

DESTRUCTION OF CHOROID PLEXUS CELLS IN VITRO: A NEW CONCEPT FOR THE TREATMENT OF HYDROCEPHALUS?

Jake Timothy, F.R.C.S.(SN)

Department of Neurosurgery,
Leeds General Infirmary, Leeds,
England

Paul Chumas, F.R.C.S.(SN)

Department of Neurosurgery,
Leeds General Infirmary, Leeds,
England

Aruna Chakrabarty, F.R.C.Path.

Department of Neurosurgery,
Leeds General Infirmary, Leeds,
England

James M. Drake, F.R.C.S.(C)

Department of Neurosurgery, Sick
Children's Hospital, Toronto,
Ontario, Canada

Ewan Morrison, Ph.D.

Molecular Medicine Unit, St.
James's University Hospital, Leeds,
England

Reprint requests:

Paul Chumas, F.R.C.S.(SN),
Department of Neurosurgery,
Leeds General Infirmary,
Great George Street, Leeds LS1
3EX, England.

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OBJECTIVE: The current treatment of hydrocephalus using ventriculoperitoneal shunts and third ventriculostomies remains problematic. We revisited the concept of destruction of the choroid plexus for the treatment of hydrocephalus by using an immunotoxin-based technique to specifically destroy this tissue. This approach was based on the observation that, as an epithelial tissue, choroid plexus expresses a number of specific cell-surface proteins that represent excellent potential targets for the creation of a choroid plexus-specific immunotoxin.

METHODS: In this study, we characterized sheep and human choroid plexus cells (including atypical and carcinoma cell lines) using fluorescence microscopy in combination with histochemical staining of rat brain and confirmed the presence of a number of epithelium-specific proteins in choroid plexus cells. Immunotoxins were then manufactured by linking these antibodies to ricin A chain and ricin A-B chain. These immunotoxins were delivered to choroid plexus-derived cells in culture, and the results were compared with results of exposure to a nonspecific immunotoxin.

RESULTS: Complete cell death of choroid plexus cells was seen after only a 1-hour exposure to the specific immunotoxin, as opposed to the minimal cell death seen with a nonspecific immunotoxin after several hours of exposure.

CONCLUSION: These results suggest that immunotoxin-mediated ablation of choroid plexus may be a viable method of treating hydrocephalus and choroid plexus-derived tumors.

KEY WORDS: Choroid plexus, Hydrocephalus, Immunotherapy

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The treatment of hydrocephalus remains difficult. Current treatment involves diversionary procedures, such as ventriculoperitoneal shunts or third ventriculostomies. These techniques have their drawbacks, which include shunt blockage and infection, overdrainage, subdural collections, and vascular damage (9, 11, 12). Shunting procedures rely only on the symptomatic treatment of hydrocephalus rather than addressing the primary problem: the decrease in absorption or occasionally the overproduction of cerebrospinal fluid (CSF). In 1918, Dandy (1) first proposed the destruction of choroid plexus as a potential treatment for hydrocephalus. His article showed that although it was possible to remove the choroid plexus from the lateral ventricles as an attempt to reduce

CSF production, the morbidity and mortality were high. More recently, endoscopic coagulation of the choroid plexus has been attempted, with limited success (7). This may reflect the fact that open and endoscopic choroid plexotomy targets only the choroid plexus in the lateral ventricles. Furthermore, the bleeding and debris associated with these procedures is likely to compromise absorption of CSF.

We revisited the concept of destruction or partial destruction of the choroid plexus as a means to treat hydrocephalus. Our concept was to design a reagent that, on injection into the ventricular system, would be able to attach specifically to the choroid plexus and destroy this tissue, thereby reducing the CSF production.

MATERIALS AND METHODS

Cell Culture

Sheep choroid plexus cells initiated from the choroid plexus of an ovine brain were obtained from the European Collection of Cell Cultures (Centre for Applied Microbiology and Research, Salisbury, Wiltshire, England). For all culture maintenance and experiments, the culture medium used was RPMI containing Glutamax (Invitrogen Corp., Paisley, Scotland), supplemented with 10% fetal calf serum and penicillin/streptomycin (Invitrogen Corp.). Cells were incubated at 37°C in the presence of 5% CO₂. Primary cultures of human choroid plexus cell lines were obtained after patient/parental consent. Solid pieces were washed with fresh medium (Ham's F-10) and left to soak for 15 to 20 minutes. The tissue was macerated with a scalpel, and single cells were released, separated, and immediately placed into culture medium in a flask and incubated in a CO₂ incubator. The solid pieces remaining were digested for 40 minutes in trypsin. The cells were centrifuged, and the trypsin was poured off. This was followed by a 1-hour exposure to collagenase; the cells were again centrifuged, poured off, and added to the culture medium. Human normal, atypical, and carcinoma choroid plexus cell lines were cultured. Only the choroid plexus carcinoma cell line was immortalized and subsequently deposited back to the European Collection of Cell Cultures for archiving and remains in their catalog.

Antibodies

Three antibodies were selected as potential choroid plexus targets on the basis that they should be monoclonal reagents that recognize extracellular epitopes on epithelial cells. We speculated that these would have a range of specificity, with epithelial membrane antigen (EMA) (Neomarkers, Fremont, CA; 1 mg/ml purified without bovine serum albumin and sodium azide) being highly specific; multidrug resistance (MDR) 1 gene product (CD243; AMS Biotechnology, Oxford, England; 500 µg/ml in phosphate-buffered saline [PBS], sodium azide-free) being moderately specific; and the anti-Na⁺,K⁺-adenosine triphosphatase (ATPase) β1 subunit (Research Diagnostics, Inc., Flanders, NJ; 200 µg in 0.238 ml of 0.1 mol/L Tris-glycine with 0.05% sodium azide) being the least specific. As a control for nonspecific binding, normal mouse immunoglobulin (IgG) (Vector Laboratories, Burlingame, CA) was used.

Immunofluorescence and Confocal Microscopy

Potential antibody-binding sites and antibody sites specific to choroid plexus tissue were investigated by confocal microscopy. Cells were grown on glass coverslips and fixed in 4% paraformaldehyde in PBS for 20 minutes to detect cell-surface antigens. To detect intracellular antigens, this fixation was followed by a brief permeabilization step using 0.1% (v/v) Triton X-100 in PBS; alternatively, cells were fixed in methanol for 5 minutes at -20°C. All secondary antibodies were Alexa Fluor conjugated reagents obtained from Molecular Probes Inc. (Eugene, OR). Coverslips were mounted on slides with

Mowiol polyvinyl alcohol (Kuraray, Frankfurt, Germany) and viewed with a Leica TCS-SP confocal imaging system (Leica, Wetzlar, Germany).

Specificity of Antibodies to Choroid Plexus

Dissected whole rat brains were fixed in 10% buffered formalin. Whole-brain coronal slices were embedded in paraffin. Representative blocks were used. Routine immunohistochemistry was performed on 4-µm-thick sections on 3-aminopropyltriethoxysilane-coated slides by use of a heat-induced epitope retrieval system with Vector antigen unmasking solution (Vector Laboratories) as pretreatment and a Dako ARK animal research kit (Dako, Cambridge, England). The antibody dilutions were as follows: EMA, 1:200; MDR 1 gene product, 1:10; and anti-Na⁺,K⁺-ATPase, β1 subunit, 1:200.

Immunotoxin Manufacture

Ricin A chain, 50 µg (Sigma Chemical Co., St. Louis, MO) was added to 100 µg of IgG, 20 µg EMA, 20 µg MDR, or 16.8 µg Na⁺,K⁺-ATPase antibodies, respectively. Sulfo-*m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester cross-linker, 100 µg (Pierce, Rockford, IL) was dissolved in 100 µl of sterile water. Of this, 10 µl was added to the antibody/ricin mixtures. These were then incubated for 2 hours at 37°C. After incubation, the mixture was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis to confirm antibody binding to ricin (Fig. 1). Because the mixture contained free antibody, ricin, cross-linker, and immunotoxin, further purification was performed with fast performance liquid chromatography (FPLC). One hundred microliters of the mixture was loaded over a Superdex 200 HR (Pharmacia, Uppsala, Sweden) FPLC column running with PBS. Fractions of 0.5 ml/min were taken. Peaks corresponding to the molecular weights of the immunotoxin and free antibody were impossible to differentiate, and therefore, the peaks were pooled. Usually, three fractions (0.75 ml) were collected, and the other

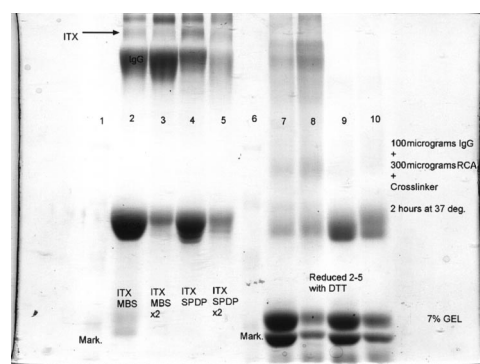


FIGURE 1. A 7% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel showing a band of protein at approximately 190 kilodaltons (arrow) corresponding to conjugated immunotoxin (ITX) using IgG as the antibody, ricin A-B (RCA) as the immunotoxin, and sulfo-*m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) as the cross-linker. DTT, dithiothreitol; SPDP, succinimidyl 3-(2-pyridyldithio)propionate.

peaks corresponding to free ricin, degraded antibody products, and possible preservatives were discarded. Pooled peak fractions were stored frozen for future use. The process was also repeated using 50 μg ricin A-B chain.

Cell Killing Assays

Cell cultures were grown on coverslips in 6-well culture dishes until confluent. The culture medium was then removed, and various concentrations of the immunotoxin mixture were added to 1 ml of fresh medium. This was added to the cell cultures for various periods of time (maximum, 120 min). The cell culture was then washed twice with PBS, and fresh culture medium was added to the cell culture. Inspection of the cell culture was performed daily for up to 10 days. Cells were then fixed in paraformaldehyde, stained with crystal violet, and mounted on slides.

RESULTS

Specificity

On rat brain sections, both EMA and MDR antibodies showed good specificity to choroid plexus tissue (Fig. 2). The antibody to the Na^+, K^+ -ATPase showed less specificity for choroid plexus and therefore was not as good a potential target for immunotherapy of the choroid plexus. It was encouraging to note that the ependymal cell lining gave no staining with the EMA and MDR antibodies.

Results of Immunostaining

Confocal microscopy results indicate that sheep choroid plexus cells are a good model to study antibody-binding sites in the human choroid plexus. Because no commercial human choroid plexus cell cultures are available and it was not possible to maintain the human cell line, we could not perform cell-killing assays on these cells. Our results from confocal microscopy staining on sheep choroid plexus correlated well with human choroid plexus staining. Sheep and human choroid plexus, including normal, atypical, and carcinoma cell lines, were positive for Na^+, K^+ -ATPase, EMA, and MDR antibody, indicating that this tissue is amenable to immunotherapy with the appropriate antibodies. It was interesting to note that although all cells express the specific antibodies tested, there was variability between even adjacent cells with respect to strength of staining. This is not artifactual, and one could speculate that there is a subpopulation of choroid plexus cells that have differing function, as also suggested by Szymdynger-Chodobska et al. (10).

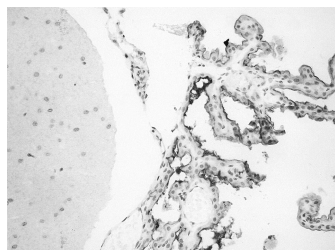


FIGURE 2. Photomicrograph of immunohistochemistry for EMA showing surface staining in rat choroid plexus epithelium. The underlying brain tissue is negative (original magnification, $\times 200$).

Cell Killing Assays

We used both ricin A chain and ricin A-B chain. There is clear evidence that the ricin A-B chain is a more potent immunotoxin than ricin A chain alone. All further experiments were then performed with ricin A-B chain.

Two observations are clear. Increasing the time of exposure of the cells to the immunotoxin correlated with increased cell death. Minimal cell death occurs with an exposure of 0 to 15 minutes; however, after 30 minutes of exposure, cell death is more obvious, with nearly complete cell death at 120 minutes of exposure of immunotoxin on cell cultures. After this experimental result, we exposed the cells to an incubation period of only 1 hour with immunotoxin. Clearly, we were able to increase cell death at lower concentrations of immunotoxin by simply increasing the incubation period with the immunotoxin.

With an increase in the dosage of the immunotoxin, cell death was also increased (Fig. 3). This was true for each of the antibodies studied apart from the control IgG antibody, in which increasing immunotoxin volume did not correlate with increased cell death. Optimum dosages of immunotoxin for each antibody-toxin conjugate were found, and the experiments were repeated twice. Minimal cell death occurred with the control IgG-ricin conjugate compared with the more specific antibodies at the same dosages. The most potent antibody-ricin conjugate was MDR, followed by EMA and then Na^+, K^+ -ATPase. MDR was five times more potent than EMA and 10 times more potent than Na^+, K^+ -ATPase antibody.

Human choroid plexus carcinoma cells were also cultured. Cell death did occur, but only at higher dosages and greater incubation periods than those of normal choroid plexus (not shown).

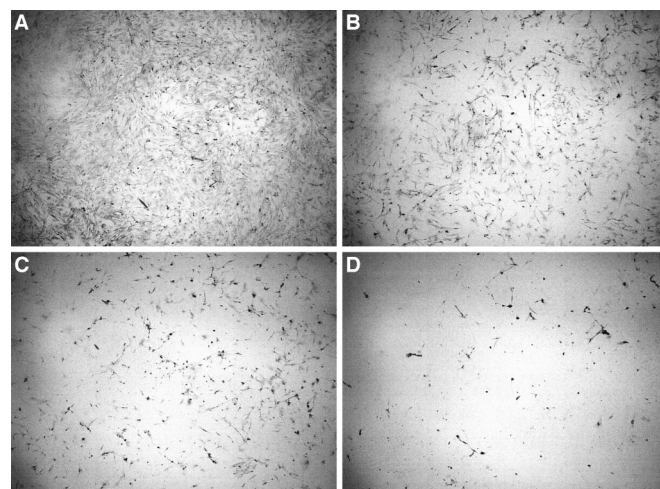


FIGURE 3. Low-power photomicrographs of cell density using an increasing dose of immunotoxin (MDR immunotoxin) on sheep choroid plexus cells. Exposure time of immunotoxin was 1 hour. At 5 μl , there is minimal cell death; at 20 μl , there is significant cell death. A, 5 μl ; B, 10 μl ; C, 15 μl ; D, 20 μl (crystal violet stain; original magnification, $\times 10$).

DISCUSSION

This is the first time the concept of targeting choroid plexus tissue by use of an immunotoxin as a potential treatment for hydrocephalus has been used. This method aims to modify the CSF production physiology rather than to treat the symptoms.

The concept of combining an antibody to a “toxin” to form an immunotoxin (IT) has been around for more than 20 years. Ricin, the toxin from the castor bean, is a glycoprotein (molecular weight, 65,000) composed of two chains, A and B, joined by a disulfide bond. The B chain binds to galactose-terminating glycoproteins and glycolipids expressed on the surface of most cells. This allows for the toxin to be taken up by the cell and for the A chain to kill the cell by inactivating the ribosomes. Immunotoxins have therefore been constructed by use of either ricin (IT-R or RCA) or only A-chain ricin (IT-A).

Monoclonal antibodies have been used as the usual ligand. Not surprisingly, an important consideration in the selection of an antibody to form an IT-A is the nature of the target antigen and the pathway by which it triggers entry into the cell. Antibodies to receptors that result in the compound entering endosomes rather than lysosomes are far more effective. Antibodies that bind close to the plasma membrane have been found to be more effective. Other factors include the affinity of the monoclonal antibody and the density of the antigen on the target cell. To circumvent some of these problems, researchers have used antibody fragments [Fab' or F(ab')₂] attached to A chain. One of the advantages of these fragment IT-As is that they are less immunogenic. IT-Rs are less dependent on the nature of the binding of the monoclonal antibody to the cell, because the B chain can interact directly with the cell wall. However, this also means that they are less specific, because they can bind to nontarget cells via the B chain. Researchers have therefore prepared IT-Rs with short cross-linkers such that the antibody blocks the galactose-binding sites on the B chain. Blocked ricin has also been generated by cross-linking fetuin-derived oligosaccharides into the galactose-binding sites of the B chain.

Three antibodies were selected as potential choroid plexus targets on the basis that they should be monoclonal reagents that recognize extracellular epitopes specific to the apical surface of epithelial cells. Although there are other antibodies specific to the choroid plexus, the following antibodies are monoclonal and commercially available and have the advantage of not being species-specific, thereby binding to sheep, rat, and human choroid plexus epitopes.

The Na⁺,K⁺-ATPase pump is found in all vertebrate cells that maintain an ionic gradient. In choroid plexus cells, it is found on the apical plasma membrane domains. This pump is an oligomer composed of stoichiometric amounts of two major polypeptides, the α - and β -subunits. The β -subunit is a polypeptide that crosses the membrane once and has a 305-amino-acid residue that is outside the cell membrane on the apical side and therefore can act as a target for immunotherapy, unlike the α -subunit, which is primarily within the cell

membrane. This antibody binds to a region in the ectodomain (approximately amino acids 62–304) of the β -subunit.

EMA was first described in 1979 by Heyderman et al. (2), who raised antisera to elements of secretory epithelia. The chemical nature of EMA has not been completely defined. Although EMA epitopes are widespread within the body, they seem to be limited to the apical surface of the choroid plexus cells within the brain and especially within the ventricle. Arachnoid cells do express EMA (5), and it is possible that the antibody toxin could interfere with CSF absorption itself. This would need to be examined in an *in vivo* environment.

Tumor cell resistance to cytotoxic drugs is considered one of the major obstacles to successful chemotherapy. Some tumors are initially resistant and never respond to cytostatic drug treatment; others initially respond well but eventually regrow and become resistant. This phenomenon may result from genetic mutations induced by the antitumor agent used or may represent the selection of preexisting resistant cell populations in the malignant tumor. MDR describes the simultaneous expression of cellular resistance to a variety of unrelated drugs primarily of natural origin. The classic form of MDR is mediated by P-glycoprotein, which acts as a drug efflux pump. Staining of human choroid plexus tissue with MRK16, a monoclonal antibody that recognizes an extracellular epitope specific to human MDR1 P-glycoprotein, showed a homogeneous expression pattern of MDR P-glycoprotein confined to the choroid plexus epithelial cells (8).

To produce cytotoxicity, it is necessary to introduce a disulfide bond between the A chain and the ligand (presumably to allow intracellular reduction to free the A chain). The linkers used to form this bond are usually heterofunctional agents that introduce an activated thiol group. To increase stability, hindered cross-linkers have been used. Some Fab' and Fab fragments have a free cysteine residue that can be used to form a disulfide bond. Adding carboxylic ionophores and lysosomotropic agents can enhance the cytotoxic effect of IT-As. The potency of IT-As can also be enhanced by the addition of free B chains in the presence of lactose, by formation of an IT-B directed at the antibody component of the IT-A, or by cotargeting the A and B chains separately to the cells using the same antibody. Although specificity is reduced by combining the antibody with ricin A-B chain (IT-R), potency is dramatically increased. The B chain facilitates uptake of the immunotoxin into the cell.

Zovickian et al. (13) studied the use of immunotoxin therapy for leptomeningeal neoplasia in an animal model and found the intraventricular route to be the best, because it reduces systemic toxicity. In this study, the dosages were well tolerated, and there was an increase in tumor cell death in the treatment group. In 1997, Laske et al. (4) reported a clinical trial of intraventricular administration of an immunotoxin for leptomeningeal neoplasia. This study addressed many of the concerns we had relating to the injection of immunotoxins into the ventricular system of the brain. Covalent bonding of ricin-antibody conjugates seemed to be stable *in vivo*. No obvious systemic toxicity was caused by intraventricular injection of

antibody, and no patient developed detectable levels of anti-conjugate antibodies. With regard to pharmacokinetics, the early-phase half-life of the immunotoxin within the CSF compartment averaged 44 ± 21 minutes, and the late-phase half-life averaged 237 ± 86 minutes. Therefore, the incubation of the sheep choroid plexus cells with immunotoxin for 1 hour in an in vitro setting in the present study seems reasonable. The pharmacokinetics and toxicology of immunotoxins administered into the subarachnoid space in nonhuman primates and rodents have also been studied (6). The only side effect was related to the local toxicity on Purkinje cells, the destruction of which can result in ataxia and lack of coordination.

Another potential problem is the fact that an inflammatory reaction is to be expected when a toxin is injected into the ventricular system, and this may further exacerbate poor CSF absorption in the hydrocephalic patient. This inflammatory response, however, seems to be dose-dependent; provided that the dose was less than $38 \mu\text{g}$, no toxicity symptoms were detected clinically, and the article did not report any patient developing hydrocephalus as a consequence of intraventricular immunotoxin injection.

Our immunotoxin preparation contained a proportion of free antibody, because we were unable to remove this by use of FPLC. Because there would be competition for binding sites, the free antibody would act to dilute the effects of the immunotoxin; therefore, a pure immunotoxin devoid of free antibody would be expected to be more potent. We chose ricin because it has been used widely in the preparation of immunotoxins and its effects have been well characterized in experimental and clinical studies.

Of the three antibody-toxin conjugates tested in this study, the Na^+, K^+ -ATPase immunotoxin is the least useful, because it is less choroid plexus-specific than the others and is also the least potent. EMA immunotoxin had the highest histological specificity for choroid plexus tissue but was not as potent as MDR in the cell-killing assays. Both of these immunotoxins will therefore be studied in an in vivo model in future studies. Although we had limited success concerning the in vitro death of human choroid plexus carcinoma cells, probably because of less expression of the specific epitopes and innate resistance of these cells, the concept may be useful in the future as an adjunct to surgical excision. The dosage and incubation time necessary for complete cell death are greater than for cell death of normal choroid plexus cells.

It is appreciated that not all CSF production is from the choroid plexus and therefore that inactivation of the choroid plexus in this way will not necessarily halt all CSF production. More recently, the choroid plexus has also been implicated in neurohumoral modulation of the brain (3), and therefore, complete destruction of all the choroid plexus may well disrupt an important physiological function of this tissue. Chemical choroid plectomy may therefore cause problems in the clinical setting. Nonetheless, even if not a "cure" for hydrocephalus, it will be a useful tool to assess CSF physiology in the laboratory. The advantage of having a dose-dependent immunotoxin is that choroid plexus physiology may need to be altered

only slightly to address the pathological condition of hydrocephalus.

CONCLUSION

It is both the specificity of the antibody conjugates and the reduction of systemic toxicity by injection into the CSF compartment that makes the use of an immunotoxin-based technique an attractive method to treat hydrocephalus. This article serves only to demonstrate the concept of specific destruction of the choroid plexus. Future studies using intraventricular injection into the congenital hydrocephalic rat model should allow the possibilities of this technology to be explored further.

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Acknowledgments

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COMMENTS

The authors of this article hypothesize that, since the choroid plexus produces cerebrospinal fluid (CSF), its destruction could lead to a treatment for hydrocephalus. Rather than trying to remove the choroid plexus by surgical methods, they attempted to achieve choroid plexus destruction by immunotoxins that were specifically targeted to choroid plexus epithelial cells.

The authors identified several choroid plexus epithelial-specific proteins. They then developed an immunotoxin to destroy these epithelial-specific proteins. In vivo, they showed staining of the rat choroid plexus using epithelial membrane antigen. In vitro, they showed increasing cell death in response to higher concentrations of multidrug-resistant immunotoxin administered to cultured sheep choroid plexus cells.

The authors present this information as a preliminary study. They are considering additional studies whereby immunotoxin would be injected intraventricularly into a congenital hydrocephalic rat model.

As the authors note, not all CSF is produced by the choroid plexus. In fact, experimental studies indicate that 20% to 30% of CSF formation is extrachoroidal. Milhorat (2) has reported that removal of the choroid plexus from the lateral ventricles in hydrocephalic patients rarely results in ventricular diminution and, in most cases, the ventricles continue to enlarge. However, in his studies, the choroid plexus was only removed from the lateral ventricles and not from the third and fourth ventricles. Whether or not the remaining choroid plexus tissue can compensate is an unknown factor. Cutler et al. (1) have noted that, as a result of the nature of the formation curve versus the absorption curve, a reduction in normal CSF production by one-third would cause the intracranial pressure to decrease by only 1 mm Hg. This helps explain why drugs to reduce CSF formation or surgical removal of the choroid plexus have not proved to be particularly useful in controlling hydrocephalus. Thus, the main problem with hydrocephalus is CSF absorption and not CSF formation.

J. Gordon McComb
Los Angeles, California

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Attempts to treat hydrocephalus by choroid plexus ablation comprise one of the more interesting chapters in the history of neurosurgery. In this study, the authors describe a technique for the in vitro destruction of choroid plexus cells using specific immunotoxins. Although immunotoxin-mediated ablation of the choroid plexus has some research interest, the implication that such an approach might be a viable method for treating hydrocephalus overlooks the lessons of history. For example, the authors fail to cite an exten-

sive body of evidence concerning the extrachoroidal formation of CSF (1, 2, 4), the inability of choroid plexectomy to dramatically reduce the rate of CSF formation (5, 6), and the utter failure of choroid plexectomy as treatment for hydrocephalus (2-4, 7). Also missing is requisite information concerning neurotoxicity and the in vivo effects of widespread epithelial sloughing, which is almost certain to accentuate CSF obstruction. Given these issues, the results of the current study do not rise to the standard of "a new concept for the treatment of hydrocephalus."

Thomas H. Milhorat
Manhasset, New York

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The hope of patients and neurosurgeons is that alternative treatments for hydrocephalus will be developed. The authors present a clever, attractive, and novel approach using well-tested immunological techniques to cause cell death of choroid plexus in culture. For the past several decades, excellent work has been undertaken in engineering laboratories to design improved CSF diversion devices, such as programmable valves. However, our future goal should include the development of strategies to improve CSF absorption or decrease production, thus avoiding the "friction" associated with maintaining a working shunt. Immunological techniques used in chemotherapy for leukemia and carcinomatous meningitis may provide us with clues for decreasing the production of CSF with immunotoxins. The proposed conjugates described in this article are attractive because they combine the potency of the toxin ricin with the specificity of the chosen ligand.

To date, the most successful treatment of immunotoxins has been in the depletion of T cells from allogeneic bone marrow grafts to prevent graft-versus-host disease. However, trials of a variety of similar immunotoxin combinations to treat solid malignancies, chronic lymphocytic leukemia, and other disease states have met with limited or poor results. The obstacles are great, including rapid systemic CSF clearance, poor delivery to target sites, and humoral immunological responses to the toxins. Whether an inflammatory response to toxins in the CSF in vivo hinders the effect of the antibody-toxin ligand is a concern. The authors predict, however, that this will be a dose-dependent effect and will thus be ameliorated by lower

doses of immunoconjugate. Furthermore, the idea of attaching an antibody with specific binding to choroid plexus epitomes to a toxin such as ricin is attractive because it has the advantages of being given intraventricularly, thus avoiding the systemic side effects that may lead to unacceptable complications. The hope is that this avenue of "immunotechnology" will potentially translate into satisfactory long-term, sustainable control of hydrocephalus through medications that can be titrated with a good safety profile.

Richard G. Ellenbogen
Seattle, Washington

This is an in vitro study in which an immunotoxin is directed against cultured choroid plexus-derived cells. The toxin resulted in cell death, whereas a nonspecific antibody toxin did not. The concept is that this could be explored as a treatment for hydrocephalus.

This is very preliminary work, and many issues need to be resolved before the findings can be applied clinically. The problem with any immunotoxin is the specificity of the antibody; the authors have attempted to address this, but there is concern that the ependymal surface or other tissues might bind to the toxin. This might cause an inflammatory reaction and further compromise CSF absorption. The authors also acknowledge that there are extrachoroidal sources of CSF production that would remain even after successful ablation of the choroid plexus.

Leslie N. Sutton
Philadelphia, Pennsylvania

In the current study, the authors report that they were able to destroy living choroid plexus cells using immunotoxin-linked monoclonal antibodies. They have shown that the antibodies can be injected into the ventricles of experimental animals without undue toxicity. They mention that the CSF is not produced only by the choroid plexus but think that the failure of choroid plexectomy to treat hydrocephalus is probably attributable to the surgeon's inability to access the choroid plexus of the third and fourth ventricles. Acetazolamide, which either markedly decreases or completely blocks production of CSF by the choroid plexus, has not been shown to prevent symptomatic CSF absorptive difficulties except in the context of hydranencephaly, in which there is a very small volume of brain parenchyma to produce CSF (3). Extrachoroi-

dal CSF production may represent as much as 50% of the total production (2), and removal of the choroid plexus from the lateral ventricles does not lead to permanent reduction in the rate of production of CSF (1). Since CSF absorption is pressure dependent, less CSF is absorbed when pressure is lowered. This controller function maintains an equilibrium in the volume of the cerebral ventricles and keeps the intracranial pressure within a relatively tight range. Obstruction to outflow will not be changed by decreasing by half or two-thirds of the volume of CSF entering the system. We await a study of the use of intraventricular monoclonal antibodies in established animal models of hydrocephalus to see whether or not this can have application in this situation.

Harold L. Rekate
Phoenix, Arizona

1. Milhorat TH, Hammock MK, Chien T, Davis DA: Normal rate of cerebrospinal fluid formation five years after bilateral choroid plexectomy: Case report. *J Neurosurg* 44:735-739, 1976.
2. Milhorat TH, Hammock MK, Fenstermacher JD, Levin VA: Cerebrospinal fluid production by the choroid plexus and brain. *Science* 173:330-332, 1971.
3. Wellons JC III, Tubbs RS, Leveque JC, Blount JP, Oakes WJ: Choroid plexectomy reduces neurosurgical intervention in patients with hydranencephaly. *Pediatr Neurosurg* 36:148-152, 2002.

The authors have applied the principles of immunotherapy to the concept of selectively ablating choroid plexus epithelium. Their careful in vitro study demonstrates that it is possible to destroy choroid plexus cells in a dose- and time-dependent manner using a specific immunotoxin. When applied to rat brain slices, the relevant antibody appears to be specific for choroid plexus tissue. As their title indicates, the impetus for this effort is to devise a safer, more effective treatment for hydrocephalus. I admire their goal and encourage them to see it to fruition. The number of potential problems along the way is formidable. The authors have alluded to some of them. As they point out, we do not really know what will happen if choroid plexus epithelium is permanently eliminated. To me, the more exciting, proximate goal of this technology is to gain a better understanding of choroid plexus function and CSF physiology.

Paul H. Chapman
Boston, Massachusetts

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