Ependymal Denudation and Alterations of the Subventricular Zone Occur in Human Fetuses With a Moderate Communicating Hydrocephalus

María Dolores Domínguez-Pinos, MD, Patricia Páez, Antonio-Jesús Jiménez, PhD, Bernardo Weil, MD, Miguel-Angel Arráez, MD, José-Manuel Pérez-Figares, PhD, and Esteban-Martin Rodríguez, MD, PhD

Abstract

In mutant rodents, ependymal denudation occurs early in fetal life, preceding the onset of a communicating hydrocephalus, and is a key event in the etiology of this disease. The present investigation was designed to obtain evidence whether or not ependymal denudation occurs in 16- to 40-week-old human fetuses developing a communicating hydrocephalus (n = 8) as compared to fetuses of similar ages with no neuropathologic alterations (n = 15). Sections through the walls of the cerebral aqueduct and lateral ventricles were processed for lectin binding and immunocytochemistry using antibodies against ependyma, astroglia, neuroblasts, and macrophages markers. Anti-caveolin was used as a functional marker of the fetal ependyma. The structural and functional molecular markers are differentially expressed throughout the differentiation of the human fetal ependyma. Denudation of the ependyma of the aqueduct and lateral ventricles occurred in all fetuses developing a communicating hydrocephalus, including the youngest ones studied. The denuded surface area increased in parallel with the fetus age. The possibility is advanced that in many or most cases of human fetal hydrocephalus there is a common defect at the ependymal cell lineage leading to ependymal detachment. Evidence was obtained that in hydrocephalic human fetuses a process to repair the denuded areas takes place during the fetal life. In hydrocephalic fetuses, detachment of the ependyma of the lateral ventricles resulted in the (i) loss of the germinal ependymal zone, (ii) disorganization of the subventricular zone and, (iii) abnormal migration of neuroblasts into the ventricular cavity. Thus, detachment of the ependymal layer in hydrocephalic fetuses would not only be associated with the pathogenesis of hydrocephalus but also to abnormal neurogenesis.

Key Words: Abnormal neurogenesis, Ependymal defect, Ependymal denudation, Fetal human hydrocephalus, Neuroblasts, Subventricular zone.

INTRODUCTION

Discontinuities of the ependymal lining the brain cavities, in particular the lateral ventricles, have been associated with hydrocephalus (1, 2). In human cases of hydrocephalus such an ependymal loss has been regarded as resulting from the ventricular dilatation due to the accumulation of cerebrospinal fluid (CSF) (1, 2). According to Sarnat, the extent of the ependymal denudation correlates with the degree of dilatation of the lateral ventricles, and alterations of the ependyma in human hydrocephalus of fetal life and early infancy are not primary in the pathogenesis (2).

However, the view that ependymal loss is a consequence of ventricular dilatation has been contested by recent investigations carried out in natural and experimental mutant mice, which provided strong evidence that hydrocephalus may result from a primary alteration of the ependymal cell lineage (3–7). Thus, in the hyh mouse, a programmed denudation of the ventricle ependymal occurring early in the fetal life precedes the onset of a moderate communicating hydrocephalus (3), and the denudation of the dorsal ependyma of the cerebral aqueduct occurring shortly after birth leads to aqueductal obliteration and severe hydrocephalus (4). Hydin is a transmembrane protein that in the brain is exclusively expressed in the ciliated ependyma (5). The experimental mutation of the hydin gene and the naturally occurring mutation of such a gene in the hy3 mutant is associated with hydrocephalus and most likely the cause (5). Musashi proteins are RNA-binding proteins; two of them, msi1 and msi2, are expressed in neural precursor cells and in all ependymal cells. The disruption of the msi1 gene causes an abnormal differentiation of the ependyma of the cerebral aqueduct, aqueductal stenosis, and hydrocephalus (6).

A transgenic mouse with an insertion that prevents the expression of a variant transcript of RFX4 (member of the regulatory factor-X family of winged-helix transcription factors) resulted in a missing subcommissural organ and the development of congenital hydrocephalus (7). The subcommissural organ is an ependymal gland located in the roof of the aqueduct (8).
Therefore, the statement by Sarnat that there are no known genetic diseases specifically affecting the ependyma should be reconsidered, at least with respect to the primary role of ependyma in animal hydrocephalus (2). Indeed, there is not yet evidence that such a primary alteration of the ependyma may also occur in human hydrocephalus.

Whether the ependymal loss in human hydrocephalus is only the result of stretching and tearing of the ependymal lining caused by ventricular dilatation in severe hydrocephalus or a primary pathologic event of the ependymal cells occurring prior to the onset of hydrocephalus needs to be clarified. Within the limitations of research in human hydrocephalus, the present investigation was designed to determine whether ependymal denudation occurs in fetuses developing a communicating hydrocephalus with moderate dilatation of the ventricular cavities. Fetuses of similar ages with no neuropathologic alterations were used as controls.

**MATERIALS AND METHODS**

**Patients**

The brains from 8 fetuses with communicating hydrocephalus and 15 fetuses with clinically normal development and no neuropathologic alterations from the Pathology Department of Carlos Haya Hospital, Málaga, Spain, were selected for the present investigation. Cases of hydrocephalus included (i) 4 isolated hydrocephalus of 16 weeks (female), 30 weeks (male), 31 weeks (male), and 36 weeks (female); (ii) 3 hydrocephalus associated with lumbosacral meningomyelocele of 20 weeks (male), 22 weeks (female), and 36 weeks (male); the latter had in addition prosencephaly; (iii) one female fetus of 40 weeks with hydrocephalus associated with Potter syndrome. The cases used as control for the present investigation included (i) 6 spontaneous abortions of 16 weeks (2 males and one female), 21 weeks (male) and 30 weeks (one male and one female); (ii) 2 therapeutic interruptions of pregnancy at 12 weeks (male) and 22 weeks (male); (iii) 5 placenta defects of 21 weeks (female), 25 weeks (male), 30 weeks (male), 31 weeks (male), and 33 weeks (male); (iv) one Potter syndrome (21 weeks, male) with no neuropathologic alterations; (v) one sudden death (37 weeks, male).

**Methods**

The brains obtained from all fetuses were fixed by immersion in 5% formaldehyde for 2 days. Coronal cuts to expose the ventricles were performed, which allowed us to evaluate the volume of the ventricular cavities and the patency of the cerebral aqueduct. Blocks of tissue containing various regions of the walls of the lateral ventricles and of the cerebral aqueduct were obtained and then processed for paraffin embedding. Serial sections from each block were obtained and mounted individually on separate poly L-lysine coated slides. Adjacent sections were stained with hematoxylin and eosin or processed for lectin binding or immunocytochemistry.

**Lectin Binding**

The lectin *Limax flavus* agglutinin (LFA) with affinity exclusively for sialic acid was used. Unlabeled LFA (Calbiochem, San Diego, CA) was used at a concentration of 7 μg/ml in 0.1M Tris buffer, pH 7.3. The lectin-binding site was revealed by the immunoperoxidase method using anti-LFA serum (raised by E. M. Rodriguez, Valdavia, Chile).

**Immunocytochemistry**

Sections to be immunoreacted were irradiated by microwave; the slides were immersed in a Coplin jar filled with Tris-HCl buffer, pH 9.5, containing 5% urea, and irradiated in a microwave oven, set at 900 W, 2 sessions, 5 minutes each. The immunoperoxidase method of Sternberger et al was applied (9). The following primary antibodies were used: (i) CD99, monoclonal, against human p30 and p32 plasma membrane glycoproteins expressed by several cell types including the ependyma (10) (Clone 12E7, Dako, Denmark); 1:50 dilution; (ii) anti-glial fibrillary acidic protein (GFAP), raised in rabbit (Sigma, St. Louis, MO), 1:100 dilution; (iii) antivimentin raised in goat (Sigma, Madrid, Spain), 1:500 dilution; (iv) a monoclonal antibody specific for human macrophages (Sigma), 1:400 dilution; (v) anti-β III tubulin, monoclonal (Sigma), 1:5000 dilution; (vi) anti-caveolin-1, raised in rabbit, affinity purified (N-20; Santa Cruz Biotechnology, INC., San Diego, CA), 1:2000 dilution. Incubation in the primary antibody was in a moist chamber for 18 hours. Anti-rabbit IgG raised in goat (Sigma), anti-mouse immunoglobulins (Sigma), and anti-goat IgG developed in rabbit (Sigma) were used at a dilution 1:50, for 1 hour. PAP complexes using anti-peroxidase developed in rabbit, goat or mouse (Sigma) were used at a dilution of 1:100, 1:100, and 1:200, respectively, for 30 minutes. 3,3’-diaminobenzidine tetrahydrochloride (DAB, Sigma) was used as electron donor. All antibodies were diluted in 0.01M buffered phosphate saline, pH 7.3. Omission of the incubation in the primary antibody was used as a control for the immunoreaction.

**RESULTS**

**Ependyma of Nonhydrocephalic and Hydrocephalic Fetuses**

The ependyma of hydrocephalic and nonhydrocephalic fetuses displayed similar structural and immunocytochemical characteristics. The study included 15 nonhydrocephalic fetuses ranging in age between 12 and 37 weeks and 8 hydrocephalic fetuses between 16 and 40 weeks. All nonhydrocephalic and hydrocephalic fetuses displayed an open cerebral aqueduct (Fig. 1D). All samples from the cerebral aqueduct and lateral ventricles from nonhydrocephalic fetuses examined were lined by a continuous ependymal lining (Figs. 2, 3). The youngest fetuses studied (12–25 weeks) displayed a pseudostratified immature ependyma formed by elongated cells with their major axis lying perpendicular to the ventricular surface (Fig. 1A, B, F, G). Most of the cells lacked cilia and few had only one or very few cilia (Fig. 2B); many cells processes originated in the stratified ependyma projected deeply into the neuropil (Fig. 1B, F). Most of the cells lining the lateral ventricles and the aqueduct displayed GFAP immunoreactivity (Fig. 1B) and were strongly reactive with the CD99 antibody.
Mitotic figures were frequently seen in deep regions of the stratified ependyma (Fig. 1G). In the 29- to 40-week-old fetuses the immature ependyma progressively differentiated into a single layer of multiciliated ependymal cells that were strongly reactive with anti-vimentin and weakly reactive with anti-CD99. This ependyma showed a distinct glycocalix rich in sialic acid residues as revealed by the lectin LFA (Fig. 3B). In the aqueduct and lateral ventricles, 2 populations of ependymal cells were visualized; one displayed long basal processes and GFAP immunoreactivity and the other was not reactive for GFAP and apparently lacked basal processes (Fig. 4G). No mitotic figures were seen in the ependyma of 29- to 40-week-old fetuses. The cholesterol-binding proteins caveolins 1 and 2 are the main component of noncoated plasma membrane invaginations known as caveolae. In all of the samples the antibody against caveolin-1 reacted exclusively with the smooth muscle cells of blood vessels and the ependyma. In the lateral ventricles of nonhydrocephalic fetuses and in the nondetached ependyma of hydrocephalic fetuses anti-caveolin (i) reacted

(Fig. 1C, F, G).
FIGURE 2. (A–H) Sections through the wall of the lateral ventricles and choroid plexus immunostained for caveolin-1. (A, B) Wall of a lateral ventricle of a nonhydrocephalic 16-week-old fetus. The immature ependyma is reactive (bracket); the immunoreaction is especially strong in the cells facing the ventricle (open arrow). Solid arrows point to cells apparently displaying only one cilium. (C, D) Wall of a lateral ventricle of a nonhydrocephalic 31-week-old fetus. Immunoreactive caveolin is present in the cell body and basal processes (arrows) of ependymal cells. (E, F) Wall of a lateral ventricle of a hydrocephalic 40-week-old fetus. (G) Choroid plexus (ChP) of a nonhydrocephalic 16-week-old fetus. E, immature ependyma. (H) ChP of a hydrocephalic 20-week-old fetus. Magnifications: (A, C, E, G, H) 500×; (B, D, F) 1,250×.
weakly with all cells forming the stratified immature ependyma of the 16- and 20-week-old fetuses; the basal processes of these cells were not reactive (Fig. 2A); (ii) immunostained strongly the more mature ependyma of the 30- to 33-week-old fetuses; the reaction was strong in the supranuclear region of the cytoplasm and in the long basal processes (Figs. 2C); (iii) in the monolayered matured ependyma of the 40-week-old fetus the immunoreactive caveolin appeared as distinct masses located in the supra- and infra-nuclear regions of the cytoplasm (Fig. 2E).

In the choroid plexus of all hydrocephalic and nonhydrocephalic fetuses studied, including the youngest ones, caveolin was strongly expressed by all choroidal ependymal cells, with the immunoreaction distributed throughout the cytoplasm (Fig. 2G, H).

Ependymal Denudation Is a Common Feature of Hydrocephalic Fetuses

The 8 hydrocephalic fetuses investigated had the lateral and third ventricles dilated (Fig. 1E), an open cerebral aqueduct (Fig. 1D) and were regarded as communicating hydrocephalus. In the younger fetuses (16–22 weeks) the areas of the walls of the aqueduct and lateral ventricles displaying ependymal denudation were consistently present, but not extensive (Fig. 1A). The denuded areas were not covered by astrocytes or other organized cell elements, leaving the neuropil directly exposed to the ventricular lumen (Fig. 1A–C); no microglia/macrophage cells (see below) were seen in the vicinity of the denuded areas. The nondenuded areas of the young fetuses were lined by a neuroepithelium/immature ependyma that did not differ from that of nonhydrocephalic fetuses of the same/similar age with respect to the multilayered arrangement and immunoreactivity of the ependymal cells (Fig. 1A–C, F, H). Indeed, the latter expressed GFAP (Fig. 1B), p30/32 glycoproteins (Fig. 1C, F, G), and caveolin.

The surface area devoid of ependyma increased as the fetus developed. In the most mature fetuses investigated (36 and 40 weeks), the surface area showing ependymal denudation roughly matched that lined by ependyma (Fig. 4A, B). In the lateral ventricle, large denuded areas alternated with nondenuded areas (Fig. 4A, B). The latter were lined by a monolayered ciliated ependyma (Fig. 4C) that displayed a thick glycocalix that was rich in sialic acid residues (Fig. 4F). In these areas the ependymal cells and many subependymal cells were reactive for CD99 (Fig. 4C, E). Approximately half of the population of the ependymal cells expressed GFAP (Fig. 4G), and all of them expressed caveolin.

In the oldest fetuses studied (36 and 40 weeks), the denuded areas of the lateral ventricles were characterized by (i) being mostly occupied by a dense plexus of cells processes reactive for CD99 and GFAP (Figs. 4D, G, 5D); (ii) the presence of numerous cells displaying short radiating processes labeled with the antibody against human macrophages, and most likely corresponding to microglia/macrophage cells (Fig. 5E–H). These cells were also seen lying on the exposed surface of the denuded areas. In the nondenuded areas, these microglia/macrophage cells were not seen in the vicinity of the ependyma but deep into the neuropil (Fig. 5E, G).
FIGURE 4. (A, B) Lateral ventricle of a 40-week-old hydrocephalic fetus. Hematoxylin and eosin stain showing nondenuded (open arrows) and denuded (solid arrows) areas. (C–J) Lateral ventricle of a 36-week-old hydrocephalic fetus. (C) Nondenuded area. Immunostaining with CD99 and hematoxylin. (D) Denuded area. Immunostaining with CD99 and hematoxylin. (E–G) Adjacent sections through denuded and nondenuded (asterisk) zones. The nondetached ependyma reacted with CD99 (E), LFA (F), and anti-GFAP (G). Vertical arrows point to border between both zones. (H–J) Immunostaining for β III tubulin. (H) Nondenuded area. (I) Denuded area. Clusters of neuroblasts are seen on the denuded neuropil. (J) A large mass of neuroblasts protrudes into the ventricle. Asterisk denotes nondenuded area. Magnifications: (A) 12×; (B) 56×; (C, D) 560×; (E–G) 280×; (H) 730×; (I) 750×; (J) 240×.
In the neuropil underlying the large denuded areas there were ependymal rosettes with a distinct lumen and cell clusters that were reactive with anti-CD99; unlike the surface ependyma, these cells did not display immunoreactive vimentin and little or no reactive GFAP.

No alterations were detected in the choroid plexus of hydrocephalic fetuses. Thus, the ependymal choroidal cells displayed normal cytologic features and, compared with those of nonhydrocephalic fetuses, showed no differences concerning the amount and spatial distribution of immunoreactive caveolin (Fig. 2G, H).

The subcommissural organ was found in some of the hydrocephalic and nonhydrocephalic fetuses and will be the subject of a special study.
In Hydrocephalic Fetuses the Subventricular Zone Becomes Disorganized and Neuroblasts Reach the Ventricular Cavity

Areas of the lateral ventricles of the 36- and 40-week-old hydrocephalic fetuses where ependymal denudation has not occurred displayed a well-organized subventricular zone. This zone is formed by (i) a single layer of multiciliated ependymal cells reactive for GFAP; (ii) a thin layer of small spherical cells reactive for β III tubulin, most likely corresponding to neuroblasts; (iii) a thick fibrous glial layer reactive for GFAP; and (iv) a fibrous neural layer reactive for β III tubulin (Figs. 4H, 5A, B). In the areas where ependymal denudation had occurred, the subventricular zone showed a distinct alteration, namely, that the β III tubulin-positive neuroblasts had apparently increased in number and where distributed throughout the GFAP-positive glial layer (Fig. 5C, D). In the areas devoid of ependyma, small clusters of neuroblasts lying on the free surface were seen (Fig. 4I). Large masses of neuroblasts protruding into the ventricular lumen through the denuded areas were frequently seen (Fig. 4J).

DISCUSSION

In the human, ependymal cells differentiate from the neuroepithelium along a caudal-rostral gradient; it starts at about the fourth week of gestation and is completed at approximately gestational week 22 (11, 12). Structural proteins such as vimentin, GFAP, and certain cytokeratins are expressed by the human ependyma following a temporal and spatial pattern (12, 13). In regions other than the lateral ventricles, ependymal cells express GFAP at early gestational period as compared with the ependyma of the lateral ventricles that expresses such a protein during the second half of the fetal life. By the time of birth only a few ependymal cells of the lateral ventricle still express GFAP (12), supporting the view that the ependyma of the lateral ventricles is the last one to complete its differentiation.

Caveolin is a distinct molecular marker of the caveolae-mediated endocytosis and transcytotic pathway (14–16). In the central nervous system of adult rats, caveolin-mediated endocytosis occurs in all ciliated ependymal cells, in the ependymal cell of choroid plexus, and in tanyctyes (16). As a key functional molecule, its expression in the immature ependyma of the youngest fetuses indicates that the complex process of endocytosis/transcytosis starts to operate in these cells at early stages of brain development. The location of immunoreactive caveolin in the apical and basal poles of the immature and immature ependymal cells suggests that these cells internalize compounds from the CSF and from the intercellular space.

Since the choroid plexus of the nonhydrocephalic and hydrocephalic fetuses showed no differences in the amount and cellular distribution of immunoreactive caveolin in the ependymal cells of the choroid plexus, and caveolin being a key functional marker, it may be postulated that fluid endocytosis/transcytosis in the choroid plexus of hydrocephalic fetuses is not affected. Furthermore, the lack of ependymal denudation in the choroid plexus of hydrocephalic fetuses resembles the situation of the hydrocephalic mutant hyh mice (3) and further supports the distinct characteristics of this ependyma as compared with the ciliated ependyma lining the ventricles (4, 17).

Denudation of the Ciliated Ependyma Occurs in All Fetuses Developing a Communicating Hydrocephalus

According to recent investigations in animals with spontaneous hereditary hydrocephalus, ependymal denudation is a phenomenon that precedes and triggers the onset of hydrocephalus early in the fetal life (3, 5, 6). In the present investigation the lateral ventricles of the 8 hydrocephalic fetuses displayed ependymal denudation, whereas this pathology was not found in any of the 15 nonhydrocephalic fetuses analyzed. This suggests that in human, as in certain animal mutants, ependymal denudation may be related with the onset and/or early stages of hydrocephalus. This possibility is further supported by the fact that the youngest hydrocephalic fetuses studied (16 weeks) already displayed ependymal denudation. Discontinuities in the ependymal lining of hydrocephalic fetuses associated with a proliferation of astrocytes have also been reported by Sarnat (11). The present findings in the nonhydrocephalic fetuses do not agree with those of Doeling et al, who studied 111 brains of human fetuses with absence of gross defects and found that they all presented small areas of focal ependymal loss without prominent subependymal gliosis (18). These authors speculated that this ependymal defect, which occurs more frequently in the early third trimester of gestation, results from a normal modeling process taking place in the growing brain. Without information regarding the extent of the denuded areas and of the gliosis occurring in these areas it is difficult to compare these findings with the present results.

In the mutant hyh mouse, ependymal denudation occurs as a programmed process that parallels the process of ependymal differentiation, so that ependymal detachment occurs at certain specific sites and times of development (3). In the human hydrocephalic fetuses, ependymal denudation appears to occur throughout the studied period (16–40 weeks), as judged by the presence of denuded areas with and without astrocyte scar. This raised the question whether the mechanism leading to the detachment of the thick multilayered immature ependyma is the same operating in the detachment of the single matured ciliated ependyma (see below).

Mechanism(s) Underlying the Ependymal Denudation

An important issue that has been raised recently is whether ependymal denudation is the result or the cause of hydrocephalus. It has long been sustained that the ependymal loss is a consequence of the expansion of the ventricular cavities occurring in hydrocephalic patients (1, 2). According to Sarnat, the extent of the ependymal denudation correlates with the degree of dilatation of the lateral ventricles and hydrocephalic human fetuses would not be primary in the pathogenesis (2).

Recent investigations carried out in natural and experimental mutant mice have provided strong evidence that hydrocephalus may result from a primary alteration of the...
and a severe hydrocephalus (4). It has recently been elucidated that in the
hyh mouse, a programmed denudation of the ventral ependymal that occurs early in fetal
life precedes the onset of a communicating hydrocephalus (3), and
the denudation of the dorsal ependyma of the cerebral
aqueduct occurring after birth leads to aqueductal obliteration
and a severe hydrocephalus (4). It has recently been elucidated
that the mutated gene in the hyh mouse corresponds to the
gene encoding for soluble N-ethylmaleimide sensitive factor
attachment protein α (αSnap) (19, 20). αSnap plays a key role in
the intercompartmental transport of proteins, including
transport to the plasma membrane. The mutated αSnap in the
hyh mouse results in altered localization of many sur-
face proteins of neural stem cells, such as E-cadherins and
β-catenin (19, 21). It seems highly likely that this defect would
also be expressed in the ependymal cell lineage of the hyh
mutant and the cause of the ependymal detachment occurring
in this hydrocephalic mouse (3, 4). The immunologic blockage
of N-cadherin by a single injection of a blocking antibody into
the CSF of chick embryos results in areas devoid of ependyma
and the progressive formation of ependymal rosettes in the
underlying neuropil (22); suggesting that both phenomena are
related and that rosettes may be the result of a primary defect
of the developing ependyma leading to its abnormal migration
into the neuropil. Interestingly, ependymal denudation coex-
isting with subependymal rosettes is found in hydrocephalic
human fetuses (2; present report). The fact that there is a
differential expression of GFAP and vimentin in the epen-
dymal cells lining the ventricular surface as compared to that
of the cells forming the rosettes indicates that the latter not
only results from an abnormal migration but also from a change
in the phenotype. This could also explain the appearance of
subependymal ependymal clusters. Sarnat has shown that in
fetuses and infants with Chiari II malformations and stenosis
of the cerebral aqueduct there are vimentin-immunoreactive
subependymal rosettes in the vicinity of the fourth ventricle,
and vimentin is focally upregulated in the ependyma of the
areas of dysgenesis, namely the aqueduct, fourth ventricle, and
central canal of the spinal cord, but not in the ependyma of
lateral ventricles (23). These observations could not be con-
firmed in the present investigation since it could not be estab-
lished whether the 3 cases of hydrocephalus associated with
lumbosacral meningocelecele studied were compatible with
a Chiari II malformation. Furthermore, the ependyma of the
cerebral aqueduct and lateral ventricle of these cases appears
to express vimentin normally.

Different genetic defects affecting the ependyma appear as a primary alteration leading to hydrocephalus (5, 6). The present findings in human fetuses with a communicating hydrocephalus, especially those obtained in early develop-
mental stages when hydrocephalus is moderate, favor the view
that in the human also an alteration in the ependyma could
precede the onset of hydrocephalus.

The fact that a primary defect in the ependyma of a given
animal mutant triggers hydrocephalus can be explained on the
basis of a gene mutation (see above). However, an explanation
(on a genetic basis) of the ependymal denudation as a primary
defect in the 8 human fetuses studied, whose hydrocephalus
could be expected to be of different etiologies, is more difficult.
The possibility that in many or most cases of human fetal hydro-
cephalus there is a common defect at the ependymal cell lin-
eage leading to ependymal detachment has to be considered.
This possibility is supported by the fact that in the four known
hydrocephalic mutant and transgenic mice displaying muta-
tions of different genes, they all shared a primary defect of the
ependymal lining (3–7, 24, 25).

**Reaction of Astroglia and Microglia
to Ependymal Detachment**

It has been established that astrocytes play a key role in
repairing the denuded areas in hydrocephalic hyh mice (4).
Indeed, they proliferate, migrate to the denuded walls, change
their phenotype (they express vimentin normally expressed
only by the ependyma), and form a lining “resembling” the
ependymal layer. Furthermore, a period of a few days elapsed
between the ependymal detachment and the astroglial lining of
the exposed surface (4). This might explain the fact that in the
youngest hydrocephalic human fetuses the denuded area
lacked astrocytes and in older fetuses such areas were occu-
pied by GFAP-positive astrocytes. It may be suggested that in
the hydrocephalic human fetuses a process to repair the de-
nuded areas takes place during the fetal life.

The increased number of microglia/macrophage cells in
the neuropil of the denuded areas was a phenomenon observed
in the oldest fetuses studied. Although the functional signif-
icance of this finding is difficult to envisage, the following
information is worth considering. Microglial cells, in addition
to being phagocytic, have multiple functions, (26). Thus, they
have been shown to regulate astrogliosis in scar formation
(27), and in mutant hydrocephalic H-Tx rat they upregulate as
soon as there is ventricular enlargement (28).

**Detachment of the Ependymal Layer May
Affect the Process of Neurogenesis in
Hydrocephalic Fetuses**

Most of the cells of the developing mammalian brain are
produced in 2 germinal zones associated with the ventricular
walls, namely, the ventricular zone and the subventricular zone.
The ventricular zone is a pseudostratified ependyma contain-
ing multipotent neural stem cells. As the brain develops, this
zone progressively transforms into immature and then into
mature ependyma. The subventricular zone is located under-
neath the ventricular zone along the lateral walls of the
lateral ventricles of the embryonic brain (29). Neurogenesis
continues in this zone throughout the animal life span (30). In
rodents and primates the postnatal subventricular zone contains the primary (stem cells) and secondary (neuroblasts)
progenitors; young neurons born in this region migrate in
chains to replace neurons of the olfactory bulb (30, 31). In
the adult human, however, the subventricular zone is a niche of
unique organization where astrocytes forming a subependymal
ribbon behave as multipotent progenitor cells and migrating
neuroblasts are missing (32). According to the present
findings, the subventricular zone of the oldest human fetuses
studied (36 and 40 weeks) is a distinct zone occupied by ast-
rocytes and neuroblasts, thus resembling that of rodents (30)
and primates (31). Although more evidence is needed to con-
firm the presence in the human fetal brain of a “conventional”
subventricular zone, the question arises of how and when the
shift from the conventional fetal to the unique postnatal subventricular zone does occur.

Detachment of the ependyma of the lateral ventricles of the hydrocephalic fetuses results in (i) the loss of the germinal ependymal zone, (ii) the disorganization of the subventricular zone, and (iii) the abnormal migration of neuroblasts into the ventricular cavity. Thus, detachment of the ependymal layer in hydrocephalic fetuses would not only be associated with the pathogenesis of hydrocephalus (see above) but also to abnormal neurogenesis. Interestingly, widespread loss and disorganization of ependyma is found in the brain of children with lissencephaly and other disorders of neuroblast migration (33).

ACKNOWLEDGMENTS

The authors are grateful to Dr. Sebastian Luna-More, head of the Department of Pathology of Hospital Materno Infantil Carlos Haya, Malaga, Spain, for providing most of the tissue samples used in the present investigation; Mr. Genaro Alvial from Instituto de Histología y Patología, Valdivia, Chile; and personnel of Department of Pathology of Hospital Materno Infantil Carlos Haya for valuable technical support.

REFERENCES

15. Tuna PL, Hubbard AL. Transcytosis: Crossing cellular barriers. Physiol Rev 2003;83:871–932