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# Injury to the Preterm Brain and Cerebral Palsy: Clinical Aspects, Molecular Mechanisms, Unanswered Questions, and Future Research Directions

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## Abstract

Cerebral palsy will affect nearly 10% of the 60,000 very-low-birth-weight infants born in the United States in the next year, and an even greater percentage will display some form of permanent neurological impairment resulting from injury to the preterm brain. The 2008 Neurobiology of Disease in Children Symposium, held in conjunction with the 37th annual meeting of the Child Neurology Society, aimed to define current knowledge and to develop specific aims for future clinical, translational, and fundamental science. A complex interplay of both destructive and developmental forces is responsible for injury to the preterm brain. Advances in imaging and histology have implicated a variety of cell types, though pre-oligodendrocyte injury remains the focus. Research into different mechanisms of injury is facilitating new neuroprotective and rehabilitative interventions. A cooperative effort is necessary to translate basic research findings into clinically effective therapies and better care for these children.

### Keywords

cerebral palsy; molecular mechanisms; translational research

## Clinical Aspects of Injury to the Preterm Brain

Moderator: Michael V. Johnston, MD, Kennedy Krieger Institute, Baltimore, MD

### Injury to the Preterm Brain: Where Are We Now?

Joseph Volpe, MD, Children's Hospital Boston, Boston, Massachusetts

Dr Volpe reviewed current understanding of injury to the preterm brain. About 60,000 babies weighing <1500 grams are born in the US each year, and with neonatal ICU advances, 90% of them survive. Unfortunately, 5% to 10% of these babies are left with motor deficits and 25% to 50% with cognitive, behavioral, and/or social deficits affecting school success.

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The main neuropathology seen in affected preterm infants is sufficiently distinct to warrant the term “encephalopathy of prematurity,” with periventricular leukomalacia (PVL) occurring in 50% of these infants. Periventricular hemorrhagic infarction, though severe, is quantitatively less important, occurring in just 5%. PVL is characterized by 2 components, a focal component that leads to loss of all cellular elements, and a more diffuse, cell-specific element characterized by pre-oligodendrocyte injury, astrocytosis, and microgliosis. Macroscopic focal lesions produce cysts readily seen with conventional imaging modalities, but this type of PVL occurs in only 5% of cases. Much more common is “noncystic” PVL with microscopic focal lesions that evolve into small gliotic scars as opposed to cysts. Pre-oligodendrocyte injury can either result in complete cell death or loss of cell processes, with both morphologies seen concurrently. Subsequently there is replenishment of a pre-oligodendrocyte population with impairment in maturation and myelination, and the outcome is a deficit in mature oligodendrocytes and hypomyelination.

The pathogenesis of this pre-oligodendrocyte injury relates to a number of interacting factors — cerebral ischemia, infection and inflammation, and a maturation-dependant vulnerability of pre-oligodendrocytes. This vulnerability in the preterm infant is a propensity for cerebral ischemia due to impaired vascular autoregulation, which generates reactive oxygen and nitrogen species. These accumulate secondary to a delay in acquisition of antioxidant defense systems by pre-oligodendrocytes and cause injury. Simultaneously, these cells are actively acquiring iron for differentiation, which leads to further production of reactive species. Intraventricular hemorrhage greatly increases the likelihood an infant will have pre-oligodendrocyte injury, likely due to release of a large amount of non-heme iron.

Increases in extracellular glutamate, which occur with ischemia, have been shown to correlate with white matter injury in animal models. The source of glutamate appears to be principally from reversal of glutamate transporters in pre-oligodendrocytes, and there is an overexpression of glutamate transporters in pre-oligodendrocytes in the premature period. Glutamate activates gluR2-deficient alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors on cell bodies and N-methyl D-aspartate (NMDA) receptors on cell processes, which leads to calcium influx and generation of reactive species. The presence of these 2 different receptors on distinct parts of the cell raises the possibility of a 2-stage process of excitotoxicity. NMDA receptors have a higher affinity for glutamate and flux calcium more readily. This could lead to loss of cell processes initially, with progression to cell death only with activation of the AMPA receptors located on the cell body.

There is clearly a relationship between infection, inflammation, and PVL, with direct activation of microglia. During development, microglia are most abundant in the white matter in the third trimester, during the window of pre-oligodendrocyte injury. Infection and inflammation are associated with release of pathogen-associated molecular products that activate microglia. These activated microglia lead to the production of reactive species. Additionally, ischemia and hypoxia are associated with molecular products that also activate microglia. Dr Hagberg and colleagues have shown potentiation between these 2 insults, and subthreshold activation of each could lead to clinically important insults.

There is a confluence of maturation-dependent factors that lead to premyelinating oligodendrocyte injury. There are upstream mechanisms of ischemia, reperfusion, and inflammation activating downstream mechanisms of excitotoxicity and free radical attack, and so there are a number of potential targets for prevention of injury. Recent work has shown minocycline to be effective as an anti-microglial agent and topiramate and memantine have been shown to block AMPA and NMDA receptor-mediated injury. There is

also experimental data suggesting efficacy for nonpeptidyl compounds, antioxidant enzyme mimetics, oxygenase inhibitors, and nitric-oxide synthase inhibitors in blocking reactive species. Additionally, data support the role of vitamins E and K and anti-apoptotic agents, such as estradiol, in preventing injury. Many of these seem to be on the threshold of translation to the infant.

However, white matter injury is not the whole story. Cognitive and behavioral deficits, without major motor deficits, are the dominant syndrome, suggesting grey matter disease. Careful clinical, correlative, and advanced magnetic resonance imaging (MRI) studies have shown that white matter injury correlates with cognitive deficits, and that premature babies with white matter injury have markedly diminished cerebral cortical grey matter volumes, as well as decreased thalamus and basal ganglia volumes. Additionally, diffusion tensor imaging (DTI) MRI shows a decreased anisotropy in cerebral white matter, suggesting axonal disease.

Neuronal loss is likely due to interplay between primary destructive lesions and secondary trophic and maturational disturbances of the preterm brain. During the third trimester, the thalamocortical unit is actively developing. Neurons from the thalamus send axons through the white matter, where they are beginning ensheathment by premyelinating oligodendrocytes, toward the cortex to synapse. However, at this stage in development the cortex is still immature, and these axons must briefly synapse with subplate neurons, a transient layer at peak development in the third trimester. Also, during this time, γ-aminobutyric acid-mediated (GABAergic) neurons, generated in the subventricular zone, are migrating to the upper cortex. In PVL, pre-oligodendrocytes may receive the initial insult, directly leading to a decrease in myelination. Because of trophic interactions between pre-oligodendrocytes and axons, this would lead to axonal degeneration, as well as cortical and thalamic degeneration. However, it is also possible that axons themselves, the thalamus, subplate neurons, or migrating GABAergic neurons are initially injured. Each of these insults would lead to both axonal injury and, through secondary trophic effects, the degeneration, or at least the failure of maturation, of pre-oligodendrocytes. So there are many possible scenarios in which primary injury leads to secondary trophic and maturational disturbances that would result in the neuronal-axonal disturbances seen both functionally and by MRI. Dr Volpe chose pre-oligodendrocyte injury as most likely, but emphasized that all of these scenarios are likely playing a role. To close, Dr Volpe reiterated that brain injury in the premature infant is a complex amalgam of both destructive and developmental disturbances, and any therapeutic intervention must address this complexity.

## Neuropathology of Periventricular Leukomalacia

Rebecca Folkerth, MD, Children’s Hospital Boston, Boston, Massachusetts

Dr Folkerth reviewed the neuropathology found in human autopsied sections of PVL. There are fundamental imaging patterns of injury in PVL, including cystic and diffuse. However, there are also microscopic injury patterns detectable only with autopsy.

There is a distinct evolution in the histology of the focal necrosis of PVL. Within the first 24 hours of insult, there is coagulative necrosis with some pycnotic nuclei, indicating irreversible injury, and vacuolization, with a relatively well-preserved penumbra. Histologically, the injury is essentially an infarct, with irreversible destruction of all cell types within it, including the axons passing through. As this transitions into subacute, or organizing, PVL, there is an influx of macrophages and reactive astrocytes with the beginning of cavitation. This either resorbs completely to form a cystic cavity, or forms a glial scar, with persistence of macrophages, reactive astrocytes, and, sometimes, mineralization.

From 24 weeks to 34 weeks of gestation, the period of susceptibility to PVL, O1+ and O4+ oligodendroglial precursors predominate. The first microscopic appearance of myelin basic protein occurs around week 30, though myelination does not occur with visibly staining myelin until the postnatal period. In vitro, studies have shown these oligodendroglial precursors are vulnerable to free radicals, oxygen-glucose deprivation, and cytokine attack.

In autopsied sections of postmortem human brain tissue, hydroxynonenal and nitrotyrosine, free radical adducts, localized to developing oligodendroglial cells. In further exploring this free radical injury, it was found that levels of superoxide dismutase are below adult levels at birth and are even lower during the period of vulnerability to PVL, whereas catalase and glutathione peroxidase levels before birth are higher than adult levels. This dyssynchrony of expression of these antioxidant enzymes may confer a vulnerability to free radical injury in these pre-oligodendrocytes.

Pre-oligodendroglial cells have also been shown to be sensitive to cytokines such as tumor necrosis factor alpha, interleukin-2, interleukin-6, and interferon gamma. There is increased expression of interferon gamma in PVL sections over controls. Interesting, interferon gamma was found in macrophages, but not in microglia. Interferon gamma receptors are also present on pre-oligodendrocytes, perhaps acting as a growth factor. The source of interferon gamma may be macrophages evolved from microglia or it may be part of the circulating cytokines in the maternal/fetal circulation. Regardless, pre-oligodendrocytes have the cytokine receptor, and are therefore susceptible to cytokine toxicity.

The consequence of this injury to the pre-oligodendrocyte is surprising. There is some detectable apoptosis, though less than might be expected. With quantitative analysis, there is an increase in olig2-positive pre-oligodendrocyte cell density. There is definite cell death occurring, yet there is increased density of oligodendrocyte precursors. Additionally, in chronic necrotic foci, there is aberrant myelin and abnormal myelin basic protein immunostaining at an age prior to the presence of normal myelination. This is present only in chronic foci, so it takes some time to develop.

A number of possible explanations exist for this preservation of density with hypomyelination. One possibility under study is cell replenishment with progenitors from the germinal matrix of the subventricular zone. This could be occurring quickly enough that there is no evidence of overall olig2-positive cell loss. Research shows there is a decrease in oligodendrocyte progenitor cells from the subventricular zone after hypoxia/ischemia, and this decrease is not associated with apoptosis. Additionally, it has been shown that these progenitor cells proliferate and contribute new oligodendrocytes to areas of demyelination in experimental autoimmune encephalitis, a possible reactive oligodendrogliosis. It is also possible that astrocytes and microglia, as well as axonal injury, promote oligodendroglial migration.

Another facet receiving attention in PVL is axonal injury, which is more prevalent than previously thought. Staining for fractin, a protein released during apoptosis, injured axons were seen far removed from focally necrotic lesions, suggesting a more widespread process than could be appreciated with older markers, such as beta-amyloid precursor protein. Furthermore, this axonal injury is occurring at the peak time of axonal elongation in the forebrain.

Lastly, there is associated neuronal loss and gliosis in the grey matter, particularly in the thalamus. Markers of oxidative stress show neurons affected by free radical adducts in regions of cortex adjacent to PVL. Similar injury is seen in subplate neurons and Cajal-Retzius cells. This grey matter neuropathology may be responsible for the cognitive deficits seen in PVL. The neuropathology of PVL is a pan-encephalopathy in which all brain cellular

elements are involved, and represents a complex interplay of free radical, excitotoxic, and cytokine injury.

## Neuroimaging of the Preterm Brain

David Edwards, FMedSci, Hammersmith Hospital, London, United Kingdom

Dr Edwards discussed technical advances and clinical value of imaging tools. Unfortunately, conventional neuroimaging with MRI is currently unable to predict with precision future achievement of preterm infants, with sensitivity and specificity ranges from 30% to 80%. This is surprising given the detail of the data acquired. However, used differently, MRI may be able to tell us much more. The most common conventional lesion seen on MRI is diffuse, excessive high-signal intensity in the white matter. Children with this lesion have worse developmental outcomes, similar to children with frank pathology, and it is this lesion that needs to be focused on to use MRI to predict outcome.

DTI has great potential. Briefly, DTI relies on the movement of water and whether this movement is random, such as in a ventricle, or constrained, such as in myelin fibers. This can be used to track nerve fibers and define tracts through the brain. With DTI a relationship between tissue microstructure, represented by the apparent diffusion coefficient, and measures of outcome can be seen. However, the relationship is relatively poor due to the subjective nature of the measuring tools currently in use and partial volume effects at the edge of the region of interest. Tract-based spatial statistics, developed by Dr Steve Smith in Oxford, eliminates this subjectivity. Contamination from grey matter is no longer a problem, and nonsubjective comparison of groups is possible. Using tract-based spatial statistics to analyze white matter differences, data show that children with poor developmental outcomes at the age of 2 have lower fractional anisotropy than children who do well, and significant differences can be seen in specific regions, such as the corpus callosum.

Further examination of these microstructure differences included the study of visual function and optic radiations. A direct relationship was seen between visual score and fractional anisotropy. Furthermore, when tract-based spatial statistics were used to analyze the brain as a whole, no other brain region showed this relationship, so the microstructure characteristics of the optic radiation was directly related to visual ability in the perinatal period.

DTI can even be used to partition tissues. Fibers running from the cortex to the thalamus seen with DTI do not run to the entire thalamus, but populate distinct areas analogous to nuclei. Expanding this, maps of connectivity within the brain can be made. Furthermore, using tractography methods to image children with white matter lesions, one finds that the decreased volume seen in the thalamus is not only structural but functional, as there is decreased connectivity between the thalamus and the cortex. This technique provides a powerful tool to look into the relationship between structure and function.

Dr Edwards went on to discuss volume relationships, and though other groups have found that brain volumes of preterm babies are smaller, Dr Edwards and colleagues do not see this in well-cared-for preterm babies; rather, there is brain area shrinkage in infants with problems in development. It is not the size, as an absolute measure, but rather the rate of growth of the brain that is important and that shows significant relationship to outcome. Data show the earlier an infant is born, the less the cortex grows per unit brain volume, and this is directly related to the Griffith’s quotient at 2 years of age. This provides an imaging correlate of the rate of growth of brain surface area to the fact that children born more prematurely, in general, do worse. It is the growth and development of the brain that matters.

Functional imaging, despite initial obstacles with infants, is beginning to produce some exciting data. Functional MRI can be combined with tractography to provide very precise structure-function relationships. Nonstimulated functional MRI looks at the brain at rest, and there is a clearly subcortical component of activation, exactly where the subplate should be. This might provide a new technique to look at damage of these subplate neurons and the effect this might have on overlying cortical growth.

## Fetal Inflammatory Response and Brain Injury

Olaf Dammann, MD, University of Hannover, Germany

Dr Dammann covered the role of the fetal inflammatory response as an etiologic factor in perinatal brain injury. Formerly, it was thought that prematurity was one of the single factors that increased the risk of brain damage in preterm babies. This has evolved to the concept that some exposure during pregnancy, such as intrauterine infection, leads to both preterm birth and white matter damage and cerebral palsy through a fetal inflammatory response that contributes to the onset of both, a causal “fork” rather than a sequence.

The view that an infection during pregnancy that gains access to the intra-amniotic compartment leads to damage by directly exposing the fetus and brain to bacteria is likely inaccurate, as there is no evidence of bacteria in the fetal brain. Rather, the fetal inflammatory response syndrome taking place on the systemic level might also be present in the brain and account for the abnormalities found.

In the 1970s, it was shown in a postmortem population of newborns that bacteremia at the systemic level led to a 5-fold increased risk for brain damage. More recent work has shown an association between placental markers of inflammation and abnormalities in the preterm brain. This led to the proposal that soluble markers of infection and inflammation, such as pro-inflammatory cytokines, may be damaging the preterm brain.

Chorioamnionitis, with its resultant inflammatory response, increases risk of brain damage, especially in preterm infants. The leukocytes of the inflammatory response in chorioamnionitis are of maternal origin. In comparison, the inflammatory response in the fetal vessels is composed of leukocytes of fetal origin. This response to fetal vasculitis, the fetal inflammatory response, is associated with an even greater increased risk (11–fold) of brain damage than chorioamnionitis.

More recently, evidence of the fetal inflammatory response was gathered through the ELGAN (Extremely Low Gestational Age Newborns) study, which provided extensive data on the brain, lung, and eye. Neonatal biomarkers and 2-year outcomes were also assessed. The data show only a minimal increase risk of ultrasound-defined white matter damage associated with chorioamnionitis and fetal vasculitis. However, there is a quite an increase in risk of spastic diplegia, especially when bacteria were present in the placenta. Interestingly, there is a high proportion of extremely-low-gestational-age newborns with 1, 2, or even 3 species of bacteria in their placenta.

Levels of C-reactive protein, one biomarker used to measure fetal inflammatory response, were much higher in those with diplegia during the first postnatal week in the ELGAN study. Although these levels drop, there is an ongoing inflammatory response throughout the first month in those who later develop diplegia.

Dr Dammann suggested that white cells play an important role as well. The fetal endothelium produces cytokines in response to a systemic exposure to infection and fetal leukocytes get activated and either go through the already damaged blood-brain barrier or

actively damage it and then go through. These leukocytes then help elicit astrocytosis and the microglial response that damages developing oligodendrocytes. In babies of low gestational weight with white matter abnormalities on neonatal MRI, not only are pro-inflammatory cytokines elevated in neonatal cord blood, but the ratio of CD45RO T-cells, which is a signal for an antenatal immune response and antenatal white cell activation, is also elevated. It is likely the interaction between the adaptive and the innate immune response that contributes to the intensity and duration of the process that results in white matter damage.

Microglial response also appears to be part of white matter damage, independent of how the damage is initiated, at least experimentally. The association between grey and white matter damage might be due to the fact that the upregulated and activated microglial cells are located in the intermediate zone where developing neurons migrate through. This could explain the cognitive abnormalities seen in babies born to mothers with signs of an inflammatory response at birth. Data show a 4-fold increased risk for a subnormal nonverbal intelligence quotient (IQ) at 9 years in preterm infants of mothers with a fever at birth.

Dr Dammann then offered the idea that it is not a single insult but an ongoing exposure to persistent neuroinflammation that is the etiology of brain damage and long-term developmental disability. Inflammation is visible in vivo via PET, which identifies upregulated microglia in multiple sclerosis, Parkinson's, and dementia. At autopsy of patients with autism, which is more prevalent among preterm infants, there is a very clear pattern of neuroinflammation happening even in adolescents and adults. Also, an acute systemic marker of astrocyte damage, S100B, is present in the blood of children with cerebral palsy at age 10. Lastly, there is experimental evidence that upregulation of a pro-inflammatory response in the brains of neonatal animals exposed to lipopolysaccharide can go on for weeks.

In conclusion, Dr Dammann reiterated there is evidence on multiple levels that an antenatal exposure to intrauterine infection appears to elicit a fetal inflammatory response that likely plays a role in white and grey matter damage in preterm newborns and their long-term disabilities.

## Hemodynamic Mechanisms of Prematurity-Related Brain Injury

Adre du Plessis, MBChB, Children's Hospital Boston, Boston, Massachusetts

Dr Du Plessis reviewed the hemodynamic model of injury in preterm infants. Current use of permissive hypotension, toleration of low blood pressure until evidence of perfusion abnormalities, emphasizes the doubts clinicians have about the ability to alter hemodynamics to prevent injury. This stems from a failure to prove a clear relationship between systemic blood pressure and brain injury in the preterm infant and the perception of a failure of pressor-inotrope medications to decrease the prevalence of brain injury. Additionally, a number of papers suggest these medications may actually create high-risk situations for brain injury.

The hemodynamic model states cerebral vascular insults result when the physiologic immaturity of the systemic and cerebral circulations conspire to act upon the anatomic vulnerability of the preterm brain. Essentially, there are regions of the brain that are undervascularized as well as regions with an extremely fragile vascular structure predisposed to hemorrhage with hemodynamic disturbances. Additionally, pressure autoregulation in the preterm infant is tenuous and easily disrupted. Lastly, there is increasing evidence that the relationship between central control and mechanical efficiency of the systemic circulation of the preterm infant is inefficient.

Animal studies show that hemodynamic factors play a role in brain injury. Specific insults result in neuropathology very similar to that seen in human infants. However, clinical data are weaker, and in only a minority of studies has there been significant relationships between hemodynamic insults defined by investigators and neurologic outcomes.

One of the major challenges in the clinical setting is defining the hemodynamic insult. In principal, insult occurs when oxygen delivery falls below oxygen demand. Unfortunately, this cannot be accurately measured. However, cerebral blood flow, as a substitute for oxygen delivery could be continuously monitored, though this is not currently available at the bedside. Dropping down another level to something that can be monitored, and with some necessary assumption, continuous arterial blood pressure can be used as a surrogate for cerebral blood flow.

The inability to identify what constitutes a significant hemodynamic insult has also been a major problem. Both hypotension and fluctuations in blood pressure are thought to put infants at risk for injury. However, there are a myriad of different definitions for thresholds of hypotension. Variability has been equally challenging to define. Furthermore, though the risk for injury is higher in immature babies, physiologic signals become paradoxically more variable with maturation.

There must be a temporal relationship between insult and injury prior to investigation of a causative relationship, and defining an injury has been equally challenging. Ideally, the imminent onset of injury can be identified to allow a therapeutic window. Unfortunately, there can be major brain injury in preterm infants without a sentinel event and without obvious acute signs. To date, injury has been measured with ultrasound, which is good for diagnosing hemorrhagic lesions, but not the diffuse white matter injury more commonly seen today.

There are compensatory mechanisms that protect the hemodynamic system from injury. As pressure begins to fall, the first response is cerebral vasodilation, in which cerebral blood volume increases while blood flow remains constant. Once vessels are maximally dilated, the second compensatory phase begins with an increase in cerebral oxygen extraction. Lastly, when oxygen extraction is maximized, oxygen metabolism starts to fail and injury occurs.

Dr Du Plessis and colleagues are interested in identifying hemodynamic thresholds that indicate when these compensatory mechanisms begin to fail. Near-infrared spectroscopy can be used to measure differences in cerebral oxyhemoglobin and hemoblogin concentration. This difference can be equated to changes in cerebral blood flow. Using a systems analysis approach, with mean arterial blood pressure as the input, a high coherence at a certain frequency with the hemoglobin difference signal would indicate that mean arterial blood pressure and cerebral blood flow are changing together, a pressure-passive circuit. Data show a high coherence, or pressure passivity, occurs in 20% of preterm infants, though it is not constantly present. It fluctuates and appears to improve with maturation. However, frequency-based coherence alone is not associated with an increased risk of intraventricular hemorrhage. Hypotension is a risk factor for pressure passivity, but the positive predictive value is very low. However, when the output signal is analyzed in terms of frequency coherence and gain, the amount of power coming through the system, in the frequency bandwidth that autoregulation is thought to work, there is an association between high gain and intraventricular hemorrhage. This is the first data wherein continuous recordings of this relationship might give insights into infants at risk for hemodynamic injury.

## Clinical Manifestations of Cerebral Palsy

Jan Brunstrom, MD, St. Louis Children's Hospital, St. Louis, Missouri

Dr Brunstrom began by thanking all attendees on behalf of those affected by cerebral palsy for coming together to hear discussions from all different aspects of expertise in the field. Dr Brunstrom then went on to discuss the manifestations of cerebral palsy.

For more than 150 years, people have tried to accurately define and classify cerebral palsy. It is not a single disease or a single disorder, it is highly variable. The hallmark of cerebral palsy is having a movement disorder. Importantly, a neurologic abnormality alone is insufficient; activity and functional participation must be limited. Many terminologies have been used to describe this group of disorders, but these do not provide an accurate description to allow comparison of one patient to another. For example, the most common type of disorder associated with prematurity and periventricular white matter injury has classically been called diplegia, but functional abilities in this group vary greatly.

One of the most pivotal pieces of work in the past decade has been efforts to better classify these patients by developing scales of motor function. In the Gross Motor Function Classification Scheme, for instance, a score of 1 is associated with patients that keep up well with peers, while a score of 5 signifies the need for complete human assistance. Hopefully, clinicians can provide more detailed descriptions of patients, which will help fuel basic science work aimed at correlating phenotype with underlying brain injury.

It is impossible for an injury to the developing brain to attack solely the motor area, and patients have multiple other problems directly attributable to their brain injury. Patients have sensory abnormalities of proprioception, general sensation, and of special senses; cognitive disabilities; epilepsy; problems with communication; and behavior issues; and many children display autistic features. Additionally, there are general medical conditions associated with cerebral palsy that are consequences of living in a body that does not move correctly, such as osteopenia, scoliosis, muscular contractures, bony deformities, and hip dysplasia due to hypertonia. Often, these associated impairments pose a bigger threat to a child's chances for a good quality of life and independence than their motor disability.

Children with cerebral palsy and epilepsy are much less likely than epilepsy patients without cerebral palsy to become completely seizure-free, with or without medications, and are more likely to require multiple medications, with more medication side effects. Importantly, prognosis varies with cerebral palsy subtype. It may often be better for children who are candidates to undergo epilepsy surgery. This is even with the consequence of worsening motor disorder, as uncontrolled epilepsy causes a bigger impact on the child's life.

Communication is another important problem. Inability to communicate affects every aspect of a patient's life. For example, one patient Dr Brunstrom saw was not even considered educable until, at 9 years old, he was able to get a communication system. Though communicating just one syllable with communication software can be equivalent to doing 20 push-ups, for some patients it is their only means of interacting with the outside world.

Visual abnormalities pose another quality of life issue in cerebral palsy, and it is unfortunate that some ophthalmologists consider some patients' vision "good enough" for cerebral palsy. Visual problems are both common and multifactorial and should be closely addressed. Recent evidence shows a correlation between type of visual disorder and severity of motor problems. Patients with very mild motor problems tend to have the same visual problems as the normal population, with congenital esotropia being the most common, though it is 2 to 3 times more common in cerebral palsy, whereas patients with more severe motor impairment

have visual problems less common in the general population, including ocular motor disorders and gaze apraxias that affect education. Furthermore, most patients without obvious vision problems still have some disturbance with visual perception, which greatly impacts their ability to be educated.

In the realm of cognition, there is little information. Children with cerebral palsy score poorly on IQ tests, but IQ tests are poorly designed to test a nonverbal child or one who does not have the fine motor skills to do certain tasks. Thankfully, progress is being made, and we are finding that even the most severely affected children, if time is taken to accurately test them, have nonverbal reasoning abilities. Conversely, highly verbal children are at risk of having undiagnosed nonverbal learning disorders masked by their high verbal ability.

Dr Brunstrom ended by discussing the comprehensive approach necessary to look after these patients. Every aspect, from motor problems to associated impairments to general health problems, must be addressed. At very best, patients can look forward to hard work, strengthening, exercise, and using assistive technology to try to get into the classroom. Hopefully, there will be a paradigm shift and there will be very specific treatment paradigms for specific populations with specific MRI findings or phenotypes. Lastly, Dr Brunstrom reminded all attendees of the importance of complete diagnostic studies to rule out other, possibly treatable, disorders that can mimic cerebral palsy.

## Questions and Answers

Audience: What is your opinion about videos to teach children sign language? I found it very helpful. I don’t know whether you’re aware of it or use it or not. It’s for different age groups.

Dr Brunstrom: In terms of sign language, it’s a useful skill if the patients actually have the manual dexterity to be able to use sign language. But the problem is many of these patients don’t even have the motor skills to use their hands to reach out and touch anything. So we have to find ways for people that can’t do that.

Audience: This is good for kids who may otherwise be better off with arms and legs but have major difficulty with their auto-musculature and have such a difficult problem at school that it seems to be helpful. And as far as that increasing inflammation is concerned I think that’s fascinating because I had a child with TORCH infection. The only thing he presented with was increasing hearing deficit and he was labeled ADHD. He came to me and we found out that he had hearing problems and when we tested it again he was going down. Gave him IVIG and that stopped his progression. Very interesting.

Audience: Dr Brunstrom, what is your opinion about the very severely involved patients with the new research on the brain computer interface that “60 Minutes” had a beautiful piece on and patients now with the EEG cap picking up the electrical discharges and converting into voice-generated responses.

Dr Brunstrom: I actually think that is very exciting and is going to likely be, if it can happen fairly quickly, the way for patients like Andrew to really have the best chance to communicate and get past all of his motor disabilities.

Audience: I have a newborn follow-up clinic targeted toward kids with neonatal injury of all types for the kids who don’t meet our newborn follow-up for under 1000 grams. And when we’re looking for kids who are at risk for cerebral palsy the first thing you see is no head control and tight arms and legs. It doesn’t seem to matter too much what the specific type of brain injury is. What’s the neurologic pathologic correlation? It’s such a classic picture.

Why don't these kids have head and trunk control? And it's the biggest problem that parents complain about, at least initially, and later on it's that they don't have good use of their arms or one arm, but initially it's head and trunk control. It's huge. And maybe it gets better over time and maybe there's a way to help it get better over time, but it doesn't seem to correlate with anything. But it's the earliest sign, at least in the clinic that I have.

Dr Du Plessis: Well, I don't know. Maybe Dr Volpe is better able to answer that. But there is a certain type of cerebral palsy, sort of the parasagittal injury, for example, where if you look at the homunculus the distribution of injury is right in an area where proximal upper limbs and the trunk are primarily affected. Other than that I can't say.

Audience: What's the localization of this? I can't figure it out. I see mostly term infants because the premature infants are followed in another clinic. But even kids I pick up along the way who were premature, a lot of these kids don't have good head control.

Dr Volpe: Well, it is the first thing that develops. I think if you're having--if part of the motor system is involved in the pathology—and it almost always is, then I think that that's what you would expect to see first. But I think in terms of the specific lesion, Dr Du Plessis' answer I would agree with completely. But I think it can be seen with other pathologies that affect the motor system, of which there are many, and I think it's because that is among the dominant early motor functions that the baby exhibits.

Audience: True and it's early, but it's persistent and problematic and I don't know if anybody's got an answer for how to help parents make that better.

Dr Johnston: Obviously needs some research. It sounds like you've stumped the experts.

Audience: Pathogenesis in the premature infant was very well-described and typically those children have a great degree of spasticity as opposed to the full-term children who turn out to have cerebral palsy that tend to be more dystonic. I was wondering if there's any speculation as to why. You showed us a lot of pathology in the thalamus and the globus pallidus in the premature infants who had PVL. How do you explain what's going on, or can you at least speculate why you see a lot of spasticity in the premature children and more dystonia in the mature children?

Dr Volpe: That's a very good question, and I think that it relates probably to the nature of the pathology as much as the location. When you look at asphyxiated term infants the most consistent pathology is in the putamen. So I'm not surprised that there should be dystonia with that kind of a syndrome. What I am perhaps surprised about is that it took us so long to really realize how consistent that is.

But I think that the premature infants' basal ganglia is not the overtly destructive phenomena that you see in the asphyxiated baby, and why should something that might be largely maturational trophic degenerative as in the premature versus the destructive disease of the term baby be manifested differently? But I think that must be at the core of it, but I can't explain it further than that.

Dr Brunstrom: I just want to say one thing, though. Even though term babies and the dystonia that you see with them, the generalized dystonia, even the athetoid movements where it's obvious they have dystonia, I would actually argue that a lot of premature babies with typical spastic cerebral palsy also have dystonia but it looks different when it's on top of spasticity than when it's dystonia by itself.

Audience: There was a recent article, I think it was in *JAMA*, talking about genetic susceptibility and predisposing a patient and prematurity. So I wonder whether anybody has some experience or more input.

Dr Brunstrom: I think that the thing is there's multiple different things that can predispose you to the brain injury and we're just in the early stages of seeing that there's genetic underpinnings that may make a baby more susceptible to any type of brain injury, whether they're premature or term.

Dr Johnston: We think one thing that is genetic is male sex makes you a little more vulnerable to cerebral palsy, so that may be one.

Audience: My question is an imaging question for Dr Edwards. That tract-based spatial statistics math that really looks amazing and fantastic—is it a DTI-based technology? My question is since it's a fiber tracking, if it's a DTI matter tracking and thalamus is a subcortical grey matter, how do you determine which fiber goes to which part of the thalamus?

Dr Edwards: Yes, it's a DTI-based technology and you need high-quality DTI images with at least 15, preferably 32 and, better, 64 directions. So you need high-quality data with no movement artifact. The conventional way or the original way of doing it was just to run a streamline, as I showed in the picture, between voxels. But in fact, that doesn't work very well because those streamlines die out very quickly for statistical reasons. So, what I showed you on the images was probabilistic tractography and purely technically those images are not tracts, they're the probability that a tract is in a certain area. So using that technique you actually get much better connection between regions you want to look at and that works pretty well. Most people are moving now away from streamlining to some form of probabilistic tractography.

There are some problems with it which are worse in the adult literature than they are in babies. One of them is the crossing fiber problem, which is the assumption that in any given voxel the fibers go in one direction, but if 2 fibers join that instead of having a direction you have a circle. But there are algorithms coming out now for that and, interestingly, babies don't have so much of a problem with that as adults do, probably because the superior longitudinal fasciculus is less well developed in the white matter. So with that technology you actually can get the probabilistic tracts to run into the grey matter a little bit. They don't go into cortex very well but they do go into the thalamus and you do actually manage to get a probability of where that tract would be based on the method which you've used, and you can adjust these to different constraints. All these techniques have assumptions behind them and I'm happy to talk about those assumptions in more detail if you'd like afterward, but they do go into grey matter if you use probabilistic techniques but not if you use streamlines.

Audience: With regard to your postmortem tissue findings, I know you compared them to control, but do you still think that the same mechanisms of microglia and inflammation are also a factor in those children or those premature infants that have very minimal medical complications? And also, regarding your olig2 staining in the area of the lesion, olig2 has also been shown to be increased and upregulated in astrocytes. That was just recently published in the past year and do you think that might actually be a factor?

Dr Folkerth: I'll answer the last one first. In our study, we did co-localization staining for olig2 and GFAP and it did not co-localize in our population, and in the study you're talking about I'm not sure if it's the one I'm thinking of, that it's strictly comparable to ours as an experimental model. The first one's a very good question. That's one of the hugest reservations we have about any of our conclusions because obviously normal babies don't

die, so what we're calling controls more properly should be referred to as cases that do not have the standard classical histologic evidence of PVL in the white matter. That isn't to say that they may not have other types of brain injury, in fact they do very often, but when we're doing the studies comparing PVL versus non-PVL that's basically the distinction we're making. And you're right. It's very hard to generalize. As I mentioned in one of my first slides, the autopsy incidence is very much higher than you might predict.

## Molecular Mechanisms in Injury to the Preterm Brain

Moderator: Pierre Gressens, MD, PhD, Hospital Robert Debré, Paris, France

### Apoptotic Mechanisms of Cell Death in the Brain

Henrik Hagberg, MD, PhD, Göteborg University, Göteborg, Sweden

Dr Hagberg discussed apoptotic mechanisms of cell death in the immature brain. There is enormous complexity in the pathophysiology of hypoxic-ischemic brain injury. The final outcome is determined by various factors, such as genetic background, developmental age, connectivity, and the presence of sensitizing or tolerance-inducing pre-exposures. Primary and secondary injury events are followed by regenerative and compensatory responses. Evidence shows there is a biphasic response to injury with an almost complete recovery of high-energy phosphates, mitochondrial respiration, and glucose-metabolizing brain tissue after primary injury, followed by secondary deterioration signifying secondary injury. Dr Hagberg and colleagues have focused on apoptotic mechanisms during this interval between primary and secondary injury, as this may be a clinically effective time to initiate therapy.

The most common morphologic response to hypoxic-ischemic injury is a mixed apoptotic and necrotic phenotype. The intrinsic pathway seems to be important, as hypoxic-ischemic injury causes permeabilization of the mitochondrial membrane. This leads to release of cytochrome-C, assembly of the apoptosome, activation of caspase-3 and caspase-9, and cell death. This activation of the intrinsic pathway is developmentally regulated, as animal studies show massive activation of caspase-3 in response to hypoxic-ischemic injury in preterm-equivalent brain that is not seen in more mature brain.

To assess the importance of the intrinsic pathway, Dr Hagberg and colleagues studied the effect of upregulation of XIAP, an efficient inhibitor of apoptosis family protein that blocks caspase-3 and caspase-9 and inhibits the intrinsic pathway. Upregulation of this anti-intrinsic pathway protein results in a remarkable decrease in caspase activation and resultant brain injury. This provides a possible target for therapy, though there have been discrepancies in the efficacy of broad-spectrum caspase inhibitors at reducing injury. The apoptotic process in the immature brain appears to be different in males and females, as data support a predominance toward the caspase non-dependent pathway in males and the caspase-dependent pathway in females. In fact, some studies show a particular caspase-inhibitor is only efficient in females.

Apoptotic-inducing factor translocates from the mitochondria to the nucleus following hypoxic-ischemic injury and correlates with development of brain injury. Like caspase activation, apoptotic-inducing factor is developmentally regulated, though it is translocation, rather than overall expression, that is increased in premature-equivalent brain in animal studies. Creation of an animal model that expresses 10% to 20% of normal levels of apoptotic-inducing factor correlated with a decrease in brain injury. Importantly, combination with a broad-spectrum caspase inhibitor provides significant additional protection, with up to a 70% total reduction of brain injury; thus, the caspase-dependent and the caspase non-dependent pathways act in parallel.

Cyclophilin A is a protein that assists apoptotic-inducing factor, and cyclophilin A-deficient mice are protected from hypoxic injury to a similar degree as apoptotic-inducing factor-deficient mice. Cyclophilin A and apoptotic-inducing factor co-translocate to the nucleus, and transgenic mice with a deletion of cyclophilin A are unable to move apoptotic-inducing factor into the nucleus. The present model suggests apoptotic-inducing factor is released from the mitochondria to the cytosol after hypoxia-ischemia, particularly in the immature brain, and binds to cyclophilin A in the cytosol. This complex transitions into the nucleus to help form the degradosome.

These studies suggest both the caspase-dependent and caspase-nondependent pathways are important, and it may be more efficient to develop a neuroprotective strategy at the level of preventing mitochondrial outer membrane permeabilization to block both pathways. There are 2 major pathways through which mitochondria could be permeabilized. The first pathway is through opening of a Bax-dependent channel in the outer mitochondrial membrane. The second is through opening of a membrane permeability transition MPT pore, as seen in ischemic insults in adult brain. However, while cyclophilin D (which regulates membrane permeability transition pore opening) knockouts showed a significant reduction in adult brain injury, the opposite was seen in immature brain injury. Deleting cyclophilin D in the neonatal brain increases injury, so cyclophilin D has an anti-apoptotic effect in immature brain tissue. Importantly, this effect is not gender-dependent, so is upstream of the gender-dependence mentioned previously. It appears that cyclophilin D deficiency evokes a greater increase in cytochrome-C release, suggesting it may increase mitochondrial permeability in the immature but not the mature adult brain. Furthermore, Bax translocation to the mitochondria is more pronounced in cyclophilin D-deficient mice, suggesting cyclophilin D prevents Bax/Bak assembly oligomerization and decreases mitochondrial permeabilization and cytochrome-C release. This evidence suggests Bax-dependent permeabilization is most important for permeability in the immature brain. Indeed, the Bax inhibitor protein provided a significant reduction of hypoxic-ischemic brain injury and caspase-3 activation.

The membrane permeability transition pore functioning is also developmentally affected. In the adult brain, membrane permeability can be elicited with 5 calcium pulses. Inhibition of cyclophilin D increases the number of calcium pulses needed. However, in the neonatal brain, 3 times as many calcium pulses were necessary to evoke mitochondrial permeabilization in control cells, and this was less sensitive to cyclophilin D inhibition. This suggests the membrane permeability transition pore is simply less sensitive in the immature mitochondria. Cyclophilin D shifts from being an inhibitor of membrane permeabilization during early life to being a critical inducer of membrane permeability transition-dependent permeabilization in the adult brain.

Lastly, to block mitochondrial outer membrane permeabilization and provide neural protection to the immature brain, Bax-dependent membrane permeabilization must be blocked. Unfortunately, the Bax inhibitor peptide discussed previously is not clinically useful. However, another regulator, caspase-2, shows promise. It is an upstream caspase and, in vitro, inhibition is able to block Bax-dependent mitochondrial outer membrane permeabilization. Fortunately, a selective caspase-2 inhibitor has been developed specifically to provide brain protection for the immature brain, and has already shown efficacy in neonatal models of excitotoxicity, hypoxia-ischemia, and stroke.

## Experimental Models of White Matter Injury

Stephen Back, MD, PhD, Oregon Health Sciences Center, Portland, Oregon

Dr Back discussed experimental animal models and theories of white matter injury. The past decade has seen great progress with determination of key triggers for injury, affected cell types, and molecular pathways. Current animal models reflect this progress and represent the now dominant forms of pathology seen in preterm infants, noncystic focal or diffuse white matter lesions. Ischemia reperfusion is an important mechanism of injury to the periventricular white matter in the preterm infant. This is for a number of reasons, such as the pressure-passive circulation of the preterm infant and the significant oxidative damage that selectively targets the oligodendrocyte lineage in the periventricular white matter. Importantly, major pathological features of white matter injury are reproduced in large preclinical animal models of cerebral ischemia.

During the past decade, Dr Back and colleagues have developed a global ischemia model in preterm fetal sheep. At two-thirds gestation, fetal sheep brain development looks very similar to premature 24- to 28-week human infants. These animals display a pressure-passive circulation, and a functional model of global cerebral ischemia is created using reversible bilateral carotid occlusion. This model generates the dominant pathology of noncystic focal and diffuse white matter lesions with relative sparing of grey matter. However, with increased ischemia, there is more extensive involvement of the cortex and basal ganglia, which can help explain the spectrum of pathology seen in patients. The strength of this model lies in the ability to monitor a wide variety of physiologic indices at the time of injury, including fetal heart rate, blood pressure, blood gases, and, importantly, cerebral blood flow. Cerebral blood flow is monitored through injection of fluorescently labeled microspheres into the fetal cerebral circulation at various study times, including basal conditions, ischemia, and reperfusion. This allows unbiased quantification and monitoring of cerebral blood flow to any region of the brain.

Using this model, it was found that blood flow under basal conditions in periventricular white matter is about two-thirds lower than overlying cortex. During ischemia, blood flow falls quite profoundly. However, the relative fall in both periventricular white matter and cerebral cortex is similar. Looking more closely at specific regions, no apparent gradients in cerebral blood flow were found when the periventricular white matter was segmented from deep to superficial layers. In comparing lesions with greater injury and cell death to regions with lower cell death, there was not a significant difference in the magnitude of cerebral ischemia. In fact, there is a lack of evidence to support the popular hypothesis that periventricular white matter is selectively vulnerable to ischemic injury due to vascular border zones; it actually appears that the relative severity of ischemia to the cortex is greater than that to the periventricular white matter.

The distribution of ischemic injury in fetal sheep white matter is not uniform; medial white matter is more damaged than lateral white matter. This may be due to cellular maturational factors. The oligodendrocyte progenitors that predominate through the high-risk period for white matter injury are homogenously distributed throughout the white matter in humans. However, in the fetal sheep model, there is significant heterogeneity of white matter development, with medial white matter, composed of predominately pre-oligodendroglial cells, more equivalent to that of a preterm infant. Conversely, the lateral white matter has greater numbers of oligodendrocytes and displays early myelination. Data illustrate that white matter injury severity coincides with oligodendrocyte maturation. The medial white matter is more vulnerable due to the predominance of pre-oligodendroglial cells, whereas the lateral, with more myelinating oligodendrocytes, is more resistant. In the context of the previous finding that there is no difference in cerebral blood flow under ischemic conditions between the medial and lateral white matter, it appears ischemia is necessary, but not sufficient, to explain the white matter injury seen. The topography of white matter injury is

related both to the maturational stage and distribution of susceptible cells, and oligodendrocyte maturation confers resistance to injury.

In acute hypoxic-ischemic injury, there is extensive, selective degeneration of pre-oligodendrocytes, but there is a surviving population. Paradoxically, however, the surviving pre-oligodendrocytes do not differentiate to regenerate myelinating cells in chronic lesions. This illustrates the importance of glial injury and chronic glial scarring in white matter lesions. The glial scar accumulates a variety of molecules and extracellular matrix, including hyaluronic acid, which have been shown to block maturation of pre-oligodendrocytes. In an ischemic-reperfusion rat model, the acute response to hypoxia-ischemia is massive degeneration of pre-oligodendrocytes, likely by a caspase-3-independent mechanism. There is also considerable delayed cell death through a caspase-3-dependent mechanism. Surprisingly though, in chronic lesions there is an increase in oligodendrocyte progenitors. Yet these oligodendrocyte progenitors are not myelinating, despite the presence of axons in these lesions. These cells have a remarkable regenerative capacity, but after injury, there is no return of function. In critically ill preterm infants, recurrent hypoxia-ischemia is a very common occurrence. Studies show a massive necrotic and apoptotic cell death response to a recurrent hypoxic-ischemic model with 2 separate insults not seen with a single insult. Normal white matter maturation confers resistance to hypoxia-ischemia, but pre-oligodendroglial cell maturational arrest in these chronic lesions confers persistent susceptibility to recurrent hypoxia-ischemia.

## Excitotoxic Mechanism of White Matter Injury

Frances Jensen, MD, Harvard University, Boston, Massachusetts

Dr Jensen presented work focusing on the role of excitotoxicity and developmental patterns of neurotransmitter expression as one of the multifactorial mechanisms of preterm brain injury. Glutamate is the major excitatory neurotransmitter in the brain. Receptors are not just in neuronal synapses, but on non-neuronal cells as well, and may play an important role in neuronal-glial and glial-glial signaling. Receptors for glutamate are variably present in the developing brain and the unique pattern present in the premature window offers potential for therapeutic targets.

Glutamate receptors are involved in important signaling cascades, usually in terms of excitatory neurotransmitters that involve calcium and downstream signaling cascades important for such things as memory and synaptic development. However, with too much excitation, these cascades can lead to upregulation of calcium-dependent pathways involved in free radical formation and apoptosis.

Many studies have shown a pooling of extracellular glutamate under conditions of both hypoxia/ischemia and sepsis, 2 clinical causes of injury to the preterm brain. In vitro evidence shows that pre-oligodendroglial cells are intrinsically vulnerable to oxygen-glucose deprivation and excitotoxicity. There is a role for glutamate receptors not only in oligodendrocyte injury, but potentially also in injury to neurons, subplate neurons, microglia, and astrocytes.

Glutamate receptors are developmentally regulated. They are present very early in radial glia and subplate neurons, are next expressed on premyelinating oligodendrocytes, microglia, and cortex, and are then finally expressed around synapses at term. In premature oligodendrocytes, data shows upregulation of many of the glutamate receptor subunits, and receptors are even functional. Importantly, the peak expression of glutamate receptors occurs during the peak window of vulnerability to oxidative stress.

Both NMDA and AMPA receptors are heteromeric, and subunit composition of glutamate receptors is important, as the presence of different subunits affects the functionality of the receptor. In the adult, NMDA receptors are the major calcium-permeable neuronal receptor; AMPA receptors lack calcium permeability. However, the premature AMPA receptor lacks GluR2 and is calcium-permeable, and can therefore play a role in calcium-regulated features of excitotoxicity and indication of oxidative stress. During development, there is upregulation of GluR2-deficient AMPA receptors on both neurons and glia. NMDA receptor subunits are also developmentally regulated, with NR2B upregulation causing increased duration of calcium channel opening, and NR3A upregulation decreasing magnesium block and causing an increase in the frequency of channel opening. There is upregulated activity of both AMPA and NMDA receptor activity during development, which suggests excitability is important for the developing brain. This increased excitability, however, lends itself to increased excitotoxicity.

With this context of increased excitability in the preterm brain, glutamate receptor antagonism is appealing as a potential therapeutic target. Animals treated with NBQX, an AMPA receptor antagonist, after a hypoxic-ischemic injury displayed preservation of white matter. Unfortunately, NBQX is not available for human use, but there is a drug with FDA-approval that is a noncompetitive AMPA receptor antagonist, topiramate. Animals treated with 30 mg/kg/dose of topiramate after ischemic injury showed relative preservation of white matter. NMDA receptor antagonists were also evaluated. Memantine is an NMDA receptor antagonist and is already being studied in dementia trials. Animals treated with memantine after ischemic injury showed significant improvement in cell survival. More importantly, there was also long-term improvement evidenced by decreased cortical thinning in response to injury.

There are already drugs on the shelf that may offer protection from excitotoxicity-mediated injury. Though safety issues have not yet been studied, data shows no constitutive apoptosis with either topiramate or memantine. Hopefully, these drugs will be considered for evaluation in further preclinical trials. However, there is a reason for the variable expression of glutamate receptors during normal development, so treatment windows may be necessarily brief.

## PET Imaging of Neuroinflammation

Diane Chugani, PhD, Children’s Hospital of Michigan, Detroit, Michigan

Dr Chugani discussed advances in PET imaging of neuroinflammation. The fetal inflammatory response to infection and inflammation causes blood-brain barrier permeability and stimulation of fetal microglia that produce cytotoxic metabolites. These damage oligodendrocytes and lead to PVL and cerebral palsy. With the development of probes that attach to activated microglia, this inflammatory process can now be imaged.

Microglia are present in high density in the white matter of developing brain, during the window of susceptibility to PVL. Microglial cells possess certain properties at rest, which change with activation, such as in the fetal inflammatory response. One such dynamic change is an unveiling of a peripheral benzodiazepine binding site on mitochondria. This site binds ligand PK11195, which is labeled with carbon 11 and used as a PET probe in a number of neurological disorders associated with brain inflammation. PK11195 normally binds minimally to normal brain with microglia in the resting state, and increased PET signal represents activated microglia and inflamed brain tissue. PET images can be co-registered with MRI images with specific markers to allow regions of activation to be seen in great detail.

Dr Kannan and colleagues used PK11195 to study the neuroinflammatory response in neonatal rabbit kits in which lipopolysaccharide had been previously injected along the maternal uterine wall. This animal model produces 1-day postnatal kits with hypertonia and deficiencies in feeding and ambulating. In control animals injected with PK11195 ligand, the PET signal is higher within 10 minutes of injection than after one hour, indicating the ligand has not bound to receptors and has been washed away. However, the opposite is seen in endotoxin-treated animals; there is increased activity later in the scan, as the PK11195 has bound to receptors in activated microglia. Interestingly, with increasing endotoxin doses, the initial PET signal decreases, indicating possible decreased cerebral perfusion. Due to this discrepancy in initial values, to accurately compare data, the slope, rather than absolute values, of the retention of the tracer was used as an index. Data showed a relationship between the slope of signal retention and neurobehavioral scores, with increasing slope correlated with increasing impairment. Hind limb hypertonia had the best correlation with the PET scan. To correlate these PET findings with pathology, brain slices were stained for microglia, and there was an endotoxin dose-dependent increase in the number of microglia cells and changes in microglia morphology.

Diffusion tensor imaging may also be useful in imaging of neuroinflammation. Endotoxin-treated animals show a significant decrease both in parallel diffusion and in fractional anisotropy, with no change in apparent diffusion coefficient. The populating of the white matter tracts with microglia may be responsible for this change in diffusivity. When parallel diffusion and fractional anisotropy are compared with the ratio of activated microglia to total microglia in different brain regions, data show that increasing number of activated microglia correlate with a greater change in parallel diffusivity.

Ongoing inflammation may be playing a role in the pathology of injury. Data from a longitudinal study shows the PK11195 retention slope is very high on day 1, is decreased on day 4 and 5, and is largely normal by day 8. If this course can be altered, cerebral structures may be able to regenerate and repair. Animals were injured with endotoxin as described previously and were then treated with minocycline for 3 days postnatally and imaged on postnatal day 5. Minocycline-treated animals showed less retention of tracer than control animals. Furthermore, neurobehavioral testing of minocycline-treated animals on postnatal day 8 showed improvement in movement deficits, with treated animals able to hop more similarly to uninjured animals.

In closing, PET imaging of neuroinflammation is starting to be applied to human infants born to mothers with a history of chorioamnionitis. In one particular case, an infant born to a mother with severe chorioamnionitis was initially asymptomatic with no evidence of sepsis; however, the infant suffered intraventricular hemorrhage within one week. PET imaging of this neonate using PK11195 showed increased labeling throughout the brain compared with control adults, with particularly increased labeling at the ventricular wall at an area of increased MRI signal intensity. Importantly, this was compared with normal adult brain, as there are not yet data from age-matched controls. There is still much work to be done before PET imaging of neuroinflammation is clinically useful.

## Controversies & Unanswered Questions

Moderator Janet A. Brunstrom, MD, St. Louis Children’s Hospital, St. Louis, Missouri

### Rehabilitative Therapies: Do They Improve Outcome?

Diane Damiano, PhD, PT, Washington University, St. Louis, Missouri

Dr Damiano described the scientific evidence for traditional and newer rehabilitation strategies for traumatic brain injury, and their potential to improve motor coordination and promote neural recovery in affected children. The International Classification of Functioning approach of considering an individual's ability to participate in daily activities in addition to body structure and function provides a practical way to measure improvement seen with physical therapy. Classically, it has been thought that physical therapy, along with medical interventions like surgery, functions to keep children with cerebral palsy on their initial developmental track. So, although children do not usually increase their motor function beyond their predicted capacity, appropriate interventions keep them from falling off their expected progress curve. New evidence suggests that with appropriate physical therapy, patients may actually surpass initial functional expectations.

Neurological motor physical therapy is the use of exercises to restore motor function by improving motor patterns and overcoming motor deficits. The goals of physical therapy include maximizing a child's function and participation given their neurological status, preventing deterioration or further deformity, educating the family on positioning and handling of their child and available resources, and recommending assistive devices and orthoses and teaching families how to use them. Many of these areas have not been studied extensively, and Dr Damiano emphasized the need for the application of evidence-based practice in physical therapy to reduce the variability between therapists and outcomes.

The first major randomized controlled trial on physical therapy was published in 1988 by the Kennedy Krieger Institute and it compared neurodevelopmental therapy, the then dominant form of physical therapy, with an infant-stimulation program not used by therapists. The study concluded that neurodevelopmental therapy offered no short-term advantage over infant stimulation and did not decrease the incidence of contractures or the need for orthopedic therapy. Again, in 2001, the American Academy of Cerebral Palsy published a study in *Developmental Medicine* showing that neurodevelopmental therapy did not confer any advantage over the alternatives. Despite this evidence, neurodevelopmental therapy is still used in cerebral palsy in the United States, which is perhaps a wasteful use of limited medical resources and time.

A more recent review of physiotherapy evidence, including conductive education, did not show sufficient evidence to support comprehensive physical therapy. Four high-quality reviews of more focused interventions showed evidence in support of strength training to improve muscle strength, upper extremity training for bimanual performance, hippotherapy to improve muscle symmetry, and training to improve reactive balance. Dr Damiano focused on muscle strengthening as one example of the benefits of evidence-based physical therapy. Muscle weakness limits mobility by both a primary and a secondary effect; primarily, there is a reduced ability to produce force due to the central nervous system insult, and secondarily, inactivity amplifies the existing weakness. This secondary effect is most pronounced in patients with decreased mobility. Treatments used to control spasticity in cerebral palsy patients may also weaken the muscles, and this is an additional source of weakness that must be avoided and addressed.

Of the impairments seen in cerebral palsy, strength has the greatest correlation with activity, gait speed, and gross motor function measures. Strength training increases muscle strength and may improve kinematics and self-perception of physical performance and participation. The results for increased gait speed, wheelchair propulsion, and gross motor function measure are less consistent. Dr Damiano stressed that muscle-strengthening exercises must become a lifestyle rather than isolated periods of physical therapy to provide their maximum benefit.

Constraint-induced movement therapy is also strongly supported by available evidence, including a review in *Neural Plasticity* that suggests that its benefits are not necessarily from the restraint but rather from mass practice. A Cochrane review of constraint-induced therapy on the upper extremities of children with cerebral palsy concluded this is a promising but still experimental approach.

Body-weight supported treadmill training is highly popular in neural rehabilitation and in pediatric practice, but little evidence is available for its use in the pediatric population. Of the 17 studies of body-weight supported treadmill training in children with central nervous system injury (the highest level of study was a cohort study with 7 children in each group), several showed support for increased gait speed or gross motor function measure and several studies showed no significant effect. The studies varied in duration, speed, additional therapies, and body weight support. These data again suggested that the time spent doing physical activity was more important that the specific activity in producing results.

Finally, Dr Damiano discussed promising results in motor-assisted cycling therapy to improve motor coordination and walking. Motorized bicycles can be used by families in their homes and are monitored with computer chips, and the action of cycling is similar to walking in frequency and shares similar neural circuits. Ten children with cerebral palsy, many with severe spasticity and dystonia, were enrolled in this motored cycle pilot study; they were cycled at 50 revolutions per minute without resistance and showed promising improvements.

In concluding, Dr Damiano suggested that the current goals of physical therapy in children with cerebral palsy should include prioritizing treatments that are supported by the highest levels of evidence and terminating those that do not work, and developing collaborations with neurologists and neuroscientists to study the function of exercise in neural recovery.

## Activity-Dependent Cortical Plasticity

Monica Perez, PhD, PT, Kennedy Krieger Institute, Baltimore, Maryland

Dr Perez discussed several studies on temporary electrophysiologic changes observed in response to activity. These may occur at every level of the nervous system, though her talk focused on the primary motor cortex, secondary motor areas, and the spinal cord. Two training paradigms were used in these studies; the first is a series of sequencing movements of the fingers that subjects learn by practicing for a half hour to one hour, and the second is a complex series of movements with the lower limb. The lower limb is particularly interesting in the context of central nervous system injury because cerebral palsy patients exhibit coactivation of antagonistic leg muscles during the stance phase of locomotion and abnormal gait reflex modulation.

Subjects were trained to perform a complex movement with the ankle muscles while in the seated position. Recording electrodes were placed at the tibialis anterior muscle, which receives more corticospinal input than the other muscles of the lower limb, and the soleus muscle. Transcranial magnetic stimulation was used to evoke a response in these 2 muscles before and after execution of the task. Three groups were used in the study; the first learned and performed this complex motor task and the second practiced the same number of repetitions and degree of displacement of the ankle joint but performed a simple task. Therapists passively performed dorsiflexion and plantar flexion in the third group. Only subjects in the complex skill training group showed a significantly decreased number of errors after training. This group also had increased amplitude and area of responses by the tibialis anterior muscle to transcranial magnetic stimulation. These changes in excitability

lasted approximately 15 to 30 minutes after training. The groups that performed the simple task and passive training did not show any similar changes in excitability.

Complex task acquisition also triggered temporary local changes at the level of the primary motor cortex. Electrical stimulation of the leg representation of the primary motor cortex produced changes in the amplitude of the motor-evoked potential similar to those produced by transcranial magnetic stimulation. This suggests that the observed changes may be mediated at the level of the primary motor cortex. Training was also shown to decrease the magnitude of intracortical inhibition in the leg representation of the primary motor cortex in one subject. The magnitude of facilitation did not change. Cortical excitability also appears to return to baseline within 30 minutes of training.

To look for similar changes at the level of the spinal cord, the soleus H-reflex was tested after posterior tibial nerve stimulation. A peak-to-peak amplitude decrease of the soleus reflex was observed in the subject who performed the complex acquisition task. The slope of the soleus reflex recruitment curve also decreased after the acquisition task in this patient, but the changes in excitability lasted only 15 to 20 minutes. No changes were observed in the subject who performed the control task. Motor skill acquisition appears to mediate altered spinal cord activity by presynaptic changes at the 1A sensory fibers. Although sensory filtering was observed at the level of the spinal cord, it does not appear to occur at the somatosensory cortex. Corticomuscular coherence was then used to assess corticospinal drive to the motorneurons, and similar temporary changes were observed in the subject performing the complex acquisition task. These findings may be applied to patients with a central nervous system lesion who may experience flexor paresis, extensor spasticity, decreased corticospinal drive to ankle dorsiflexors, and decreased presynaptic inhibition between agonist and antagonist muscles.

A similar set of studies focusing on a task performed with the digits of the dominant upper extremity showed an increased peak-to-peak amplitude of responses in the motor cortex that participates in active learning, but not in the contralateral motor cortex. Conversely, intracortical inhibition tended to decrease bilaterally. Overall, this work suggests that modulation of corticomuscular excitability of specific muscles that are engaged in a motor task occurs in motor learning, and that these changes may serve to filter some sensory inputs that can impede learning.

## Neuroprotective Interventions: Is it too Late?

Doe Jenkins, MD, Medical University of South Carolina, Charleston, South Carolina

Dr Jenkins spoke about the time course of hypoxic-ischemic central nervous system injury as it relates to neuroprotective interventions. In general, hypoxic-ischemic injury occurs as a series of known steps beginning with ATP depletion and disruption of oxidative metabolism. Next, reperfusion results in continued free radical damage, cytotoxic edema, necrosis, and glutamate toxicity. A variable latency period follows, during which phosphate stores recover. Lastly, during the secondary energy phase, oxidative stress and glutathione depletion, along with loss of glial function and neurotropin, leads to apoptotic and necrotic cell death. It is now clear that neuroprotective mechanisms must be initiated before this secondary phase to successfully prevent massive cell death.

The actual time window for effective neuroprotection is difficult to define. In term infants, hypothermia studies have shown that the neuroprotective interval is approximately 0 to 6 hours after moderate to severe hypoxic-ischemic injury. This may be shorter in preterm infants because of their lack of antioxidant reserve and other complicating disease processes.

Dr Jenkins stressed that each neuroprotective agent will have a unique time course and effective window based on its mechanism of injury.

Several animal trials of neuroprotective interventions have shown that early intervention provides maximal effectiveness. A rat study of NF-kb accumulation in 7-day-old rats who were subjected to hypoxic-ischemic injury showed a biphasic mode of NF-kb induction, at 0.5 hours to 6 hours after injury and again at 12 hours to 24 hours after injury. Significant neuroprotection was observed in animals treated with the NF-k inhibitor NBD, an NF-kb antagonist, at 0 hours, and at 3 hours of reperfusion. However, if the same agent was given at 0, 6, and 12 hours, the neuroprotective effect was lost and the injury was actually more severe than in sham-treated animals. This suggests that neuroprotection by NF-kb inhibition is only effective as a very early intervention. The same pattern was observed for cytokine expression—including tumor necrosis factor-alpha and interleukin-1β—and for microglial activation, with very little activation with early NF-kb inhibition and no difference in activation from the vehicle in early and late-treated animals. It is important to avoid interfering with reparative processes while attempting to achieve optimal neuroprotection.

Several antecedent processes in utero may change the time course of hypoxic-ischemic injury and neuroprotection. These include chronic hypoxia and chorioamnionitis, which is especially significant in preterm infants. Chorioamnionitis causes a significant fetal inflammatory response and is the major perinatal risk factor for white matter injury in newborns. It leads to a 9-fold increase in otherwise idiopathic cerebral palsy in term infants and a 3-fold increase in preterm babies. In a model for chorioamnionitis, rat pups exposed to E. coli lipopolysaccharide in utero who then sustained a hypoxic-ischemic injury at postnatal day 7 had a large area of infarct compared with the selective neuronal necrosis observed in non-lipopolysaccharide exposed pups. A similar study in sheep showed that lipopolysaccharide-treated sheep did not recover from a short hypoxic-ischemic injury as well as control animals. These findings suggest that lipopolysaccharide endotoxin, and by extension chorioamnionitis, may sensitize the central nervous system to hypoxic-ischemic injury, which is common during normal delivery. Early neuroprotective intervention, especially in neonates with chorioamnionitis or another prenatal inflammatory condition, may attenuate potential hypoxic-ischemic injury.

Dr Jenkins then described studies of N-acetylcysteine, a glutathione precursor and an antioxidant, as a potential neuroprotective agent. N-acetylcysteine significantly reduced the area of injury and pathology score of the tissue when given 0 versus 24 hours after hypoxic-ischemic injury in lipopolysaccharide-exposed animals. No neuroprotective effect was observed when N-acetylcysteine was given 2 hours after injury, so the window for treatment after injury is very short. Additionally, significant neuroprotection was observed if the treatment was applied before injury, and lipopolysaccharide-induced interleukin-6 expression in amniotic fluid was attenuated if N-acetylcysteine administration preceded the exposure. N-acetylcysteine given shortly after hypoxic injury in 4-day-old piglets also showed effects on preserving cardiac output and cerebral autoregulation compared with saline-treated animals. In this way, the secondary insult of hypoxic-ischemic injury may be reduced or prevented. N-acetylcysteine provides nearly complete neuroprotection if given at immediately after injury, and the neuroprotective effect declines with time. It inhibits lipopolysaccharide-induced inflammatory mediators and apoptosis of oligodendrocyte precursor cells, which remain functional.

Lastly, Dr Jenkins described the challenges of designing a clinical trial of antenatal N-acetylcysteine administration and neuroprotection. This agent is already FDA-approved for pregnant women, and some pharmacokinetic data is available for it in preterm infants. However, the pharmacokinetics in adults and its distribution throughout various

compartments, including the mother's blood, the placenta, the fetal blood, and the fetal central nervous system, are not clearly understood. These will be evaluated by a National Institutes of Health-funded pilot trial of N-acetylcysteine administered prenatally within 4 hours of onset of maternal fever. Preterm and term infants will also be treated, and inflammatory markers, white matter damage, and their neurologic outcomes after 12 months will be measured. The goals of this study are to safely delineate the pharmacokinetics and any neuroprotective effects of N-acetylcysteine in this population.

## Possible Role for Stem Cell-Based Therapies

John McDonald, MD, PhD, Kennedy Krieger Institute, Baltimore, Maryland

Dr McDonald discussed a variety of roles for stem cells as potential treatment and research tools for nervous system injury. Regardless of its cause, the demyelination, cell death, and loss of connections observed in brain and spinal cord injury are shared features. Although a lesion may appear grossly localized, microscopic injury often extends far from the lesion and progresses over time. This continued damage may result in accelerated nervous system aging or impaired development in the pediatric population. Even in the most severe cases of spinal cord injury, functional connections are still present across the lesion and can be identified with diffusion tensor imaging. The initial goals of stem cell therapy should include finding ways to protect and use these remaining connections, overcoming the inhibitory scar that exists around a lesion, and stimulating regrowth or neuronal sprouting by growth factor release and environmental modulation.

Dr McDonald first focused on the goal of optimizing remaining connections. Dysmyelination and resulting misinformation seem to impair function beyond what is suggested by the anatomical damage. Current phase 1 clinical trials are investigating the potential of stem cells, both embryonic and other, to facilitate remyelination and restore appropriate function to remaining connections. Transplanted cells can be used to replace damaged nervous system cells, including neurons, glia, and vasculature, but they also appear to have a significant neuroprotective role. Cell transplants after a central nervous system injury can reduce delayed cell death by producing growth factors such as brain-derived neurotrophic factor. This function does not require prolonged stem cell graft survival, making it more immediately feasible than nervous system element replacement. When transplant is done in the acute phase of injury, the neuroprotective effects of stem cells are similar to using NMDA or AMPA receptor antagonists. Enzyme replacement also holds a great deal of promises in diseases such as Batten disease or non-ketotic hyperglycinemia, where an enzyme rather than a cellular element is needed.

As a research tool, stem cells are offering the potential to create models for human diseases that could not previously be modeled in animals. A technique called induced pluripotency allows us to create cells similar to embryonic stem cells from human-based disease cells. In mice, stem cells from the inner cell mass of a state similar to the blastocyst are differentiated and purified into highly migratory, tri-potential neural progenitor cells, which when transplanted into an adult rat with spinal cord injury almost completely fill the lesion and can migrate up to a centimeter one week after implantation. The majority of these cells become oligodendrocytes, but they may promote neuronal and astrocyte growth. Using MRI imaging, it was observed that these cells can afford the animal a level of behavioral recovery beyond what is expected for the numerical change in cell numbers. The mechanism of this behavioral recovery is not yet clearly understood.

Stem cells can further be used to optimize the environment at the site of nervous system injury and to promote survival of endogenous cells. Several possible mechanisms have been suggested, including modulation of the inflammatory response and production of

metalloproteases that digest the inhibitory extracellular matrix, allowing for migration of endogenous and implanted cells. In a rat spinal cord injury model, animals that received an embryonic stem cell transplant one week after injury had a dramatically reduced cellular inflammatory response. This effect may be important in the functional recovery observed with stem cell-transplanted animals. A series of studies have demonstrated that the transplanted cells make metalloproteases, which allow them to tunnel through extracellular matrix inhibitors like chondroitin sulfate and to travel through normally inhibitory white matter scars. Endogenous cells can also migrate in the presence of engrafted stem cells.

Modulating neural activity at the zone of injury may also optimize the nervous system environment for stem cell transplantation and recovery. Reduction in neural activity below the level of a spinal cord injury reduces activity-dependent cell growth, survival, migration, myelination, and maintenance of connections. In an animal model with complete spinal cord transection, functional electrical stimulation below the level of the lesion stimulates the activity-dependent indices of repair listed above. Conversely, baclofen, which is commonly used to manage spasticity, reduces these indices significantly. It reduces the birth and survival of new cells and impairs behavioral recovery and the remyelination typically observed after acute injury. This effect persists after termination of baclofen treatment.

A human trial of functional electric stimulation in patients with severe spinal cord injury also showed promise in terms of behavioral recovery. Patients had complete impairment, ASIA level A spinal cord injury, and used a functional electric stimulation bike for one hour per day, 3 days per week over a 3-year period. Test patients experienced reduced spasticity, and 50% were able to discontinue baclofen use, while 90% were able to reduce their therapy. Additionally, 70% of patients experienced improved motor neurologic scores compared with the control group.

In summary, Dr McDonald highlighted promising applications of stem cells that are on the horizon. He stressed that they can be used in a variety of ways, including nervous system cellular replacement, enzyme replacement, growth factor repair, and neuroprotection. However, for any of these uses to reach maximal effectiveness, the environment at the site of injury must be optimized.

## Questions and Answers

Audience: Two questions. Number one, we've heard that hypothermia works in full-term babies, has it been tried in preemies and if so how far down? And my second question is, has N-acetylcysteine been used or can it be used for patients who have just had hypoxic injury without cytokine superimposition?

Dr Jenkins: Systemic hypothermia or whole-body cooling is probably not the best idea for preterm babies. A long time ago we learned that preterm babies who were cold don't survive as well. But the head cooling is being investigated in that manner. Dave Edwards could probably answer that better than I could. We do whole-body cooling but there are still safety issues involved with that as well. As far as N-acetylcysteine for hypoxic-ischemic injury, we are investigating that in the lab right now.

Audience: I had a question for John McDonald. I've had a couple of patients, one of whom went to China for a stem cell transplant. What is your opinion about this? How is this being done in China, and how long before this comes to fruition here in the United States? And, what do we tell our patients in the meantime regarding these issues?

Dr McDonald: I'll tell you what I actually tell them. The majority of the access to stem cell transplantation around the world is not through a clinical trial; you have to pay for it, which

means that there is no proven efficacy yet. There are risks. And it's important that the patients understand that there's actually enhanced risks. So in many of the sites, their rate of infection and the rate of surgical complication is much higher than we're achieving here, and they need to understand that in helping them make their decision. They also need to understand that participating in something like that may obviate them from participating in clinical trials later.

Dr Johnston: John, how about going to Duke for cord blood cells?

Dr McDonald: Yes, it's the same approach for cord blood cells.

Audience: There were some talks today suggesting that the injury that occurs to the preterm brain can be an ongoing one and, in fact, there was mention of a persistent oligodendroglial cell type that allows for ongoing injury. What effect does exposure to hyperbaric oxygen therapy have in these children? And do we have an opinion about hyperbaric oxygen therapy for the children with cerebral palsy?

Dr Johnston: I think there is actually a new paper on hyperbaric oxygen killing cells through oxidative stress. I think it has the potential, certainly in young infants. Maybe in older children it wouldn't matter, but there's certainly that potential downside.

Dr McDonald: Yes, I think it's a double-edged sword and we don't have a really good understanding of the exact positive effects depending on when the treatment is done. So it falls into a similar category as stem cell transplantation, and I would approach it with a patient in the same way. Thinking of cell death, you brought up that cells are dying long after the injury, and remember, cell death is a constant feature of the nervous system—so potentially, these treatments can impact either of those things.

Dr Edwards: I have a question for Dr Jenkins, if I may. Since the LPS endotoxin you used is primarily E. coli, can you provide any evidence that exposure to E. coli is common in babies who have chorioamnionitis?

Dr Jenkins: In our country, it's very common. It is now the number 1 cause of sepsis in newborns in the U.S.

Dr Edwards: So can I refer you to Romero's recent paper, which shows no E. coli?

Dr Jenkins: I understand that, but when we culture the newborn blood, E. coli is the most common.

Dr Edwards: That's not the question I asked.

Dr Jenkins: I understand. There is a break between actually culturing it in utero and culturing it in the baby. So you are right. We have a gap there in our knowledge, but your point is well-taken.

Audience: Dr McDonald, your stem cells transplant talk focused on just embryonic stem cells, but any thoughts about neural progenitor cells, mesenchymal stem cells, the cord blood you mentioned at Duke, or bone marrow? Also, can you offer any thoughts on a time window for treatment from the point of injury and a route of administration?

Dr McDonald: Yes, in my talk I used embryonic stem cells as an example because they are the best scientific tool of discovery because we can make modifications. However, the concept that this is not just replacement but that the cells can do many other functions is a common feature of all of these cells. I think in terms of timing, the majority of cell

transplantation in the animal studies has been done acutely. Less has been done in the subchronic and even less has been done in the chronic phase. Then, under any of those conditions, tracking those cells for a long time hasn't been done yet. The majority of the studies only track them for a short period of time. I think we should be cautious about assuming what the mechanisms are.

Audience: This is also for Dr McDonald. I am wondering whether human leukocyte antigen (HLA) incompatibility between stem cell and the host plays a role in the cell death.

Dr McDonald: Yes, the question is about HLA involvement in efficacy of transplant or cell death. Many studies have demonstrated that it is important. I know that the Geron clinical trial suggests that it's not very important, they developed one early immunocompetent embryonic stem cell lacking HLA markers and even when the cells become differentiated they lack markers. It's likely it's going to be an issue, and I think the largest volume of research clearly demonstrates that some relative matching is going to be important. I think that's accomplishable. It is doable, for example, to develop embryonic stem cell lines like blood groups. We may need many more than 4 groups to transplant most people, but the big advantage of embryonic stem cells is once all the work has gone into that human genome-like project, those cells can be used for studying every organ and can be used for transplantation across all organs. I think that's really going to be a key goal.

Dr Maria: This is more of a clinical or applied question for Dr Brunstrom and Dr Damiano. Many families have very high expectations of what their children can accomplish and pour a lot of resources into therapies and assistive devices in terms of communication and multidisciplinary efforts and all. Jan, I sent you several of my patients who had high expectations who were very happy with the visit. So the question is what are reasonable expectations that we can be sharing with our patients regarding the return on physical therapy and occupational therapy, and in trying to encourage the use of electronic boards for communication? Is it reasonable for the family to notice dramatic gains in the way of speech and language, communication, PT (physical therapy), and OT (occupational therapy) intervention?

Dr Damiano: I will just answer for physical therapy. The one thing that I think is reasonable now is changes in activity. So the key thing that I tell parents is that they just need to get their kids active from the youngest possible age and find ways to do that. I think that's very reasonable and then to make lifestyle changes—this does not mean you're sent to therapy for the rest of your life, but this is something you need to do the rest of your life. The reason a lot of the things we do are important is evident when we look at adults who stopped walking at young ages. So we have to really think of longer-term strategies that people can make part of their lives. The problem is that parents do want to do everything they can, but sometimes that may just be letting your child have the most normal childhood possible despite the fact that they have a disability.

Dr Brunstrom: I think we have grossly underestimated the ability of a lot of these patients, even the ones who are very, very severely impaired, and that is because of all the layers of things that are wrong with them that all get attributed to this big black box. By selectively doing our best to treat what we can, but still trying to keep a balance, we do see dramatic improvements in patients from a whole host of patients that have learned how to use communication devices and are shocking teachers and other people in terms of their ability to learn, to patients that are Gross Motor Function Classification System (GMFCS) 5 that have been relegated to wheelchairs and are now able to take steps with assistive walkers. And no, they're not running around as their primary means of mobility, but it improves their

overall health, it reduces their risk for bone fractures, and multiple other things, but this is still something that needs a lot of research.

The other point here, as we have seen with the stem cell discussion, is that these families are desperate. I get asked at every single clinic about stem cells, and sometimes they don't even listen to me because they're going to do it anyway because they're so desperate. So we really have to work harder to answer these families and give them something to hold onto with evidence behind it, because they're going to do what they think they have to do anyway.

Audience: I just wondered if Dr McDonald could expand on his comments about baclofen and the double-edged sword of baclofen therapy exacerbating weakness and yet helping spasticity. Certainly, the children who benefit the most from baclofen are the ones who are the most spastic and usually pretty distant from the time of injury so probably beyond a time when there's a lot of active regeneration going on, I would guess.

Dr McDonald: Well, I'm sorry if I appeared anti-baclofen, but I just mean we need to use it carefully. As you know, many of the most incomplete patients, those who are beginning to walk or who have a lot of function oftentimes have the worst spasticity. I think using baclofen under conditions where that individual gains function is good. I think there's other ways to manage spasticity in the majority of individuals that don't gain function by taking those medications. Obviously, the baclofen pump has been a revelation in our field and it has aided a lot of people with spinal cord injury. It allows a lot of people to walk and it's a good use there, but we don't want to overuse it. The majority of individuals are willing to put up with some spasticity and we don't need to send them home on the maximal dose, but oftentimes individuals are already taking the maximal dose by the time they first come to your clinic. Their dose has been increased in the hospital, so it can be slowly decreased and activity and alternative approaches to control the spasticity can be tried as well.

Audience: I have 2 questions about stem cells. First, should parents now be told to bank stem cells or blood from their babies? Second, now that we know so much about epigenetic modulators of gene expression in imprinting, is that a factor that you consider as you're considering stem cell therapy?

Dr McDonald: We don't recommend to our patients that they should bank their cells, but we often get calls from people that absolutely want to bank them and they can afford the amount of money that they're spending, and then I think it's OK. Epigenetics is a major area of interest and a giant impact on these cells and is going to play a very, very, very big role in how well the transplanted cells do what we want them to do and even in immunorejection. I think that realization just over the last 5 years gives epigenetics a dramatically more important role.

Mindy Aisen, MD: This also applies to cord blood. I'm from the Cerebral Palsy International Research Foundation so my day is full of these kinds of phone calls like Jan Brunstrom mentioned, especially because it's been on television and there's going to be a People magazine article about cord blood. I'm not sure it's a bad idea to bank cord blood because it's clearly valuable in the child whose bone marrow is wiped out in chemotherapy. It seems that stem cells don't cross the blood-brain barrier and there's so few of them in the cord blood that it doesn't seem like an appropriate therapy for the not thoroughly irradiated. What do you think the mechanism of improvement is in these isolated cases we're seeing on the news? Do you think it's a growth factor? Children develop, including children with cerebral palsy, and they improve spontaneously and placebo is a powerful effect. So what do you think is happening with cord blood infusions that are not causing infections and are not cord blood?

Dr McDonald: Well, let me handle different things. The general things around the world, where they're using cord blood like an injection of a molecule, it's not clear at all what's associated. Those are uncontrolled things. There is a placebo effect. Oftentimes, kids are participating in rehabilitative treatment that itself can provide those benefits. But obviously, the cells don't necessarily need to get into the nervous system to potentially have an effect, even in enzyme replacement or altering immune response. There is not strong data that transdifferentiation or those cells getting into the nervous system is the clear benefit, even in the animal studies that have shown positive effects. For the purposes of enzyme replacement, just participating in chimeric vascular formation may be adequate. Beyond that it's hard to comment. There are multiple potential mechanisms.

Audience: Diane, about 30 years ago I started out as an occupational therapist and it was during the heyday of neurodevelopmental therapy. In occupational therapy with cerebral palsy, it went almost from trying to make the child functional to only being able to do functional things if it was within the realm of neurodevelopmental therapy practice. Have you seen a change in this approach? I know you're not an occupational therapist, but have you seen a change in the approach of occupational therapy in the last decade? What is the feeling right now about function and making the child as functional as possible?

Dr Damiano: I think occupational therapy is probably ahead of the curve with looking at everyday life, so I think that part of the profession is still very strong. I have found that neurodevelopmental therapy is actually very strong even at the higher level people in OT as well, and I'm not really sure why that is. What I think is happening in the field, though, is that we have this division, which is the real problem. It's that we have a group of therapists saying one thing or practicing one stream and a group that are really trying to keep up with the evidence and to adjust their practice on a daily basis just like physicians are required to do. I think both professions are very similar. We both have a lot of opportunities we need to pursue and to try—basically, for 40 years neurodevelopmental therapy has been shown to not be better than anything else, so we really need to reevaluate it.

## Executive Summary for the Day

Donna Ferriero, MD, UCSF Children's Hospital, San Francisco, California

Dr Ferriero presented a closing talk summarizing the major points of the day and highlighting some remaining questions for the future directions discussion. The first session focused on clinical aspects of injury to the preterm brain. It is clear that what is often considered just a white matter injury also involves neuronal damage and is the result of the combined effects of infection or inflammation with hypoxia-ischemia. Axonal damage has been shown to be prominent with gliosis and microgliosis, and the interactions between microglia, oligodendrocytes, and cytokines are very important in mediating this process. Better methods for cell identification and for delineation of dying cells are necessary to develop our understanding of the associated pathology. Connectivity and trophic factors for each cells type are also important aspects of the injury to be considered. In terms of imaging, MRI may not give a complete understanding of the injury, and newer techniques such as diffusion tractography and fractional anisography should be optimized to correlate anatomic and functional damage.

Preterm central nervous system injury does not only occur perinatally; prenatal and postnatal infections may contribute to continued neuroinflammation in children with cerebral palsy. Cerebral perfusion and oxygenation are also important factors in injury, and developing better techniques to monitor these variables may help us understand their role and optimize treatments. Finally, improved classification and scoring systems for cerebral palsy are necessary to standardize clinical care.

Animal models of hypoxic-ischemic central nervous system injury have suggested that cerebral blood flow is not decreased in white matter lesions. Instead, the immaturity of oligodendrocytes at the areas of the lesion coupled with inflammation may lead to a myelination failure. These oligodendrocytes may potentially be a target for stem cell replacement. Apoptosis plays a major role in secondary brain injury, and understanding the molecular elements involved, especially at the mitochondrial permeability pore, may allow us to target these with specific inhibitors. Excitotoxicity also has a major effect in the preterm brain. Cells in the immature brain show altered patterns of receptor expression, which may make them more vulnerable to excitotoxicity and may serve as treatment targets.

Strong evidence exists for constraint-induced and resistance exercise as well as strength training in the field of physical therapy. Standardization and measurement of outcomes are still necessary to define the best treatments. Activity-dependent changes in the brain and spinal cord appear to mediate motor improvement with training, and studying these over longer periods of time may offer the greater understanding of their long-term benefits. In terms of neuroprotection, early intervention appears to be key and should be incorporated into future clinical trial design. Finally, stem cell therapies hold a great deal of promise in this field, but more understanding is necessary of the cell types to be used and of combination therapies that may optimize the environment for transplant and endogenous neuronal survival.

## Future Directions

Moderators: Deborah Hirtz, MD, NINDS Program Director

### Panel Discussion

Sidhartha Tan, MD, Northwestern University, Evanston, Illinois

Steven Miller, MDCM, MAS, FRCPC, BC Children’s Hospital, Vancouver, British Columbia

Pam Follett, MD, MPH, Tufts University School of Medicine, Boston, Massachusetts

Mindy Aisen, MD, UCP Research and Education Foundation, Washington, DC

Carina Mallard, PhD, Göteborg University, Göteborg, Sweden

Alec Hoon, MD, MPH, Kennedy Krieger Institute, Baltimore, Maryland

The panel discussion focused on addressing the questions and challenges highlighted in Dr Ferriero’s executive summary and on highlighting major ideas from each of the panelists. The panel suggested the following topics as important issues for future research:

### Clinical Management

- All professionals who treat children with cerebral palsy must serve as advocates for these children to garner more funding for treatments and research. Also, they must attempt to allocate funding efficiently to effective and necessary treatments.
- There is an increasing adult population with cerebral palsy, and these individuals face problems associated with extended inactivity. It is now even more necessary to consider the long-term effects of any childhood intervention for cerebral palsy, to eliminate useless therapies, and to ensure that patients maintain the best possible quality of life into adulthood.
- A standard and detailed system of classification is necessary to describe patients with preterm central nervous system injury. Also, nonmotor deficits in cognition, language, behavior, and function must be recognized and addressed in any comprehensive classification scheme.
- Interprofessional collaboration, perhaps in the form of centers of excellence around the country, is necessary to establish cohesive and standardized intervention protocols and to help families navigate this process.

**Translational Research**

- Cerebral palsy is not a disease but a complex syndrome with various contributing etiologies, including environment, genetics, and the physiologic effects of prematurity. All these factors should be considered in identifying possible treatment targets and fully elucidating the contributing factors of this syndrome.
- There is a need for objective, quantitative estimates of injury. These clinical measures of motor function and muscle tone can be defined in animal models and applied to patients.
- Computational neuroscience may be a promising technique to model changes in connectivity based on controlled variables at the system level.

### Clinical Trials

- Because of limited resources, collaboration and cooperation are key in prioritizing and studying relevant therapies. Specifically, communication between those investigators focused on pathogenesis and molecular mechanisms of central nervous system injury and those performing clinical trials may help to focus research on the most promising interventions and outcomes.
- Clinical imaging may be very helpful in anticipating associated problems as a child develops. Diffusion tensor imaging holds potential for correlating injury and clinical phenotype. These methods must be studied and optimized statistically to understand why some children have adverse outcomes despite normal imaging and some children with abnormal imaging do very well.
- Improved statistical methods are necessary for combining and analyzing data across centers to maximize what is learned about the success of various neuroprotective and rehabilitative interventions in a broad patient population.

The overall message of the panel was to continue to place an emphasis on collaboration to more effectively improve the quality of life of children with preterm central nervous system injury. Teaching young investigators and physicians how to effectively design clinical trials and identify potential treatment targets was also identified as a major goal.