Non-human primate species have been selectively used in the scientific investigation of adult and newborn neurological diseases. The rhesus monkey has been utilized in models of term asphyxial insults, accurately reflecting the mechanisms and neuropathology demonstrated in the newborn human infant. More recently, a premature baboon model developed for evaluation of bronchopulmonary dysplasia has been applied to the investigation of cerebral development and injury, revealing high similarity in neuropathology to the premature human infant. Given the differences in the outcomes of neuroprotective therapies between lower order species, such as the rat, and human trials in disorders such as stroke, nonhuman primate models may provide an invaluable resource for safety and efficacy testing before trials in human newborns. This article summarizes both models of brain injury. The histologic findings from the models are compared with neuropathological studies in human infants.

Semin Perinatol 28:396-404 © 2004 Elsevier Inc. All rights reserved.

The animal species most suitable for modeling human neurological disease remains controversial. The use of nonhuman primates for the study of neurological diseases is clearly not appropriate for all disorders because of the cost in time and resources necessary to develop and use these models combined with ethical considerations. Further, many important fundamental pathophysiological mechanisms are being successfully addressed in other species. Rats are the most commonly used species for the investigation of neurological disease in adults and newborns (Table 1; see later chapter “Experimental models of perinatal brain injury”). Although there are undeniable differences between rats and primates with respect to the complexity of cortical motor organization, there is an increasing recognition that there are marked functional similarities including the cortical and basal-ganglia circuits controlling the development, maintenance and selection of adaptive motor response. Thus, many aspects of neurological disease and its functional correlates can be tested in lower order species. However, nonhuman primate models, such as the baboon model described below, provide unprecedented fidelity to the human condition. Consequently, they may be appropriate for testing agents and interventions proven successful in lower order species before their evaluation in human studies. Thus, lower order and nonhuman primate models can serve complementary roles in the evaluation of neurologic disorders.

Primate Taxonomy

The order primate includes humans, apes, monkeys, prosimians and related animals. All of the 190 or more species of primates are classified into one taxonomic order because they are mammals with close evolutionary roots. Nonhuman primates are limited in their natural habitats to the tropical and subtropical areas of the New World (Americas) and Old World (Africa and Asia). There are striking morphometric similarities in primates, including the retention of pentadactylysm and erectness in posture, while intellect and lively success in complex environmental situations are recognizable higher cognitive skills. The primate family is illustrated in Fig. 1. The hominoidea superfamily, which represents the highest order in the primate family, includes gibbons and orangutans as well as gorillas, chimpanzees and humans (Fig. 1). The majority of nonhuman primate research is performed in a limited number of species: the squirrel monkey, the rhesus monkey, the macaque monkey and the baboon, with such research being undertaken predominantly for vaccine work, pharmacological and toxicological studies, and im-
munology. Less than 10% of studies in nonhuman primates occur for neuroscience.4

Animal Models for Neurological Injury in the Newborn

Before investigating the role of any animal species in the study of neurological diseases in the newborn brain, it is important to answer several fundamental questions:

1. What is the normal ontogeny of this species, and is the model developmentally appropriate for the neonatal brain injury research paradigm?
2. Are there similarities in biochemical response, physiology and neuropathology that accurately mimic the human condition?
3. Are there functional correlates in chronic outcomes, relevant to human premature and term born infants, that support the relevance of the model and the utility of therapeutic neuroprotective interventions?

Ontogeny of the Nonhuman Primate Brain

Fetal studies of the macaque and baboon have demonstrated that they share the same multivillous placental blood flow pattern shown by the human and are highly relevant to studies of altered placental function.5,6 There are, however, differences between the species in the patterns of amniotic fluid composition and in fetal breathing movements with the baboon most closely resembling the human fetus.

We have recently assessed the ontogeny of cerebral development over the last third of gestation in the baboon species to establish a background for studies of the effects of prema-
the internal capsule, alveus, and lateral geniculate nucleus. Subcortical myelination further proceeds in a dorsomedial to lateral direction, with staining apparent in the gyri flanking the lateral fissure, including the superior temporal gyrus.

The extent of hemispheric gyral folding, cortical growth, astrocyte development and myelination can be used to establish the gestational ages at which equivalent brain development occurs in the baboon and human. At 125 dg in the baboon, all layers are present in the cerebral cortex, the main sulci and gyri have developed, and myelination has commenced in the internal capsule and diencephalon. In humans, the six cortical layers are established by at least 28 weeks and the primary gyr by 26 to 28 weeks. Taken together, this suggests that development of the baboon brain at 0.7 of gestation (125 dg) equates to human development at 26 to 28 weeks (182-196 dg). In baboons, there is a brain growth spurt from 125 to 140 dag, followed by a slower rate of growth from 140 to 160 dg. However, cortical thickness increases more significantly during the latter time period. By 140 dag, myelin is present in the posterior limb of the internal capsule and extends into the dorsal gyri of the frontal and parietal lobes in the region around the central sulcus. Fetal human brains display a growth spurt around 28 weeks, and there is prominent growth in cortical gray matter between 29 and 40 weeks. Myelogenesis begins in the periventricular white matter at around 30 weeks in the human although mature myelin is not evident in the forebrain until about 44 to 52 weeks postconception, appearing first in the posterior limb of the internal capsule at about 44 weeks, in the temporal gyrus at about 48 weeks and the optic radiation at 52 weeks. Taken together, this implies that the baboon brain at 140 dag is equivalent to 32 to 34 weeks, and 160 dag to term, in humans. Overall, there is a marked similarity in the sequence of development, albeit with a somewhat more advanced pattern of myelination in the baboon. Thus, as has been previously noted, the “term” nonhuman primate baboon (184 dg) is much more advanced in cerebral structural development and may differ in neuropathology and physiology in comparison to the “term” human infant. The baboon would be more appropriately studied at 0.8 to 0.85 of full term, or 160 dag, for comparison with term human infants. An accurate understanding of the appropriate development neurobiology of the period of cerebral development is clearly crucial to selecting the correct model for investigation, but is often sadly lacking in full definition in many animal species studied for neonatal neuropathology. Of note, many aspects of cerebral development have been studied in rhesus and macaque monkeys.

**Are There Similarities in Nonhuman Primate Models to the Human Infant with Term Perinatal Hypoxic–Ischemic Insult?**

The major cause of neurological morbidity in the term-born human infant is hypoxic–ischemic brain injury. The se-
quency of pathophysiological events leading to neonatal hypoxic–ischemic brain injury is ultimately dominated by the occurrence of global cerebral ischemia. The neuropathological features of human neonatal hypoxic–ischemic encephalopathy are determined by factors such as the severity, pattern and type of insult, as well as the gestational age and metabolic status, including temperature, of the human infant.

The most common variety of neuronal injury resulting from hypoxic-ischemic injury in the term infant is selective neuronal necrosis. Neurons demonstrate the greatest sensitivity to hypoxic-ischemic injury in the term infant. Among term born human infants, neurons of the CA1 region of the hippocampus (Sommer’s sector), deeper layers of the cerebral cortex, and cerebellar Purkinje cells are injured most frequently by hypoxic–ischemic insult.16–18 Neuronal necrosis is most prominent in the watershed regions of the cerebral cortex and in the depth of the sulci, reflecting the greater effect of ischemia in these regions. Parasagittal cerebral injury is the principal ischemic lesion of the full term infant with injury to the cerebral cortex and subcortical white matter in the parasagittal and superomedial aspects of the cerebral convexities. The injury is bilateral and usually symmetrical. Status marmoratus describes a pattern of injury involving the basal ganglia and thalamus, characterized initially by neuronal loss, gliosis and hypermyelination. Other patterns of hypoxic–ischemic injury consist of focal and multifocal ischemic brain necrosis, including infarction, porencephaly, hydranencephaly and multicystic encephalomalacia.17

**Nonhuman Primate Studies of Term Hypoxic–Ischemic Injury**

Studies defining the perinatal patterns of brain injury occurring during experimental asphyxia in the nonhuman primate with associated cardiovascular and blood gas alterations have been conducted exclusively by Myers and Brann.19–23 Four patterns of perinatal brain damage resembling that of asphyxial insults to the human term infant have been described.21

The first pattern of injury described in the rhesus monkey by Myers is that of “total asphyxia”—a pattern of brainstem injury noted when respiratory gas exchange of the fetus is completely stopped. The brain structure that is most vulnerable is the inferior colliculus, with damage to the superior olives, Purkinje cells, vestibular nuclei, posterior and lateral ventral nuclei of the thalamus, and gracile/cuneate nuclei appearing sequentially with increasing duration of asphyxia. Of interest in this model is that the duration of asphyxia insult necessary to produce microscopic brain injury increased dramatically with decreasing gestational age, ie, 12 minutes at term compared with 30 minutes at midgestation.

The second pattern of injury is that of “partial asphyxia with acidosis,” for which the monkey fetus is subjected to partial asphyxia by impairment of placental gas exchange in a chronic fashion. In eight Rhesus monkeys, following prolonged partial insult, there is clinical behavior similar to the term asphyxiated newborn infant, with a prolonged time to first gasp and clinical seizure activity.20 The average time of first seizure is 23 hours of age. Neuropathology reveals pale or hemorrhagic necrosis involving the entire cortex of both hemispheres or restricted to a parasagittal posterior parietal distribution. Animals subjected to partial asphyxia of shorter duration tend to survive and later show focal cortical injury with predominance in the posterior parietal parasagittal regions. Rarely these animals survive beyond the newborn period. With later survival there is asymmetrical bilateral porencephaly or cerebral necrosis with cystic degeneration of gray and white matter, centered in the middle paracentral region.

The third pattern of injury described is that of “partial asphyxia without acidosis,” which is associated with selective white-matter injury. This pattern of injury appears when respiratory gas exchange of the fetus is diminished gradually and maintained for long periods of time. In contrast to the low oxygen levels, pH and pCO2 levels were virtually unaffected in this model. The neuropathological findings are similar to those noted in juvenile monkeys exposed to cyanide or carbon monoxide. Both processes are known to impair cerebral oxidative metabolism without altering CO2 or pH.

The final pattern described is that of “partial asphyxia in association with total asphyxia.” In this model, partial asphyxia in association with total asphyxia leads to selective basal ganglia injury. Necrosis and later gliosis affect the caudate, putamen, and globus pallidus, with less conspicuous lesions of the neocortex.21 These pathological findings of status marmoratus bear a striking similarity to human neuro-pathological lesions in neonatal hypoxic–ischemic encephalopathy. The relative degrees of partial and total asphyxia are reflected in varying degrees of involvement of cerebral cortex. Animals subjected to severe partial asphyxia and relatively short episodes of total asphyxia show lesions of both basal ganglia and cerebral cortex, whereas those subjected to more severe episodes of total asphyxia show more striking brain-stem abnormalities.

**Correlations with the Human Model**

The parasagittal distribution of lesions described by Brann and Myers20 with partial asphyxia correlate with the parasagittal distribution of cerebral injury recognized in the human infant by both technetium brain scan24 and positron–omission tomography.25 There is some variation in the functional clinical correlate to the parasagittal lesions between the human and nonhuman primate models. In the human infant, the clinical correlation is of a greater degree of weakness of the proximal limbs, upper greater than lower extremities. In contrast, long-term evaluation of the monkey revealed spastic paraparesis. Hypotonia was also noted in the monkeys, as recognized in human infants who had manifest prolonged periods of abnormal fetal heart rate patterns.26 The white matter lesions with minimal gliosis are well described in premature human neonates and correlate with the midgestational rhesus model of oligoacidotic hypoxemia.27 Human neonates with apparent acute total asphyxia and brain stem
injury have also been reported. Thus, the clinical and pathological findings described by Myers and coworkers are remarkably similar to those that have been noted in the human infant, rendering this a model of potential high utility, particularly for neuroprotective drug trials for birth asphyxia before human trial.

Are There Similarities in Nonhuman Primate Models to the Human Infant with Preterm Cerebral Injury?

Very low birth weight (VLBW) infants now represent approximately 1.5% of all live-born infants, increasing slightly as a proportion of total births over the last 3 decades.28,29 More notably, the survival of VLBW infants has increased to close to 90% with recent improvements in obstetric and neonatal management.29-31 This increased survival has been greatest in the extremely low birth weight (ELBW) cohort of infants who weigh less than 1000 g and are at greatest risk of later neurological deficits.30-32 With this improved survival, there has been no reduction in the documented rates of neurological disability occurring in premature infants, with a recognized increase in the absolute numbers of infants with long term neurological sequelae.33 For all VLBW survivors, 5 to 15% later exhibit major spastic motor deficits, while an additional 25-50% exhibit less prominent developmental disabilities, involving not only motility but also cognition and behavior, with school disturbance a nearly uniform result.33-38 Improvement in the detection, diagnosis and management of neurological injuries and, ultimately, formulation of strategies to prevent the accompanying morbidity require an understanding of the pathogenesis of glial and neuronal injury in the brain of the premature infant.

The principal neuropathological substrates for the neurological disturbances in the human premature infant appear to involve the cerebral white matter. These lesions include periventricular hemorrhagic infarction and periventricular leukomalacia (PVL). The most common neuropathology found at autopsy in the premature infant is PVL. PVL refers to necrosis of white matter in a characteristic distribution, ie, dorsal and lateral to the external angles of the lateral ventricles, involving particularly the centrum semiovale (frontal horn and body), optic radiation (trigone and occipital horn), and acoustic radiation (temporal horn).37 The incidence of the disorder at autopsy varies considerably from one medical center to another, ie, from approximately 25% to 75%. Despite this variation, several facts remain clear: the lesion is observed particularly often in infants who (1) are prematurely born (<30 weeks gestation), (2) survive more than a few days, (3) have intraventricular hemorrhage (IVH), (4) have evidence of cardiorespiratory disturbance, and (5) have evidence of antenatal/placental/fetal infection.37,39-41 The clinical consequences of PVL are a significant increase in the risk of cerebral palsy. In a meta-analysis, it was found that 58% of 272 infants with cystic PVL developed cerebral palsy, compared with 2.6% of 655 infants with normal head ultrasound scans.32 The etiology of white matter injury is not known, but clinical data suggest that ischemia–reperfusion and/or infection–inflammation are important factors.53

Other major forms of neuropathology in the human preterm infant, less common than PVL, include IVH, selective neuronal injury, and focal cerebral ischemic lesions. IVH, in addition to being associated with an increased risk of PVL,57,58 is also intimately associated with the occurrence of periventricular hemorrhagic infarction. Apart from these major forms of neuropathology, it is also likely that the extremely immature infant is at risk for more subtle alterations in cerebral structure and development. Our research has described alterations in cortical gray matter development in premature infants with PVL,57,58 and in premature infants receiving dexamethasone.39 These more subtle alterations may include a reduction in cortical gyral development, impaired or delayed cerebral myelinization, impairment in cortical laminar organization, and/or a reduction in axonal and dendritic growth and synaptogenesis. Such subtle abnormalities have not been extensively examined on previous human neuropathology studies, but MR studies of premature infants are increasingly suggesting that such alterations in cerebral structure are common, particularly in the extremely immature infant.60-63

Nonhuman Primate Studies of Cerebral Injury in the Premature Infant

We have evaluated the premature nonhuman primate as a model of cerebral injury in the immature brain. Employing a model of premature delivery of the baboon, we have attempted to determine the nature and severity of the cerebral injury resulting from prematurity delivery followed by intensive care similar to that received by the human premature infant. In this regard, the experimental conditions for this baboon model are nearly identical to those experienced by premature human infants. The respiratory and cardiovascular development of premature baboons54,65 suggests that 125 dg corresponds to 26 to 28 weeks in humans. Our pilot work on the ontogeny of cerebral development also confirm that 125 dg is equivalent to 26 to 28 weeks human gestation (vide supra). Thus, the experimental cohort of baboons we have studied was delivered at 125 dg and subsequently nursed for two weeks in an intensive care facility with euthanasia at 139 to 140 dg. The neuropathological examination included assessment of white matter damage, neuronal necrosis, presence of reactive astrogliosis or activated microglia, ventricular size, and the presence of subarachnoid, germinal matrix or intraventricular hemorrhage. The pattern of cerebral injury correlates with that seen in the preterm human infant, validating the prematurely born baboon as a highly appropriate model for the study of preterm human birth.

Preterm Baboon Model

Infants are delivered by elective hysterotomy under general anesthesia at 125 ± 2 days gestation. The infants were weighed,
anesthetized with intramuscular ketamine (5-10 mg/kg) and intravenous valium (0.1-0.5 mg/kg), and intubated with a 2.0 to 2.5 mm diameter endotracheal tube. All animals received surfactant replacement therapy (Survanta or Curosurf) and were ventilated on a standard, time-cycled, pressure-regulated infant ventilator (Infant Star) with humidifier maintained at 36 to 37°C. Hypotension was managed with volume replacement, dobutamine, dopamine and/or epinephrine infusions as needed. For all animals, brains were removed at post-mortem after euthanasia at 139 dg and placed immediately into 10% formalin. Sections were stained for hematoxylin and eosin (H&E), glial fibrillary acidic protein (GFAP) immunohistochemistry, and lectin histochemistry.

**Histological Findings in the Premature Baboon Model**

Histological examination revealed various neuropathologies, the two most common of which were white matter injury and hemorrhage.

Evidence of white matter injury was found in 50% of the prematurely delivered animals. The injury ranged from small patches of reactive astrocytosis (Fig. 3) to more extensive damage with activated microglia, small cystic lesions with dense cellular borders, and endothelial hypertrophy. The extent of white matter injury ranged from approximately 0.5-2.5% of total white matter. White matter injury occurred most frequently in the parietal and occipital lobes. There was often ventriculomegaly present in those animals with significant white matter injury. One quarter of the prematurely delivered neonates displayed enlarged lateral ventricles (Fig. 4) in relation to the surrounding brain tissue when compared with gestational control brains. In particular, the posterior and inferior horns were markedly enlarged.

Subarachnoid hemorrhages were observed in 40% of the prematurely delivered neonates. Hemorrhages also occurred in the lateral ventricles (5%), germinal matrix (9%), white matter (28%) and cerebellum (9%). Distinct regions of cell loss were seen in the CA2/3 region of the pyramidal cell layer in the hippocampus in some animals, and reactive astrogliosis was observed in association with this cell loss.

**Correlations with the Human Model**

Neonatal white matter injury, the most common form of damage in the baboon, is also the most common form of injury in human infants. In baboon, this damage is usually the diffuse form, with cystic infarction occurring rarely. Diffuse white matter injury is associated with the attraction of infiltrating cells, although the injury is not as extensive as that reported in humans, which may be due to the absence of potentiating or priming factors such as perinatal infection or hypoxia.

The frequency of intraventricular and subarachnoid hemorrhage also closely correlates with human data. Hippocampal cell loss occurs in about 20% of the premature baboons and may be directly relevant to the recent observations of hippocampal atrophy in older, prematurely born children.

Overall, there are marked similarities in the pattern of cerebral injury between prematurely delivered baboons and premature human infants. Most notably, the premature baboon displays a marked predominance of cerebral white matter injury in the absence of an experimental interventional insult. There is also striking similarity in the distribution and frequency of cerebral injury to that seen in the prematurely born human infant (Table 2). Thus, this model has the potential to shed light on the nature of cerebral injury in the prematurely delivered human. Clearly there are important differences between this model and the human setting, such as the presence of potentiating or priming factors precipitating preterm labor, including perinatal fetal infection, although these may be possible to replicate in the model in the future. The functional correlates of the neuropathologies in the prematurely born baboon are unknown, but clearly worthy of study.

**Figure 3** The presence of astrogliosis in the periventricular white matter in the premature baboon delivered at 125 days and receiving neonatal intensive care for 14 days demonstrated by glial fibrillary acidic staining (A, B). Note also the presence of micro-hemorrhage in the white matter (B).

**Figure 4** Ventricular size was enlarged in 25% of experimental animals as compared here in two experimental animals without (A) and with (B) ventriculomegaly. One can also note the relative white matter atrophy in the animal displaying significant ventriculomegaly.
Disadvantages of the Nonhuman Primate Models

It is important to point out the disadvantages in the use of these models, which must be balanced against their advantages. They include:

1. The requirement for a facility specifically staffed and equipped for nonhuman primates
2. The significant cost of the animals and their care in full neonatal intensive care setting
3. Ethical considerations in the use of a higher order primate species
4. The need for professional individuals with high surgical, medical, and laboratory skills with nonhuman primates.

One means of reducing cost is to perform studies of multiple organ systems in a single experimental setting. For example, the baboon data described above were derived from brains removed from animals that underwent various ventilation protocols for optimization of mechanical ventilation strategies. Thus, the same model can be used to evaluate brain injury and ventilation schemes. Another approach is to apply these models in a selective fashion. For example, therapies and interventions could be screened in lower species. Those that prove successful would be evaluated in nonhuman primate models before their application to human studies.

Conclusion

Nonhuman primate models of term and preterm brain injury have a remarkably high similarity to human brain injury in etiological mechanism, neuropathological lesion and clinical outcome. Though their implementation is resource intensive, their selective use for evaluation of brain injury and protective strategies is well justified.

References


<table>
<thead>
<tr>
<th>Regional of Cerebral Injury</th>
<th>Premature Human Infant</th>
<th>Premature Baboon (125 dg treated for 14 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periventricular leukomalacia (white matter injury)</td>
<td>Cystic PVL (5%)</td>
<td>Cystic PVL (5%)</td>
</tr>
<tr>
<td></td>
<td>Diffuse PVL (20%)</td>
<td>Diffuse PVL (50%)</td>
</tr>
<tr>
<td></td>
<td>Moderate ventriculomegaly (40-50%)</td>
<td>Moderate ventriculomegaly (25%)</td>
</tr>
<tr>
<td></td>
<td>(Vermont–Oxford)</td>
<td>(Vermont–Oxford)</td>
</tr>
<tr>
<td></td>
<td>Subarachnoid haemorrhage, isolated 30%</td>
<td>Subarachnoid haemorrhage, isolated 40%</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>Intraventricular hemorrhage 20%</td>
<td>Intraventricular hemorrhage 6%</td>
</tr>
<tr>
<td></td>
<td>Subarachnoid haemorrhage, isolated 30%</td>
<td>Subarachnoid haemorrhage, isolated 40%</td>
</tr>
<tr>
<td>Hippocampal injury</td>
<td>Recent MR studies highlight significant hippocampal atrophy in older prematurely born children and the subiculum of the hippocampus vulnerable to injury in premature infants</td>
<td>Hippocampal cellular injury and reactive astrocytosis (20%)</td>
</tr>
<tr>
<td>Cortical and deep nuclear gray matter injury</td>
<td>Injury to the thalamus, globus pallidus. Volumetric MR studies reduction in cortical gray matter volumes in preterm infants by term and later</td>
<td>Gray matter injury in gray matter (25%) and basal ganglia (6%)</td>
</tr>
<tr>
<td></td>
<td>Further subtle cortical laminar study under investigation</td>
<td>Further subtle cortical laminar study under investigation</td>
</tr>
</tbody>
</table>

Table 2 The Neuropathologic Findings in the Premature Baboon (125 days surviving with neonatal intensive care 14 days) in Comparison to Those Found in the Human Premature Infant


63. Inder TE, Wells SJ, Mogridge NB, et al: Defining the nature of
the cerebral abnormalities in the premature infant: A qualitative magnetic resonance imaging study. J Pediatr 143:171-179, 2003

