstituted powdered Dulbecco's minimum essential medium (DMEM) and Ham's F-10 which had been supplemented with 0.09 U/ml insulin, 10 mM HEPES, 28 mM NaHCO3, 300 mg% glucose, and 15% (v/v) fetal calf serum, hereafter referred to as "DMEM-F10 medium." The serum was formulated as previously described for the supplemented Waymouth's medium. After 2 days of in vitro culture, the serum was reduced to 10% (v/v) in all preparations. Some cultures with both types of media were supplemented (125 mg/ml) with 7S nerve growth factor (NGF).* The cultures were maintained in a humidified 4% CO2 incubator** at 36°C, and the medium was replaced daily or every other day according to the pH shift. Viable coverslip cultures were maintained up to 21 days and matrix cultures up to 34 days in vitro.

Electron Microscopy Study

Fresh tumor specimens were obtained from Case 8 at the first and third craniotomies. The tissue was rapidly trimmed and fixed at room temperature in 2% glutaraldehyde-2% paraformaldehyde buffered in 0.04 M sodium cacodylate (pH 7.2). After fixation, the tumor was rinsed in 0.04 M cacodylate buffer (pH 7.2) and processed and embedded in an epoxy resin for transmission electron microscopy. Sections 1 μm thick and ultrathin sections were stained and examined using standard methods employed previously.14,25

After 10 and 31 days, in vitro Gelfoam cultures (see above) were examined by standard electron microscopy. Cultures were rinsed in neutral phosphate-buffered saline (PBS), fixed for 24 hours in a similar fixative (see above) at 4°C, rinsed in cacodylate buffer (as above), and then processed, embedded, and examined as for the specimens of the in situ tumor.

Immunohistochemical Techniques

Glia Fibrillary Acidic Protein. Sections 6 μm thick were deparaffinized in xylene for 10 minutes and rehydrated through graded ethanol to water. After blockage of the endogenous peroxidase activity with hydrogen peroxide (0.5% in methanol for 30 minutes), immunohistochemical staining was performed using the peroxidase-antiperoxidase (PAP) method of Sternberger31 and a polyvalent glial fibrillary acidic (GFA) protein rabbit antiserum.† The staining involved sequential incubations with 10% normal goat serum, anti-GFA protein rabbit serum (1:1400 dilution), goat anti-rabbit immunoglobulins (1:50 dilution), and rabbit

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* Waymouth's MB752/1 medium obtained from Grand Island Biological Co., Grand Island, New York; fetal calf serum obtained from Reheis Chemical Co., Chicago, Illinois.
† Insulin and HEPES obtained from Sigma Chemical Co., St. Louis, Missouri.
‡ Unfiltered serum obtained from Reheis Chemical Co., Chicago, Illinois; filtered serum, Lot 110451, obtained from HyClone Laboratories, Inc., Logan, Utah.
§ Organ culture dishes, No. 3037, obtained from Falcon Labware, Oxnard, California.
** Forma Model 3157 incubator obtained from Forma Scientific Co., Marietta, Ohio.
† GFA protein rabbit antiserum provided by Dr. L. F. Eng, Stanford University School of Medicine, Stanford, California.
PAP-complex (1:200 dilution). All reagents were dissolved in 0.05 M Tris-buffered saline (pH 7.6) containing 1% normal goat serum. The sections were incubated with the primary antibody for 18 hours at 4°C. All other steps were carried out at room temperature for 15 minutes each. The reaction was developed in freshly prepared 3,3'-diaminobenzidine tetrahydrochloride (5 mg in 6.5 ml of 0.05 M Tris-buffered saline containing 0.015% hydrogen peroxide). Specificity of the staining was determined by substituting normal rabbit serum for the primary antiserum or by eliminating the primary antiserum from the staining procedure.

**Neurofilament Polypeptide.** An anti-neurofilament mouse monoclonal antibody (Tp-NFP 1A3) was used to define an epitope of the neurofilament triplet polypeptide in the 200K-dalton region (anti-neurofilament polypeptide (anti-NFP)). This antibody (an immunoglobulin (Ig)G molecule) was produced by the immunization of BALB/c mice with bovine NFP isolated according to Zackroff, et al., 26 and the hybridomas produced by fusion with the myeloma line SP 2/0 as previously described. 3 The antibody reacts specifically with a neurofilament protein at approximately 210K daltons (Fig. 1) in the crude NFP extracts from bovine brain as demonstrated by SDS-PAGE followed by electrotransfer 5 and immunoblotting. The immunoblotting was carried out as follows: after electrotransfer of the proteins and the blocking of nonspecific sites on the paper with 5% bovine serum albumin for 1 hour, the paper strip was incubated overnight with colloidal gold-labeled anti-mouse IgG§ and silver-enhanced according to Moeremans, et al. 15 Extensive immunohistochemical surveys of human, mouse, and rat tissues have demonstrated specific labeling of neurons and their axons/dendrites (VP Collins, et al., in preparation).

Sections 6 μ thick were deparaffinized in xylene for 10 minutes and delipidized in chloroform for 24 hours. After rehydration through graded methanol to water and blockage of the endogenous peroxidase activity with hydrogen peroxide (see above), immunohistochemical staining for the neurofilament triplet polypeptide epitope as defined by Tp-NFP1A3 was performed. The four-step PAP method comprised sequentially, mouse anti-NFP monoclonal antibody (1:100 dilution), rabbit anti-mouse immunoglobulins (1:25 dilution), swine anti-rabbit immunoglobulins (1:25), and rabbit PAP-complex (1:100). Prior to treatment with the primary antibody, the sections were saturated with 10% normal swine serum. All reagents were dissolved in 0.05 M Tris-buffered saline (pH 7.6) containing 1% normal

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‡ Goat anti-rabbit immunoglobulins obtained from Cooper Biomedical, Malvern, Pennsylvania; rabbit PAP-complex obtained from Dako Corp., Santa Barbara, California.

§ Colloidal gold-labeled anti-mouse immunoglobulin G obtained from Tanssen Life Sciences, Beerse, Belgium.

¶ Rabbit anti-mouse immunoglobulins and swine anti-rabbit immunoglobulins obtained from Dako Corp., Santa Barbara, California.

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Fig. 1. Left: Immunoblot demonstrating the reactivity of the mouse monoclonal antibody Tp-NFP1A3 with only a single band at approximately 210K daltons, following SDS-PAGE (9%) preparation and electrotransfer to nitrocellulose paper of a crude neurofilament preparation from bovine spinal cord. Right: Coomassie blue-stained gel of the same neurofilament preparation following SDS-PAGE (9%) study. Note that the gel contains many proteins other than the neurofilament polypeptide triplet, including glial fibrillary acidic protein (at approximately 50K daltons) and tubulin (at approximately 55K daltons). Positions of molecular weight standards in K daltons are given on the left.

swine serum. The sections were incubated with the primary antibody for 18 hours at 4°C, and all other steps were carried out at room temperature for 15 minutes each. The reaction was developed in freshly prepared 3,3'-diaminobenzidine tetrahydrochloride (see above). Negative controls were obtained by incubation in normal mouse serum in place of the monoclonal antibody or by omitting the primary antibody.

**Double-Labeling Technique.** For simultaneous recognition in the same section of epitopes defined by both the anti-NFP (Tp-NFP1A3) antibody (applied first) and the polyvalent GFA protein rabbit antiserum (applied second), a double-immunostaining technique was used. 3,3'-Diaminobenzidine-tetrahydrochloride (see above) and 4-chloro-1-naphthol* (2.5 mg in 6.5 ml of 0.5 M Tris-buffered saline containing 1% absolute ethanol and 0.015% hydrogen peroxide) were used as chromogens for the first and the second antibody reactions, respectively.
TABLE 1
Clinical features of desmoplastic infantile gangliogliomas

<table>
<thead>
<tr>
<th>Case No. (Accession No.)</th>
<th>Sex &amp; Age at Presentation</th>
<th>Location of Tumor</th>
<th>Treatment</th>
<th>Survival After First Op</th>
<th>Survival &amp; Recurrence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (C87)</td>
<td>M, 9 mos</td>
<td>rt frontoparietal</td>
<td>resection</td>
<td>14 yrs</td>
<td>dead, NR</td>
</tr>
<tr>
<td>2 (C1258)</td>
<td>M, 3 mos</td>
<td>rt frontoparietal</td>
<td>resection, radiation</td>
<td>10.5 yrs</td>
<td>alive, NR</td>
</tr>
<tr>
<td>3 (C2021)</td>
<td>M, 10 mos</td>
<td>lt parietal</td>
<td>resection, radiation</td>
<td>10.5 yrs</td>
<td>alive, NR</td>
</tr>
<tr>
<td>4 (C4002)</td>
<td>M, 18 mos</td>
<td>lt frontoparietal</td>
<td>resection, radiation</td>
<td>4.2 yrs</td>
<td>alive, NR</td>
</tr>
<tr>
<td>5 (C3562)</td>
<td>F, 2 mos</td>
<td>lt temporoparieto-occipital</td>
<td>resection, radiation</td>
<td>3.5 yrs</td>
<td>alive, NR</td>
</tr>
<tr>
<td>6 (C4805)</td>
<td>F, 3 mos</td>
<td>rt temporoparietal</td>
<td>resection, radiation</td>
<td>2.7 yrs</td>
<td>alive, NR</td>
</tr>
<tr>
<td>7 (C5013)</td>
<td>M, 3 mos</td>
<td>lt frontoparietal</td>
<td>resection</td>
<td>1.5 yrs</td>
<td>alive, NR</td>
</tr>
<tr>
<td>8 (84-5929)</td>
<td>F, 4 mos</td>
<td>rt frontoparietal</td>
<td>resection,† chemotherapy, radiation</td>
<td>1.5 yrs</td>
<td>alive</td>
</tr>
<tr>
<td>9 (C1286)</td>
<td>M, 11 mos</td>
<td>rt posterior parietal</td>
<td>biopsy only</td>
<td>0</td>
<td>dead</td>
</tr>
<tr>
<td>10 (C1865)</td>
<td>M, 4 mos</td>
<td>lt frontotemporal</td>
<td>resection,† radiation</td>
<td>3 wks</td>
<td>dead</td>
</tr>
<tr>
<td>11 (C3712)</td>
<td>M, 3 mos</td>
<td>lt temporoparietal</td>
<td></td>
<td></td>
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</tbody>
</table>

* NR = no recurrence.
† Multiple resections with only partial resection at the first operation.
‡ Death in the immediate second postoperative period due to coagulopathy.

After 34 days in vitro, three matrix cultures (see above) were processed for localization of GFA protein and NFP as described above.

Immunofluorescence Detection of GFA Protein

Coverslip cultures, established from explants obtained from Case 8 at the third operative tumor resection (see above), were stained for GFA protein as previously described. The cultures were examined at 12, 19, and 21 days after having been grown in either Waymouth’s medium or DMEM-F10 medium with or without NGF. Smears from adult mouse cerebellum, fixed in the same manner, were used as positive controls. Fluorescence microscopy was performed using a Leitz Ortholux II epifluorescence microscope, and photographs were taken with Kodak Tri-X film at 1600 ASA.

Electron Microscopic Immunocytochemistry Study

A fresh surgical specimen obtained from Case 8 at the first resection was immediately placed in Waymouth’s medium (as above) for transport from the operating room. Within 20 minutes of excision, the tissue was fixed according to the following protocol. It was placed for 1 hour in freshly prepared 4% paraformaldehyde with 8.5% sucrose, 0.2% glutaraldehyde, and 1 mM CaCl2 in 0.1 M phosphate buffer (pH 7.4), and then overnight at 4°C in freshly prepared 4% paraformaldehyde with 1 mM CaCl2 in 0.1 M sodium bicarbonate buffer (pH 10.4), as previously described. The specimen was transferred to PBS (with 1 mM CaCl2) and stored at 4°C for 9 days before 50-μm sections were cut on a Vibratome. The sections were stained for GFA protein or NFP by a previously described method. The following dilutions of antibodies were used: 1:1000 anti-GFA protein (polyclonal rabbit antiserum)† and 1:100 Tp-NFP1A3 (anti-NFP). Staining for anti-NFP was done by one of two methods following incubation with the primary antibodies: 1) 1:25 rabbit anti-mouse immunoglobulins (1 hour 30 minutes), 1:400 mouse PAP diluted in 1% normal goat serum in 0.05 M Tris, pH 7.6 (2 hours 20 minutes); or 2) 1:25 rabbit anti-mouse immunoglobulins (45 minutes), 1:25 swine anti-rabbit antiserum (45 minutes), 1:100 rabbit PAP diluted in 1% normal swine serum in Tris buffer (2 hours 20 minutes). In the control preparations for both methods, normal rabbit serum was substituted for rabbit anti-mouse immunoglobulins.

The stained sections and their controls were processed for electron microscopy, osmicated, flat-embedded, and photographed as previously described. Selected ultrathin sections were counterstained on the grid with uranyl acetate and lead citrate.

Results

The chief clinical features of the 11 cases in this series are shown in Table 1. Cases 2 and 5 were lost to follow-up monitoring 10.5 and 3.5 years after surgery, respectively, at which time the patients were free of any clinical manifestations of recurrent disease. One patient (Case 7) has peripheral neurofibromatosis. The following clinicopathological characteristics can be summarized from Table 1.

Patients’ Age and Sex

Seven of the 11 patients presented with evidence of an intracranial mass at or before the age of 4 months. The oldest patient presented at the age of 18 months. There were eight boys and three girls.

† Polyclonal rabbit antiserum supplied by courtesy of Dr. L. F. Eng, Stanford University School of Medicine, Stanford, California.

** Vibratome, Series 1000, manufactured by Lancer, St. Louis, Missouri.
FIG. 2. Case 8. Computerized tomography (CT) scan with contrast enhancement prior to the first craniotomy, demonstrating the massive size and cystic spaces of the right frontoparietal tumor. No continuity with the ventricular system was shown in any CT plane.

Tumor Site

The frontal and parietal lobes were the regions most frequently involved, with relative sparing of the occipital lobe.

Gross Tumor Features

The gross features of the lesions were similar in all cases, the growths being characteristically massive (as shown in Fig. 2, Case 8), and usually occupying two hemispheric lobes. They were invariably cystic, the cysts often accounting for a significant fraction of the tumor volume. Another striking feature was the remarkably firm texture of their solid component. This was, however, associated with lack of any definite cleavage plane from the surrounding brain. Despite their large size, none of the tumors communicated with the ventricle.

Part of their more superficial portions had a variable attachment to the dura.

Microscopic Features of the Tumors

All the tumors were virtually identical in that routine preparations most frequently showed abnormal tissue with a wavy pattern of relatively benign-appearing fibroblastic cells accompanied by a dense network of connective tissue (Fig. 3A). The first impression was therefore that of a moderately cellular fibroma, thus accounting for the firmness of the gross specimen. However, careful examination of these desmoplastic areas and extensive sampling of the tumor revealed variable numbers of neuroepithelial cells and their processes intermingled among the connective tissue elements either singly or in small groups (Fig. 3B), as described below.

In fields distinct from, but adjacent to, the desmoplastic areas, neoplastic neuroepithelial cells in varying stages of differentiation infiltrated the surrounding brain. Well-defined islands of reactive astrocytes were readily distinguished in the marginal zones, especially in the areas of intense desmoplasia (Fig. 3E). In 10 of the 11 tumors, a number of poorly differentiated neuroepithelial cells, presumably of the primitive type, were identified (Fig. 3D), being found in moderate numbers in seven of the tumors. They were especially numerous in one case only (Case 6), where they were accompanied by mitotic figures and associated with small foci of necrosis.

While in some of the tumors the glial nature of a number of neoplastic cells in both the desmoplastic and the non-desmoplastic areas could already be recognized in routine preparations (Fig. 3B), the immunoperoxidase stain for GFA protein demonstrated to an unexpected extent astrocytic differentiation in all tumors, including the extensively desmoplastic areas (Fig. 3E, F, and I). Neoplastic ganglion cells, identified with the usual neurohistological techniques, were present in four cases (Fig. 3C). In all five cases in which the Bielschowsky silver technique could be applied to frozen-section material, delicate argyrophilic neuritic processes emerging from tumor cells were visualized (Fig. 3G). In two cases in which paraffin-embedded tissue only was available, silver-positive neurites were likewise demonstrable. In eight of the 11 cases, a number of tumor cells and their processes stained positively by immunoperoxidase for the presence of NFP (Fig. 3H and I); with the exception of one case (Case 9, in which additional tissue from the same area of the tumor was not available), this positivity was found in all the tumors.

Fig. 3. Microscopic appearances of the tumors. A: The desmoplastic component of the tumors had the characteristic appearance of a dense wavy pattern of fibroblastic cells at the usually distinct interface with the surrounding brain. Case 3. H & E, x 180. B: Neoplastic neuroepithelial cells were usually intermixed as small clusters or single cells amid connective tissue. Case 8. H & E, x 280. C: Fields of mature ganglion cells with a fibrillary neuropil were present in specimens from four cases. Case 4. H & E, x 350. D: Moderate numbers of primitive, poorly differentiated neuroepithelial cells were present in most tumors in this series. Several arrangements of cells suggest incompletely formed rosettes, but no distinctive rosette formation was found in any of the tumors. Case 9. H & E, x 350. E: Glial fibrillary acidic (GFA) protein immunohistochemistry demonstrating the typical appearance of well-defined islands of reactive astrocytes in the marginal zones of the tumor. Note several GFA-positive tumor cells adjacent to the island of reactive astroglia. Case 8. GFA protein-peroxidase-antiperoxidase (PAP) immunoperoxidase staining with hematoxylin counterstain, x 280. F: Study with GFA protein immunohistochemistry showing numerous neoplastic astrocytes with well-developed processes. Note GFA protein-negative cells with prominent, round cytoarchitecture (upper middle continued→
that exhibited silver-positive neuritic processes and/or neoplastic ganglion cells.

Immunohistological positivity for the 200K-dalton NFP was often demonstrable in large rounded cells (Fig. 3H and I) that were invariably negative for GFA protein (Fig. 3F and I). These neuroepithelial cells and their processes, immunohistochemically distinct for GFA protein and 200K-dalton NFP, respectively, were always intimately admixed (Fig. 3I). As summarized in Table 2, divergent differentiation along astrocytic and/or ganglion cell lines was therefore established by neurohistological and immunohistochemical techniques in nine of the 11 cases. In two cases (Cases 5 and 7), only astrocytic differentiation could be documented, but the possibility that other areas of these tumors might have shown neoplastic differentiation as well could not be excluded because of the unavailability of additional paraffin-embedded or wet formalin-fixed material.

Electron Microscopic Studies

Numerous blocks from the first and third craniotomy specimens of Case 8 were examined by transmission electron microscopy. The findings were most notable for the presence of elongated cells with abundant cytoplasm and often lobulated nuclei (Fig. 4A and B), with usually prominent nucleoli. The cytoplasm was rich in organelles, which included numerous mitochondria, abundant ribosomes, and stacks of granular endoplasmic reticulum (Fig. 4B and C inset). These cells contained numerous cytoplasmic filaments (Fig. 4C and inset) measuring 9 to 10 nm in diameter. A frequent feature was the partial covering of their arborizing, sometimes club-shaped processes by a basal lamina (Fig. 4A inset and C) that was often juxtaeposed to collagen fibrils. These cells also showed numerous cytoplasmic infoldings in their more peripheral portions (Fig. 4), and the infoldings were often partly covered by a basal lamina (Fig. 4A inset).

Interspersed with the tumor cells were other cells that were more attenuated and often electron-dense; these were interpreted as fibroblasts (Fig. 4A and B). They had abundant ribosomes and granular endoplasmic reticulum, and showed variable numbers of cytoplasmic tags, but no basal lamina.

No axons, myelin sheaths, dense-core vesicles, synapses, or synaptic ribbons were identified. A single cilium with a 9+0 configuration was found in one of the filament-containing cells.

Electron Immunocytochemistry

Glia fibrillary acidic protein immunoelectron microscopy demonstrated the filamentous distribution of the protein in the cells that contained abundant cytoplasmic filaments (Fig. 5A left and right). The free surface of some of the GFA protein-immunopositive cells was partially covered by a basal lamina (Fig. 5A right). A number of cell bodies and processes, not covered by a distinct basal lamina, stained positively with the monoclonal antibody against NFP (Fig. 5B), and the filamentous distribution of the protein was demonstrated (Fig. 5B inset).

Many areas of the tumor were unstained with either anti-GFA protein serum or anti-NFP monoclonal antibody. The unstained areas included pools of basal lamina material and collagen (Fig. 5B) and the intervening fibroblasts. Cells morphologically similar to those positive for GFA protein or the NFP were often negative with each antiserum. The tissue processing employed precluded the examination of the same cell by serial sections using different immunocytochemical techniques.

Tissue Culture Studies

Immunofluorescence studies of the tissue from Case 8 demonstrated GFA protein in elongated, multipolar cells that had migrated from the solid explants on the glass coverslips after 12 to 21 days in vitro. After 12 days, some astrocytes developed extensive arborizing processes. After 21 days in culture, the fibroblasts from the tumor became the predominant cell type, with concomitant loss of GFA protein reactivity in the coverslip cultures. Light microscopic examination of the sponge-foam explants showed mostly fibroblasts, with the exception of cultures after 10 and 31 days, which, in plastic-embedded sections, demonstrated viable intercalated neural cells. Electron microscopy of these two cultures revealed hypertrophic astrocytes (Fig. 6A) with numerous filament-packed processes whose free surfaces were often convoluted and covered by a basal lamina (Fig. 6B). The latter was frequently redundant and convoluted (Fig. 6C). The filaments had the same measurements in vivo and in vitro. In addition to those packed with filaments, some of the processes contained
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Fig. 4. Electron micrographs of original tumor tissue from Case 8 (osmicated tissue counterstained on ultrathin sections with lead citrate and uranyl acetate). A: Overview of the tumor cells shows lobulated nuclei and numerous cytoplasmic infoldings, often covered with a basal lamina. Electron-dense cell bodies and processes were presumably fibroblastic. × 3750. Inset: Detail of cytoplasmic infoldings covered by basal lamina. × 20,250. B: Another similar tumor cell showing prominent stacks of rough endoplasmic reticulum. × 4125. C: Higher magnification of another tumor cell containing numerous cytoplasmic filaments. Cytoplasmic membrane covered by a conspicuous basal lamina is adjacent to extracellular collagen fibrils. × 15,000. Inset: Details of another tumor cell showing stacks of rough endoplasmic reticulum, numerous cytoplasmic filaments (measuring 9 to 10 nm in diameter), subsurface densities near the cytoplasmic membrane, and a surface basal lamina. × 30,000.
Fig. 5. Immunoelectron micrographs of original tumor tissue from Case 8 (osmicated tissue, unstained thin sections). A: Glial fibrillary acidic (GFA) protein cytochemical studies. Left: A neoplastic astrocyte contained numerous filaments staining positively with a rabbit polyclonal anti-bovine GFA protein antiserum at a 1:1000 dilution. × 7500. Right: Higher magnification of another tumor astrocyte, partially covered by a basal lamina (arrows) and showing filamentous distribution of reaction product. × 29,500. B: Anti-neurofilament polypeptide (NFP) immunocytochemical study showing three tumor cell processes stained positively with a 1:100 dilution of anti-NFP. Other tumor cells and the intervening collagen fibers were negative. × 9500. Inset: Detail of a cell process showing the filamentous distribution of the reaction product. × 30,000.
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**Fig. 6.** Electron micrographs of tumor tissue from the third craniotomy of Case 8 grown *in vitro* on sponge foam for 10 days in Waymouth’s medium with nerve growth factor (osmicated tissue, thin sections counterstained with uranyl acetate and lead citrate). 

A: A hypertrophic neoplastic astrocyte containing numerous filaments and profiles of rough endoplasmic reticulum. \( \times 4875 \)  
B: Numerous cell processes packed with 9- to 10-nm filaments. Their free surfaces were often convoluted and covered by a basal lamina (asterisks). \( \times 6000 \)  
C: Redundant convoluted basal lamina material adjacent to tumor cell processes and spilling into the extracellular space. The material was continuous in places (asterisks) with the basal lamina covering the free edge of the processes. \( \times 18,750 \).
microtubules and others a mixture of filaments and microtubules (Fig. 7A). As in the original tumor, some of the tumor astrocytes contained cytoplasmic infoldings covered by a basal lamina (Fig. 7B). Occasional long-space collagen fibrils were present in the dense desmoplastic matrix of the explants after 31 days.

Treatment

Surgical resection was performed in 10 of the 11 cases in this series. One patient (Case 10) was only subjected to biopsy because of the extensive size of the tumor at the time of clinical presentation. Two patients (Cases 8 and 11) underwent repeated tumor resections after initial partial removal. One of these patients (Case 11) had two resections and died of a coagulopathy immediately following the second craniotomy; the other (Case 8) has had three resections and presently shows no evidence of recurrent disease. Postoperative irradiation delivered to the tumor site, whole brain, and/or entire neuraxis was given in all but two of the successfully operated cases (Cases 1 and 7). In Case 8, radiation was administered following the third resection. Chemotherapy was administered only in Case 8, following the first partial resection.

Survival

Follow-up data indicate that the prognosis for survival is very good after successful surgical resection. Eight of nine patients with complete or near-total surgical resection have survived 1.5 to 14 years following operation without clinical evidence of further or recurrent tumor growth. The one death in this group (Case 1) occurred 14 years after resection and was unrelated to any tumor recurrence. None of the patients had evidence of metastatic dissemination.

Discussion

The 11 cases reported here comprise a distinct clinicopathological group of supratentorial embryonal tumors with an overall favorable prognosis following successful surgical resection. The predominant clinical features are presentation within the first 18 months of life (usually within the first 4 months) and a massive size suggesting a fetal or perinatal origin. Multiple cysts and a distinctive firmness are hallmarks of their gross characteristics. Principal microscopic features include a predominating dense fibrous desmoplasia with a usually divergent neuroepithelial cell population composed of differentiating astrocytes and ganglionic cells. Astrocytic differentiation was documented in all the tumors by the presence of GFA protein. Whenever sufficient tissue was available to permit the distinction of brain parenchyma from the leptomeningeal compartment, the areas of intense desmoplasia were found to coincide with tumor localization in the leptomeninges. In almost all patients, therefore, leptomeningeal extension of the growth was a salient characteristic, a feature that has been well documented in other forms of neuroepithelial tumor such as the desmoplastic medulloblastoma and the pleomorphic xanthoastrocytoma.

The conspicuous presence of connective tissue in these tumors seems therefore partly due to predominant involvement of the leptomeninges by the neoplasm, the increased fibrous tissue being presumably produced by the meningeal fibroblasts. Another interpretation should, however, be raised in light of increasing evidence currently documenting the synthesis, by some astrocytic and other neuralglial tumor cells, of extracellular matrix proteins that are more commonly associated with cells of mesenchymal origin. In the one case we studied by electron microscopy, a characteristic feature of the neoplastic astrocytic cells was that their free surfaces were often invested by a basal lamina. This strongly suggests that, as in the pleomorphic xanthoastrocytoma, some of the tumor elements may have originated from cells that might normally differentiate into subpial astrocytes, the free surface of which has a well-defined basal lamina. Exceptional examples of a tumor termed "gliofibroma" have also been described in young subjects, the case described in the latter report being presumed to be congenital. In both instances, the neoplasm consisted of a dense mixture of closely intermingled astrocytic and fibroblastic elements of mature composition, with the added feature in the case of Iglesias, et al., that both types of cell were invested by a common basal lamina. The possibility of aberrant neoplastic glial metaplasia resulting in the synthesis of extracellular products more usually associated with mesenchymal cells is further strengthened by the exceptional development of cartilage in astrocytomas. The in vitro synthesis of heterogeneous collagen types by human neuroblastoma cell lines is well documented. However, in that case the neural-crest origin of peripheral neuroblastomas may confer to their cells an increased potential for diverse phenotypic differentiation, including the capacity for collagen synthesis; thus, a tripartite capacity of differentiation of neuroblastoma cells in culture to neuronal, Schwann, and melanocytic cell phenotypes based on the synthesis of extracellular matrix proteins has been suggested. The speculation that neoplastic central neuroepithelial cells, present in the tumors described in this report, might be directly involved in the production of extracellular connective tissue is neither supported nor excluded by our morphological observations. This phenomenon deserves further investigation.

The massive component of the dense desmoplasia may also influence the clinical behavior of these tumors by increasing tumor size. A report demonstrating the stimulation by substances P and K of deoxyribonucleic acid (DNA) synthesis in cultured human skin fibroblasts suggests a mechanism by which the desmoplastic response to neoplastic neural cells may develop. Thus, a therapeutic approach aimed at controlling this reaction may be of clinical value when total resection is not possible.

Neuronal cell populations, sometimes including ma-
Desmoplastic infantile gangliogliomas

FIG. 7. Electron micrographs of tumor tissue from Case 8 (third craniotomy) grown in vitro on sponge foam for 31 days in Dulbecco's minimum essential medium and Ham's-F10 medium without nerve growth factor (osmicated tissue, sections counterstained with uranyl acetate and lead citrate). A: Some cell processes were packed with filaments, others contained microtubules, and others contained both. × 26,500. B: A tumor cell is shown, filled with filaments and displaying cytoplasmic infoldings covered by basal lamina. × 25,000.

ture ganglion cells in addition to the more numerous immature cells, were documented in nine of our 11 cases by a modified Bielschowsky silver impregnation technique for neurites and by an immunoperoxidase staining procedure for the 200K-dalton neurofilament triplet polypeptide. One of the two negative cases did not have enough tissue available for performing silver impregnations or immunoperoxidase staining for NFP. The absence of neuronal differentiation in the other case may have resulted from a scant neoplastic neuronal population combined with inadequate sampling. Alternatively, the absence of neuroblastic elements may have been a reflection of the variable potential of the transformed cell population, in which differentiation might, exceptionally, have been restricted to the astrocytic line. According to the classical scheme of central neurocytogenesis, astrocytic differentiation has been considered to occur at a relatively late stage of neurocytogenesis. However, there is evidence suggesting that, in the primate brain, it may occur earlier in central neural development, GFA protein being already expressed in midgestation in some of the mitotically active cells of the ventricular matrix. In cultures of multipotential precursor cells derived from a mouse experimental teratomatous line, GFA protein is expressed in neural cell populations differentiating from these cells before markers of neuronal differentiation. Therefore, in the context of this presumably congenital (and therefore embryonal) tumor, restriction of differentiation along astrocytic lines only, without demonstrable neuronal elements, is still compatible with the hypothesis of neoplastic transformation having taken place in early fetal life.

Immunohistochemistry and immunocytochemistry with the mouse monoclonal antibody Tp-NFP1A3 against a 210K-dalton neurofilament triplet polypeptide were important in helping to confirm the neuronal component of the tumors, especially when no appropriate wet tissue was available for silver impregnation or for electron microscopy. In this study, dual labeling techniques with the anti-NFP monoclonal antibody and the polyvalent GFA protein antiserum at the light microscopy level have shown no commonly labeled tumor cell types, thus indicating that in these tumors the epitopes defined by the neuronal and glial markers are not mutually expressed.

Two other groups of supratentorial tumors arising in young subjects with a presumptive superficial neuro-related lesions had a diffuse white matter distribution and a lack of distinct borders, which were difficult to distinguish from the surrounding gliosis.
epithelial cortical cell of origin have been reported: the pleomorphic xanthoastrocytoma and the superficial cerebral astrocytoma. The 11 cases presented here share with the pleomorphic xanthoastrocytoma the common features of: 1) an astrocytic tumor cell population; 2) the presence of basal lamina covering part of the cytoplasmic membrane of the tumor cells; 3) a predominantly leptomeningeal invasion; 4) the presence of desmoplasia; and 5) a favorable prognosis. The differences are: 1) a different age incidence; 2) a different localization (the pleomorphic xanthoastrocytoma being most frequently found in the temporal region); and 3) an absence of neuronal differentiation in the pleomorphic xanthoastrocytoma.

The superficial cerebral astrocytoma reported by Taratuto, et al., shows many features in common with the tumors reported here, including their occurrence in infancy, their voluminous size with the presence of cystic areas, the intense leptomeningeal reaction, and their favorable prognosis. A notable difference lies in the demonstration of neuronal tumor cell populations in the great majority of our patients. The group of tumors reported by Taratuto, et al., may therefore represent examples of restricted astrocytic differentiation occurring in a tumor entity that is in practice identical with our's, or their cases may possibly have included a neuronal component that may not have been specifically identified. A review of our series so far suggests that, in this overall group of tumors, restriction of differentiation to the astrocytic line only is exceptional. Documentation of further cases may be necessary to determine to what extent all the examples of this apparently distinct entity can be established to exhibit bipotential differentiation, or whether some cases deserve to be retained among the purely astrocytic tumors.

One example of a complex infantile supratentorial tumor with divergent neuronal, glial, and Schwann cell differentiation has also been documented. The 11 cases of desmoplastic infantile ganglioglioma presented here appear to be distinct from that tumor. The electron microscopic study of one case (Case 8) showed no evidence of synapses or of neuritic processes that were invested by Schwann cells.

Designation of these 11 cases as "desmoplastic infantile gangliogliomas" is meant to emphasize their salient clinicopathological features and their kinship to the ganglioglioma. Their distinctions from classical ganglioglioma include their clinical presentation in infancy, the more frequent inclusion of immature neuroepithelial cells, and the invariable presence of the characteristic dense desmoplasia. Another difference is their predominant localization, which does not include the marked selectivity of classical gangliogliomas for the temporal lobe. In one of our cases (Case 6), the ominous features of a large component of primitive cells, abundant mitoses, and necrosis were indistinguishable from those of a glioblastoma. However, early follow-up results in that case (2.7 years postoperatively) have so far shown no evidence of recurrence.

The divergent differentiation of neuronal and glial tumor cells often associated with primitive cell populations suggests an origin from bipotential precursor cells. The resemblance of the differentiating glial tumor cells to normal subpial astrocytes and the massive leptomeningeal direction of tumor growth further point to a superficial neuroepithelial cell of origin. From the cytogenetic viewpoint, it is tempting to speculate that these tumors may be related to potential foci of continuing neurocytogenesis situated in the subpial granular layer of the cerebral hemispheres. In man, this layer has substantially disappeared by the 8th month of fetal life, but remnants have been found in the frontal lobes 6 months after birth. In any event, the massive size of these tumors presenting in infancy clearly points to their pre- or perinatal development and thus justifies their inclusion among the embryonal tumors of the CNS. However, their biological behavior makes them more akin to the gangliogliomas than to the poorly differentiated forms of embryonal CNS tumor: hence the term proposed here for their designation.

Finally, these tumors illustrate the need for special diagnostic techniques and extensive tissue sampling in complex cerebral tumors, especially those arising in the younger age groups. Their successful identification is important because they form a distinct clinicopathological group with a favorable prognosis after successful surgical resection. This necessarily involves thorough histological study of the tumor. Such a study should include multiple blocks of fixed tissue, appropriate silver impregnation techniques applied to formalin-fixed, unembedded tissue, and immunohistological characterization with both glial and neuronal cell markers.

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Address reprint requests to: Scott R. VandenBerg, M.D., Ph.D., Division of Neuropathology, Department of Pathology, University of Virginia School of Medicine, Charlottesville, Virginia 22908.