Characterization of a model of hydrocephalus in transgenic mice

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Object. The purpose of this study was to elucidate the pathophysiological characteristics of hydrocephalus in a new transgenic model of mice created to overexpress the cytokine transforming growth factor–β (TGFβ) in the central nervous system (CNS).

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

One hundred twelve (49.6%) of 226 mice developed clinical manifestations of hydrocephalus, characterized by dorsal doming of the calvaria, spasticity, limb tremors, ataxia, and, ultimately, death. The presence of the TGFβ transgene was determined by performing polymerase chain reaction (PCR) analysis of sample tail slices. Animals with the hydrocephalic phenotype consistently carried the transgene, although some animals with the transgene did not develop hydrocephalus.

Alterations in brain structure were characterized using magnetic resonance (MR) imaging, gross and light microscopic analysis, and immunocytochemical studies. Magnetic resonance imaging readily distinguished hydrocephalic animals from nonhydrocephalic controls and 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 TGFβ, 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 TGFβ gene in the brains of transgenic mice, and enzyme-linked immunosorbent assay confirmed overexpression of the TGFβ cytokine in brain, cerebrospinal fluid, and plasma.

Conclusions. The transgenic murine model provides a reproducible representation of congenital hydrocephalus. The authors hypothesize that overexpression of TGFβ, 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 should also help in the search for new 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 dismal, 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 pathophysiological characteristics of hydrocephalus should aid the search for new 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–β (TGFβ), in the central nervous system (CNS). Transforming growth factor–β, a multigene cytokine that plays a broad range of regulatory roles in cell growth, differentiation, and tissue repair. The TGFβ, transgenic mouse model was initially developed by investigators studying the response of astrocytes to injury in vivo. 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 TGFβ, 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 TGFβ, cytokine and its receptor.

Materials and Methods

Transgenic Murine Model of Hydrocephalus

The present transgenic model of hydrocephalus was originally
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created by Galbreath and colleagues\(^4\) in 1995. Their aim was to develop a transgenic mouse that overexpressed TGF\(_\beta\), in which they could study the effects of this regulatory cytokine on the astrocyte response to injury in vivo. The transgene was produced by incorporating porcine TGF\(_\beta\), complementary DNA downstream from a human glial fibrillary acidic protein (GFAP) fragment, thus using 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 × SJL mice (B6SJLJ). The eggs were transferred to pseudopregnant FVB/N foster mice.

The consistent finding of hydrocephalus in founder animals was unexpected. A hydrocephalic line, established by performing ovarian transplantation from an affected female founder animal that overexpressed TGF\(_\beta\), was designated TgN4Mes. We have perpetuated this line and used it as a model of congenital hydrocephalus 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 polygamously harem-breeding scheme. Each breeding male was placed with two or three wild-type females for a period of 5 days to ensure that the females would be in estrous and become inseminated. Thus, offspring were heterozygous for either the transgene or the wild type. Breeder males were removed after 5 days to ensure that the postpartum estrus was not used.

Polymerase Chain Reaction

Analysis of the integration of the transgene into subsequent generations was conducted using the polymerase chain reaction (PCR) on DNA derived from tail-cut samples obtained in pups at the time of weaning. The DNA was obtained by cutting 15-mm slices from the tails of weaned pups. This tissue was protease digested, and the DNA was placed on an affinity spin column and eluted. The DNA bank Accession No. M13177) and transgenic porcine (Genbank Accession No. X12373) TGF\(_\beta\) sequences. These PCR primers amplify a region of 195 bp. Sequences of primers used are as follows: 5’-TGGAATCTCGTGCCATCAAAC-3’ (forward), 5’-AAACGCAGCGATGAACTG-3’ (reverse) for β-actin; and 5’-GCCCTCTGCTCTAACAT-3’ (forward), 5’-TGCGATGATGCCCTTG-3’ (reverse) for TGF\(_\beta\). Cycling conditions consisted of 25 rounds (β-actin and TGF\(_\beta\)) of 1 minute at 94°C; 1 minute at 60°C (β-actin) or 1 minute at 55°C (TGF\(_\beta\)); 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 TGF\(_\beta\)

A sandwich-based commercially available TGF\(_\beta\), enzyme-linked immunosorbent assay (ELISA) was used for the quantification of TGF\(_\beta\), 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 ethylenediamine tetraacetic acid as an anticoagulant. For tissue lysates, snap-frozen brains obtained from 2-day-old hydrocephalic and nonhydrocephalic mice (weighing approximately 50 mg) were rapidly homogenized on ice in ristocetin-induced platelet agglutination buffer (1 × phosphate-buffered saline, 1% Igepal CA-630, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate). Total protein concentration in the tissue lysates was determined using 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 TGF\(_\beta\), and stored at −20°C until the assay was performed according to the manufacturer’s instructions. A standard curve using TGF\(_\beta\), was established, and the concentration of TGF\(_\beta\), in CSF was expressed as picograms per milliliter, the concentration of TGF\(_\beta\), in plasma was expressed as picograms per milliliter.
nanograms per milliliter, and the quantity of TGF-β1 in tissue lysates was expressed as picograms of TGF-β1 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 OCT medium was obtained from Tissue-Tek, Sakura-Finetec (Torrence, CA). The reaction buffer, Taq polymerase, and RNaseeasy Lysis Buffer 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 the 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: typically there were between eight and 12 pups per litter, and 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 the 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 TGFβ, transgene was determined by performing PCR analysis of tail slices. 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 light anesthesia, 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 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.

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 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 TGFβ, 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 TGFβ, cytokine was demonstrated in 17 of 17 hydrocephalic animals and in five of seven
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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 nonhydrocephalic mice (Fig. 4). No receptor staining was noted at the ventricular ependyma for either the hydrocephalic or control mice.

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 TGFβ, 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.

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 of the brain accessible to the CSF compartment.

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 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 TGF$_{β_1}$ ($p = 0.03$; 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 TGF$_{β_1}$ within the brains of transgenic mice. The detection of TGF$_{β_1}$ in the brains of nontransgenic mice suggests that the samples used may have been laced with blood and therefore platelets, a potent source of TGF$_{β_1}$.

To assess the specificity of the expression of the transgene, plasma was assayed by ELISA for TGF$_{β_1}$ in a transgenic breeder animal, and TGF$_{β_1}$ was found to be approximately twofold higher in the transgenic mouse compared with the control mouse (Fig. 6C). This finding suggests that the overexpression of TGF$_{β_1}$ 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 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 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. Shunt-related complications include malfunction due to underdrainage (by obstruction of the ventricular catheter, valve, or distal catheter) or overdrainage (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.

It has been estimated that approximately 50,000 CSF shunts are placed in patients each year in the United States and that such procedures account for almost $100 million of national healthcare expenses. 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 an 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, who placed a cotton pledget in the sylvian aqueduct of a dog and observed enlargement of the ventricles proximal to the site of obstruction. Subsequently Milhorat 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, mycobacterium tuberculosis, blood, lampblack, India ink, Thorotrast, silicone, and Silastic.

A great number of animal models have been developed in 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. The results of subsequent studies have shown that virus-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. More recently, a number of inherited models of hydrocephalus have been reported in the rat, the mouse, and the hamster, although the precise mechanism responsible for hydrocephalus in these models remains unclear.

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 TGFβ, transgene was found, as measured by PCR analysis of tail-cut samples, 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 TGFβ, 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 TGFβ, 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 TGFβ, on the CNS (specifically, the astrocytic response to injury). Unexpectedly, the founder animals consistently developed hydrocephalus, with increased deposition of the ECM proteins, laminin, and fibronectin preferentially in perivascular locations.

**Transforming Growth Factor–β**

Transforming growth factor–β, is a multifunctional regulatory cytokine with diverse effects on cellular growth and differentiation. 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, TGFβ, possesses broad activities in embryological development, wound healing, hematopoesis, bone development, ECM formation, and in modulating the immune response. It is considered the prototypical member of a group of structurally related polypeptides referred to collectively as the TGF superfamily. Members of the superfamily include the TGFβs, activins, inhibins, bone morphogenetic proteins, müllerian-inhibiting substance, *Drosophila decapentaplegic* gene complex, and the *Xenopus Vg-1* gene.

Five isoforms of TGFβ have been characterized in vertebrates, three of which (TGFβ1, TGFβ2, and TGFβ3) are closely related and occur in mammals. These isoforms have been identified in the human CNS and peripheral nervous system.

Transforming growth factor–β, is synthesized as a large 391–amino acid precursor that is secreted in an inactive form, and it requires activation by removal of the N-terminal proregion, called the latency-associated peptide. The active form of TGFβ, is a 25-kD homodimer that is stored in the ECM and at the cell surface. The precise mechanism of conversion to active TGFβ, is unknown.

Transforming growth factor–β was originally isolated from platelets and was named for its ability to stimulate the anchorage-independent growth of nontumorigenic fibroblasts. As the prototypic member of this family, TGFβ, elicits potent, diverse, and often opposing cellular responses depending on the cell type and the nature of the local environment. The current information on the effects of TGFβ 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.

There is abundant evidence in situ localization of the adverse effects of excessive TGFβ on various somatic organ systems. Elevated levels of TGFβ 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.

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

Further insight into the role of TGFβ in normal development has been provided by targeted disruption of the gene (knock-out studies). Animals that were homozygous for the disrupted TGFβ allele (TGFβ, 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 TGFβ, null mice can receive maternal TGFβ, through the placenta, perhaps explaining why the deleterious effects develop in a delayed fashion and why early organogenesis is not disturbed. The role of TGFβ in the CNS is not well understood. In the developing brain, the TGFβ, cytokine and its receptors are expressed in the meninges and choroid plexus. Cultured astrocytes have been shown to express all three isoforms of TGFβ and their receptors, and increased levels of TGFβ have been found in rodent brains after creating focal cerebral lesions. In fact, in a rat tissue–culture model in which hippocampal neurons were used to evaluate brain injury, the addition of TGFβ, promoted axonal regeneration in a dose-dependent fashion.

Transforming growth factor–β has been identified in human leptomeningeal cells and in CSF, prompting investigation into its role in a wide spectrum of neurological disorders including stroke, acquired immune deficiency–related dementia, Alzheimer’s disease and Down’s syndrome pathological entities, Parkinson’s disease, multiple sclerosis, Guillain–Barré syndrome, chronic schizophrenia, and bacterial meningitis in children.
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Serendipitous Finding of the Transgenic Murine Model of Hydrocephalus

To understand better the role of TGFβ in the CNS, two independent laboratories created transgenic mice to over-express TGFβ. Both groups were interested in the astrocytic response to injury, and expression of TGFβ was targeted to astrocytes by using a promoter that contained regulatory elements from the human GFAP gene. 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 TGFβ 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 TGFβ in the pathogenesis of hydrocephalus. Transforming growth factor–β, 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. A likely source of CSF of patients after suffering subarachnoid hemorrhage is the ECM. It is well known that TGFβ 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. The aim of the present study was to perpetuate a colony of transgenic mice that were created to overexpress TGFβ, 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 TGFβ and the ECM, the model should prove useful in elucidating the pathophysiology of hydrocephalus and the role of TGFβ in the CNS in health and disease.

Attempts to block the effects of TGFβ 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 TGFβ, that serve as the basis for quantitating its presence in biological fluids by sandwich-based ELISA. Neutralizing antibodies can be used to block the effects of TGFβ in vivo. In animal models, the exogenous addition of TGFβ had the effect of reducing inflammation in a diverse set of conditions, including cutaneous wounds, glomerulonephritis, brain injury, and experimental allergic encephalomyelitis, a model of multiple sclerosis.

If TGFβ overexpression and its effect on the ECM plays a role in the generation of hydrocephalus, then blocking TGFβ might be useful in the treatment or prevention of hydrocephalus. It is possible to test this hypothesis by using the transgenic mouse model, administering TGFβ-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 TGFβ 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 TGFβ, has been identified as part of a feedback system that regulates cell growth. Decorin offers a possible mechanism for treating a variety of conditions associated with overproduction of TGFβ, for which current therapies are inadequate. It is a small chondroitin–dermatan sulfate 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 immunological reaction. When decorin was administered to glomerulonephritic rats, the production of ECM was inhibited, and clinical manifestations of disease were attenuated. Thus, decorin might have a role in the pharmacological treatment of congenital hydrocephalus, and its effects on TGFβ inhibition could be elucidated using the present transgenic murine model.

Future Directions

We hypothesize that the overexpression of TGFβ 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. The present study was to perpetuate a colony of transgenic mice that were created to overexpress TGFβ, 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 TGFβ and the ECM, the model should prove useful in elucidating the pathophysiology of hydrocephalus and the role of TGFβ in the CNS in health and disease.

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References

49. Grande JP, Melder DC, Ziemenske AR: Modulation of collagen gene expression by cytokines: stimulatory effect of trans-
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forming growth factor-β1, with divergent effects of epidermal growth factor and tumor necrosis factor α on collagen type I and collagen type IV. J Lab Clin Med 130:476–486, 1997


70. Jones HC, Harris NG, Inglis BA, et al: Reduced cortical me-

A. R. Cohen, et al.

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