Characterization of a model of hydrocephalus in transgenic mice

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The purpose of this study was to elucidate the pathophysiology of hydrocephalus in a new transgenic model of mice created to overproduce the cytokine, transforming growth factor-β1 (TGFβ1), in the central nervous system (CNS).

Galbreath and colleagues generated transgenic mice that overexpressed TGFβ1 in the CNS in an effort to examine the role of this cytokine in the astrocytic response to injury. Unexpectedly, the animals developed severe hydrocephalus and died. The authors perpetuated this transgenic colony to serve as a model of congenital hydrocephalus, breeding asymptomatic carrier males that were heterozygous for the transgene with wild-type females.

One hundred twelve (49.6%) of 226 mice developed clinical manifestations of hydrocephalus, which was characterized by dorsal doming of the calvaria, spasticity, limb tremors, ataxia, and ultimately death. The presence of the TGFβ1 transgene was determined by performing polymerase chain reaction (PCR) analysis of sample cuts of tail. The animals with the hydrocephalic phenotype consistently carried the transgene, although some animals with the transgene did not develop hydrocephalus. Animals without the transgene did not develop hydrocephalus.

Alterations in brain structure were characterized using magnetic resonance (MR) imaging, gross and light microscopic analysis, and immunocytochemistry. Magnetic resonance imaging readily distinguished hydrocephalic animals from nonhydrocephalic controls, and it demonstrated an obstruction at the outlets of the fourth ventricle. Gross and light microscopic examination confirmed the MR findings. The results of immunofluorescent staining of brain tissue slices revealed the presence of the TGFβ1 cytokine and its receptor preferentially in the meninges and subarachnoid space in both hydrocephalic and control mice. Reverse transcriptase-PCR analysis demonstrated tissue-specific expression of the TGFβ1 gene in the brains of transgenic mice, and enzyme-linked immunosorbent assay confirmed the occurrence of overexpression of the TGFβ1 cytokine in brain, cerebrospinal fluid, and plasma.

The transgenic murine model provides a reproducible representation of congenital hydrocephalus. The
authors hypothesize that overexpression of TGFβ₁ in the CNS causes hydrocephalus by altering the environment of the extracellular matrix and interfering with the circulation of cerebrospinal fluid. A model of hydrocephalus in which the genetic basis is known should be useful for evaluating hypotheses regarding the pathogenesis of this disorder and additionally, should help in the search for novel treatment strategies.

**Key Words** *transforming growth factor* *cerebral ventricle* *cytokine* *hydrocephalus* *magnetic resonance imaging* *animal model* *transgenic mouse*

Hydrocephalus, the pathological accumulation of cerebrospinal fluid (CSF) within the ventricles of the brain, is the most common neurosurgical disorder of childhood. The natural history of untreated congenital hydrocephalus is poor, with less than one third of affected children surviving beyond 18 months of age. Insertion of a shunt within the ventricles is the current surgical standard of care, but this surgery is associated with more complications than any other neurosurgical procedure currently performed. Further elucidation of the pathophysiology of hydrocephalus should aid the search for novel treatment strategies, but existing experimental models are cumbersome or flawed.

In the present report we focus on a new experimental model of hydrocephalus in transgenic mice, which was created to overexpress transforming growth factor-β₁ (TGFβ₁) in the central nervous system (CNS). Transforming growth factor-β₁ is a multigene cytokine with broad regulatory roles in cell growth, differentiation, and tissue repair. The TGFβ₁ transgenic mouse was initially developed by investigators studying the astrocytic response to injury in vivo.[45] Unexpectedly, founder animals consistently developed hydrocephalus. We have perpetuated this colony to serve as an experimental model of hydrocephalus.

Our hypothesis was that overexpression of TGFβ₁ in the CNS caused hydrocephalus by altering the environment of the extracellular matrix (ECM). To characterize hydrocephalus in this transgenic colony, we used magnetic resonance (MR) imaging, gross and light microscopic analysis, and immunohistochemical analysis to identify the TGFβ₁ cytokine and its receptor.

**MATERIALS AND METHODS**

**Transgenic Murine Model of Hydrocephalus**

The present transgenic model of hydrocephalus was originally created by Galbreath and colleagues[45] in 1995. Their aim was to develop a transgenic mouse that overexpressed TGFβ₁ so as to study the effects of this regulatory cytokine on the astrocyte response to injury in vivo. The transgene was produced by incorporating porcine TGFβ₁ complementary DNA downstream of a human glial fibrillary acidic protein (GFAP) fragment, thus utilizing GFAP as the promoter. Transgenic mice were produced according to standard techniques by microinjecting the DNA solution into the male pronucleus of fertilized eggs obtained from mating F1 hybrid C57BL/6J x SJL mice (B6SJLF1). The eggs were transferred to pseudopregnant FVB/N foster mice.

The consistent finding of hydrocephalus in founder animals was unexpected. A hydrocephalic line was established by performing ovarian transplantation from an affected female founder animal that overexpressed TGFβ₁ and assigned the designation TgN4Mes. We have perpetuated this line and used it
to serve as a model of congenital hydrocephalus and to study the pathogenesis of this disorder.

**Transgenic Mouse Colony**

Male mice that were heterozygous for the transgene but in which the hydrocephalic phenotype was not present (asymptomatic carriers) were crossed with wild-type female mice (C57BL/6J) by using a polygamous harem breeding scheme. Each breeding male was placed with two or three wild-type females for a period of 5 days to ensure that females would be in estrous and become inseminated. Thus, offspring were either heterozygous for the transgene or wild type. Breeder males were removed after 5 days to ensure that the postpartum estrous was not utilized.

**Polymerase Chain Reaction**

Analysis of the integration of the transgene into subsequent generations was conducted using the polymerase chain reaction (PCR) on DNA obtained in tail-cut samples from pups at the time of weaning. The DNA was obtained by taking 15-mm cuts from the tails of weaned pups. This tissue was protease digested, and the DNA was placed on an affinity spin column and subsequently eluted. The DNA content was confirmed by spectroscopy. The isolation of DNA and PCR was performed. The necessary thermal cycles were performed in a thermal cycler, and samples were then placed on a 2% agarose gel and subjected to electrophoresis. Gels were evaluated under ultraviolet light.

**Magnetic Resonance Imaging**

Hydrocephalic and control mice underwent MR examination after induction of thiopental sedation. High-resolution proton-density gradient-echo MR images were obtained using a specially designed 1-cm-diameter solenoidal receiver coil and a 1.5-tesla magnet. The brains of hydrocephalic animals were compared with brains of nonhydrocephalic controls on three-dimensional volumetric scans.

**Morphological Analysis**

Gross analysis of hydrocephalic and control brains was performed on sections cut in the coronal plane. Brains were fixed in 4% paraformaldehyde and then embedded in paraffin. Ten-micron slices were obtained, and tissue was subsequently stained with hematoxylin and eosin for light microscopic examination.

**Volumetric Analysis of Ventricular Size**

Qualitative analysis of ventricular size was performed on frozen brains. The mice received a light anesthetic of halothane and were killed by submersion in liquid nitrogen. The heads were then placed in optimum cutting temperature (OCT) medium and sliced in the coronal plane up to the region of the third ventricle. Photographic records were obtained of hydrocephalic and nonhydrocephalic brains.

**Immunohistochemical Analysis**

Immunohistochemical analysis was performed to identify the TGFβ1 cytokine and its receptor. The animals received an anesthetic of halothane and were subsequently decapitated. The heads were then submerged in isopentane cooled with liquid nitrogen. Entire heads were then placed in OCT medium and 8-µ slices were cut using a cryomicrotome. The tissue was then fixed in cold acetone for 10 minutes. A preblock of 10% normal serum in phosphate-buffered saline was used to suppress nonspecific binding of immunoglobulin G (IgG). The tissue was then incubated with the primary antibody. Cytokine analysis
was conducted using a polyclonal anti-TGFβ₁ affinity-purified chicken IgG, and receptor analysis was performed using a polyclonal anti-TGF-β₁ receptor purified rabbit IgG. For detection of the cytokine, a fluorescein-conjugated secondary antibody, rabbit anti-chicken immunoglobulin Y, was used. For receptor detection, a fluorescein-conjugated secondary antibody, goat anti-rabbit IgG, was used. Tissue samples were examined using light, darkfield, phase-contrast, and fluorescent microscopy. Photographs were made from the slides for future examination.

**Isolation of RNA and Reverse Transcriptase PCR**

Using the test kit, total RNA was obtained by following the manufacturer's instructions. Briefly, whole brains (approximately 50 mg) from 2-day-old neonatal mice were rapidly homogenized in guanidine isothiocyanate (RNeasy Lysis Buffer) and subsequently processed according to the manufacturer's instructions. Samples of RNA were stored in diethyl pyrocarbonate-treated water at -20°C until needed. Agarose gel electrophoresis was used to analyze total RNA qualitatively for its integrity, noting the receptor RNA bands. For reverse transcriptase (RT) PCR, 250 ng of total RNA was reverse transcribed in the presence of poly-dT primers for 50 minutes at 42°C by using Superscript II reverse transcriptase. Samples were subjected to PCR in the presence of 0.5 µM of appropriate primers in a reaction mixture containing 1 X reaction buffer, 200 µM dNTPs, 1 U Taq polymerase, and 1.5 mM MgCl₂. For the determination of β-actin, intron-spanning primers were used such that the presence of genomic DNA in the RNA preparation would result in the amplification of a PCR product at approximately 500 bp, whereas complementary DNA would result in a 349-bp product. For TGFβ₁, primers were designed such that they would anneal to a completely conserved region of both the endogenous murine (Genbank Accession Number M13177) and transgenic porcine (Genbank accession #X12373) TGFβ₁ sequence. These PCR primers amplify a region of 195 bp. Sequences of primers used are as follows: 5'-TGG AAT CCT GTG GCA TCC ATG AAA C-3' (forward), 5'-TAA AAC GCA GCT CAG TAA CAG TCC G-3' (reverse) for β-actin; and 5'-GCC CTT CCT GCT CCT CAT-3' (forward), 5'-TTG GCA TGG TAG CCC TTG-3' (reverse) for TGFβ₁. Cycling conditions consisted of 25 rounds (β-actin and TGFβ₁) of 1 minute at 94°C; 1 minute at 60°C (β-actin) or 1 minute at 55°C (TGFβ₁); and 1 minute at 72°C. The PCR products were separated on a 2% agarose gel and stained with ethidium bromide for ultraviolet visualization.

**Enzyme-Linked Immunosorbent Assay for TGFβ₁**

A sandwich-based commercially available TGFβ₁ enzyme-linked immunosorbent assay (ELISA) was used for the quantification of TGFβ₁ in CSF, plasma, and brain tissue lysates. For the collection of fluids, CSF was obtained from the hydrocephalic ventricles of snap-frozen brains. Control CSF was obtained from the cisterna magna of a nonhydrocephalic rat. Plasma was collected from the tail veins of hydrocephalic and nonhydrocephalic mice by using ethylenediaminetetraacetic acid as an anticoagulant. For tissue lysates, snap-frozen brains obtained from 2-day-old neonatal hydrocephalic and nonhydrocephalic mice (weighing approximately 50 mg) were rapidly homogenized on ice in ristocetin-induced platelet agglutination buffer (1 X PBS: 1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate). Total protein concentration in the tissue lysates was determined by the method of Lowry, and 40 µg of total protein obtained from each sample was used for the ELISA. Samples were acid treated to liberate the detectable biologically active form of TGFβ₁ and stored at -20°C until the assay was performed according to the manufacturer's instructions. A standard
curve using TGFβ₁ was established, and the concentration of TGFβ₁ in CSF was expressed as picograms per milliliter, the concentration of TGFβ₁ in plasma was expressed as nanograms per milliliter, and the quantity of TGFβ₁ in tissue lysates was expressed as picograms of TGFβ₁ per milligram of total protein.

Sources of Supplies and Equipment

The transgenic mice used in these experiments were a gift from Dr. A. Messing, School of Veterinary Medicine, University of Wisconsin, Madison, WI.

The reaction buffer and Taq polymerase were produced by Qiagen (Valencia, CA). The affinity-purified chicken IgG was obtained from R&D Systems (Minneapolis, MN). Jackson ImmunoResearch (West Grove, PA) manufactured the rabbit anti-chicken IgY. We obtained the anti-TGF₁ receptor purified rabbit IgG and the goat anti-rabbit IgG from Santa Cruz Biotechnology (Santa Cruz, CA). The intron-spanning primers used in tests to determine β-actin were obtained from Maxim Biotech (So. San Francisco, CA).

We performed the DNA isolation and PCR test by using a kit (QIAGEN TAC Core Kit) manufactured by Qiagen. The DNA thermal cycler (480) was obtained from Perkin Elmer US Instrument Division (Norwalk, CT). To obtain brain slices, we used the Jung cryomicrotome (CM 1000), which is made by Leica Instruments GmbH (Nussbloch, Germany). For the RT-PCR analysis we used the Superscript II kit produced by Life Technologies (Rockville, MD).

RESULTS

Clinical Observations

To date we have examined 226 pups, of which the hydrocephalic phenotype was present in 112 (49.6%). Multiple litters were examined, and typically there were between eight and 12 pups per litter, usually between eight and 10. Clinical manifestations of hydrocephalus were noticeable shortly after birth. At postpartum Day 1 or 2, animals developed dorsal doming of the calvaria, and a bright glow was noted when heads were transilluminated with a fiberoptic light. The mice developed progressive signs including macrocrania, spasticity, limb tremors, ataxia, and ultimately, death (Fig. 1). Mice with hydrocephalus died between 3 weeks and 4 months after birth (median survival 1 month). The mice were weaned at age 21 to 28 days. Typically, the hydrocephalic mice survived for only a few days after weaning.
The presence of the TGFβ₁ transgene was determined by performing PCR analysis of tailcuts. Mice with the hydrocephalic phenotype consistently carried the transgene. Some animals with the transgene did not develop hydrocephalus. Animals without the transgene did not develop hydrocephalus.

**Magnetic Resonance Imaging Observations**

High-resolution proton density gradient echo MR images were obtained in the axial, coronal, and sagittal planes. After induction of a light anesthetic the images were obtained in mice placed in a specially designed, small solenoidal receiver coil that was coupled with a 1.5 tesla magnet. Imaging was performed on days postpartum Days 2, 7, 14, and 150. Although there was some degradation of the images related to motion, MR imaging provided an accurate representation of intracranial pathological entities, readily distinguishing between hydrocephalic animals and nonhydrocephalic controls.
Fig. 2. Magnetic resonance imaging studies obtained in an anesthetized 2-month-old mouse with advanced hydrocephalus. The high-resolution proton-density gradient-echo images demonstrate massive enlargement of the lateral, third, and fourth ventricles. Left: Sagittal image. Center: Coronal image. Right: Corresponding coronal cut frozen section of the same animal after it was killed.

In hydrocephalic animals massive enlargement of the lateral, third, and fourth ventricles was demonstrated (Fig. 2). The aqueduct of sylvius appeared narrow but patent. In control animals normal-sized ventricles without intracranial abnormalities were observed (Fig. 3).
Morphological Observations

Analysis of frozen cut sections in the coronal plane confirmed the MR findings of diffuse enlargement of the lateral, third, and fourth ventricles (Figs. 2 and 3). Significant ventriculomegaly was present in the hydrocephalic mice in which the transgene was present as early as day postpartum Day 2, the time of the earliest radiological and morphological examination.

Immunohistochemical Observations

Immunohistochemical analysis of brain tissue slices was performed on frozen sections of 17 hydrocephalic and seven control brains (whole-head mounts) to determine the presence and location of the TGFβ1 cytokine and its receptor. For both the cytokine and receptor assays, a conjugated secondary antibody was attached to a fluorescent probe, fluorescein isothiocyanate.

The presence of the TGFβ1 cytokine was demonstrated in 17 of 17 hydrocephalic animals and in five of
seven nonhydrocephalic controls examined at 2 days postpartum. In these animals, the presence of fluorescence was noted in the vicinity of the meninges and, sometimes, the ependyma of the lateral ventricles. The presence of the TGFβ₁ receptor was observed in the meninges in all 17 hydrocephalic mice and in five of the seven the nonhydrocephalic mice (Fig. 4). No receptor staining was noted at the ventricular ependyma for either the hydrocephalic or control mice.

Fig. 4. Immunohistochemical stain demonstrating presence of the TGFβ₁ receptor in the convexity meninges (m) of a 2-month-old nonhydrocephalic control mouse. The meninges demonstrated a bright green fluorescence. s = scalp, c = calvaria, b = brain, * = subarachnoid space. Magnification X 400.

A negative control test was performed to ensure reliability of the assays for both the TGFβ₁ cytokine and its receptor. This was conducted using the identical protocol for each assay and simply substituting chicken IgG for the primary antibody for detection of the cytokine. For the receptor analysis, we used rabbit IgG for the primary antibody. No evidence of fluorescence was demonstrated, eliminating the possibility of nonspecific binding.

Levels of TGFβ₁ Messenger RNA in the Brains of Transgenic Mice

To determine the level of messenger (m)RNA expression in the brains of transgenic mice, RT-PCR was performed. Figure 5 demonstrates that the expression of TGFβ₁ mRNA was substantially increased on RT-PCR tests in the brains of the 2-day-old transgenic hydrocephalic mice as compared with nontransgenic control littermates. This is in contrast to the expression of β-actin, which was relatively constant among the samples tested. Worthy of note is that we did not detect the 500-bp PCR product, even after 35 PCR cycles, which would have been expected had genomic DNA been present in the samples. These data suggest that the presence of the TGFβ₁ transgene results in the upregulated tissue-specific expression of TGFβ₁ mRNA in the brain.
Fig. 5. Products of RT-PCR analysis of TGFβ₁ (upper) and β-actin (lower) mRNA expression in the brains of transgenic and nontransgenic mice. Total RNA was obtained from each of four separate mice (two nontransgenic controls and two transgenic hydrocephalic mice) and subjected to RT-PCR. A substantial increase in the expression of mRNA for TGFβ₁ in the transgenic mice is noted, whereas there is no apparent difference in β-actin expression among the groups. In the β-actin gel (lower), note the absence of the 500-bp PCR product, thus confirming the absence of genomic DNA in the samples.

Protein Levels of TGFβ₁ in the Brains of Transgenic Mice

To detect the presence of TGFβ₁ in the brains of transgenic mice, CSF was collected from the ventricles of hydrocephalic 2-day-old transgenic mice. It has been demonstrated that TGFβ₁ is absent from the CSF under normal conditions.[72] As determined by ELISA, CSF from transgenic mice demonstrated strong levels of TGFβ₁ protein (Fig. 6A), whereas the control CSF obtained from rat cisterna magna failed to show appreciable levels of TGFβ₁. This suggests that TGFβ₁ is produced in an area in the brain accessible to the CSF compartment.
Fig. 6. Graphs illustrating the results of ELISA of TGFβ₁ protein expression in the CSF (A), brain tissue lysate (B), and plasma (C) in transgenic mice.

In an effort to confirm the expression of TGFβ₁ in the brains of transgenic mice, we performed Western blot analysis of lysates obtained from the brains of 2-day-old neonatal mice. Because we were unable to detect the 12-kd protein from any of the samples (data not shown) we proceeded to analyze such samples
by ELISA. Brain lysates collected from transgenic mice had an approximately 2.5-fold increase in TGFβ₁ (p = 0.03; unpaired Student's t test) as compared with their nontransgenic littermates (Fig. 6B). This finding appears to confirm that the presence of the transgene upregulates the expression of TGFβ₁ within the brains of transgenic mice. The detection of TGFβ₁ in the brains of nontransgenic mice suggests that the samples used may have been laced with blood and therefore platelets, a potent source of TGFβ.

To assess the specificity of the expression of the transgene, plasma was assayed by ELISA for TGFβ₁ in a transgenic breeder animal, and TGFβ₁ was found to be approximately two-fold higher in the transgenic mouse compared with control normal mouse plasma (Fig. 6C). This finding suggests that the overexpression of TGFβ₁ within the brains of these mice does not remain contained within the CNS. Alternatively, the GFAP promoter, which controls the transgene, may allow for expression in regions outside of the CNS.

**DISCUSSION**

There is a clear need for a reliable experimental model of hydrocephalus. Hydrocephalus is the most common neurosurgical problem of childhood[20,93] and is diagnosed in adults with increasing frequency.[21] Despite this, knowledge of the pathophysiology of ventricular enlargement in hydrocephalus has continued to elude scientists and clinicians.[90,100]

**Natural History of Hydrocephalus**

The natural history of untreated hydrocephalus is dismal, with a substantial mortality rate, and there is significant intellectual impairment among survivors. In a review of 182 children with untreated congenital or acquired hydrocephalus, Laurence and Coates[79] reported that two thirds of the patients died by age 18 months, and 80% died by age 20 to 25 years. In another series of children in whom hydrocephalus was untreated, Yashon, et al.,[119] reported that half of the children were dead by age 3 years. In a series of patients with myelodysplasia and untreated hydrocephalus, Eckstein and Macnab[33] found a mortality rate of 85 to 96%.

**Treatment of Hydrocephalus**

The treatment of hydrocephalus dates back to the fifth century B.C., when Hippocrates recognized that the head could swell in response to an accumulation of fluid within it, and drainage was attempted by puncturing the fontanelle.[27] Hippocrates believed that hydrocephalus was the result of chronic epilepsy and that water collected when the diseased brain became corroded and began to melt. Over the years, countless medical and surgical therapies have been applied and ultimately abandoned as ineffective. As recently as the early 1950s, hydrocephalus was considered a fatal condition that generally went untreated.[95] In 1952, Nulsen and Spitz[92] published a landmark article in which they described the successful management of infantile hydrocephalus by using a valved shunt to divert CSF from the enlarged cerebral ventricles into the vascular system via the jugular vein. Ventricular shunting marked a turning point in the treatment of hydrocephalus, and children with this once-fatal condition were then able to survive.

Despite this important advance, the treatment of hydrocephalus remains a formidable undertaking for the neurosurgeon today. Ventriculoperitoneal shunt insertion, the current surgical standard of care for
hydrocephalus and the most common operation performed by pediatric neurosurgeons, is associated with a higher complication rate than any other neurosurgical procedure.[94] Shunt-related complications include malfunction due to underdrainage (that is, by obstruction of the ventricular catheter, valve, or distal catheter) or overdrainage (that is, by subdural hematoma, premature suture closure, secondary stenosis of the sylvian aqueduct, slit ventricle syndrome with high pressure, and slit ventricle syndrome with low pressure). Additionally, because shunts are implanted foreign bodies, they are associated with a risk of infection, with an average infection rate as high as 10 to 15% among large series.[9] It has been estimated that approximately 50,000 CSF shunts are placed in patients each year in the United States, and such procedures account for almost $100 million of national healthcare expenses.[10] It is disturbing that approximately half of this expenditure is for CSF shunt revision procedures, which underscores the troublesome nature of these devices. Thus, although the placement of extracranial CSF shunt represents the mainstay of treatment for hydrocephalus, it is far from an ideal solution. Attempts to optimize the treatment of patients with hydrocephalus have been limited by the current lack of detailed knowledge concerning the basic nature of the disorder. In a sense, placement of ventricular shunts has provided a crude solution to the problem of hydrocephalus before the details of its pathogenesis could be determined. The more that is learned about the pathophysiology of hydrocephalus, the greater is the chance of finding novel treatments and methods of prevention.

**Existing Experimental Models of Hydrocephalus**

Hydrocephalus results from a complex interaction between CSF formation and absorption, and most existing animal models of hydrocephalus are based on creation of an obstruction to the flow of CSF, either within the cerebral ventricles (noncommunicating hydrocephalus) or within the subarachnoid space (communicating hydrocephalus). The first model of experimental hydrocephalus was developed in 1914 by Dandy and Blackfan,[25] who placed a cotton pledget into the sylvian aqueduct of a dog and observed enlargement of the ventricles proximal to the site of obstruction. Subsequently Milhorat[89] has induced hydrocephalus in primates by inserting a rubber catheter into the fourth ventricle and inflating a balloon. Hydrocephalus has been produced by injection of multiple substances into the ventricles or subarachnoid space, including bacteria,[42] mycobacterium tuberculosis,[28] blood,[5] lampblack,[114] India ink,[91] thorotrast,[104] silicone,[113] and silastic.[62]

A great number of animal models have been developed with which to study hydrocephalus, although many are difficult to maintain or do not accurately reflect the clinical disorder in humans. Hydrocephalus that is associated with aqueductal stenosis has been induced by intracerebral injection of mumps, parainfluenza 2, and influenza viruses.[65-67,85] The results of subsequent studies have shown that viral-induced hydrocephalus probably results from encephalitis with inflammatory changes in the ependyma and leptomeninges and that stenosis of the sylvian aqueduct is more likely the result of hydrocephalus than its cause.[87] More recently, a number of inherited models of hydrocephalus have been reported in the rat,[68-71] the mouse,[15,97] and the hamster,[120] although the precise mechanism responsible for hydrocephalus in these models remains unclear.

A fairly reproducible animal model of hydrocephalus, and the model most widely studied, is based on the intracisternal injection of kaolin.[7,8,22,29,31,32,34,36,43,47,50,51,55-60,77,84,88,101,102,115] Despite the abundance of experimental models, the pathogenesis of hydrocephalus remains largely not understood. Many of the existing models are flawed, expensive to maintain, or difficult to use. Even the commonly studied kaolin-injected model has significant limitations because of the associated severe...
diffuse inflammatory meningitis encircling the base of the brain and brainstem.

A New Transgenic Model of Hydrocephalus

The present report represents our attempt to elucidate the pathogenesis of hydrocephalus by studying a new model in transgenic mice that were generated to overexpress TGFβ1. When male mice that were heterozygous for the transgene were crossed with wild-type females, one half of the offspring reliably developed progressive symptomatic communicating hydrocephalus. In all animals with the hydrocephalic phenotype the TGFβ1 transgene was found, as measured by PCR analysis of tail-cut samples[44] although not all animals with the transgene developed hydrocephalus. We were able to perpetuate the colony by using asymptomatic male carriers as breeders. This model is inexpensive, easy to maintain, and highly reproducible. Our aim in the present study was to use this transgenic murine colony to describe the clinical, radiological, and pathoanatomical findings in a model of congenital hydrocephalus, as well as the role of the cytokine, TGFβ1, in the genesis of this condition.

This new model of transgenic murine hydrocephalus is, to our knowledge, the only one of its kind in existence. The generation of hydrocephalus in transgenic mice that were created to overexpress TGFβ1 was actually a serendipitous finding. It was described independently by two groups of investigators, each of which created a transgenic preparation to evaluate the effects of TGFβ1 on the CNS (specifically, the astrocytic response to injury).[45,117] Unexpectedly, the founder animals consistently developed hydrocephalus, with increased deposition of the ECM proteins, laminin and fibronectin, preferentially in perivascular locations.[117]

Transforming Growth Factor-β1

Transforming Growth Factor-β1 is a multifunctional regulatory cytokine with diverse effects on cellular growth and differentiation.[48,86,108] Cytokines, extracellular signaling peptides or proteins that mediate cell-to-cell communication locally, are unlike conventional hormones that can act at distant sites. This local communication is coordinated by autocrine and paracrine loops, such that the effects of cytokines may be stimulatory or inhibitory.

With pleiotropic effects on multiple cell types,[35,61] TGFβ1 possesses broad activities in embryological development, wound healing, hematopoiesis, bone development, ECM formation, and in modulating the immune response.[53,76,86] It is considered the prototypic member of a group of structurally related polypeptides referred to collectively as the TGF superfamily.[107] Members of the superfamily include the TGFβs, activins, inhibins, bone morphogenic proteins, müllerian-inhibiting substance, Drosophila decapentaplegic gene complex, and Xenopus Vg-1 gene.[14,86,107] Five isoforms of TGFβ have been characterized in vertebrates, three of which (TGFβ1, TGFβ2, and TGFβ3) are closely related and occur in mammals.[4,76] These isoforms have been identified in the human CNS and peripheral nervous system.[41,111]

Transforming growth factor-β1 is synthesized as a large 391-amino acid precursor that is secreted in an inactive form, and it requires activation by removal of the N2-terminal proregion, called the latency-associated peptide.[6,11,35,86] The active form of TGFβ1 is a 25-kD homodimer that binds to a number of cell surface receptors.[4,16,19,30,46,54,116] Latent TGFβ1 is stored in the ECM and at the cell surface. The precise mechanism of conversion to active TGFβ1 is unknown.
Transforming growth factor-ß was originally isolated from platelets, and it was named for its ability to stimulate the anchorage-independent growth of nontumorigenic fibroblasts.[2,48,52,98] As the prototypic member of this family, TGFß1 elicits potent, diverse, and often opposing cellular responses depending on the cell type and the nature of the local environment.[107] The current information on the effects of TGFß is somewhat confusing, and although this cytokine has been implicated as playing a key role in the initiation of tissue repair, its sustained production can be harmful and cause scarring in many organs.[11,14]

In the literature, evidence of the adverse effects of excessive TGFß on various somatic organ systems abounds. Elevated levels of TGFß have been implicated in the pathogenesis of fibrosis in diverse tissues throughout the body, both in animal models and human disease. The affected sites include skin, kidney, liver, lung, arteries, joints, and the CNS.[11,14,37,38,73,122]

The effect of TGF-ß overexpression on different organ systems has been studied extensively in transgenic mice by coupling the TGFß gene to various tissue-specific genes.[48] For example, targeting expression of TGFß1 to the epidermis resulted in inhibition of skin development.[105] Targeting expression to the mammary gland in pregnant animals resulted in inhibition of alveolar development and lactation.[63] Targeting expression to osteoblasts led to widespread bone loss and an osteoporosis-like phenotype.[39] Pancreatic islet cell overexpression of TGFß by the use of an insulin promoter led to chronic pancreatitis and pancreatic fibrosis.[80] Hepatic overexpression caused the development of multiple organ lesions, including hepatic fibrosis, glomerulonephritis, arteritis, myocarditis, and pancreatic and testicular atrophy.[103]

Further insight into the role of TGFß in normal development has been provided by targeted disruption of the gene (knock-out studies).[48,76,103,107] Animals that were homozygous for the disrupted TGFß1 allele (TGFß1 null mice) developed a lethal wasting syndrome with massive multifocal inflammatory infiltrates and died several weeks after birth. No gross morphological abnormalities were detected, although it has been demonstrated that TGFß1 null mice can receive maternal TGFß1 through the placenta, perhaps explaining why the deleterious effects develop in a delayed fashion and why early organogenesis is not disturbed.[81]

**Transforming Growth Factor-ß1 and the CNS**

The role of TGFß1 in the CNS is not well understood.[41] In the developing brain, the TGFß1 cytokine and its receptors are expressed in the meninges and choroid plexus.[40,53,74] Cultured astrocytes have been shown to express all three isoforms of TGFß and their receptors,[23] and increased levels of TGFß1 have been found in rodent brains after creating focal cerebral lesions.[82,83] In fact, in a rat tissue-culture model in which hippocampal neurons were used to evaluate brain injury, the addition of TGFß1 promoted axonal regeneration in a dose-dependent fashion.[1]

Serendipitous Finding of the Transgenic Murine Model of Hydrocephalus

To understand better the role of TGFβ1 in the CNS, two independent laboratories created transgenic mice to overexpress TGFβ1.[45,117] Both groups were interested in the astrocytic response to injury, and expression of TGFβ1 was targeted to astrocytes by using a promoter that contained regulatory elements from the human GFAP gene.[17,18] Because GFAP is an intermediate-filament protein that is expressed almost exclusively in CNS astrocytes, use of systemically administered GFAP as the promoter had the effect of eliminating bioavailability problems due to the blood-brain barrier, as well as mechanical trauma-related problems associated with local injections into the brain.

Surprisingly, founder animals harboring the TGFβ1 transgene developed severe progressive hydrocephalus and died after several weeks. Ovarian transplantation from an affected female founder animal permitted continuation of the model, with hydrocephalus expressed in heterozygous carriers of the transgene. We have perpetuated this line (TgN4Mes) to serve as a model of congenital hydrocephalus. Compared with previous experimental models of hydrocephalus, the murine transgenic model is simple, inexpensive, and highly reproducible. It is currently the only transgenic model of hydrocephalus in existence, and we believe it is the superior to previous experimental models of hydrocephalus.

A clinical rationale exists for examining the effects of TGFβ1 in the pathogenesis of hydrocephalus. Transforming growth factor-β1 has been shown to be elevated in the CSF of patients after suffering subarachnoid hemorrhage, with higher levels found in those who developed hydrocephalus than in those who did not.[72] A likely source of TGFβ1 is release from platelet storage,[2] although the multifactorial sources may be implicated. In an animal model, progressive communicating hydrocephalus has been induced in mice following the intrathecal injection of human recombinant TGFβ1.[108] Thus, there is clinical and experimental evidence to support a role for TGFβ1 in the induction of hydrocephalus.

Future Directions

We hypothesize that the overexpression of TGFβ1 in the CNS causes hydrocephalus by modulating the environment of the ECM. It is well known that TGFβ promotes ECM deposition through a variety of mechanisms, stimulating the synthesis of matrix proteins and blocking matrix degradation by inhibiting the synthesis of proteases and increasing the synthesis of protease inhibitors.[14,48,49,96] The aim of the present study was to perpetuate a colony of transgenic mice that were created to overexpress TGFβ1 in the CNS and to use this model to provide clinical, radiological, morphological, and immunohistochemical data regarding the origin and evolution of congenital hydrocephalus. Although the present experiments neither corroborate nor disprove a relationship between TGFβ1 and the ECM, the model should prove useful in elucidating the pathophysiology of hydrocephalus and the role of TGFβ1 in the CNS in health and disease.

Attempts to block the effects of TGFβ1 in the CNS can be tested using the transgenic murine model. Such a paradigm can help facilitate the search for newer methods for preventing or treating hydrocephalus. Neutralizing antibodies have been raised against TGFβ1 that serve as the basis for quantitating its presence in biological fluids by sandwich-based ELISA.[26,41] Neutralizing antibodies can be used to block the effects of TGFβ1 in vivo. In animal models, the exogenous addition of TGF-β
had the effect of reducing inflammation in a diverse set of conditions, including cutaneous wounds,[106] glomerulonephritis,[13] brain injury,[83] and experimental allergic encephalomyelitis, a model of multiple sclerosis.[78]

If TGFβ₁ overexpression and its effect on the ECM plays a role in the generation of hydrocephalus, then blocking TGFβ₁ might be useful in the treatment or prevention of hydrocephalus. It is possible to test this hypothesis by using the transgenic mouse model, administering TGF-ß neutralizing antibodies to pregnant mice carrying the transgene in an attempt to prevent the development of hydrocephalus in the offspring. The ability to block the formation of hydrocephalus in this model by using antibodies to TGFß would have significant therapeutic implications, but such a study might be limited by the inability of large proteins to cross the blood-brain barrier.

Decorin, a natural inhibitor of TGFß, has been identified as part of a feedback system that regulates cell growth.[118] Decorin offers a possible mechanism for treating a variety of conditions associated with overproduction of TGFß, for which current therapies are inadequate. It is a small chondroitin-dermatan sulphate proteoglycan that has a core protein and a single glycosaminoglycan chain. A naturally occurring human compound that can be produced in recombinant form, it is unlikely to elicit a significant immunologic reaction. When decorin was administered to glomerulonephritic rats, the production of ECM was inhibited, and clinical manifestations of disease were attenuated.[12] Thus, decorin might have a role in the pharmacological treatment of congenital hydrocephalus, and its effects on TGFß inhibition could be elucidated using the present transgenic murine model.

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