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The Encephalopathy of Prematurity: One Pediatric Neuropathologist's Perspective

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Abstract

A major challenge in understanding brain injury in the premature brain is the establishment of the precise human neuropathology at the cellular and molecular levels, as such knowledge is the foundation upon which the elucidation of the cause(s), scientific experimentation, and therapies in the field is by necessity based. In this essay, I provide my perspective as a pediatric neuropathologist upon pathologic studies in the developing human brain itself, including a review of past, present, and future aspects. My focus is upon the path that has brought us to the current recognition that preterm brain injury is a complex of white and gray matter damage that results in the modification of key developmental pathways during a critical period, which in turn defines the adverse clinical outcomes as important as the primary insult itself. The evolution of this recognition, as well as the introduction of the term “encephalopathy of prematurity” for the complex of gray and white matter damage because of acquired and developmental mechanisms, is discussed. Our enhanced understanding of the fundamental neuropathology of the human preterm brain should bring us closer to more effective therapy as the need to prevent and treat injury to developing oligodendrocytes and neurons in combination is appreciated.

My charge as a pediatric neuropathologist long interested in brain injury in the premature infant is to provide a perspective upon pathologic studies in the developing human brain itself. At the request of Dr Adre DuPlessis, the editor of this series, I review here past, present, and future aspects of the neuropathology of the human premature brain in the context of its specific “challenges, advances, detours, and quantum leaps” (his words). This perspective, on the basis of personal insights gained over a two-decade career in the field, is hoped to benefit the readers. I am particularly fortunate in that my own research has been inspired and guided by 4 giants in the field who share in common a love of children, a fascination with the beauty of the developing nervous system, a passion to further our understanding of pediatric brain injury, and an unwavering commitment to mentor and with kindness: Drs Joseph J. Volpe, Miguel Marine-Padilla, Dawna Armstrong, and Floyd Gilles. Below I have highlighted the lessons they have taught me individually, as well as to the field as a whole.

Significantly, the major challenge in understanding brain injury in the premature brain, in my opinion, is the establishment of the precise human neuropathology at the cellular and molecular levels, as such knowledge is the foundation upon which the elucidation of the cause(s), scientific experimentation, and therapies is by necessity based. To me, the major “quantum leap” in our understanding, in the last few years, has been the recognition that preterm brain injury is a complex of white and gray matter damage that results in the modification of key developmental pathways during a critical period, which in turn defines

the adverse clinical outcomes as importantly as the primary insult itself. The concept of the devastating intersection between development and disease is not unique to any one investigator in perinatal brain injury; indeed, this intersection is the sine qua non of all pediatric disorders. Nevertheless, this concept as applied to premature brain injury is brilliantly synthesized and elegantly articulated by the world-renown “thinker” in neonatal neurology, Dr Volpe,¹ in his already classic review published but a few months ago. This quantum leap forward in our conceptualization of premature brain injury is best captured in the label “encephalopathy of prematurity” that was coined by Dr Volpe² to emphasize the combined acquired tissue loss and altered developmental trajectories in combined white and gray matter sites in the developing human brain. Moreover, the constellation of cognitive, motor, and emotional impairment in long-term survivors of prematurity reflects the particular patterns of white and gray matter damage in combination with arrested developmental programs—patterns that depend upon the severity, timing, and chronicity of the injury, as well as an individual confounding factors.^{1,3} The neuropathology of the encephalopathy of prematurity, as well as its causes and pathogenesis, has been reviewed by Dr Volpe¹ and by Dr Volpe in collaboration with me;³ it is also summarized by Dr Volpe elsewhere in this series. In the following perspective, I focus rather upon advances, detours, roadblocks, and future directions towards understanding this entity in its entirety and ultimately abolishing it.

Advances

The Concept of Preferential White Matter Vulnerability in the Fetal Brain

A major advance in our understanding of the preterm brain injury was the early recognition (formally beginning in the 19th century) that the developing cerebral white matter is particularly vulnerable to insult in the human fetus. Historical reviews mention the key roles of Little, Virchow, Parrot, and Schwartz in focusing attention upon brain damage in the premature infant and the predominance of white matter pathology in the perinatal period.^{4,5} Parrot’s studies at the end of the 19th century in particular emphasized yellow or chalky plaques, 5 to 6 mm in diameter, in the periventricular white matter and sparing of the gray matter.⁴ In their historical review, Banker and Larroche⁵ point out that Parrot ascribed a “particular vulnerability to the immature white matter, attributable to the fact that the affected zones are farthest from the blood supply”, because the lesions were centrally placed in the white matter and the “cerebral cortex and basal ganglia were spared”, and that Parrot further suggested the causes of the white matter damage were “circulatory and nutritional disturbances” occurring in an “actively developing brain”.⁵ From the outset, controversies abounded, including about the role of anoxia vs infection in its pathogenesis, the nature (normal or pathologic?) of diffuse fatty change or “metamorphosis” of glial cells, and the significance of certain periventricular cells that were variously interpreted as inflammatory or periventricular germinal rests.^{4,5} In 1962, the comprehensive study of “periventricular leukomalacia” (PVL) in 51 infants dying at the Children’s Hospital Boston was published by Banker and Larroche.⁵ In this landmark study, (1) they reported that the clinical and pathologic features were correlated, leading to the conclusion that PVL is the basis of many instances of mental retardation and spasticity; (2) the name “periventricular leukomalacia” for this type of perinatal white matter damage was recommended; (3) the temporal sequences of the histopathology of PVL were delineated; (4) a consistent topography of the necrotic lesions in the periventricular white matter was described (Fig 1); (5) axonal damage (“retraction clubs”) in the focally necrotic lesions in the periventricular white matter was highlighted; and (6) a key causative role for ischemia relative to border zones of the vascular supply to the deep white matter and inadequate blood flow complicating systemic hypotension was emphasized.⁵ Of note, astrogliosis in the white matter surrounding or distant from the necrotic foci was not described, nor was diffuse microglial activation.

The next conceptual advance in our understanding of the neuropathology of the preterm brain, in my opinion, was the recognition of diffuse astrogliosis in association with the necrotic foci by Dr Gilles and colleagues^{6,7} in the 1970s and 1980s, leading them to introduce the terms “perinatal telencephalic leukoencephalopathy” (PTL) or “acquired perinatal leukoencephalopathy”. Dr Gilles’ observations were based in large part upon his monumental analysis of almost 200 fetal and neonatal brains accrued under the auspice of the national collaborative perinatal project (NCPP) sponsored by the National Institute of Neurological and Communicative Disorders^{6,7} and during his time at Children’s Hospital Boston. Dr Gilles considered 4 histologic features evidence of perinatal white matter damage under the rubric of PTL, ie, necrotic foci (PVL), hypertrophic astrocytes (see below), perivascular amphophilic globules, and cells described as “acutely damaged glia”.⁶ In a sample of newborns enrolled in the NCPP, necrotic foci were found in 10%, hypertrophic astrocytes in 42%, amphophilic globules in 29%, and acutely damaged glia in 16%.⁶ Of note, Gilles postulated that the acutely damaged glia are comprised of “glial cells destined to become oligodendroglia and to lay down and maintain myelin,” and that they are “either destroyed or transformed by ... insults”.⁶ He further suggested that “gliosis represents a transformation of pluripotent glia into glia-fibril-producing cells, rather than into myelin-producing and -supporting oligodendroglia”.⁶ Remarkably, these seminal observations by Dr Gilles were made before the introduction of immunocytochemical markers for astrocytes (glial fibrillary acidic protein [GFAP]) and pre-myelinating OLs (pre-OLs) and/or mature OLs [O4, O1, OLIG2, and myelin basic protein (MBP)]. Dr Gilles’ other contributions to our understanding of preterm brain injury include (1) the application of large population (epidemiologic) analysis to determine the risk factors for the combined or separate features of PTL,^{6,7} (2) the recognition based upon such risk factor analysis of the potential role of endotoxin in the pathogenesis of perinatal white matter injury,⁶ and (3) the emphasis upon the testing of causative hypotheses generated from human pathology in developmental animal models, as exemplified by his analysis of endotoxin toxicity to white matter in kittens.⁸

As a neuropathology fellow in training with Dr Gilles, I came to appreciate the full extent of astrogliosis in preterm brain injury in the daily sign-out of autopsy cases with him. These teachings were supplemented by the neuroimaging observations emphasized by my other mentor, Dr Volpe,⁹ in which periventricular necrotic lesions are accompanied by diffuse signal intensity in the surrounding white matter. The combined neuroimaging and autopsy teachings convinced me of the validity of the formulation that white matter damage in the preterm infant is comprised of 2 key components, which are defined as (1) the “focal” component of periventricular necrosis, and (2) the “diffuse” component of gliosis in the surrounding white matter.^{3,10} The identification of diffuse astrogliosis in the white matter in preterm brain injury (termed PVL) is important because it suggests a penumbra of diffuse (reversible) injury to pre-OLs in the surrounding white matter that is more amenable to intervention, repair, and recovery than the global injury to tissue in the core (irreversible) infarcts of the periventricular regions³—an idea for testing in animal models.

Advances in our understanding of preterm brain injury reflect in large part the application of immunocytochemistry, computer-based quantitation and graphics, western blotting, in situ hybridization, and other modern methods directly to the postmortem human brain. Under the visionary leadership of Dr Volpe in a NINDS-funded program beginning in 2000, our group in neuropathology at Children’s Hospital Boston began concentrated efforts to define systematically the cellular basis of preterm injury directly in the human brain at autopsy.^{11–22} The application of immunomarkers for OL cell lineage in conjunction with computer-based quantitation by our group revealed that: (1) the number and density of pre-OLs are not reduced in the subacute and chronic stages of the diffuse component of PVL; (2) rather, they are increased immediately adjacent to the periventricular necrotic foci, suggesting the

possibility of attempted regeneration and repair; and (3) dysfunctional myelin synthesis in preserved pre-OLs likely accounts in large part for subsequent impairment in myelination (Fig 2).¹² We observed, for instance, abortive attempts at myelin formation around the necrotic foci, as well as intense MPB immunostaining in OL perikarya, suggestive of a “hang-up” in MPB trafficking from its site of synthesis in the cytoplasm to its deposition in processes involved in axonal ensheathment (Fig 2). During this same period, Dr Stephen Back et al²³ reported reduced numbers of pre-OLs in the white matter in PVL cases with “acute” lesions in a smaller sample size, raising the possibility that pre-OLs may undergo cell death immediately after injury but, in conjunction with our findings, be “replaced” in the subacute and chronic stages. This possibility is supported by an earlier report by our group of striking (qualitative) pre-OL depletion in 2 PVL cases compared with controls.²¹ Of note, Iida et al²⁴ reported previously a loss of OLs with the non-specific marker ferritin in PVL cases in which there was associated myelin-staining in tissue sections. Taken together, these human data suggest that the number of pre-OLs on balance may depend upon the stage at which the histopathology is captured at the time of death, and that the “snapshot” is taken relative to the timing of the acute insult, progression of tissue damage, and extent and type of OL regeneration and repair. The application by us of CD68 antibody as an immunomarker for the microglial/macrophagocytic lineage revealed diffuse activation of the microglia in the diffuse component of PVL,²¹ of major importance given that a role for microglia in the pathogenesis of PVL was previously not described (see above), as well as the known roles of microglia in the inflammatory response and cytokine and free radical toxicity to pre-OLs.^{3,25–27}

The application by us of the fraction antibody as an immunomarker for axonal damage revealed a striking degree of axonal injury in the diffuse component of PVL, of major importance in potentially redefining our concept of preterm white matter injury as a disorder not exclusively of developing OLs but also of developing axons, and raising the possibility that this white matter injury even reflects a primary axonopathy^{1,3,13} (Fig 3). The application by us of immunomarkers of oxidative and nitrative stress indicated extensive free radical injury in the diffuse component of PVL, including particularly to pre-OLs, which demonstrate coexpression of these markers by double-labeling techniques.²¹ These observations concerning free radical injury in PVL was supported by those of Dr Back et al²³ in PVL cases compared with controls with tissue measurement of $F_{(2)}$ -isoprostane.

The application of cytokine immunomarkers by us¹⁶ and Dr Kadhim et al²⁹ indicated the marked expression of interleukins, tumor necrosis factor- α , interferon- γ , and other cytokines in astrocytes and macrophages in both the diffuse and focal components of PVL, suggesting an important role for them in the pathogenesis of the white matter injury, which must be accounted for in the development of experimental models and that may prove useful in discovery of therapeutic agents. Moreover, the demonstration of receptors for interferon- γ on pre-OLs indicated that the vulnerability of these cells to cytokine toxicity reflects at least in part receptor-mediated interactions.^{16,30} The application of immunomarkers for the glutamate transporter GLT1 indicated its expression in the reactive astrocytes and macrophages of the inflammatory response of PVL, suggesting that these cells play, as yet to be defined, role in preventing excitotoxic damage to pre-OLs.¹⁷ Finally, the application of immunomarkers for stem and progenitor cells by us¹⁰ and others³¹ is opening major new avenues of research by indicating that the cerebral white matter in human premature infant has the innate capacity for regeneration and repair, and that therapeutic strategies to augment this capacity may prove immensely effective in the prevention of long-term neurological disabilities. Of note, our autopsy analysis of brain injury in the early preterm period compared with the late preterm period reveals that the late preterm infant is at risk for the same types of damage that occurs in early preterm infants, ie, the encephalopathy of prematurity.^{18,19} Moreover, it suggests that the underlying

neuropathologic substrate of the subtle cognitive and emotional problems increasingly recognized in the late preterm infant³² are likely the result of milder degrees of injury than the severe injury that results in major clinical problems in low birth weight infants.

In addition to pathologic insights, the application of modern techniques to the human fetal brain at autopsy has revealed multiple developmental factors that contribute to the biological susceptibility to PVL. Of particular interest are the factors that increase vulnerability to cerebral ischemia in the setting of the sick premature infant with pulmonary immaturity, respiratory distress syndrome, and impaired cerebral vascular autoregulation.^{1,3} Work by our group indicates that these factors in the white matter in the last half of gestation, ie, the peak time frame of PVL, include (1) the predominance of pre-OLs in the white matter at the peak age for PVL, which are known to be especially vulnerable to excitotoxic and free radical injury compared with mature OLs,^{33,34} (2) the developmental delay in the maturation of the superoxide dismutases,³⁵ (3) the transient expression of the glutamate transporter GLT1 in the cerebral white matter,³⁶ (4) transient expression of AMPA receptors, with the lack of the relative expression of GluR2,³⁷ (5) the transient elevation in the density of ameboid microglia,³⁸ and (6) immaturity of axonal projections and cellular structure (neurofilaments).²⁸ The work of Dr Takashima and Tanaka⁴⁰ also indicates the morphologic immaturity of the arterial vasculature supplying white matter over this same period, thereby increasing the white matter's vulnerability to ischemia, which complicates deleterious hemodynamic changes in the sick premature infant.³⁹ Thus, the basis of the vulnerability of the fetal white matter to ischemic injury alone is complex and involves multiple factors that collide in time and space to form the "perfect storm" of susceptibility.

Morphological Evidence for the Interaction Between Injury and Development in the Encephalopathy of Prematurity

Perhaps the best illustration of this principle is the demonstration by Dr Marin-Padilla⁴¹ of the consequences of white matter damage upon the subsequent development of the cerebral cortex (Fig 4). In his landmark Golgi study of the cerebral cortex in long-term survivors of severe white matter necrosis, Dr Marin-Padilla⁴¹ showed secondary neuronal abnormalities, including in Layer V (Fig 4). He found that the developing cortex was deprived of afferent terminals due to their severance from the cortex by the extensive white matter necrosis.⁴¹ Moreover, some of the cortical neurons failed to reach their subcortical targets because their axons also were destroyed by the white matter lesion.⁴¹ Of major interest was his stunning observation that the axotomized pyramidal cells transformed from long-projecting into local-circuit neurons⁴¹ (Fig 4). On the basis of the observations by Golgi, Marine-Padilla⁴¹ proposed that the neurological sequelae of perinatal white matter lesions are a direct consequence of postinjury, gray matter "transformations". Dr Marin-Padilla personally taught me that there is no substitute for careful observation with the microscope in which the mind continuously works to correlate structure with function in 3 dimensions as the eye sees two-dimensional cells on the slides in wonderment.

Detours

The major detour, in my opinion, in our understanding of the neuropathology of the preterm brain was the historical focus upon white matter injury to the almost total exclusion of gray matter injury. Indeed, the conventional teaching became that gray matter structures, particularly the cerebral cortex, are spared in preterm brain injury and are developmentally resistant to hypoxic-ischemic damage in particular in the fetal period. Although gray matter injury in the preterm brain was indeed recognized,^{3-5,10,42} it was downplayed, and the conventional wisdom emerged, as evidenced in 3 representative quotations:

1. "The human fetal brain seems to be extremely vulnerable to injury and the patterns of injury are distinct from those seen in adults and older children. In particular, the cerebral white matter is unusually vulnerable to injury whereas the gray matter is less frequently affected".⁴²
2. "Hypoxic-ischemic injury during the prenatal period tends to lead to white matter injury and subsequent loss in gray matter during maturation. On the other hand, hypoxia-ischemia in the term infant, ie, neonatal or postnatal exposure, leads preferentially to damage in the deep gray matter and parasagittal regions of the CNS".⁴³
3. "Though these differences are not absolute, the preterm brain exhibits a higher degree of susceptibility of the white matter whereas the term brain exhibits primarily gray matter injury".⁴⁴

The use of modern neuroimaging to study preterm brain injury and its consequences was the crucial event that refocused, in my opinion, the neuropathologist's attention upon the gray matter. Volumetric MRI studies in living infants over a period have reproducibly demonstrated deficits in multiple cortical regions, thalamus, basal ganglia, hippocampus, and cerebellum at term equivalent and into childhood and adolescence.^{1,45-50} Moreover, these studies have shown that these neuroimaging deficits correlate with multiple cognitive, memory, or emotional problems upon follow-up evaluation.^{1,46,50} Cognizant of the overwhelming evidence for widespread (and indeed profound) gray matter injury in the preterm brain by neuroimaging, our group undertook a "re-look" at the entire spectrum of the histopathology of prematurity through the systematic survey of all gray and white matter brain regions in 41 premature infants dying between 1997 and 1999, ie, during the modern era of the premature intensive care nursery.¹⁴ We divided the cases into 3 groups according to white matter histopathology, ie, PVL (n = 17), diffuse white matter gliosis (DWMG) without focal periventricular necrosis (n = 17), and neither PVL nor DWMG (n = 7). We applied the most important tool of all neuropathology to this study, ie, the eye through the microscope. We found that over one-third of the PVL cases also demonstrated gray matter lesions which were characterized by neuronal loss and/or gliosis⁴¹ (Fig 5). Neuronal loss of any degree of severity by visual assessment occurred exclusively in the PVL cases compared with non-PVL cases; its incidence in the PVL cases was 38% in the thalamus, 33% in the globus pallidus and hippocampus, and 29% in the dentate nucleus.⁴¹ Gliosis without obvious neuronal loss was more common than neuronal loss and gliosis combined in the thalamus (56% of cases), globus pallidus (60%), hippocampus (47%), basis pontis (100%), inferior olive (92%), and brainstem tegmentum (43%). Not unexpected were the findings of obvious neuronal loss in only 13% of the cerebral cortex (frontal only) and gliosis alone (31%) in the PVL cases, reinforcing the long-time observation that the cerebral cortex (but not the thalamus or other gray matter sites) seems histologically spared relative to the white matter. In a survey of the frequency of histopathologic features of brain injury in low birth weight infants (n = 67) by Dr Gilles and colleagues,⁵¹ "neuronal loss" (not further defined) was found infrequently compared with white matter abnormalities; the specific gray matter sites were not reported.

Our neuropathologic survey of the entire preterm brain indicated to us that neuronal loss and/or gliosis are the histopathologic hallmarks of gray matter injury in PVL and is widespread, affecting virtually all gray matter sites, albeit in variable combinations and degrees.³ Moreover, it reinforces the finding of the neuroimaging studies that gray matter injury is common in PVL, occurring in our series collectively in over one-third of the cases.¹⁴ This survey has formed the basis of our current strategy, to pursue with computer-based quantitation and immunocytochemistry in detail the neuropathologic findings in all the major gray matter sites affected, beginning with the thalamus¹⁵ and the axons coursing

through the white matter.¹³ In-depth analysis by us of the thalamus, for instance, in 22 PVL cases and 16 non-PVL cases revealed that 59% of PVL cases demonstrated thalamic damage compared with 16% of non-PVL cases ($P = .01$). Moreover, this damage occurred in 4 different patterns, ie, diffuse gliosis with or without neuronal loss, microinfarcts with focal neuronal loss, macro-infarcts in the distribution of the posterior cerebral artery, and status marmoratus.¹⁵

These heterogeneous patterns likely reflect different mechanisms, including diffuse hypoxia-ischemia and focal embolism,¹⁵ that require pursuit each in their right. Although the damage involved all thalamic nuclei, it was noteworthy in the mediodorsal nucleus because of key role in working memory and the report of deficits in this function in preterm survivors, as well as in the reticular nucleus because of key role in attention and arousal and the report of deficits in these functions in such survivors.¹⁵ The question arises: how did we in preterm brain injury get side-tracked to focus upon white matter injury almost to the exclusion of gray matter injury? The answer lies, I believe, in part in the subtlety of the gray matter lesions, ie, microscopic neuronal loss and gliosis, which are “overshadowed” by the striking cystic cavities of PVL obvious to the naked eye at brain cutting. Dr Volpe has also suggested to me the possibility that early ultrasound imaging demonstrated large periventricular cysts; once the clinical incidence of these radiographic cysts substantially decreased over the last 2 decades,⁵² we were able to “see” the gray matter damage, no longer distracted by cystic PVL recognition (Dr Volpe, personal communication); the fortuitous introduction of volumetric MRI over this same period also helped. In addition, historically we did not have specific tools to “enhance” the detection of subtle neuronal loss and gliosis, such as computer-based methods to determine gray matter volumes and neuronal number and density or immunocytochemical methods with the GFAP antibody to highlight reactive astrocytes, in tissue sections. It is also likely that the discernment of subtle injury is behind the capabilities of current tools, particularly in autopsy tissue with lengthy postmortem intervals. Detection of astrocytes (comprised of subtypes with protoplasmic morphology in the cerebral cortex and fibrillary morphology in the cerebral white matter) illustrate this issue well.⁵³ Recently, a study of the adult human cortex found that protoplasmic astrocytes could be detected only in freshly resected specimens obtained at surgery, for instance, temporal lobe resections, which were fixed in paraformaldehyde, and that they could not be detected in autopsy specimens fixed in formalin.⁵³ Consequently, the concept of the preferential involvement of white matter over cortex in preterm brain injury may “simply” reflect an inability to immunostain cortical protoplasmic astrocytes, as opposed to white matter fibrillary astrocytes, for unknown biological or technical reasons in postmortem tissue. The failure to detect gray matter injury in the preterm brain may also reflect the involvement of subcellular structures, such as dendrites, axonal terminals, and spines in neuropil that require for their elucidation certain tools that are not conventionally applied at autopsy. Marine-Padilla’s findings of dendritic and axonal pathology in the cerebral cortex associated with white matter injury (see above) illustrates this point well, as this pathology was detected only with the application of the labor-intensive Golgi’s stain that is not standard in clinical neuropathology.⁴¹ Recognizing the potential role of subcellular pathology in gray matter, Dr Gilles wrote in his review of acquired perinatal leukoencephalopathy with Dr Leviton in 1984:

“Disorders with gray matter damage are not a focus of this review because there is little epidemiological knowledge about them. To a large extent this might reflect a lack of data from routine postmortem examination about disorders of synaptogenesis, which probably contribute more to functionally important deficits than does frank neuron destruction”.⁶

The detection of “disorders of synaptogenesis” in the pre-term brain will require the application of immunocytochemistry and western blotting to assess synaptic proteins, as

well as of Golgi and other special stains to visualize dendrites and spines, the major synaptic sites.

Yet, a major consideration of the reasons that substantial gray matter injury was not historically recognized in the pre-term brain was because it was not there. In my view, this has to be a consideration given the impressive skills of the publishing investigators to recognize the whole spectrum of human developmental neuropathology. Perhaps gray matter injury has become more pronounced in the era of modern intensive premature care—an era which coincides with that of modern neuroimaging and neuropathology—due to the emergence of new diseases in association with the prolonged survival rates of premature infants, for example, opportunistic infections, or to complications of the new technologies themselves, for example, oxygen toxicity secondary to the inadvertent use of nonphysiologic levels of oxygen with mechanical ventilation. To take but one example, thalamic damage is not mentioned at all by Banker and Larroche⁵ in their comprehensive study of perinatal brain injury in 1962 compared with multiple reports of substantial volume loss and pathology in this structure in perinatal brains over a period.^{1,3,45–50} Indeed, neuronal loss and gliosis are nonspecific consequences of cell injury and result from oxygen toxicity, bilirubin toxicity, hypoglycemia, and infection,^{3,4,10} in addition to hypoxia-ischemia. Perhaps the substantial thalamic injury in the preterm brain reported by us in 2008—almost a half century following the report of Banker and Larroche—is a manifestation of other metabolic/infectious insults in the modern day nursery in isolation or in combination with hypoxia-ischemia. Indeed, we have already seen secular trends in the neuropathology of preterm brain injury over the last 2 to 3 decades in the decline of cystic (as opposed to noncystic) PVL as detected by neuroimaging in living infants.² We can only speculate that this decline reflects the temporal introduction of more effective treatments for hypotension and hypoxia, among other possibilities. Our group at Children's Hospital Boston needs to go back to the archives in the Department of Pathology and reexamine the same microscopic slides reviewed by Banker and Larroche to test the hypothesis that the severity and type of gray matter injury we (the current neuropathologists at this venerable hospital) observe was indeed not present in their day, particularly in the thalamus as a case in point. In the ever-wise words of Dr Volpe (elsewhere in this series), we need to go “back to the future”.

“What's in a name?” My answer, Juliet, is “Everything”—as witnessed by the subtle effect of the name “periventricular leukomalacia”, in my opinion, upon research in preterm brain injury. In their seminal report, Banker and Larroche did indeed describe gray matter damage in association with the white matter damage, reporting that most of their cases showed “minor changes” in the gray matter that was characterized by mild neuronal loss and gliosis, as well as neuronal karyorrhexis in the acute stages.⁵ The affected sites listed by them were the cerebral cortex (lower layers), hippocampus (Sommer's Sector), subiculum, griseum pontis, dentate nucleus, and cerebellar Purkinje cells; germinal matrix hemorrhages were also present in 5 premature infants.⁵ In their summary, Banker and Larroche stated: “Attention is drawn to a unique disease of the cerebral white matter that we have encountered in 19% of all infants who died under 1 month of age ... In addition, there was a diffuse loss of nerve cells in the cerebral cortex”.⁵ Thus, although they recognized the association of cortical injury, they recommended the term periventricular “leukomalacia”, ie, “white matter softening”, thereby emphasizing the pathology of the white matter to the neglect of that in the gray matter. Consequently, brain injury in the preterm infant became synonymous, I believe, with PVL although Banker and Larroche were emphasizing the predominate and not exclusive feature of perinatal brain injury in their experience. One major exception to the historical focus upon white matter injury was an insightful report in 1987 by my mentor, the pediatric neuropathologist Dr Arm-strong.⁵⁴ In this report, Dr Armstrong observed that intraventricular hemorrhage in 20 infants who survived more than 1 postnatal week did not occur in isolation but rather was associated with choroid plexus

hemorrhages in 46% of the cases and additional brain findings in 92% of the cases, including PVL, brainstem necrosis, hydrocephalus, and cerebellar necrosis.⁵⁴ Thus, she pointed out that PVL occurred with germinal matrix hemorrhages and other brain abnormalities. The recognition of a complex of hemorrhagic and nonhemorrhagic lesions in the white and gray in perinatal brain injury was the basis of her visionary advice that the entire spectrum of injury be considered in attempts to prevent and treat any one of the lesions, and that their combined presence should be “suspected during clinical assessment of survivors” (Armstrong, 1987). Indeed, Dr Armstrong always taught us to step back from the 1 slide under the microscope and “observe” the whole picture, a gift she herself so greatly possessed.

On the basis primarily of neuroimaging studies, Dr Volpe in 2005 first introduced the term “encephalopathy of prematurity” to emphasize the constellation of abnormalities in the preterm brain, including PVL, germinal matrix hemorrhages, hydrocephalus, and neuronal/axonal pathology.² The specific neuropathologic features of the neuronal/axonal abnormalities in particular were substantiated by systematic studies of gray matter sites and axons by our group under his inspiration.^{13–15,19} The value of the use of the term “encephalopathy of prematurity” cannot, in my opinion, be underestimated for it conveys the real complexity of preterm brain injury without focus upon one specific feature (white matter pathology) over another (gray matter pathology), and thereby crystallizes awareness of this complexity for the conceptualization of comprehensive strategies to address causation, treatment, and prevention. In short, treatment of white matter injury without the simultaneous treatment of gray matter injury will likely not prevent the complex outcomes of preterm survivors; both treatments are needed for the “encephalopathy of prematurity”. Of note, Dr Volpe and I have debated the proper term for brain injury in early life, particularly because the constellation of gray and white matter damage is not unique to premature infants but is also observed in full-term infants, particularly those with congenital heart disease.^{3,55} Indeed, the term “perinatal encephalopathy” may ultimately prove more accurate, with the application of “perinatal encephalopathy in premature infants” in reference particularly to premature infants.³ One additional note on nomenclature: Dr Volpe and I have often discussed the best abbreviation for the tongue-tying appellation “encephalopathy of prematurity”; we suggest the abbreviation “EP”.

In the underlying mechanism(s) of the encephalopathy of prematurity, the pathogenesis of white matter abnormalities is likely to be the same in the gray matter, with the key differences that the targeted cell in the gray matter is the developing neuron, as opposed to the pre-OLs in the white matter. Thus, we propose that the white and gray matter injury triggered simultaneously by hypoxia-ischemia occurs in the sick premature infant with respiratory compromise and systemic hypotension, leading to glutamate, free radical, and cytokine toxicity to developing OLs and neurons, with different topographic patterns of injury on the basis of their developmental and genetic susceptibilities.³ The search for a single or even primary cause of the encephalopathy of prematurity seems outmoded in light of the evidence for multiple metabolic and infectious insults bombarding simultaneously sick premature infants in multiple organ systems and multiple time points.³ In addition, a growing body of multi-disciplinary evidence suggests that infection/inflammation and hypoxia-ischemia potentiate each other to produce PVL.³ Targeting the shared pathways to necrosis and apoptosis in developing OLs and neurons is an important strategy to prevent all cell death.³

Roadblocks

The road blocks to advancement in our understanding of the neuropathology of the preterm brain are unfortunately multiple and daunting. Below I have highlighted the major road blocks that effect every day upon human research in preterm injury in the human brain.

Incomplete Knowledge of Normal Human Brain Development

In 1973, Sidman and Rakic⁵⁶ published their landmark chapter in the textbook *Cytology and Cellular Neuropathology* (edited by RD Adams and W Haymaker) about the development of the human brain, a chapter that provided the foundation, in my opinion, for all subsequent studies of human brain development and developmental pathology. In this comprehensive chapter, they laid out the major milestones and sequences of the morphologic development of the cerebral cortex, thalamus, hippocampus, basal ganglia, cerebellum, brainstem, and spinal cord.⁵⁶ In a way, all subsequent studies of human brain development have been but a “filling-in” of the biochemical, cellular, and molecular details of this beautifully delineated, structural ground plan. The application of modern immunocytochemical, western blotting, in situ hybridization, and autoradiographic tools to human brain development has been instrumental in supplementing classical Golgi, myelin, and cell stains in this endeavor. Although we have made tremendous strides in understanding human brain development, including in regards to the cellular factors underlying white matter susceptibility to hypoxia-ischemia (see above), we still do not know much about even such basic events as when in the fetal period neuronal migration to the cerebral cortex is complete.

A prime example of how the lack of normative information hinders insights into developmental neuropathology concerns astrocytes. The presence of “hypertrophic astrocytes” without associated periventricular necrosis has long been recognized in the cerebral white matter of premature infants, and Dr Gilles and his colleagues emphasized their potential pathologic significance under the PTL rubric.^{6,7} Indeed, hypoxic-ischemic white matter injury may follow a continuum of damage from mild gliosis (hypertrophic astrocytes) alone to severe (periventricular necrosis combined with gliosis).³ Yet, the possibility exists that astrocytes may normally undergo hypertrophy in the late fetal and perinatal white matter as an obligatory developmental change, potentially due to the “physiological oxidative stress” of active myelin sheath synthesis, and thus “hypertrophic astrocytes” may not be a marker of pathology at all⁵⁷ (Fig 6). On the basis of brain analysis in the NCPP, Dr Gilles and colleagues reported that hypertrophic astrocytes were present in the white matter of 11.4% of fetuses at 20 to 27 gestational weeks and increased to 67.7% at 36 to 44 weeks with a progressive increase from midgestation to term.⁶ This observation suggests that the normative morphology of astrocytes changes with age and becomes increasingly “hypertrophic”, as opposed to the conventionally held alternative interpretation that fetal white matter at term is more susceptible to injury than at midgestation.⁶ In our survey of the neuropathology of prematurity, we found gliosis (without associated focal necrosis) in the white matter of 25% of cases at 23- to 29 weeks, 86% at 30-to 36 weeks, and 100% at term,¹⁴ again indicating a progressive increased incidence with age; the attainment of “gliosis” in all the term brains raises further the interesting possibility that the finding is not pathologic but rather “normal” because all cases share it, perhaps as myelin synthesis and its concomitant physiological oxidative stress triggering astrocytic hypertrophy is increasing⁵⁷ (Fig 6). Further studies are needed to examine the significance of astrocytic hypertrophy in developmental neuropathology. This challenge is heightened by the unavoidable fact that live-born infants dying during the last half of gestation are not “normal” but rather die in intensive care units with multiple complications of prematurity that are known to adversely affect the brain (see below).

Lack of the “Perfect Control”

The selection of controls for neuropathologic analysis in preterm brains is problematic given that *all* infants who die in the late fetal and neonatal period, ie, the period of risk for PVL, die in extremis such that most have received some form of mechanical ventilation. The control brain without any injury in this time frame is simply a myth. Given that all autopsied preterm infants experience some degree of hypoxia-ischemia and other metabolic derangements at birth or during their hospital course, their brains may not be truly representative of those of “normal” living infants. Pathologic changes in preterm brains must be interpreted in light of potential (minor) abnormalities in the “control” brains. Given this caveat, we have found robust differences between PVL and control brains in markers, for example, of thalamic and axonal damage, oxidative and nitrative stress, and cytokine expression that suggest disease-specific phenomenon.^{11–22} In addition, modern neuroimaging techniques have the novel potential to verify neuropathologic findings directly in living infants with mass spectrometry, tractography, and diffusion tensor imaging for myelin ensheathment abnormalities.⁴⁵ In addition, these neuroimaging techniques are able to verify sequences of normative development that were determined in autopsy brains, as illustrated for the sequences of myelination.^{58,59}

Decline in Autopsy Rates in Fetal and Infant Cases

These declining rates are a major road block that is not fully appreciated, in my opinion, by the neonatology community. At Children’s Hospital Boston, for example, the overall autopsy rate has plummeted in half, to 30%, between 1976 and 2007 (Dr HPW Kozakewich, personal communication). Moreover, the autopsy rate in infants dying in our neonatal intensive care nursery in 2007, the last year in which such data were finalized, was only 22%, representing 5 of 23 deaths (Dr HPW Kozakewich, personal communication). From this statistic alone, one can appreciate the difficulties in accruing a large sample size of both case and control brains for comparative statistical analysis. The reasons for the decline in autopsy rates include (1) fear of “upsetting” parents further at the time of great loss; (2) fear of litigation related to potential significant findings “missed” clinically; (3) delegation of autopsy consent to the most junior (inexperienced) physicians; and (4) lack of training in physician communication around autopsy-related issues, including about specific autopsy procedures.⁶⁰ In addition, societal changes over the last 2 decades has led to an almost total lack of availability of embryonic and early fetal (< 20 gestational weeks) tissues for study that were previously attained from therapeutic abortions. In short, there is a serious crisis in the capability to attain human brain tissues in the embryonic, fetal, and neonatal periods for research—made all the more distressing by the potential missed opportunity to study these brains with modern tissue techniques. This crisis needs to be systematically addressed, and in order not to jeopardize the progress in our understanding of preterm brain injury, which is the critical first step in developing strategies to prevent or ameliorate it.

Lack of Realistic Quantitative Tools in Autopsied Brain Tissues

The most valuable resource we have for obtaining tissue samples for analysis of preterm injury in the human brain is the archives of pathology departments throughout the country; indeed, these archives at pediatric hospitals in particular are “national treasures”. To use this resource to its fullest potential, it is essential to devise quantitative tools that are valid in archival (paraffin-embedded) tissue sections. Stereology, for example, is argued to be the most reliable method to count neurons,⁶¹ but it depends upon the accrual of fresh tissue that is subsequently fixed and stained according to methods that are not standard in pathology departments. Indeed, stereology necessitates the prospective collection of the brains of rare cases and controls, which is logistically difficult in the daily operation of the autopsy room. Thus, we need to be open, albeit without compromise for validity, to two-dimensional cell counting approaches.⁶² It is argued, however that three-dimensional counting provides

“unbiased” counts of neurons, whereas two-dimensional approaches are “assumption-based” and therefore potentially yield inaccurate results.⁶² Yet, all approaches are assumption-based and involve inherent biases, and thus the selection of two-vs three-dimensional approaches for a particular study is based upon relative strengths and weaknesses.⁶² Due to our dependence on archival tissue in which stereology is not an option, coupled with the validity of two-dimensional methods in general,⁶² the use of a two-dimensional approach is reasonable and valid.

Future Directions

To advance further our understanding of the neuropathology of brain injury in the premature infant, several future directions are recommended. First and foremost, there needs to be more crosstalk between basic scientists and neuropathologists to ensure that the spectacular insights made in developmental neuroscience are applied to human preterm pathology. These insights relate, for example, to the relevant issues of glutamate, free radical, and cytokine toxicity; neuronal, oligodendrocyte, and astrocytic cell lineage; synaptogenesis; axonal path finding; subplate neurons; neuronal migration; and stem cell biology. Moreover, such crosstalk is needed to ensure hypotheses generated from human studies are tested in animal models that precise mechanisms for the cellular abnormalities in the human brain are determined. The observation of diffuse microglial activation in the white matter surrounding focal periventricular necrosis in the encephalopathy of prematurity, for example, focuses attention of basic research upon the means to ameliorate such activation to prevent pre-OL injury.⁶³ The human findings need to guide the experimental studies to ensure the relevance of the latter to the former.

Second, there needs to be more crosstalk between neuroradiologists and neuropathologists to ensure that the spectacular insights made by modern neuroimaging guide future neuropathological studies, and vice-versa. The autopsy finding of diffuse axonal injury in the encephalopathy of prematurity, for example, needs to be correlated with axonal pathology by modern tractography to translate into clinical usefulness in diagnosis and treatment.⁶⁴ Imaging of autopsy brains, although logistically demanding, is needed to correlate directly pathology with neuroimaging.

Third, more studies of normative human brain development at the cellular and molecular levels are needed, particularly in regards to the arterial vasculature, subplate neurons, late migrating GABAergic neurons, astrocytes, stem cells, and synapses.

Fourth, modern tissue techniques, for example, immunocytochemistry, receptor autoradiography, western blot analysis, high-performance liquid chromatography, computer-based quantitation, and in situ hybridization, need to be increasingly applied but nevertheless in creative combinations with classical histologic techniques that are the basis of all neuropathological insights—witness the contributions of Drs Gilles, Marian-Padilla, and Armstrong.

Fifth, we must train and mentor the pediatric neuropathologists of the future with passion, as I have luckily been mentored, to bridge the gap between bedside and bench.

Sixth, the decline in the autopsy rate must be reversed. Finally, the neuropathological issues so beautifully presented by Dr Volpe in the Lancet article¹ and reiterated in this series need to be systematically addressed. We must study in-depth the cerebral cortex, white matter, thalamus, basal ganglia, amygdala, basal forebrain, hippocampus, cerebellum, and brainstem—one by one—to characterize fully the neuropathologic substrate of the encephalopathy of prematurity.

At the end I wonder: what do Banker and Larroche think, and what will we think a half-century from now? Let the future be for us as truly amazing as today must be for them.

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Figure 1. Classic diagram from the seminal report of the neuropathology of periventricular leukomalacia by Drs Banker and Larroche in 1962,⁵ showing the distribution of the necrotic foci in the periventricular leukomalacia. (Color version of figure is available online.)

Perikaryon MBP Expression

Figure 2.

(A) A key feature of the white matter damage in the encephalopathy of prematurity is the accumulation of myelin basic protein (MBP) staining in the perikarya of oligodendrocytes (OL) (arrows), as indicated with an antibody to MBP using immunocytochemistry.¹² (B) The diagram indicates schematically that the putative arrest of MBP trafficking in the mature (or developing) OL contributes to impaired ensheathment of the axons and a functional abnormality in OL-axonal interaction (red lines). (Color version of figure is available online.)

Figure 3.

Diffuse axonal injury in the encephalopathy of prematurity (A,B) in the context of normative axonal development (C). (A) In this diagram of a representative section of the posterior frontal lobe (level of the body of the corpus callosum) in a preterm infant with the encephalopathy of prematurity, there is axonal fragmentation (small lines) in the white matter surrounding the microcystic foci (microcyst).¹³ Note the fragmentation in the corpus callosum indicative of involvement of the intrahemispheric crossing fibers. (B) Axonal damage throughout the white matter is demonstrated with an antibody to fractin, marker of apoptotic injury, with immunocytochemical methods.¹³ (C) During the peak period of the encephalopathy of prematurity and through infancy, axonal elongation occurs in the cerebral white matter, as indicated using the growth associated protein-43 marker with western blot analysis.²⁸ The growth associated protein-43 levels are expressed as the percentage of an adult standard.²⁸ (D) The axonal damage in the encephalopathy of prematurity may reflect: (1) secondary degeneration to the axon because of a primary insult to the neuronal cell body (antegrade degeneration) that is located in the thalamus or cerebral cortex; and/or (2) primary insult to the axons coursing through the white matter to and from the cortex with secondary degeneration to the neuronal cell body (dying-back or retrograde degeneration). (Color version of figure is available online.)

Figure 4. Classic Golgi drawing by Dr Marin-Padilla⁴¹ of neuronal abnormalities in the cerebral cortex overlying severe white matter necrosis in a human infant. Some neurons are transformed into small stellate neurons post injury, whereas others show alterations suggestive of centripetal dendritic reabsorption. Some pyramidal cells also seem to have lost contact with Layer I with progressive distal absorption. The reader is referred to the original publication for the in-depth explanation of the figure.⁴¹

Figure 5.

Summary diagram of the topography of lesions in the encephalopathy of prematurity as determined in a cohort of 41 premature infants.¹⁴ The incidence of neuronal loss or gliosis in each brain region is indicated. The large red circle represents the periventricular foci of necrosis; the small red dots represent reactive astrocytes in the diffusely gliotic component of the white matter pathology. (Color version of figure is available online.)

Figure 6.

The hypothesis that the hypertrophic astrocyte in the human fetal brain is normative rather than pathologic is illustrated. (A) There is a peak in the density of astrocytes with pronounced immunostaining of glial fibrillary acidic protein, the major astrocytic marker, in the cerebral white matter around the time of birth and during the transition period between Stage 1 (OL growth, migration, and proliferation) and Stage 2 (active myelin sheath synthesis) of myelination. This peak coincides with a peak in the density of immunostained morphologic astrocytes expressing markers of oxidative [4-hydroxynonenal (HNE)] and nitrative (inducible nitric oxide), suggesting that these astrocytes are involved in physiological processes related to free radical injury potentially relevant to myelin synthesis.^{21,47} (B) The changing presence of HNE-immunostaining astrocytes is indicated in the human white matter in Stage 1 and Stage 2 of myelination, compared with their absence in the fully-myelinated adult white matter.⁴⁷ A negative control indicates the specificity of the HNE antibody used in this study.⁴⁷ (Color version of figure is available online.)