The Pathogenesis of Neonatal Post-hemorrhagic Hydrocephalus

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Hydrocephalus after intraventricular hemorrhage (IVH) has emerged as a major complication of preterm birth and is especially problematic to treat. The hydrocephalus is usually ascribed to fibrosing arachnoiditis, meningeal fibrosis and subependymal gliosis, which impair flow and resorption of cerebrospinal fluid (CSF). Recent experimental studies have suggested that acute parenchymal compression and ischemic damage, and increased parenchymal and perivascular deposition of extracellular matrix proteins—probably due at least partly to upregulation of transforming growth factor-β (TGF-β)—are further important contributors to the development of the hydrocephalus. IVH is associated with damage to periventricular white matter and the damage is exacerbated by the development of hydrocephalus; combinations of pressure, distortion, ischaemia, inflammation, and free radical-mediated injury are probably responsible. The damage to white matter accounts for the high frequency of cerebral palsy in this group of infants. The identification of mechanisms and mediators of hydrocephalus and white matter damage is leading to the development of new treatments to prevent permanent hydrocephalus and its neurological complications, and to avoid shunt dependence.

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INTRODUCTION

The prematurely born human infant is acutely vulnerable to intraventricular hemorrhage (IVH) (Figure 1A). This reflects a combination of anatomical and physiological factors (17, 39, 46, 66). At 24 to 32 weeks' gestation, the subependymal germinal matrix is highly proliferative, metabolically very active, and has a rich blood supply. The capillaries and venules are not yet supported by astrocytic processes or adventitia, and not yet protected by autoregulation. Many infants born preterm have severe respiratory problems, which result in fluctuating intrathoracic and intravascular pressures as well as unstable CO2 concentrations. In sick preterm infants, the combination of fragile subependymal vessels, swinging increases of arterial and venous pressure, high metabolic demand, and periodic hypoxia, often causes bleeding into the germinal matrix and thence into the ventricles. Advances in perinatal medicine, including the maternal administration of corticosteroids, have produced a dramatic fall in the incidence of IVH over the last 20 years, from 35% to 50% to 12% in infants with birth weights below 1500 g, but the incidence remains above 20% in infants weighing under 1000 g (60, 73).

Fifteen percent of infants with IVH develop post-hemorrhagic ventricular dilatation (PHVD) (Figure 1B) and over half of these either die, or become dependent on a surgical shunt system (45). The term post-hemorrhagic hydrocephalus is generally applied when the ventricular enlargement is progressive and accompanied by enlargement of the head. Ventriculoperitoneal shunting is the most frequently used surgical treatment but is fraught with complications, and carries a life-long risk of recurrent shunt failure and infection. Non-surgical treatment such as early repeated lumbar or ventricular tapping, administration of diuretics, or intraventricular injection of a fibrinolytic agent have proven ineffective (67, 68, 70). Infants who develop PHVD have a high frequency of neurodevelopmental disabilities; approximately two-thirds develop motor dysfunction, and approximately one-third has cognitive impairment and other neurological abnormalities (9, 54, 63). A major reason for the disabilities associated with PHVD is the high frequency of associated periventricular white matter damage (64).

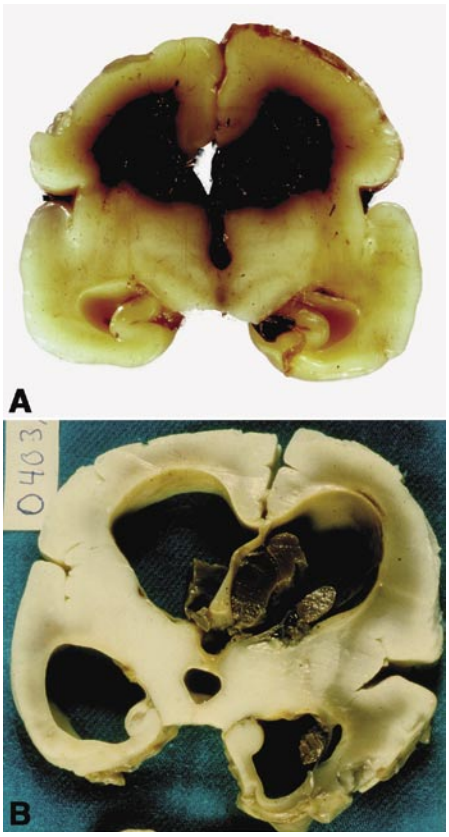

Figure 1. Intraventricular hemorrhage (**A**) in a preterm infant who died aged 3 days. Post-hemorrhagic hydrocephalus (**B**) in a preterm infant who died aged 4 weeks.

PATHOGENESIS OF PHVD

Impaired resorption of CSF. The conventional explanation for the impaired resorption of cerebrospinal fluid (CSF) after ventricular hemorrhage is that channels in the arachnoid villi are initially obstructed by microthrombi and subsequently by obliterative, fibrosing arachnoiditis, and that meningeal fibrosis and subependymal

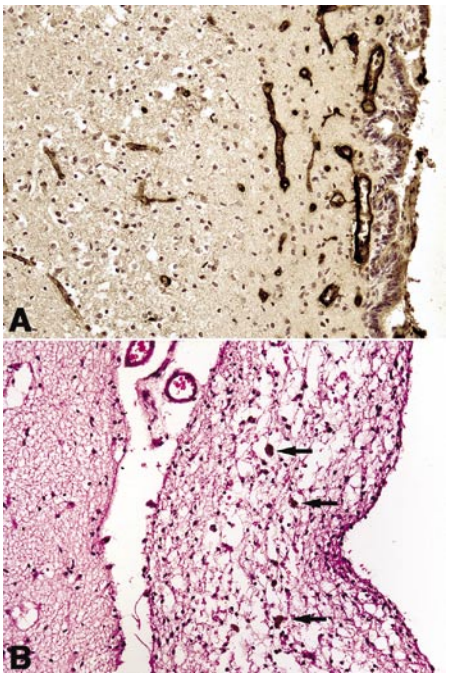

Figure 2. Periventricular and subarachnoid deposition of extracellular matrix and collagenous connective tissue in PHVD. **A.** Immunostaining reveals increased perivascular deposition of laminin in the subependymal region, in an infant with PHVD who died aged one month. **B.** A section through the leptomeninges of an infant with PHVD who died aged one month shows a thick layer of collagenous connective tissue in the subarachnoid space, which also contains haemosiderin-laden macrophages (arrows) and red cells.

gliosis also obstruct the outflow foramina of the fourth ventricle and the aqueduct of Sylvius (23, 31). Recently, the central role of arachnoid villi in the resorption of CSF has been questioned and it has been suggested that much resorption may take place across the ependyma and into blood vessels within the brain (20). This may be impeded by parenchymal and perivascular deposition of extracellular matrix (ECM), which can be demonstrated in infants with PHVD (Figure 2).

Inefficient fibrinolysis. A contributing factor may be inefficient fibrinolysis in the CSF, due to low levels of plasminogen and high levels of plasminogen activator inhibitor (21, 71). Although the formation of blood clot within the CSF is potentially reversible, in the absence of effective fibrinolysis the blood clot induces the deposition of collagenous connective tissue, and this is probably irreversible (Figure 2).

Enhanced synthesis of collagen. As noted above, PHVD is associated with meningeal and arachnoid fibrosis. Heep et al (22) found that levels of the carboxyterminal propeptide of type I procollagen were significantly higher in the CSF of neonates with PHVD, 3 to 4 weeks after IVH, than in those with congenital hydrocephalus that was not due to ventricular hemorrhage. These findings were interpreted as indicating that PHVD is associated with an increase in local synthesis of collagen.

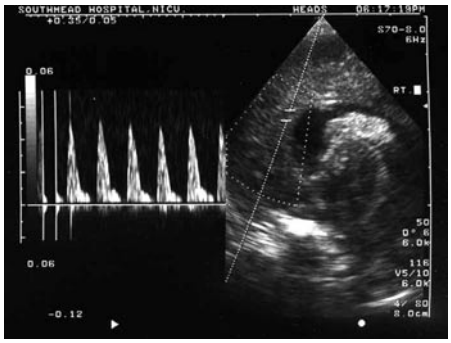

Figure 3. On the left is the Doppler velocity wave form of the anterior cerebral artery and on the right the cranial ultrasound scan of an infant with PHVD whose intracranial pressure was 15-mm Hg. The Doppler trace shows cessation of anterior cerebral artery blood flow during each diastole. Continuous blood flow was restored when intracranial pressure was reduced to 6-mm Hg.

Possible role of transforming growth factor-β (TGF-β) in PHVD. The transforming growth factor (TGF)-β superfamily of cytokines comprises several homologous polypeptides that transduce a range of signals involved in cell growth and differentiation, and the response to inflammation and tissue damage. The family members TGF-β1, -β2 and -β3 are present in all mammalian tissues, including the CNS. They have potent desmoplastic activity, through increased synthesis of collagen and several other components of the ECM (56), and are involved in a number of serious diseases involving excessive deposition of collagen and ECM, including diabetic nephropathy (4) and cirrhosis (6).

Concentrations of TGF-β1 are elevated in the CSF of adults with ventricular dilatation due to subarachnoid and intraventricular hemorrhage (13), and of both TGF-β1 and -β2 in infants with PHVD (69). Ventricular dilatation has been produced in mice by injecting TGF-β into the cerebral subarachnoid space on postnatal days 10 and 11 (61). Transgenic animals that express high levels of cerebral TGF-β1 develop communicating hydrocephalus associated with seizures and motor incoordination, and die at an early age (74) (see also paper by Masliah and Wyss-Coray in this symposium).

PATHOGENESIS OF WHITE MATTER INJURY IN PHVD

The mechanisms of damage to the periventricular white matter in PHVD are poorly understood. As in periventricular leukomalacia, a lack of autoregulation, and poor vascularization of the watershed region between the territories of supply of the penetrating leptomeningeal and deep perforating arteries contribute (36, 37, 39, 44). In addition, damage to periventricular white matter is probably exacerbated by ischemia due to raised intracranial pressure and parenchymal compression, by oxidative stress due to the generation of free radicals, and by the actions of inflammatory cytokines.

Raised intracranial pressure, parenchymal compression and ischemia. PHVD raises CSF pressure to, on average, three times the normal level (28). Figure 3 shows that an intracranial pressure of 15-mm Hg was high enough to prevent cerebral blood flow during diastole. Cerebral perfusion was restored when the pressure was reduced to 6-mm Hg. Clearly, a reduction of perfusion of this magnitude substantially increases the risk of ischemic injury. There is also evidence that distortion of periventricular axons due to ventricular dilatation may cause injury independently of ischemia (12).

Free radical-mediated injury. Non-protein bound iron is readily detectable in the CSF of neonates with PHVD (59). Hemoglobin that enters the CSF as a result of IVH releases large amounts of iron, which is likely to exceed the protein-binding capacity of the CSF and lead to the generation of hydroxyl free radicals from hydrogen peroxide via the Fenton reaction. Inder et al (25) demonstrated products of lipid peroxidation in the CSF of infants with periventricular leukomalacia. Whether these are also present in PHVD has not yet been investigated. Further evidence of potential oxidative stress comes from the finding of raised concentrations of hypoxanthine in the CSF of infants with PHVD (3). Normally, xanthine and hypoxanthine

are oxidized by xanthine dehydrogenase to form uric acid, with NAD^+ as the electron acceptor. However, under conditions of ischemia, xanthine dehydrogenase is modified to form xanthine oxidase, which uses oxygen as the electron acceptor (24, 38, 49). On restoration of cerebral perfusion, xanthine oxidase-mediated oxidation of xanthine and hypoxanthine generates superoxide and hydrogen peroxide, which cause oxidative damage. Oligodendrocyte progenitors are relatively abundant in the periventricular white matter of premature infants and are highly susceptibility to oxidative damage (2, 42, 48).

Pro-inflammatory cytokines. Clinical evidence suggests that inflammation causes damage to immature white matter (33). The concentration of tumor necrosis factor-α interleukin-1β, interleukin-6, interleukin-8 and interferon-γ are significantly elevated in the CSF of infants with PHVD (58). Tumor necrosis factor-α and interleukin-1β have both been implicated in the development of periventricular leukomalacia (11, 27), and it seems likely that these pro-inflammatory cytokines also contribute to white matter damage in PHVD.

NEUROPATHOLOGY

Human studies. Mortality in PHVD is now relatively low and modern studies of the neuropathology of PHVD have been very limited, although the classic descriptions and reviews (31, 57, 65) continue to be invaluable in stimulating research and validating information obtained from imaging studies and experimental models. Fukumizu et al (14, 15) observed hemosiderin deposits in the ventricular wall, nodular gliosis, ependymal cell loss and sub-ependymal rosette formation, and noted that these changes were more marked in PHVD than in congenital hydrocephalus or in infants in whom IVH had not been complicated by ventricular dilatation. Takano et al (62) reported that ependymal cell loss in the hydrocephalic infants caused overexpression of nestin and vimentin by immature glial cells in the regions of ependymal cell loss.

Early animal models of IVH and PHVD. Early animal models of IVH relied on the development of spontaneous subependymal hemorrhages following premature delivery, or after a variety of insults (eg, hypotension or hypercarbia) in rabbit pups, preterm fetal sheep and beagle pups (18, 35, 43, 47, 55,). The hemorrhages that developed were similar to those in the newborn infant but ventricular dilatation was not investigated, as survival was only short-term. Injection of blood mixed with thrombin into the lateral ventricles of adult pigs did not lead to post-hemorrhagic ventricular dilatation at 42-day follow-up (41). Xue et al (75) injected blood into the periventricular region in one-day-old mice but did not observe ventricular dilatation. The only previous reports of PHVD in experimental studies were those of Pang et al (51, 52, 53), who stereotactically injected enough autologous clotted blood into the lateral ventricles to distend the ventricular system of adult mongrel dogs. Eight of 10 dogs developed hydrocephalus 6 weeks later.

A rat model of neonatal PHVD. We recently developed a neonatal rat pup model of PHVD (8). Mixed sex Wistar rats were given 80-µl stereotactic injections of citrated rat blood or artificial cerebrospinal fluid (aCSF) into alternate lateral ventricles on postnatal day 7 and 8. Fourteen days later, 65% of pups injected with blood and 50% of those injected with aCSF had developed ventricular dilatation with visible enlargement of the head (Figure 4). Histological and immunohistochemical examination of these hydrocephalic rats revealed a patchy loss of ependyma, and marked astrocytic gliosis and rarefaction of the periventricular white matter (Figure 5). Apart from the presence of haemosiderin-laden, Perls'-positive macrophages within the ependyma and subependymal white matter of the pups injected with blood, there were no differences between these and the hydrocephalic rats that had been injected with aCSF. Injection of Indian ink into the lateral ventricles of animals with ventricular dilatation on postnatal day 21 revealed slowed passage of the CSF through the ventricular system; however, ink was present in the subarachnoid space 24 hours later, indicating that this was a communicating hydrocephalus.

Loss of white and gray matter. In the rat model of PHVD, there is a significant negative correlation between the extent of ventricular dilatation and both the thickness of the corpus callosum and that of the frontal cortex. The development of hydrocephalus is associated with a mean reduction in the thickness of the corpus callosum of 48%, and of the frontal cortex of 31%. Loss of white matter is also marked in the lateral periventricular region (Figure 5), where we have shown that loss of myelin and axons is associated with a reduced density of oligodendrocytes.

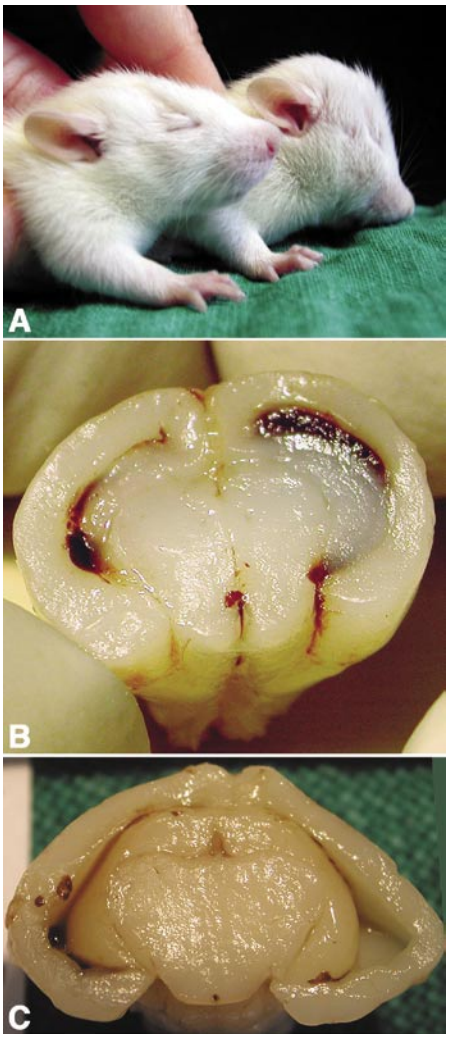

Figure 4. Hydrocephalus induced experimentally by 80-µl intraventricular injections of citrated rat blood into alternate lateral ventricles on postnatal days 7 and 8. **A.** Domed head of hydrocephalic rat pup on right, normal littermate on left. **B.** Residual blood and mild ventricular dilatation after second injection. **C.** Marked hydrocephalus at postnatal day 21.

Further research is needed into the effects of PHVD on cerebral development in man. Volumetric brain MRI studies have shown very premature infants to have reduced surface area and volume of cortical gray matter at term gestation (1) and adolescence (50). Inder et al (26) reported that premature infants with periventricular leukomalacia

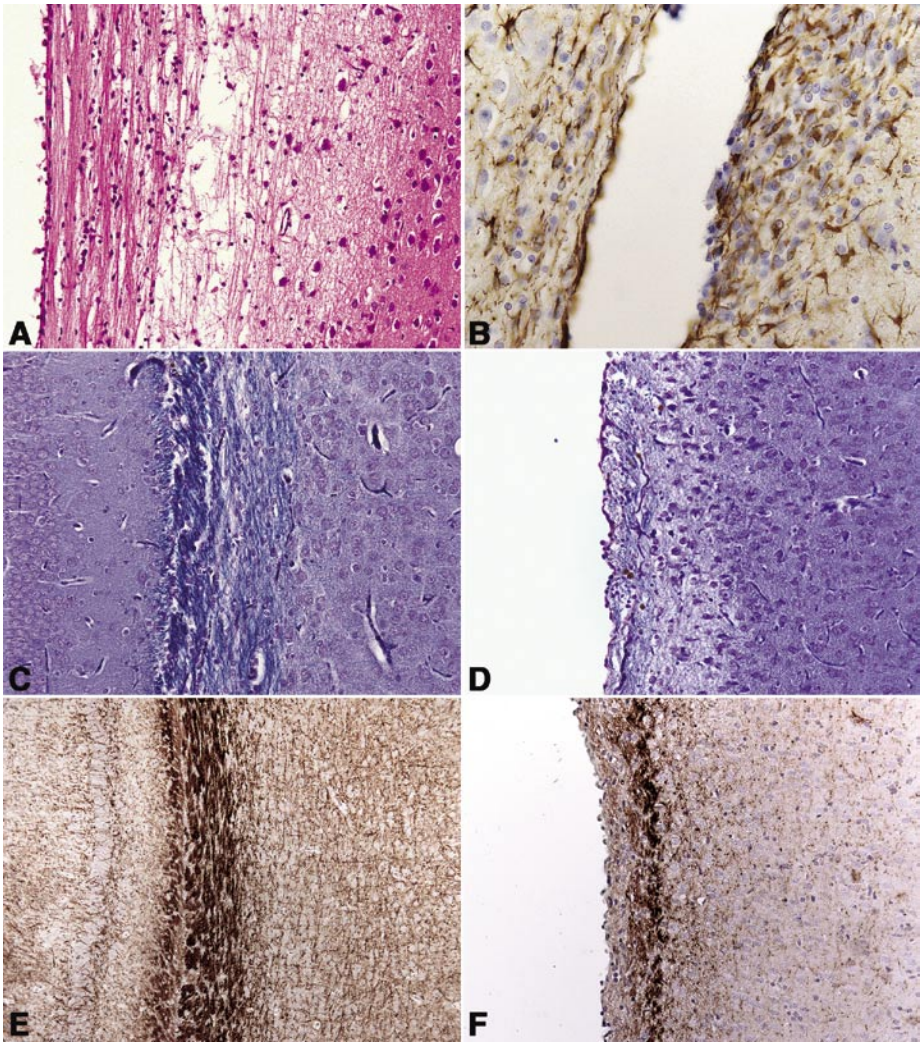

Figure 5. Damage to periventricular white matter in rat model of PHVD. **A.** Obvious rarefaction of white matter in a hydrocephalic rat on postnatal day 21. **B.** Periventricular astrocytic gliosis in a mildly hydrocephalic rat, as shown by immunostaining for glial fibrillary acidic protein. **C.** Solochrome cyanin staining of myelin sheaths in the normal periventricular white matter of a non-hydrocephalic rat. **D.** Contrast this with the paucity of solochrome cyanin-stained myelin in the white matter of a hydrocephalic rat at postnatal day 21. The small brown structures are hemosiderin-laden macrophages. **E.** Normal pattern of immunolabeling for neurofilament proteins in a non-hydrocephalic rat. (F) Marked reduction in labelling for neurofilament proteins at postnatal day 21 in a hydrocephalic animal.

had reduced volumes of cortical gray and myelinated white matter at term. However, other evidence suggests that both intraventricular hemorrhage and periventricular leukomalacia may be associated with accelerated postnatal growth of gray-matter structures (10).

Acute ventricular distension. The 80 µl of blood or aCSF injected into the lateral ventricles in the rat model of PHVD represents 12% of the circulating blood volume of a rat pup, a proportion not dissimilar to the clinical situation in newborn infants. Our finding that injection of an equivalent volume of aCSF was nearly as likely as blood to cause ventricular dilation was not expected. When we measured intracranial pressure during injection, we found a transient increase of approximately 8-fold; once again there was no difference between blood- and aCSF-injected rats (8). We have previously demonstrated raised intracranial pressure in premature infants that develop PHVD (28). Our findings suggest that acute ventricular distension and raised intracranial pressure are likely to be important pathogenetic mechanisms in the development of PHVD. Indirect supporting evidence comes from the observations that 40% of adults who sustain a large IVH go on to develop PHVD (19), and that the single most important predictor of the likelihood of development of PHVD in premature infants is the size of the IVH (45).

TGF-β in the rat model of PHVD. We have recently used the rat pup model of PHVD to look at the possible role of TGF-β in PHVD (7, 40). Very little TGF-β1, -β2 or -β3 is immunohistochemically demonstrable in the brain of normal 21-day-old normal rats. In contrast, in 21-day-old rats that had received intraventricular injections of blood or aCSF, immunoreactivity for all 3 isoforms of TGF-β was significantly elevated. TGF-β1 was present in the periventricular white matter and in the deep cortical neuropil (Figure 6A), TGF-β2 in neuronal perikarya and periventricular oligodendrocytes, and TGF-β3 in oligodendrocytes and reactive microglia. TGF-β1 and -β2 levels were elevated in injected animals irrespective of whether or not they had developed hydrocephalus, although the levels of both isoforms tended to be highest in animals that did have hydrocephalus and that of TGF-β1 was significantly related to the severity of hydrocephalus. The increase in TGF-βs was accompanied by an increase in phosphorylated p44/42 MAP kinases (P-Erk1/2)—downstream intracellular mediators of several of the effects of TGF-βs—in a distribution similar to that of TGF-β1 and -β2, and by deposition of the extracellular matrix proteins fibronectin, laminin (Figure 6B) and vitronectin (7).

Several previous studies have shown that injury affects the expression of TGF-βs in rodent brain. TGF-β1 expression increases after hypoxia/ischemia (30, 32), and penetrating cranial trauma(34). Lehrmann et al (32) found the increase to last for several days, as occurred after ventricular injection of blood or aCSF. The observations in PHVD in the rat are in keeping with clinical findings (29, 69) and those in transgenic mice which over-express TGF-β1 (16, 74), and raise the possibility of a role for TGF-βs in the development and/or maintenance of hydrocephalus after ventricular hemorrhage. However, further work is needed to establish the time-course of these changes and to assess the effects of interventions that interfere with the production and/or actions of TGF-βs, before their role can be established.

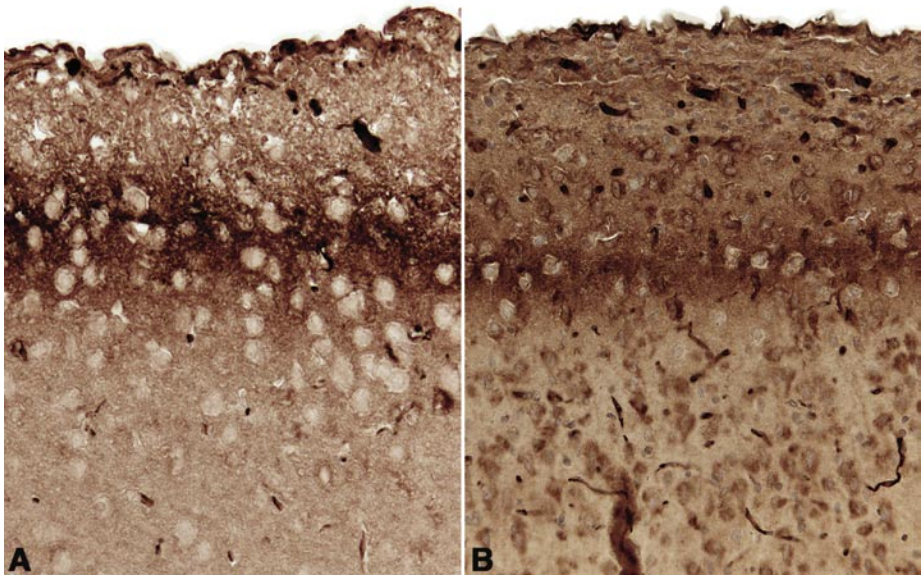

Figure 6. Expression of TGF-β and laminin are increased in the rat model of PHVD. **A.** TGF-β1 in the ependyma, periventricular white matter (particularly in relation to blood vessels) and in the deep cortical neuropil of a hydrocephalic rat on postnatal day 21. **B.** Increased periventricular laminin. The amount of laminin is much greater around blood vessels close to the ventricle than around blood vessels further away. These appearances closely resemble those in human PHVD, as illustrated in Figure 2A.

NEW APPROACHES TO TREATMENT

As current treatment strategies carry a high morbidity and are of very limited effectiveness, we have been evaluating new forms of treatment for PHVD. We have piloted drainage, irrigation, and fibrinolytic therapy (DRIFT), which involves trying to wash out as much as possible of the intraventricular blood as well as the cytokines and free iron in the CSF before irreversible hydrocephalus is established. As TGF-β1 is stored in platelets, intraventricular blood clot acts as a reservoir for its gradual release into the CSF. Infants identified as having progressive ventricular enlargement after IVH by ultrasound are anesthetized in the neonatal intensive care unit, have right frontal and left occipital ventricular catheters inserted and 0.5 mg/kg tissue plasminogen activator injected into the ventricles. After 8 hours, the ventricles are gently irrigated with aCSF (20 ml/hour) until clear fluid is drained—on average this takes 3 days. After a promising pilot study on 25 infants (72), we are currently conducting a randomized trial of DRIFT. The rat PHVD model lends itself to the preclinical evaluation of other interventions, such as the use of drugs that block the release or action of TGF-β. Indeed, the importance of careful preclinical assessment of new therapeutic approaches is highlighted by recent observations on the developmental consequences of inactivation of the gene for TGF-β1 in the mouse (5) (see also paper by Masliah and Wyss-Coray in this symposium). It will be crucial to determine whether or not the administration of TGF-β inhibitors for only a short postnatal period avoids the deleterious effects seen in the TGF-β1 knock-out mouse. We suggest that it is only by combining information gained from experimental models of PHVD with that from human studies, that it will be possible significantly to reduce the number of infants with IVH who develop hydrocephalus, white matter injury and disability.

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