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Cerebral white and gray matter injury in newborns: New insights into pathophysiology and management

Stephen A. Back, M.D., Ph.D.

Professor of Pediatrics and Neurology Oregon Health & Science University Clyde and Elda Munson Professor of Pediatric Research Director, Neuroscience Section, Pape' Family Pediatric Research Institute

Preterm survivors display an evolving spectrum of brain injury

Although major advances in the care of premature infants have resulted in striking improvements in the survival of very low birth weight (VLBW) infants (< 1.5 kg), enhanced survival has been accompanied by a significant increase in the number of pre-term survivors with long-term neurodevelopmental morbidity.^{1, 2} In the United States, the rate of preterm birth continues to rise with prematurity now complicating 1 in 8 deliveries, and VLBW infants comprise about 1.5% of the 4 million live births in the U.S. each year.³

Despite improved outcomes of children born preterm, there continues to be wide variation in functional disabilities, even among those born at the same gestational age.⁴ Premature birth alone is associated with a greater risk for reduction in both cerebral white and gray matter volume, which is associated with poorer cognitive development.⁵⁻¹² In the setting of cerebral injury, 5-10% of preterm survivors sustain permanent major motor impairment including cerebral palsy (CP) that ranges from mild motor dyspraxia to severe spastic motor deficits.¹³⁻¹⁷ By school age, approximately half display a broad spectrum of cognitive dysfunction that involves various aspects of learning, memory, language, vision, hearing, attention and socialization¹⁸⁻³³. Disabilities in multiple neurodevelopmental domains often co-occur³⁴ and persist to young adulthood.^{25-27, 35-38}

The diverse spectrum of cognitive and motor outcomes in preterm survivors has led to increasing recognition that widely distributed abnormalities in brain maturation occur. Until recently, preterm infants were at high risk for destructive brain lesions that resulted in cystic white matter injury (WMI) and secondary cortical and subcortical gray matter degeneration. These brain abnormalities lead to substantial deletion of axons and glia from necrotic white matter lesions and secondary loss of neurons in developing gray matter. However, the last decade has been accompanied by a growing number of studies that support a shift to milder forms of chronic injury where tissue destruction is the minor component. Nevertheless, these milder forms of injury are also associated with reduced cerebral growth and adverse outcomes. As discussed below, recent human and experimental studies support that this

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Correspondence to: Stephen A. Back, M.D., Ph.D. Oregon Health & Science University Department of Pediatrics, Division of Pediatric Neuroscience Mail Code: L481; Biomedical Research Building 317 3181 S.W. Sam Jackson Park Rd. Portland, Oregon 97239-3098 backs@ohsu.edu Phone: 503-494-0906.

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impaired growth is related to distinctly different forms of pathology that involve aberrant responses to injury that disrupt the maturation of neurons and glial progenitors. These emerging findings suggest that brain injury in the majority of preterm survivors involves a primary cerebral dysmaturation disorder that may ultimately be amenable to a variety of rehabilitative strategies directed at promoting brain maturation and improved neurodevelopmental outcome.

What defines an insult to the developing brain and why does this matter?

Although the full impact of preterm cerebral insults is often not fully defined until a childhood neurodevelopmental assessment occurs, there is a critical need for improved means to identify insults closer to the time of occurrence in order to implement potential therapies to prevent early injury³⁹ or promote regeneration and repair of chronic lesions.⁴⁰ However, the critical windows for interventions remain poorly defined, because of our limited tools to define primary or secondary insults in terms of their *timing/recurrence, severity and progression*. Identification of the *timing* of the early phase of insults remains challenging because the approaches are limited for real-time monitoring of cerebral blood flow, CNS tissue oxygenation, levels of CNS metabolites and biomarkers of CNS injury. Such tools are vitally important to develop, because therapies are likely to have variable impact depending upon the timing of their implementation during the course of neonatal brain development. Human brain development is a moving target that involves cellular activity-dependent events that coincide with multiple waves of neurogenesis, gliogenesis, glial and neuronal maturation, synaptogenesis, myelination and the ultimate establishment of neural networks and connectivity. Hence, the timing of an insult to the developing brain, in a large measure, defines its potential impact on multiple key neurodevelopmental events that sculpt the complex neural substrates that ultimately define brain function in later life.

Definition of the *severity* of insults is an emerging challenge that reflects the recent shift to milder forms of injury. Although MRI is the optimal imaging modality to define cerebral injury in preterm survivors,⁴¹⁻⁴⁴ the histopathological features of MRI signal abnormalities have mostly been defined for WMI where more severe necrotic injury predominates⁴⁵⁻⁵⁰. More severe insults result in tissue destruction that coincides with regional brain atrophy and signal abnormalities defined by MRI. More severe lesions also trigger glial barriers to regeneration and repair that may, for example, impede the efficacy of neural stem cell therapy.⁵¹ Despite the pronounced shift to milder forms of human cerebral injury, which have been defined by quantitative and diffusion-weighted MRI, the pathological signatures of these MRI findings remain mostly undefined in the human. As discussed below, experimental studies have begun to bridge the gap between MRI-defined cerebral injury and the corresponding histopathological features. Recent studies also support that the lack of tissue destruction or cell death also does not necessarily rule out the occurrence of a prior cerebral insult. As discussed below, we have recently identified that cerebral hypoxia-ischemia can have a widespread impact on neuronal maturation in the absence of overt neuronal degeneration.

Lessening the impact of factors that modify injury *progression* represents an important direction to improve neurodevelopmental outcomes. It is increasingly appreciated that there are many genetic/epigenetic, systemic or iatrogenic factors that adversely influence primary injury progression (e.g., nutritional status, infection, endocrine status/steroid exposure, peripheral organ dysfunction, and exposure to anesthetics, sedatives or drugs of abuse) with potentially reversible or irreversible consequences. One recently identified factor associated with abnormal microstructural cortical growth in human preterm neonates is impaired somatic growth (weight, length, and head circumference), even after accounting for co-existing brain injuries on MRI and other aspects of systemic illness such as infection.⁵²

Procedural pain and stress has also been recently linked to altered brain maturation that involves gray and white matter structures, as well as impaired brain function.^{53, 54} Procedural pain in preterm neonates has also been associated with impaired postnatal growth,⁵⁵ a predictor of poor cortical development.⁵²

In the remainder of this chapter we will review recent advances in our understanding of the role of hypoxia-ischemia in the pathogenesis of preterm cerebral injury, which begin to shed light on the mechanisms that underlie the shifting spectrum of cerebral injury in preterm survivors. The shift toward less severe injury appears to be related to changes in the timing, severity and progression of hypoxic-ischemic insults.

A maturation-dependent role for hypoxia-ischemia (H-I) in the pathogenesis of cerebral injury

Multiple lines of evidence support that cerebral ischemia is often the major factor that initiates cerebral injury in VLBW infants.⁵⁶⁻⁵⁸ Given the limitations of human studies to directly link blood flow disturbances with injury, experimental studies in fetal sheep and other animal models have greatly strengthened our understanding of the contribution of cerebral hypoxia-ischemia to gray and white matter injury. The *timing* of cerebral blood flow disturbances during development is a critical factor that contributes to CNS susceptibility to hypoxia-ischemia. For example, under conditions of prolonged global cerebral ischemia, acute injury to the cerebral cortex is relatively low in the preterm fetal sheep, whereas severe pan-laminar cortical necrosis occurs in the term animal.^{59, 60} In the near-term fetus, even brief severe global asphyxia, caused by a 10-minute occlusion of the umbilical cord, can result in prolonged hypoperfusion to cerebral gray and white matter.⁶¹ Studies of global cerebral hypoperfusion found that the mid-gestation animal displayed a predilection to periventricular and subcortical WMI, whereas the near term animal displayed predominantly parasagittal cortical neuronal injury.^{59,60, 62} In contrast to near term animals with global cerebral hypo-perfusion, near term animals with severe umbilical cord occlusions displayed more global cerebral injury. Systemic hypotension arising from intermittent or partial umbilical cord occlusion produced a variable degree of WMI in addition to primary damage to the cerebral cortex.^{63, 64, 65}

The importance of cerebral ischemia is further supported by studies where WMI was detected only infrequently in models of hypoxemia in which a restriction in utero-placental blood flow resulted in decreased oxygen delivery and mild acidemia to the fetus *without* systemic hypotension or cerebral hypoperfusion.⁶⁶⁻⁶⁸ A model of fetal metabolic acidemia induced by maternal hypoxemia similarly produced mild-to-moderate injury in mid-gestation and near-term sheep.⁶⁹ By contrast, preterm ovine WMI was only detected after repeated systemic fetal endotoxin exposure that triggered both transient hypoxemia and hypotension.^{70, 71} Hence, cerebral hypoperfusion in conjunction with hypoxia appears to be a critical factor to generate significant preterm WMI.

Do disturbances in cerebral autoregulation play a role in the pathogenesis of cerebral injury from hypoxia-ischemia?

A complex interplay of factors related to cerebrovascular immaturity predispose preterm cerebral white matter to injury from hypoxia-ischemia. Central among these factors is a disturbance in cerebral autoregulation. Cerebral autoregulation refers to the maintenance of constant CBF over a range of changes in arterial blood pressure or cerebral perfusion pressure.^{56, 72, 73} This autoregulatory range has both upper and lower limits; above or below these limits, CBF does not remain constant but instead increases or decreases passively,

along with changes in arterial blood pressure. Cerebral autoregulation has been demonstrated in several species and across developmental stages. Although the mechanism remains elusive, it appears to involve an intrinsic property of arterial smooth muscle cells. Changes in transmural pressure modify muscle tone, by affecting the activation of potassium and calcium channels in smooth muscle cells, thereby affecting membrane potential.⁷⁴ Autoregulation is also mediated by a fine balance between endothelial cell-derived constricting and relaxing factors.⁷⁵ In adults, CBF remains constant over an autoregulatory range of mean blood pressures from 50 to 150 mm Hg.⁷³ In near-term fetal sheep, the range is lower and narrower (40 to 80 mm Hg), but more importantly, normal blood pressure is no more than 5 to 10 mm Hg above the lower limit of the autoregulatory curve.⁷⁶ Preterm fetal lambs show even less autoregulatory capability.⁷⁷

Impaired cerebral autoregulation in clinically unstable premature infants was initially studied by means of xenon clearance and doppler and more recently by near infrared spectroscopy (NIRS) and spatially resolved spectroscopy.^{56, 78} Severe perinatal asphyxia, hypoxia, head trauma, and hypercapnic acidosis, even when relatively mild, attenuate or even abolish autoregulation.⁷⁹⁻⁸¹ Nevertheless, considerable controversy remains regarding the role of pressure passivity in the pathogenesis of various forms of brain injury in the sick preterm neonate. This is illustrated, for example, by studies that failed to support a role for impaired autoregulation in the pathogenesis of intraventricular hemorrhage.^{82, 83} Hence, basic questions regarding cerebral autoregulation remain unanswered, including determining the optimal clinical practices for blood pressure regulation.⁵⁶

Do vascular end and border zones play a role in the pathogenesis of cerebral injury from hypoxia-ischemia?

Cerebral vascular development, particularly in the periventricular region, is clinically relevant, because of the propensity of the premature infant to both cerebral WMI and hemorrhage in the germinal matrix and ventricles.^{84, 85} The role of cerebral vascular immaturity in the pathogenesis of preterm WMI has been difficult to define in preterm infants. Analysis of the vascular supply to the periventricular white matter has yielded conflicting results. The periventricular white matter has two major blood supplies. Perforating arteries branch from leptomeningeal arteries, penetrate the cerebral cortex and terminate as capillary beds adjacent to the ventricles. Branches of choroidal and striate arteries project toward the lateral ventricles and then deviate away from the ventricle toward their final termination in vascular capillary beds in the periventricular white matter. One early hypothesis proposed that these vascular beds collectively form vascular endzones and border zones that render the periventricular white matter particularly susceptible to ischemia. However, the existence of these border zones remains controversial.⁸⁶⁻⁸⁸ The presence of these vascular zones would provide a mechanism for WMI based on the notion that when periventricular white matter flow falls below a critical threshold, this region would display greater susceptibility to WMI relative to a putatively better-perfused cerebral cortex.

To seek evidence for these vascular zones, we measured blood flow in histopathologically-defined regions of injury in cerebral cortex and white matter in preterm fetal sheep.⁸⁹ While white matter basal blood flows were lower than cerebral gray matter (Fig. 1A), there was no evidence for pathologically significant gradients of fetal blood flow within the periventricular white matter under conditions of global partial ischemia or reperfusion (Fig. 1B, C). White matter lesions did not localize to regions susceptible to greater ischemia; nor did less vulnerable regions of cerebral white matter have greater flow during ischemia. An alternative explanation for the topography of cerebral white matter lesions is the distribution

of susceptible cell types (see below), particularly late oligodendrocyte progenitors (preOLs), that are particularly susceptible to hypoxia-ischemia.⁶⁰

Relative contributions of hypoxia-ischemia and OL lineage immaturity to WMI

The preterm fetal sheep (0.65 gestation) displays heterogeneous maturation of the oligodendrocyte (OL) lineage in periventricular white matter, which allowed us to define the relative contributions of OL maturation and vascular factors to acute WMI.⁶⁰ OL lineage maturation in medial cerebral white matter was similar to human (~23-28 weeks gestation) in that preOLs were the major OL stage present. By contrast, lateral cerebral white matter was more differentiated and contained predominantly pre-myelinating and early myelinating OLs. Surprisingly, we found that moderate cerebral ischemia did not uniformly damage these adjacent regions of white matter. Rather these adjacent regions sustained differing degrees of acute injury even though they sustained a similar degree of low flow during prolonged ischemia-reperfusion. Hence, while global ischemia was necessary for WMI, no regional differences in blood flow were found within the white matter under basal or ischemic conditions to account for the differences in cell death between adjacent white matter regions. Rather, differences in the topography of WMI were closely correlated with the distribution of vulnerable preOLs. Interestingly, in regions of preOL degeneration, other neural cell types (astrocytes, microglia and axons) were markedly more resistant to injury.^{60, 90, 91}

The timing of appearance of preOLs during white matter development also plays a major role in the severity of WMI. In a rabbit model of placental insufficiency, significant global fetal hypoxia-ischemia caused minimal WMI at fetal day 22, but a similar insult three days later in gestation caused pronounced WMI.⁹² The relative susceptibility of the white matter at these two developmental ages coincided with the timing of appearance of susceptible preOLs. Taken together, these findings suggest that perturbations in cerebral blood flow are necessary but not sufficient for WMI. The developmental predilection for WMI appears to be related to both the timing of appearance and regional distribution of susceptible preOLs. These findings predict that some near-term infants with delayed OL differentiation and myelination might also be more susceptible to WMI. Interestingly, a variable degree of WMI was detected in near term sheep after several insults^{62, 63, 65, 68, 69, 93}. Moreover, near term and term infants with congenital heart disease are also at high risk for WMI⁹⁴. Hence, the targeted death of preOLs from hypoxia-ischemia could contribute to the pathogenesis of acute WMI across a broad range of gestational ages and regions of white matter.

Patterns of WMI in the immature brain thus result from “selective vulnerability” of preOLs that are enriched in cerebral white matter during restricted windows in development.⁹⁵ The timing of appearance and spatial distribution of susceptible OL lineage cells coincides with the magnitude and distribution of acute ischemic injury in several experimental models of WMI. In particular, preOLs are highly susceptible to hypoxia-ischemia and inflammation⁹⁶ whereas earlier and later OL stages are markedly more resistant.^{60, 97, 98} The enhanced susceptibility of preOLs is a cell intrinsic property that is independent of the perinatal age of the animal or the location of these cells in the forebrain. The increasing developmental resistance of cerebral white matter to hypoxia-ischemia is related to the onset of preOL differentiation to pre-myelinating OLs that display reduced susceptibility to hypoxia-ischemia.⁹⁷

The changing spectrum of human white matter injury (WMI)

Cerebral WMI is the major form of brain injury recognized in survivors of premature birth.⁹⁹ The period of highest risk for WMI is ~23-32 weeks post-conceptual age. Perinatal WMI, including periventricular leukomalacia (PVL), was the most common finding, seen in almost half (42.5%) of affected children.¹⁰⁰ MRI-defined WMI but not gray matter injury manifests in the first months of life as abnormal movements that are predictive of CP.¹⁰¹⁻¹⁰³

The spectrum of white matter pathology includes three major identifiable forms: focal cystic necrosis, focal microscopic necrosis and diffuse non-necrotic lesions. Cystic necrotic lesions are the most severe and have been widely appreciated since the classical descriptions of PVL by Banker and Larroche.¹⁰⁴⁻¹¹¹ The cysts are typically much larger than a millimeter in diameter and comprise degeneration of all cell types including glia and axons.^{91, 112} In several series, focal cystic lesions were detected by MRI in less than 5% of cases.¹¹³⁻¹¹⁸ Moreover, the overall burden of human necrotic WMI (cystic necrosis and microcysts), defined by pathology, was decreased by ~10-fold in contemporary cohorts relative to retrospective cases from earlier decades.¹¹⁹

Despite the pronounced reduction in cystic PVL, small foci of necrosis continue to be defined by neuropathological examination of contemporary cases. These discrete foci of microscopic necrosis (microcysts) typically measure less than a millimeter.¹²⁰ Similar to cystic necrosis, microcysts evolve to lesions enriched in cellular debris, degenerating axons and phagocytic macrophages.¹¹⁹ In recent human autopsy cases from archival and contemporary cases,^{119, 120} microcysts were observed in at least 30% of cases. However, they comprised only ~1-5% of total lesion burden.¹¹⁹ Hence, microscopic necrosis occurs with high incidence, but the burden often appears to be low.

Diffuse WMI is the characteristic pattern of brain injury most frequently observed in contemporary cohorts of premature newborns.¹¹⁹ Recent human autopsy studies quantitatively analyzed the magnitude and distribution of diffuse WMI and found that these lesions are much more extensive than previously appreciated from conventional neuropathological approaches.¹¹⁹ The hallmark of these lesions is a chronic diffuse reactive gliosis comprised of activated astrocytes and microglia that extend beyond foci of necrosis. Diffuse WMI targets the oligodendrocyte (OL) lineage with selective degeneration of preOLs, whereas axons are mostly spared except in necrotic foci.^{91, 112} Human preOLs are particularly susceptible to oxidative damage¹²¹ that causes WMI with features consistent with hypoxia-ischemia.^{60, 97}

Current limitations for neuroimaging of WMI

Although cranial ultrasound is the preferred bed-side imaging technique for diagnosing cystic necrotic WMI, it has limited sensitivity for diagnosing diffuse WMI.^{113, 116, 117} MRI provides a noninvasive means for diagnosis of injury in the developing brain by conventional T1-weighted, T2-weighted, and diffusion-weighted images.^{42, 122} Nevertheless, there remains unexplained variability in the nature of lesions detected at different centers, which may reflect differences in clinical management, clinical acuity or the modes of detection by MRI. On diagnostic MRI scans, WMI is indicated by discrete focal or more diffuse areas of MR signal abnormalities. There are currently limitations to diagnostic MRI at regularly used clinical field strengths (e.g., 1.5 or 3 Tesla), which restrict its utility for diagnosis and prognosis. Chief among these is the limited detection of microscopic necrosis. At a field strength of 3T, MRI currently cannot resolve these lesions, which are typically a millimeter or less in diameter. The significance of microcysts, thus, remains an important but clinically inaccessible question. These lesions may be clinically silent or a significant contributor to motor disabilities, depending upon the extent to which

they localize to functionally important regions of white matter tracts. Microscopic necrosis may also contribute to the burden of cognitive dysfunction in preterm survivors, since gray matter injury was seen in association with microscopic necrosis, but not in cases where diffuse WMI occurred in isolation.¹²⁰

Microcysts can be visualized by MRI at ultra-high magnetic field strength (12T), as we recently demonstrated in a global ischemia model of preterm WMI in fetal sheep (Fig 2, upper panel).¹²³ This preparation generated a spectrum of WMI very similar to that observed from human autopsy studies, as well as a reduction in cerebral white matter volume similar to that observed in preterm survivors.⁴²⁻⁴⁴ We developed registration algorithms that defined the histopathological features of three distinct forms of MRI-defined WMI (Figure 2).⁹⁰ Microcysts were not detected at 1 week after ischemia, but by 2 weeks, evolved to discrete lesions that were visualized by MRI at high field strength and confirmed by pathology.⁹⁰ Similar to the human autopsy studies discussed above,¹¹⁹ microcysts were observed in 50% of animals and comprised only 1.5% of total lesion volume.⁹⁰ Hence, microscopic necrosis occurs with high incidence, but the amount of involved white matter appears to be low. The ability to resolve microcysts by MRI at clinical field strengths would be a significant advance to define the contribution of these lesions to neurodevelopmental disabilities in childhood survivors of prematurity.

Current clinical MRI field strengths also may be a limiting factor to detect the full extent of diffuse WMI as the lesions become progressively more chronic. In particular, MRI may not fully define early diffuse lesions enriched in reactive astrocytes and microglia.¹²³ We recently analyzed diffuse WMI by ultra high field MRI in our preterm fetal sheep model where animals survived for 1 or 2 weeks after global cerebral ischemia. At 1 week after ischemia, high field MRI (12 Tesla) identified a novel diffuse hypo-intense signal abnormality on T2-weighted images with high sensitivity and specificity for lesions with astrogliosis (Fig. 2, middle panel). These lesions displayed MRI and histopathological features that were distinctly different from the focal necrotic lesions typically seen in PVL (Fig. 2, lower panel). These unexpected findings suggest that current clinical MRI field strength may be a limiting factor to detect diffuse WMI, as well as microscopic necrosis. Additional clinical-pathological studies are needed to determine whether high-field MRI can provide greater sensitivity to identify diffuse WMI than is currently feasible at lower field strengths.

Dysmaturation of glial progenitors in chronic WMI and myelination failure

The propensity for myelination failure is a central feature of chronic diffuse WMI, the primary white matter lesion in preterm neonates.¹¹⁹ The major cellular elements that contribute to myelination failure are the axon and the preOL. Recently, it was shown that axons also display maturation-dependent vulnerability to oxidative stress and hypoxia-ischemia.¹²⁴ Larger caliber axons, in preparation for myelination, are particularly susceptible to injury in contrast to smaller caliber unmyelinated axons, which are more resistant. The major sites of axonal degeneration are lesions that display cystic necrosis or microcysts, and pre-myelinating axons appear to be intact in diffuse WMI.⁹¹ Hence, axonal degeneration does not appear to be a major component of diffuse WMI prior to active myelination.

Volpe initially proposed that myelination failure in chronic WMI arises from a persistent loss of pre-OLs that are the precursors to myelinating OLs.¹²⁵ Consistent with this hypothesis, preOLs were found to be markedly depleted in acute WMI in preterm human autopsy cases,¹²⁶ in perinatal rodents⁹⁷ and fetal sheep.⁶⁰ In contrast to normal white matter (Fig. 3A), chronic WMI displayed diffuse reactive astrogliosis and a striking loss of myelin

(Fig. 3B), which appeared consistent with the hypothesis that myelination failure resulted from preOL death. Although pronounced selective degeneration of preOLs occurs in acute WMI, a persistent loss of preOLs was surprisingly not found in chronic lesions in a preterm-equivalent rat model of hypoxia-ischemia.⁹⁸ This was the case even though pronounced preOL degeneration was observed for at least a week after hypoxia-ischemia. This paradox was explained by the rapid onset of a glial proliferative response to acute WMI (Fig. 3C). Within 24 hours after WMI, surviving preOLs rapidly increased in number to regenerate depleted preOLs.^{98, 127-129} This preOL expansion was driven mostly by early OL progenitors that proliferated locally at the sites of WMI⁹⁸ or cortical injury¹³⁰ rather than from the subventricular zone, where less robust generation of OL lineage cells has been observed.¹³¹⁻¹³³

Further analysis of the fate of the newly generated preOLs demonstrated that they failed to mature to myelinating OLs within lesions enriched in reactive gliosis (astrocytes and microglia). Thus, regeneration of preOLs from the surviving preOL pool compensates for preOL death, but surviving preOLs display persistent arrested differentiation in chronic lesions (Fig. 3C). More recently, arrested maturation of preOLs was also shown to contribute to myelination failure in diffuse WMI in both preterm fetal sheep and human.^{90, 119} In contrast to earlier studies,¹³⁴ a robust expansion of human preOLs was also recently defined in chronic lesions,¹¹⁹ which was unexpected, given the significant loss of these cells during the acute phase of WMI.¹²¹ Hence, chronic diffuse WMI is characterized by an aberrant response to acute injury, which involves a disrupted regeneration and repair process where preOLs are regenerated but they remain dysmature.

A role for arrested preOL maturation in chronic WMI is consistent with studies in adult animal models that have defined aberrant responses of the OL lineage in multiple sclerosis models of chronic demyelination. Myelination failure was also shown to involve a potentially reversible process linked to arrested pre-OL maturation. Reactive astrocyte-derived hyaluronic acid (HA), for example, accumulates in chronic WMI and reversibly inhibits preOL differentiation and myelination.¹³⁵ HA digestion by a CNS-enriched hyaluronidase, PH20 generates bioactive HA fragments that block OL differentiation in vitro and in vivo.^{136, 137} Interestingly, HA is also highly enriched in preterm diffuse WMI, as is its putative receptor, CD44,¹¹⁹ which suggests a role for HA in blocking regeneration and repair of chronic WMI during development.

Although neuro-imaging studies have defined impaired growth of central white matter pathways in preterm survivors, additional studies are needed to determine the temporal evolution and extent of impaired myelination in chronic lesions. It will be critical to define the developmental window over which diffuse inflammation in the white matter generates inhibitory factors that sustain preOL arrest. Recent studies also support that viable OLs and myelination are critical for axon survival,¹³⁸ raising the possibility that preOL arrest could adversely affect the functional integrity of axons in chronic lesions. A wide variety of other inhibitory factors may also contribute to preOL arrest in chronic WMI.¹³⁹⁻¹⁴² Definition of these factors could also have important therapeutic relevance for a wide variety of other disorders of myelination failure where preOL arrest is also implicated—including vascular dementias.¹⁴³

Clinical Implications of Potential Arrested White Matter Development

As illustrated in figure 3C, the severity of an ischemic insult defines whether myelination failure arises primarily from necrotic WMI (upper pathway) or preOL maturation arrest (lower pathway). As discussed above, contemporary preterm survivors display less severe WMI, which coincides with preOL arrest. The potential for therapeutic interventions appears

much more promising for this form of injury than for WMI dominated by necrosis, where essentially all cell types degenerate.

Chronic WMI thus coincides with an expanded developmental window over which preOL maturation-arrest persists, which may enhance the risk for recurrent and potentially more severe brain injury in critically ill premature infants. As noted above, preOLs are selectively more vulnerable to hypoxia-ischemia than are earlier or later stages of the OL lineage. The persistence of preOLs in chronic WMI suggests the possibility that these lesions would be more susceptible to recurrent hypoxia-ischemia than more mature normal white matter that is enriched in OLs and myelin. In fact, in rat chronic lesions with preOL maturation-arrest, the selective vulnerability of preOLs to acute hypoxia-ischemia not only persisted, but markedly increased.⁹⁸ A recurrent second episode of hypoxia-ischemia triggered a massive selective degeneration of preOLs that was much more severe than that observed after the initial episode. Arrested preOLs displayed a potentiated susceptibility to massive apoptotic degeneration, which rendered chronic white matter lesions susceptible to more severe injury. Taken together, these findings suggest that an initial insult, such as hypoxia-ischemia or infection, may trigger WMI that becomes progressively more severe with recurrent insults. Serial neuro-imaging studies are thus needed to define the progression of WMI in preterm infants as well as term infants^{41, 144} that are at risk for recurrent insults. Such studies have identified clinical features that identify infants at risk for exacerbation of initial cerebral injury (e.g., preterm newborns with postnatal sepsis). Recurrent and systemic illness is an important risk factor that may increase susceptibility to progressively more severe WMI.¹⁴⁵

Future studies are needed in relevant experimental models and from human autopsy studies to define the evolution of cerebral white matter lesions over months to years. Such information is of critical importance to define the period over which the glial scar remodels in cerebral palsy and to identify the responses of the cell types that persist during the process of myelination failure. These data will also inform the therapeutic potential of strategies such as stem cell therapy^{146, 147} or therapies aimed at reversing preOL arrest to promote regeneration and repair of injured white matter in preterm survivors with cerebral palsy or related neurological disabilities.

An emerging spectrum of gray matter injury and neuronal dysmaturation

As proposed by Volpe with the concept of an “encephalopathy of prematurity,” the cerebral gray matter of preterm survivors may involve both destructive and developmental disturbances.¹⁴⁸

Although several large human neuroimaging studies have identified significant reductions in the growth of cortical and subcortical gray matter structures including the basal ganglia, thalamus, hippocampus and cerebellum,^{52, 149-152} the relative contributions of destructive and developmental processes in contemporary preterm survivors is not yet clear. Significant neuronal loss has been reported in the human cortex, basal ganglia, thalamus and cerebellum in association with necrotic WMI from archival autopsy cases.^{120, 153-155} In addition, subplate neurons, a transient cell population, required to establish thalamocortical connections, are vulnerable to perinatal H-I in rodents¹⁵⁶ and were found to be reduced in human autopsy cases that were also diagnosed with necrotic WMI.¹⁵⁵

Neuroimaging studies of contemporary cohorts of preterm survivors support, however, that premature newborns have more extensive gray matter abnormalities than “injuries” identified by signal abnormalities on conventional MRI. Moreover, children and adults born preterm with normal neurocognitive function express altered cortical activation and functional connectivity during language and visual processing.¹⁵⁷⁻¹⁶¹ Thalamo-cortical connections are also disrupted in preterm newborns with WMI, resulting in visual

dysfunction.¹⁶² Consistent with these structural studies, preterm newborns at term-age also exhibit reduced functional connectivity between the cortex and thalamus on fMRI.¹⁶³ Altered functional connectivity in children and adolescents born preterm is now recognized as a critical risk factor for adverse neurocognitive outcomes.^{158, 159, 164}

Susceptibility of preterm cerebral gray matter to hypoxia-ischemia

The susceptibility of the preterm gray matter to injury from hypoxia-ischemia appears to be fundamentally different from that at term. Studies that employed biomarkers of oxidative damage provide indirect support for preterm cerebral injury from hypoxia-ischemia.^{108, 121} The magnitude of oxidative stress in preterm white matter was similar to that sustained by gray matter from hypoxia-ischemia at term. Under these conditions of oxidative stress, we analysed human autopsy cases with diffuse WMI and preOL degeneration, and found that neither the preterm gray matter nor the white matter displayed evidence of significant oxidative stress or cellular degeneration that involved neurons or axons.¹²¹ The resistance of the preterm gray matter to neuronal degeneration from hypoxia-ischemia is further illustrated by studies in preterm fetal sheep where the magnitude of global ischemia was very similar in superficial cortex and deeper cerebral structures including the caudate nucleus and periventricular white matter.^{60, 89} This similar degree of ischemia in gray and white matter resulted in diffuse WMI and significant preOL degeneration, but largely spared neurons in the gray and white matter.^{60, 89} Widespread neuronal degeneration was only observed under conditions of severe ischemia that caused severe white matter necrosis.⁶⁰ Notably, significant neuronal loss was seen in the human cortex, basal ganglia, thalamus and cerebellum in association with necrotic WMI,^{120, 153-155} but not in cases where diffuse WMI occurred without significant necrosis.¹²⁰ Neuronal loss appears to principally arise from retrograde axonal degeneration in necrotic white matter lesions.^{91, 112} In the preterm gray matter, significant loss of neurons thus appears to be related to more severe ischemia that causes destructive lesions.

Impaired cerebral growth from preterm cerebral hypoxia-ischemia can occur without neuronal loss

Hypoxia-ischemia to the preterm brain also causes disturbances in growth of gray matter structures. Surprisingly, this reduced growth of the cerebral cortex and the basal ganglia can also occur in response to conditions that disrupt neuronal maturation without neuronal or axonal loss. In response to cerebral ischemia, preterm fetal sheep acquired diffuse WMI, as well as a progressive reduction in cortical growth that was not explained by neuronal loss.¹⁶⁵ The basis for this unexpected result was explained by detailed analysis of the maturation of the dendritic arbor of pyramidal neurons, the major population of cortical projections neurons. During normal development, pyramidal neurons are highly immature in the preterm cerebral cortex (Fig. 4A) but in near term animals the dendritic arbor becomes much more complex (Fig. 4B), which coincides with a marked increase in cortical volume and measures of neuronal arbor complexity (Fig. 4C). In response to preterm ischemia, cortical growth impairment was accompanied by a significant reduction in the complexity of the dendritic arbor; consistent with the notion that neuronal maturation was disrupted in the setting of cerebral ischemia. Compared to controls (Fig. 4D), the ischemic animals displayed neuronal dysmaturation (Fig. 4E) that was reflected in a reduction in the total dendritic length as well as the number of branches, branch endings and branch points. Notably, the dendritic arbor was most simplified closer to the cell body where synaptic integration occurs (Fig. 4F).

A role for neuronal dysmaturation in cognitive and behavioral disturbances in preterm survivors is suggested by analysis of dendritic spines,¹⁶⁵ the key sites for synaptic activity.

We have observed reduced numbers of spines on the dysmature dendrites of projection neurons in both the cortex and caudate, which suggests that widespread disturbances in neuronal connectivity may contribute to the global disturbances in neurodevelopment seen in preterm survivors. Since disturbances in neuronal maturation occur at a critical window in the establishment of neuronal connections, even transient neuronal dysmaturation may have persistent global effects on the subsequent development of CNS circuitry.

Consistent with this notion, two recent studies found that the normal progressive loss of cortical fractional anisotropy (FA) was delayed in human preterm survivors with impaired postnatal growth,⁵² and with reduced cortical growth.¹⁶⁶ We made similar observations in preterm fetal sheep exposed in utero to global cerebral ischemia. Cortical FA was significantly higher in the ischemic animals relative to controls. To explain this observation, we developed a mathematical model to calculate FA based upon the morphology of control and ischemic neurons and found that the FA values derived from MRI and neuronal morphology were very similar.¹⁶⁵ Hence, despite a lack of overt gray matter injury, the cortex displayed a delayed loss of cortical FA that was related to widespread immaturity of the dendritic arbor of cortical projection neurons. Interestingly, one factor associated with abnormal microstructural cortical growth in human preterm neonates was impaired somatic growth (weight, length, and head circumference); even after accounting for co-existing brain injuries on MRI (e.g., WMI) and other aspects of systemic illness (e.g., infection).⁵² Hence, multiple factors including nutritional status and exposure to cerebral ischemia may contribute to the pathogenesis of neuronal dysmaturation in preterm survivors.

Conclusions

Our understanding of the pathogenesis of brain injury in the premature infant has recently undergone significant redefinition, which coincides with advances in neonatal care that have markedly reduced the overall severity and extent of the destructive processes associated with cerebral injury. Significant improvement in the care of premature neonates has also coincided with the emergence and application of improved brain imaging, which has provided better resolution of some of the key features of cerebral injury during the period of most rapid changes in brain growth and maturation. Nevertheless, further progress is needed to resolve important features of early and progressive WMI by MRI—particularly microscopic necrosis and diffuse WMI. Despite continued progress with many potentially promising therapies for the hypoxicischemic term neonate,^{39, 51} the development of a comparable therapeutic armamentarium for the preterm neonate has been limited by the greater fragility of these infants and the need for improved tools to identify the timing, distribution, severity and evolution of preterm cerebral lesions in both white and gray matter.

Despite the reduced severity of brain injury for many babies, a lack of overt tissue destruction does not exclude a lack of insults to the developing brain. Sub-injurious insults have been under studied, because the approaches required to identify cellular dysmaturation processes are laborious and time-consuming. Disruption in glial and neuronal maturation may have adverse consequences on multiple aspects of cerebral development given that the timing of dysmaturation coincides with the period of most rapid brain growth and enhanced neuronal connectivity related to elaboration of the dendritic arbor and synaptogenesis, as well as myelination. The fact that preterm survivors commonly display disability in multiple neurodevelopmental domains suggests that widespread disturbances in neuronal dysmaturation may be involved. Thus a critical next step is to identify the disrupted neuronal networks that contribute to the widespread neurobehavioral impairments that commonly persist throughout life in preterm survivors.

These new findings raise the possibility that those chronic disabilities that arise primarily from diffuse cerebral dysmaturation may be amenable to strategies directed at promoting brain maturation and improved neurological outcome. The activation of dysmaturation processes appears to coincide with disrupted regeneration and repair processes. Glial progenitors respond to WMI by partially but incompletely mounting a repair process that regenerates and expands the preOL pool, which is blocked from maturation. Similarly, some populations of immature projection neurons fail to normally mature. In contrast to mature neurons in the full term neonate that degenerate from hypoxia-ischemia,¹⁶⁷ these immature neurons survive with a simplified dendritic arbor that contributes to reduced cerebral growth.

The timing and nature of future interventions to prevent or reverse cellular dysmaturation may differ for gray and white matter. Factors such as improving infant nutrition, preventing infections, reducing neonatal stress and implementing earlier behavioral interventions may all play a role in mitigating the impact of neuronal dysmaturation. Pharmacological interventions aimed at blocking the inhibitory pathways that sustain preOL maturation arrest may reverse or prevent myelination failure with the potential for enhanced connectivity of CNS pathways. There is thus a wealth of potential new opportunities to promote enhanced brain maturation and growth that were not feasible even a decade ago.

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KEY POINTS

- Preterm infants were previously at high risk for destructive brain lesions that resulted in cystic white matter injury and secondary cortical and subcortical gray matter degeneration. Contemporary cohorts of preterm infants commonly display less severe injury that does not appear to involve pronounced neuronal or glial loss. These milder forms of injury are still associated with reduced cerebral growth
- Myelination disturbances are one of the hallmarks of chronic white matter injury arising from hypoxia-ischemia. Myelination disturbances are related to aberrant regeneration and repair responses to acute death of pre-myelinating oligodendrocytes (preOLs). In response to preOL death, oligodendrocyte progenitors rapidly proliferate and differentiate, but the regenerated preOLs fail to mature to myelinating cells.
- Although immature neurons appear to be more resistant to cell death from hypoxiaischemia than glia, they display widespread disturbances in maturation of their dendritic arbors, which provides an explanation for impaired cerebral growth.
- Numerous immature neurons and preOLs fail to fully mature during a critical window in development of neural circuitry. These recently recognized forms of cerebral gray and white matter dysmaturation raise new diagnostic challenges and suggest new therapeutic directions centered on reversal of the processes that promote dysmaturation.

Figure 1.

Preterm fetal cerebral blood flow (CBF) does not display gradients of flow under basal or ischemia-reperfusion conditions that is consistent with vascular border zones or endzones. (A) Quantification of regional fetal cerebral blood flow in vivo under conditions of basal flow. The top image in panel A represents a 3-D surface reconstruction of fluorescence images of a 0.65 gestation control ovine brain that indicates the frontal and parietal levels to which the lower blood flow images correspond in 1 and 2. Representative pseudocolor scale basal flow images show higher blood flow (arrows) in the pons (image 1) and sub-cortical gray matter (image 2) and lower flow (dark blue) in the periventricular white matter (arrowheads). (B) The fetal cerebral white matter was segmented into medial and lateral sections, both of which were further segmented into inferior, middle, and superior regions. No differences were found between basal CBF values in medial and lateral white matter. (C) Basal CBF values (mean $\pm$ SEM) for the entire inferior, middle and superior PVWM. No differences in CBF were seen between superior and inferior regions of cerebral white matter, which supported a lack of gradients of CBF during basal or ischemia-reperfusion conditions. Adapted from Riddle A, Luo N, Manese M, et al. Spatial heterogeneity in oligodendrocyte lineage maturation and not cerebral blood flow predicts fetal ovine periventricular white matter injury. *J Neurosci.* 2006;26:3045-55 and McClure M, Riddle A, Manese M, et al. Cerebral blood flow heterogeneity in preterm sheep: lack of physiological support for vascular boundary zones in fetal cerebral white matter. *J Cereb Blood Flow Metab.* 2008;28(5):995-1008 with permission.

Microscopic Necrotic WMI

Diffuse WMI

Focal Necrotic WMI

Figure 2.

Three forms of high field MRI-defined perinatal WMI with corresponding histopathological features that were generated in the 0.65 gestation fetal sheep brain at 1 or 2 weeks after global cerebral ischemia Adapted from Riddle A, Dean J, Buser JR, et al. Histopathological correlates of magnetic resonance imaging-defined chronic perinatal white matter injury. *Ann Neurol.* 2011 Sep;70(3):493-507; with permission. **Upper Panel. Microscopic necrotic WMI.** (A) Representative appearance of a focal hypointense (F-hypo) lesion seen on a T₂w image at 2 weeks after injury. Note the substantial difference in the F-hypo lesion relative to a diffuse gliotic lesion at 2 weeks, which appears more hyperintense (D-hyper). (B) A typical microscopic necrotic lesion defined by a discrete focus of immunohistochemical staining for reactive microglia and macrophages with Iba1 (red and inset) and a paucity of staining for astrocytes with glial fibrillary acidic protein (GFAP; green). Nuclei in the inset are visualized with Hoechst 33342 (blue). Bar in B, 100 μ m. **Middle Panel: Diffuse WMI** (A) Representative appearance and distribution of diffuse hypointense (D-hypo) lesions seen on a T₂w image at 1 week after injury. (B) Diffuse WMI had pronounced astrogliosis defined by immunohistochemical staining of reactive astrocytes with glial fibrillary acidic protein (GFAP; green) and a lesser population of Iba1-labeled microglia/macrophages (red) with a reactive morphology (inset). Nuclei in the inset are visualized with Hoechst 33342 (blue). Bar in B, 100 μ m. **Lower Panel. Focal Necrotic WMI.** (A) Representative appearance from the largest focal hyperintense (F-hyper) lesion seen on a T₂w image at 1 week after injury. These lesions typically localized to subcortical white matter. Note the

substantial difference in the F-hyper lesion relative to the diffuse gliotic lesions, which appears much more hypointense (D-hypo). (B) A typical macroscopic necrotic lesion defined by diffuse dense staining for reactive microglia and macrophages with Iba1 (red and inset) and a paucity of GFAP-labeled astrocytes. Nuclei in the inset are visualized with Hoechst 33342 (blue). Bar in B, 100 μ m.

Figure 3.

Numerous late oligodendrocyte progenitors (preOLs) accumulate in chronic myelin-deficient perinatal white matter lesions. Lesions were generated in response to unilateral hypoxia-ischemia in the postnatal day 3 (P3) rat with the contralateral hemisphere serving as control.⁹⁸ (A) Normal early myelination (O1-antibody; green) in control subcortical white matter (corpus callosum/external capsule) at P10 is seen with low levels of GFAP-labeled astrocytes (red) mostly concentrated over the white matter. (B) Absence of myelin in the contralateral post-ischemic lesion coincided with a diffuse glial scar that stained for GFAP-labeled astrocytes. (C) Distinctly different pathogenetic mechanisms mediate impaired myelination in necrotic lesions (PVL; upper pathway) vs. lesions with diffuse gliosis (diffuse WMI; lower pathway). Hypoxia-ischemia (H-I) is illustrated as one potential trigger for WMI. More severe H-I triggers white matter necrosis (upper pathway) with pan-cellular degeneration that depletes the white matter of glia and axons. Severe necrosis results in cystic PVL, whereas milder necrosis results in microcysts. Milder H-I (lower pathway) selectively triggers early preOL death. PreOLs are rapidly regenerated from a pool of early OL progenitors that are resistant to H-I. Chronic lesions are enriched in reactive glia (astrocytes and microglia/macrophages) that generate inhibitory signals that block preOLs differentiation to mature myelinating OLs. Myelination failure in diffuse WMI thus results from preOL arrest rather than axonal degeneration. The molecular mechanisms that trigger preOL arrest are likely to be multifactorial and related to factors intrinsic and extrinsic to the preOLs. Note that the lower pathway is the dominant one in most contemporary preterm survivors, whereas the minor upper pathway reflects the declining burden of white matter necrosis that has accompanied advances in neonatal intensive care.

Figure 4.

The preterm brain is enriched in immature neurons that do not degenerate in response to ischemia, but are highly susceptible to impaired maturation that manifests as a less mature dendritic arbor with reduced spine density.¹⁶⁵ (A) A typical pyramidal neuron from the preterm cerebral cortex of a control fetal sheep. Note the paucity of processes in contrast to the highly complex dendritic arbor of a pyramidal neuron from a near-term animal (B). (C, D) In response to preterm ischemia, cortical pyramidal neurons display disrupted maturation. Note that the typical control cell (C) is more highly arborized in contrast to the response to transient cerebral ischemia that resulted in a more simplified dendritic arbor (D). The relative complexity of the cells can be appreciated from the overlay of the red concentric Scholl rings, which illustrates that the processes of the dysmature neurons intersect less frequently with the rings. The yellow, white, pink, green and blue lines represent first-, second-, third-, fourth- and fifth-order branches, respectively, from the soma. Note the overall reduction in the size and complexity of the branching pattern in D.