Periventricular leukomalacia (PVL) is the predominant form of brain injury and the leading known cause of cerebral palsy and cognitive deficits in premature infants. The number of low-birth-weight infants who survive to demonstrate these neurologic deficits is increasing. Magnetic resonance imaging–based neuroimaging techniques provide greater diagnostic sensitivity for PVL than does head ultrasonography and often document the involvement of telencephalic gray matter and long tracts in addition to periventricular white matter. The neuropathologic hallmarks of PVL are microglial activation and focal and diffuse periventricular depletion of premyelinating oligodendroglia. Premyelinating oligodendroglia are highly vulnerable to death caused by glutamate, free radicals, and proinflammatory cytokines. Studies in animal models of PVL suggest that pharmacologic interventions that target these toxic molecules will be useful in diminishing the severity of PVL.

Ischemic and inflammatory injuries to the developing brain can lead to devastating neurologic consequences. The pattern of perinatal brain injury is highly age-dependent. In term infants, such injuries predominantly affect neurons in the cerebral cortex, yielding watershed or stroke-like distributions of damage, whereas in premature infants, immature oligodendroglia in the cerebral white matter and subplate neurons immediately below the neocortex are especially vulnerable, resulting in periventricular leukomalacia (PVL).1-2

The magnitude of the problem posed by brain injury in the premature infant is extraordinary. Improved neonatal intensive care has led to nearly 90% survival of the approximately 50,000 infants in the United States yearly with birth weight less than 1500 g. Between 5% and 10% of survivors subsequently exhibit substantial motor defects, and 25% to 50% of the remainder exhibit sensory, cognitive, and behavioral deficits. Periventricular leukomalacia is the predominant form of brain injury underlying this neurologic morbidity and is the most common cause of cerebral palsy in premature infants.

BRAIN IMAGING IN PVL

Periventricular leukomalacia is most often diagnosed in the neonatal intensive care unit by means of head ultrasonography, which demonstrates increased periventricular white matter echogenicity with or without cystic abnormalities (Figure 1). Occasionally, PVL is detectable by means of ultrasonography at birth or even in utero; however, cystic abnormalities often do not become visible at ultrasonography until 1 week or longer after birth. Periventricular leukomalacia can be all but excluded with normal findings at postnatal cranial ultrasonographic examinations performed at 1 week and 1 month after birth. In premature infants in whom repeated ultrasonography shows only increased periventricular echogenicity without cysts, less than 5% will subsequently develop overt cerebral palsy, although substantially more will show evidence of cognitive dysfunction.
The incidence of cerebral palsy, sometimes complicated by refractory complex partial seizures, is much higher in infants with ultrasonically demonstrable cystic lesions. Magnetic resonance imaging, while not always feasible in unstable premature infants, is often successful in visualizing PVL even earlier in the neonatal period than ultrasonography and, especially with repeated studies and diffusion tensor imaging, can provide prognostic information by documenting gray matter atrophy and tract degenerations. In such patients, PVL is clearly a disease of both gray matter and white matter.

Premature infants, in particular those born before 32 weeks of gestation, are vulnerable to germinal matrix hemorrhages, which often occur in conjunction with PVL. Hemorrhages confined to germinal matrix do not have a major effect on prognosis; however, if blood penetrates into the ventricles, cerebrospinal fluid dynamics may be impaired, giving rise to hydrocephalus. Hydrocephalus, if severe, can distort the corticospinal tracts and intensify the spastic diplegia that is a frequent consequence of PVL.

Epidemiologic studies indicate that the incidence of PVL is highly correlated with prematurity and less strongly correlated with chorioamnionitis. Neonatal hypocarbia and hypotension increase the incidence of PVL in premature infants. Prolonged cardiac surgery is associated with a high incidence of PVL even in term infants. Continuous near-infrared spectroscopic recording demonstrates frequent episodes of failure of arteriolar autoregulation of cerebral perfusion in sick premature infants; this increases their vulnerability to forebrain ischemia when systemic blood pressure decreases as a result of sepsis or other causes. Vulnerability of periventricular white matter to impaired perfusion in premature in-
fants is compounded by the relative sparseness of periventricular vascularity during the third trimester of gestation. Most cells of oligodendroglial lineage in the human telencephalon in the third trimester of gestation are premyelinating oligodendroglia; these cells are selectively depleted in the periventricular regions in premature infants with PVL. Microglia are numerous in human telencephalic periventricular and subplate regions in the third trimester of gestation and become activated in PVL. Toxic products of these activated microglia likely contribute to death of premyelinating oligodendroglia. The failure of reconstitution of the periventricular oligodendroglial lineage from spared germinal matrix progenitors after the acute phase of PVL is as yet unexplained.

Subplate neurons, which lie just below the developing cerebral cortex until removed by a normal process of programmed apoptosis during the third trimester of gestation, have an essential role in the axonal targeting required for formation of mature thalamocortical connections. These neurons, like premyelinating oligodendroglia, are vulnerable to ischemia, and accelerated loss of subplate neurons in PVL may contribute substantially to subsequent motor, visual, and cognitive deficits.

PREVENTION OF PVL: CURRENT CLINICAL PRACTICE

Careful attention to blood gas values is valuable in diminishing the severity of PVL: prevention of arterial PCO2 levels that fall below 35 mm Hg substantially lowers the risk of cerebral palsy. In one large study, the incidence of PVL in low-birth-weight infants with respiratory failure was also substantially reduced by inhalation of low concentrations of nitric oxide, possibly owing to improved oxygenation resulting from this therapy. Magnesium sulfate administered shortly before delivery of premature infants does not substantially diminish the incidence of cerebral palsy. Cooling of the head or whole body may reduce neurologic disability in moderately asphyxiated full-term neonates, but has not yet been critically evaluated in premature infants. Clearly, new approaches are needed to diminish PVL and the burden of neurologic disability it causes in premature infants. Attention has turned, therefore, to studies of the pathophysiology and therapy of PVL in experimental animals.

EXPERIMENTAL MODELS OF PVL

Two methods elicit selective periventricular white matter lesions in the forebrains of immature experimental animals: induction of central nervous system hypoxia-ischemia, customarily by combining unilateral carotid occlusion and reduced ambient oxygen (Figure 2), and activation of the innate immune system by administration of bacterial lipopolysaccharide. Both approaches elicit diffuse microglial activation, overproduction of proinflammatory cytokines and reactive oxygen species, and depletion of periventricular premyelinating oligodendroglia.

Unlike in human beings, in whom mature oligodendroglia begin to appear in forebrain and initiate myelination in the third trimester of gestation, terminal oligodendrogial differentiation and myelination in the mouse and rat begin postnatally. Most neonatal mouse and rat telencephalon oligodendroglial lineage cells are premyelinating oligodendroglia. Like human premyelinating oligodendroglia, these cells have exited the mitotic cycle, elaborated multiple processes, and synthesized galactolipids characteristic of myelin but have not yet translated substantial amounts of structural myelin proteins such as proteolipid and myelin basic protein. Premyelinating oligodendroglia are more susceptible than oligodendroglial progenitors to macromolecular calcium-induced calcium loading and excitotoxic death. This is a consequence of their heightened assembly of edited glutamate receptor 2–free, calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPARs). Oligodendroglial progenitors also persist in the third trimester of gestation in human and neonatal rat and mouse telencephalon. These motile, actively mitosing cells, though less susceptible to glutamate toxicity than premyelinating oligodendroglia, are especially vulnerable to the toxic effects of proinflammatory cytokines produced by activated microglia.

As in PVL in human beings, experimental PVL involves neurons and glia. Subplate neurons undergo premature apoptosis in neonatal rodents subjected to ischemic injury. In addition, whereas routine magnetic resonance images in these animals demonstrate only bilateral periventricular lesions, manganese-enhanced magnetic resonance images reveal that small cortical lesions are also present ipsilateral to the carotid occlusion. These cortical lesions, at histologic analysis, are depleted of neurons.

THERAPEUTIC INTERVENTIONS IN RODENT MODELS OF PVL

Pharmacologic manipulation of premyelinating oligodendroglial glutamate receptors is a promising method of PVL therapy. In neonatal rats subjected to hypoxia af-
ter unilateral carotid occlusion, the severity of periventricular depletion of premyelinating oligodendroglia is diminished by systemic administration of NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione) or tiorapam immediately after the hypoxic episode.22,23 Both of these drugs inhibit AMPAR-mediated calcium loading and mitochondrial dysfunction. In vitro experiments with oligodendroglial lineage cultures have shown marked antiexcitotoxic effects of specific inhibitors of GluR2-free AMPARs19 and activators of group 1 metabotropic glutamate receptors24; however, the in vivo efficacy of these drugs in PVL models has not yet been evaluated. The recent discoveries that cells of the oligodendroglial lineage in vivo, though not in vitro, express highly calcium-permeable N-methyl-D-aspartate glutamate receptors (NMDARs)25 and that ischemia in adult white matter causes NMDAR-mediated calcium accumulation in myelin and myelin disruption26 argue that NMDAR blockers should also be evaluated in PVL. A cautionary note, however, is that in vivo administration of high-affinity NMDAR blockers can induce apoptosis of immature neurons. Low-affinity use-dependent NMDAR blockers such as memantine may be safer.

A second potential method of PVL therapy is prevention of free radicals and inflammatory mediators from killing premyelinating oligodendroglia. For example, pre-treatment in the third trimester of gestation of maternal rats with the antioxidant and glutathione precursor N-acetylcysteine inhibits central nervous system induction of proinflammatory cytokines and diminishes loss of oligodendroglial progenitors elicited by administration of lipopolysaccharide.27 Because glutamate, free radicals, and proinflammatory cytokines all have a role in the pathophysiology of PVL, treatments that inhibit microglial activation and thereby prevent production of these toxic molecules might be particularly useful in preventing or ameliorating PVL. Minocycline, a second-generation tetracycline derivative, crosses the blood–brain barrier and has a proved clinical track record as an antibiotic and anti-inflammatory drug. Minocycline suppresses microglial activation and has demonstrated neuroprotective qualities in a variety of neurologic disease models including experimental PVL.27,28 However, 2 observations argue for caution before administering minocycline to prevent PVL in premature infants. First, minocycline suppresses oligodendroglial regeneration from progenitors in a myelinoxic model system,29 and second, in a large stage 3 trial in amyotrophic lateral sclerosis, weakness unexpectedly progressed more rapidly in patients receiving minocycline than in control subjects receiving placebo.30

Rather than inhibiting microglial activation, which may exert deleterious as well as desired consequences for the oligodendroglial lineage, a more desirable approach may be to identify drugs that exert exclusively trophic effects on premyelinating oligodendroglia and immature neurons, thus enhancing their capacity to resist oxidant, excitotoxic, and inflammatory mediator-induced damage. A candidate for this role is erythropoietin, which antagonizes the toxic effects on oligodendroglial and neuronal lineages of free radicals, excitotoxins, and inflammatory mediators and is efficacious in both lipopolysaccharide- and ischemia-induced PVL models.22,23

TRANSLATION OF PVL THERAPIES FROM ANIMAL MODELS TO PREMATURE INFANTS

In comparison with PVL therapeutic investigations in large animals such as fetal lambs and newborn piglets, modeling the effects of interventions on PVL in rodents is straightforward and inexpensive. Mouse PVL models, in particular, have the important advantage that contributions of specific genes to the disease process can readily be investigated by knockout and overexpression techniques. However, a significant limitation of rodent PVL models is that cerebral white matter in rats and mice comprises less than 15% of the brain volume, whereas white matter in human beings or nonhuman primates comprises more than 50% of the brain volume. For faithful preclinical modeling of novel therapeutic interventions such as glutamate receptor antagonists, inhibitors of microglial activation, and oligodendroproteic proteins in PVL, the animal chosen ideally should have a large enough volume of cerebral hemispheric white matter to enable study of both focal and diffuse components of PVL at acute, organizing, and chronic phases of evolution because the pathogenetic mechanisms in play at each stage may be different. In addition, the anatomic and functional maturation of cerebral vasculature should mirror that in the human infant. Functional studies of surviving animals should quantify motor and perceptual deficits to round out the suitability of the model. Only when the preponderance of these features is present can we be confident that results obtained with a given model will be translatable to premature infants at risk of PVL.
REFERENCES