Historically the major focus in neonatal neurology has been on brain injury in premature infants born less than 30 gestational weeks. This focus reflects the urgent need to improve the widely recognized poor neurological outcomes that occur in these infants. The most common underlying substrate of cerebral palsy in these premature infants is periventricular leukomalacia (PVL). Nevertheless, PVL also occurs in near-term (late preterm), as well as term, infants, as documented by neuroimaging and autopsy studies. In both very preterm and late preterm infants, gray matter injury is associated with PVL. In this review, we discuss the cellular pathology of PVL and the developmental parameters in oligodendrocytes and neurons that put the late preterm brain at risk in the broader context of brain development and injury close to term. Further research is needed about the clinical and pathologic aspects of brain injury in general and PVL in particular in late preterm infants to optimize management and prevent adverse neurological outcomes in these infants that, however subtle, may be currently underestimated.

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It has long been recognized that the majority of severely premature born infants have poor long-term neurological outcomes, with significant cognitive, behavioral, and/or motor deficits. In the United States alone, for example, approximately 55,000 live newborns are born very low birthweight (<1500 g birthweight) each year, and nearly 90% survive: approximately 10% of these infants, however, later exhibit cerebral palsy, and about 50% develop cognitive and behavioral deficits. Thus, nearly 5000 cases of cerebral palsy and 10,000 to 20,000 cases of serious learning disabilities result each year from early premature deliveries. The common perception, on the other hand, is that “near-term” or “late preterm” infants (variably defined as 34-37 or 35-37 gestational weeks at birth) are spared substantial brain injury, and are neurologically “normal” or “almost normal” in the neonatal period and beyond. (According to the general agreement of the conference participants, we will hereafter use the word “late preterm” rather than “near-term”.) Periventricular leukomalacia (PVL) is the most common substrate of neurological disabilities in the very prematurely born infant. This disease affects the immature white matter of the cerebral hemispheres, and peaks at 24 to 32 gestational weeks. Nevertheless, it is not restricted to the very prematurely born infant, but also occurs in the late preterm (and term) infant. Below, we review the problem of PVL in the late preterm infant, and the maturational factors of the cerebral white matter that put the late preterm brain at risk. We focus on the similarities and differences to those in the very premature brain. We begin with a general consideration of brain development and its relationship to injury in the late preterm infant.

Brain Development and Injury in the Late Preterm Infant

The last half of gestation is a critical period in the growth and development of the human brain (Figs. 1–3). A “critical period” is defined as a time-sensitive, irreversible “decision point” in the development of a neural structure or system in which deprivation of the normal environment interrupts the maturational trajectory of the structure/system. As a result, there are profound consequences for later brain maturation and behavior that cannot be corrected. Critical brain periods are characterized by rapid and/or dramatic changes in one or
more molecular, neurochemical, and/or structural parameters, such as the major “burst” of active brain growth in the last half of human gestation (Fig. 1). A “vulnerable” period is the developmental window in which the neural structure/system is susceptible to deprivation of normal environmental influences, or to adverse environmental factors, eg, cerebral ischemia/reperfusion in PVL (see below). At 20 gestational weeks, the brain weighs 10% of the brain at term; between 20 and 40 weeks, the overall brain weight increases 90% in a relatively linear fashion (Fig. 1). Strikingly, the late preterm brain at 34 weeks weighs only 65% of the term brain, with a 35% increase in growth still needed to reach term weight. At 20 to 24 gestational weeks, neuronal proliferation and migration to the cerebral cortex are considered to be complete. At 20 weeks, however, the brain is smooth, with formation only of the Sylvian fissure; in contrast, by 40 weeks, all of the primary, secondary, and tertiary gyri and sulci have formed (Fig. 2). Gyral and sulcal development is still incomplete late preterm. Volumetric magnetic resonance determinations during late gestation also demonstrate a dramatic increase in the volume of the total gray matter from 29 to 41 gestational weeks, with a linear increase of approximately 1.4% or 15 mL in absolute volume per week. This pronounced increase in total gray matter volume primarily reflects a four-fold increase in the volume of the cerebral cortex. Of note, the cortical volume in the very preterm infant at 28 gestational weeks is 13% of term volume. Nevertheless, the cortical volume in the late preterm infant is only 53% of the term volume, with approximately half the volume to be obtained in the last 6 weeks before term, ie, 40 gestational weeks. In addition, minimal “myelinated” white matter is present in the very preterm infant (around 29 weeks), but increases dramatically in volume as term is approached, with a five-fold increase between 35 and 41 weeks. Underlying the overall growth of the brain in the last half of gestation are major organizational events in the development of neurons and glia at the cellular and molecular level (Fig. 2). Synaptogenesis and dendritic arborization are occurring, and are likewise incomplete in the late preterm brain compared with the term brain, but not to the degree seen in the very premature brain (Fig. 2). Of note, these developmental changes continue to occur beyond the neonatal period (Fig. 2). In addition, axons to and from the cerebral cortex undergo elongation throughout the second half of gestation and into infancy, as demonstrated by us with the marker Growth-Associated Protein-43 (GAP-43) (Fig. 3). GAP-43 is a major phosphoprotein of neuronal growth cones in the developing and brain, and is involved in axonal outgrowth among other functions. Thus, axonal elongation in the cerebral white matter is ongoing in both the very premature and late preterm brain, and even in the term brain, and thus is potentially vulnerable to injury. Of major relevance to the pathogenesis of PVL, the maturation of oligodendrocytes and myelination are likewise incomplete in the term brain, as discussed below.

In addition to developmental changes within the cerebral cortex itself, there are concomitant changes in its connectivity. Subplate neurons are the earliest differentiated neurons originating from the ventricular zone in the first trimester of intrauterine life. They underlie the cortical plate and are actively involved in the path finding of axons from and to the thalamus. Subplate neurons have been implicated in the refinement of thalamocortical innervation via activity-dependent mechanisms whereby electrical activity (action potential) patterns influence the in-growing thalamocortical axons. Ablation of subplate neurons at a specific time-point in feline gestation (E43 in a gestation of 65 days) leads to the absence of Layer IV of the cerebral cortex as in-growing thalamocortical axons fail to reach it. Depletion of subplate neurons in...
early postnatal life in animal models also has age-specific detrimental effects, eg, prevention of thalamic axons into ocular dominance columns in Layer IV of the visual cortex, and impairment in synaptic transmission of visually-driven ocular dominance columns in Layer IV of the visual cortex, detrimental effects, eg, prevention of thalamic axons into early postnatal life in animal models also has age-specific (Top) GAP-43 expression levels in the developing white matter were examined by western blot analysis. (Bottom) These levels were grouped according to ages reflecting epochs of human brain development and plotted as a percent of adult human standard. GAP-43 expression in the white matter remains high, relative to the adult standard, suggesting that axons are still growing from 19 to 64 gestational weeks. Thus, hypoxic–ischemic damage to the subplate neurons—completely formed at this age, analogous to the preterm brain—may be adversely affected by surrounding white matter injury in PVL, may play a role in altered thalamocortical axonal development and cortical innervation in this disorder, and thereby may contribute to the cognitive deficits in long-term survivors. We emphasize that subplate neurons are still prominent in the late preterm brain, and that axonal interconnections between the thalamus and cerebral cortex, as assessed by the high level of GAP-43 in the cerebral white matter, are likely not completely formed at this age, analogous to the preterm brain. Thus, hypoxic–ischemic damage to the subplate neurons—yet to be demonstrated in human PVL—may potentially contribute to cognitive deficits in late preterm, as well as preterm, infants.

Recognition that the brain reaches only 65% of term weight by 34 weeks underscores the immaturity of the late preterm brain and its potential vulnerability to multiple insults that interfere with basic mechanisms of neuronal and glial maturation during this time-frame. Brain insults in the late preterm brain can also alter the trajectory of specific programs in neuronal and glial development, as they do in the very premature brain, thereby contributing to the neurological disabilities of the survivors. In PVL, for example, necrosis in the cerebral white matter causes axonal injury and deafferentiation of the overlying cerebral cortex, with subtle secondary abnormalities in neuronal differentiation and process outgrowth.

Figure 3 (Top) GAP-43 expression levels in the developing white matter were examined by western blot analysis. (Bottom) These levels were grouped according to ages reflecting epochs of human brain development and plotted as a percent of adult human standard. GAP-43 expression in the white matter remains high, relative to the adult standard, suggesting that axons are still growing from 19 to 64 gestational weeks.13

Definition of PVL

PVL is defined morphologically by two histopathologic components: (1) a “focal”, necrotic component in the periventricular region of the cerebral white matter; and (2) a “diffuse” component characterized by reactive gliosis in the surrounding white matter. Each component has different histopathologic outcomes: (1) the necrotic foci, involving all tissue components, evolve into cysts that typically collapse and eventually form focal scars; and (2) the diffuse lesion appears to involve preferential injury to premyelinating oligodendrocytes (pre-Ols) in the surrounding central white matter, leading to a global delay in myelination. Although the focal necrotic lesions correlate well with cerebral palsy (motor deficits), it has been suggested that the diffuse white matter lesion correlates with cognitive and behavioral abnormalities observed with PVL. These latter disorders may also be related to concomitant injury to the gray matter, eg, cerebral cortex, thalamus, and basal ganglia. Indeed, there is increasing recognition that PVL is associated with gray matter injury. Neuroimaging studies have demonstrated reduced cortical volumes in premature infants at term equivalent. Quantitative MRI studies in premature infants also demonstrate reduced thalamic volumes with or without associated white matter damage. MRI analysis in 119 consecutively born premature infants demonstrated that a low index of mental development (Bayley scales) at 1 postnatal year correlated most strongly with decreased deep gray matter (thalamus and basal ganglia combined) volume at term equivalent age.

Our studies of the neuropathology of prematurity indicate that neuronal loss and gliosis, albeit subtle, occurs in multiple gray matter sites, particularly the thalamus and, to a lesser extent, the cerebral cortex. We found that gliosis occurs in the thalamus in 33% of premature infants (n = 41), and neuronal loss in this site occurs in 15%. Damage to the thalamus raises the possibility of additional abnormal functioning in the cerebrocortical–subplate–thalamic “unit” comprised of thalamic neurons that project to the cerebral cortex and the subplate neurons that are critical in the targeting of axons between the thalamus and cortex during cortical development (see above). The thalamus plays a key role in cognition, not only via certain nuclei relaying sensory information from the external world, but also via associative nuclei positioned as critical components of distributed neuronal networks. The idea that an individual thalamic nucleus is a...
critical component of a distributed neural network that subserves a particular cognitive ability is supported by clinical observations that lesions in a specific thalamic nucleus can result in functional impairments similar to damage in the cortical region itself. 33 Although neuronal loss and gliosis in gray matter sites are nonspecific findings, their topography and histopathologic features in the premature brain are consistent with hypoxia-ischemia. The finding of gliosis and neuronal loss indicates subacute/chronic injury, and likely is the substrate, at least in part, of reduced volumes of gray matter structures in survivors of prematurity. We postulate that the combined gray and white matter damage in the premature infant is due to hypoxia-ischemia, infection, and/or as yet undefined factors in a vulnerable period in the development of OLs and neurons, and that the combined lesions in the susceptible white and gray matter sites reflect interactions between oxidative-, nitrate-, glutamate-, and cytokine-toxicity.

Incidence of PVL in Late Preterm and Term Infants

The incidence of PVL in preterm and term infants is not completely known. In our study of the neuropathology of congenital heart disease in infants dying after cardiopulmonary bypass surgery (n=38), the mean gestational age was 39.3 ± 1.4 weeks (term), with a mean birth weight of 3126 ± 53 g and median postnatal age of 21 days (range: 1-398 days). 7 In this cohort, the overall incidence of PVL was 61%, with this lesion representing the most common neuropathologic finding. 7 Of note, 32% of the PVL lesions were acute, ie, characterized by coagulative necrosis without inflammatory cell (astrocytic and microglial) reaction, a lesion considered to be 24 to 48 hours in duration and therefore histopathologic evidence of origin in the term time-frame. PVL has also been reported in living preterm and term infants with congenital heart disease following cardiac surgery with the use of magnetic resonance imaging. 8,9 Nevertheless, the overall incidence of PVL and long term outcome in living late preterm infants are not known. Of relevance to the clinical outcome of brain injury in late preterm and term infants, a recent report from the western Swedish study of the prevalence and origin of cerebral palsy indicated that there was a decreasing overall trend between 1995 and 1998 in both children born preterm and at term. 34 Nevertheless, there was an increase in dyskinetic cerebral palsy in children born at term, defined in this study as birth after 36 gestational weeks. In this group of children with dyskinetic cerebral palsy, 71% of the cases had hypoxic-ischemic encephalopathy in the perinatal period. 34

Pathogenesis of PVL

The major causes of PVL are considered to be: (1) cerebral ischemia/reperfusion in the critically ill premature infant with cerebral vascular immaturity and arterial end-zones in the periventricular region, coupled with the propensity for impaired vascular autoregulation; and (2) bacterial infection in the mother and/or fetus that triggers a cytokine response in the fetal brain, either by entry of maternal and/or fetal cytokines across the fetal blood–brain barrier that then directly injure pre-OLs, or by entry of bacterial components, eg, endotoxin, into the fetal brain and stimulation of the local cytokine production by microglia and/or astrocytes. 5 These two causes, ie, cerebral ischemia and infection, likely act synergistically to produce cerebral white matter damage, such that the infant previously exposed to intrauterine infection may be especially vulnerable to pre-OL injury by ischemic insults that are not sufficient to cause injury alone. Of note, late preterm infants, like very premature infants, can require management in intensive care nurseries, and have a higher incidence than term infants of respiratory distress syndrome, apnea, and infection—all defined risk factors for PVL. 5

In our laboratory, the over-riding hypothesis concerning the pathogenesis of PVL is that pre-OLs are the targeted cell population due to preferential vulnerability to free radical, glutamate, and cytokine toxicity in the developmentally immature white matter. The genesis of the idea that pre-OLs are targeted in PVL is the demonstration by neuroimaging studies of reduced white matter volume, ventriculomegaly, and impaired myelination. 33 The finding of a diffuse component of PVL cases. 37 Taken together, these data suggest the possibility that injury to pre-OLs occurs in the diffuse component of severely affected cases of PVL.

Neuropathologic studies by us and others suggest a major role for reactive nitrogen species (RNS) and/or reactive oxygen species (ROS) in pre-OL cytoxicity. 33,37,38 There is also marked activation of microglia in the diffuse component of PVL, suggesting the possibility that activated microglia are key contributors of ROS and RNS that injure and potentially kill pre-OLs. 33 In PVL, we propose that the underlying basis of ROS and RNS generation includes cerebral ischemia/reperfusion. Of direct relevance to PVL, data from perinatal rat models and rodent cell culture indicate pre-OLs are preferentially vulnerable to ROS in comparison to MBP+ OLs. 38,39 The finding of a diffuse activation of microglia further suggests the possibility that ischemic and infectious insults, the latter potentially through the activation of toll-like receptors by bacterial products, converge to activate microglia in the inflammatory response, and thereby augment free radical production and exacerbate white matter damage that may have been minimal with either insult alone. 5
Maturational Factors of the Cerebral White Matter in the Late Preterm Infant

A major focus of our laboratory has been to determine the developmental factors at the cellular level that underlie the vulnerability of the fetal white matter to injury in PVL. The first step we took in our laboratory toward understanding these factors was to delineate the developmental profile of OL cell lineage during the peak period of risk (Fig. 4). In 26 control autopsy brains, OL lineage progression was defined by us in the parietal white matter at the level of the atrium, a known region of predilection for PVL. Three successive OL stages, ie, late OL progenitor, immature OL, and mature OL, were characterized between 18 and 41 weeks with anti-NG2 proteoglycan, O4, O1, and anti-MBP antibodies. We found that NG2+O4+O1- late progenitors were the predominate stage throughout the last half of gestation. Between 18 and 27 weeks, O4+O1+ immature OLs were a minor population (9.9 ± 2.1% of total OLs, n = 9) (Fig. 4). Before 28 and 41 weeks, an increase in immature OLs to 30.9 ± 2.1% of OLs (n = 9) was accompanied by a progressive increase in MBP+ myelin sheaths that were initially restricted to the periventricular white matter, and first detected by immunocytochemistry around 30 weeks. Thus, the developmental window of the peak occurrence of PVL precedes the onset of active myelin sheath synthesis, ie, the time-frame when the late progenitors (NG2+O4+O1-) predominate in the cerebral white matter. The predominance of one major population of pre-OLs during the peak period of risk for PVL suggests that this OL stage is the potential target for injury in the diffuse component of PVL. Moreover, the decline in the incidence of PVL beyond late gestation coincides with the first appearance of MBP+ OLs, followed by active myelin sheath synthesis in early infancy. These data suggest that differentiation of pre-OLs to mature OLs underlies the increased resistance of the mature cerebral white matter to ischemia/reperfusion. Yet, in considering the occurrence of PVL in the late preterm brain, it should be emphasized that pre-OLs still dominate the late preterm cerebral white matter and active myelin sheath synthesis with MBP production has yet to occur (Fig. 4). Indeed, active wrapping of axons by the myelin sheath, does not occur in the cerebral white matter until 3 to 5 postnatal months. Understanding the precise timing of the sequences of myelination in the developing cerebral white matter helps to explain the vulnerability of the late preterm, as well as very premature brain to PVL. Recognition of the different stages of pre-OL differentiation and active myelin sheath synthesis across the last half of gestation, with more advancement in the late preterm than very premature brain, also helps explain the increased vulnerability of the very premature compared with the late preterm brain.

In our laboratory, we tested the hypothesis that the vulnerability of pre-OLs to free radical attack results, at least in part, from a relative developmental lack of the antioxidant enzymes (AOEs) in the cerebral white matter during the peak period for PVL in the very premature infant. We analyzed the developmental profile of selected AOE isozymes (catalase, copper/zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, glutathione peroxidase) in the cerebral white matter of the human brain from midgestation through the perinatal period (Fig. 5). During the period of greatest PVL risk (24-32 gestational weeks) and through term, including late preterm, we found that expression of both SODs (necessary for conversion of superoxide to hydrogen peroxide), as determined by western blot analysis, significantly lagged behind that of catalase and GPx (necessary for breakdown of hydrogen peroxide), which, in contrast, superseded adults levels by 30 weeks (Fig. 5). Animal studies indicate that the balance of all the AOE isozymes in the brain must be optimized for proper protection from oxygen free radicals in hypoxic-ischemic injury, with the caveat that this balance may differ between the mature and immature brain. Thus, the developmental lag in the expression of the SODs relative to catalase and GPx represents, in our opinion, a likely crucial “rate-limiting” factor in the protection of pre-OLs from superoxides.

Additional factors that likely contribute to the vulnerability of the fetal cerebral white matter to ischemic/inflammatory injury that we and others have found in autopsy studies of the brains of premature and term infants include: (1) transient over-expression of AMPA receptors on pre-OLs; (2) transient over-expression of the EAAT2 glutamate transporter, with documented expression in pre-OLs; (3) transient over-expression of activated microglial density, and (4) immaturity of the vasculature of the deep and periventricular white matter. Clearly there is no single factor, but rather, a convergence of multiple factors that accounts for the maturational vulnerability of the fetal white matter to hypoxic-ischemic injury.
In summary, different events in cerebral white and gray matter development, e.g., OL differentiation, myelination, antioxidant enzyme production, gyration, synaptogenesis, and axonal elongation, follow different sequences of maturation with different tempos in the human brain over the last half of gestation, thereby defining differential periods of vulnerability to injury. The maturation of the cerebral white matter is incomplete in the late preterm brain, and thus it is vulnerable to PVL, as established by the documentation of PVL in the late preterm (and term) brain in neuroimaging and autopsy studies. Yet, the peak of PVL occurs in the very premature infant, and likely reflects, at least in part, a greater degree of immaturity of the various factors that put the white matter at risk. Indeed, the increasing advancement in the developmental confluence of cellular and molecular factors in the cerebral white matter helps explain the “hierarchy of vulnerability” to PVL, with the greatest vulnerability in the very premature brain, followed by the late preterm and term brain, and the greatest resistance in the heavily myelinated brain in infancy and beyond. Nevertheless, developmental programs may not all be “linear” in their trajectory, and thus, the development of different parameters may “peak” at different times, and not just simultaneously at one particular age. Given the substantial overall maturation still to be undergone by the late preterm brain, injury at this time-point also has the potential to adversely affect the trajectory of different developmental programs, with lasting consequences for neurological disabilities in survivors.

More research is needed about the neurochemical, cellular, and molecular development of the human brain at the end of gestation. We need to define in particular the sequences of neuronal and glial maturation by week-to-week dates from 34 to 40 gestational weeks. Given the applicability...
of modern tools in neuroscience to the study of the human brain at autopsy, this goal is attainable, and its fulfillment will greatly aid in our understanding of the factors that put the late preterm human brain at risk for multiple types of insults. More information is also needed about the incidence and long-term neurological outcome of PVL and gray matter damage in late preterm infants, as distinct from very premature and term infants. Finally, research is needed about the long-term neurological outcome of healthy infants born at 34, 35, and 36 gestational weeks compared with infants born at 39 to 40 weeks. The under-recognized degree of immaturity of the late preterm brain relative to the term-brain begs the question: does the apparently healthy late preterm infant demonstrate compromised cognitive, behavioral, and/or motor skills, however subtle, at school age and beyond? Underlying this question is concern about potential (unknown) detrimental effects on brain development of extraterrestrial compared with intrauterine life during the last 3 weeks of gestation, ie, from 34 to 37 gestational weeks, when the brain is still undergoing rapid and dramatic development. The answer to this question is crucial to our ultimate understanding of the impact of birth close to, but not at, our current definition of term.

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