Greenfield's Neuropathology

CLINICAL INVESTIGATIONS

In the assessment of epilepsy patients, the history takes primacy, ideally in conjunction with an eyewitness account. The diagnosis of epilepsy is clinical in the vast majority of cases, but a syndromic diagnosis is also possible on the basis of clinical symptoms and signs. Clinical evaluation also guides appropriate investigations and management. The clinical formulation, ideally with identification of an underlying cause or disease process, is the best guide to prognosis, as this is determined largely by aetiology.

Among the investigations that are likely to be indicated in an individual diagnosed with new-onset epilepsy are those directed at cause and those that further stratify the type of epilepsy or syndrome. Any of these investigations may also facilitate further assessment during the course of the disease. They include tests on venous blood (typically assays of haematopoietic, renal and hepatic function), a routine 12-lead electrocardiogram (ECG) and neuroimaging (ideally a high resolution MR imaging brain scan but rarely positron-emission tomography [PET] or single photon-emission computed tomography [SPECT]). Electroencephalography (EEG), optimally undertaken with video recording, may assist in confirming the diagnosis and type of epilepsy (focal versus generalized) and in localizing the area of seizure onset. Defining the epilepsy type is worthwhile, both for determining prognosis and making treatment decisions.

Typically, a routine EEG will be undertaken in the outpatient setting, but failure to acquire useful data may necessitate a further recording, possibly with sleep deprivation, or a 24-hour ambulatory EEG including periods of sleep. Abnormalities on EEG are often brought out during sleep, and such recordings are often more revealing than further routine recording.

The ability of high resolution MR imaging to identify most (approximately 80 per cent) causes of focal epilepsy has added significantly to our understanding of the disease. The most common abnormalities in patients with focal epilepsy are cerebrovascular disease, trauma, tumours, developmental and vascular abnormalities, and hippocampal sclerosis. Clearly, there are distinct implications for patient management, depending on the underlying pathology. Some disease processes require management in their own right.

Antiepileptic drugs produce satisfactory seizure control in most patients with epilepsy, but when seizures are intractable the possibility of surgical treatment should be considered. In this situation, the therapeutic aims should be to identify the underlying pathology (usually with a combination of clinical history, neuropsychometry and MR imaging), to prove that the pathology is related aetiologically to the epilepsy (usually with EEG, often prolonged scalp EEG video telemetry), to establish that resection of the focal pathology is unlikely to produce significant adverse effects (e.g. loss of motor, sensory, cognitive or mnemonic functions) when balanced against anticipated benefits, and then to offer focal resection. Most commonly, this takes the form of anterior temporal lobectomy for temporal lobe epilepsy due to hippocampal sclerosis. A range of other pathologies and surgical approaches is possible, sometimes informed by other investigations, including functional imaging (with functional magnetic resonance imaging [fMRI], PET or SPECT) and intracranial EEG recording. Seizure-free rates without significant morbidity of the order of 70 per cent (6–12 months after surgery) are reported following hippocampectomy for hippocampal sclerosis; only slightly lower seizure-free rates are reported for the second most commonly resected pathology, focal cortical dysplasia. A range of other surgical interventions exist for epilepsy, including biopsy, lesionectomy, multilobar resection, hemispherectomy, corpus callosotomy, multiple subpial transection, radiosurgery and the extracranial implantation of a vagus nerve stimulator.

EFFECTS OF SEIZURES

The effects of seizures on the brain are complex and have to be distinguished from the consequences of any primary neurological disease process that has led to seizure susceptibility. Although there is strong evidence to support the direct and detrimental effects of seizures on brain function and structure, brain impairment is not inevitable, for example in the 'benign' epilepsies.[190] Furthermore, injurious effects of seizures should be evaluated not only in terms of histologically evident structural changes, such as neuronal loss and gliosis, but also by assessing alterations at the cellular, synaptic and molecular levels, some of which may be reversible and others permanent. The potential clinical effects of seizure-related injury include worsening seizures or neurological, psychological and cognitive disability. Effects of seizures are influenced by the maturity of the brain (i.e. whether seizures are occurring in developing or adult brain) as well as their frequency, duration and cause. Epilepsy-induced neuronal injury is therefore an area of intense research with the ultimate goal of identifying neuroprotective treatments or other interventions to prevent or reverse functional deterioration.

Mechanisms of Neuronal Injury

The concept of excitotoxicity was first proposed in 1969 as a toxic effect of prolonged activation by excitatory amino acids, mediating neuronal injury in various neurological conditions. Following a prolonged seizure, there is excessive excitatory neurotransmitter release, with overstimulation of glutamate (*N*-methyl-D-aspartate [NMDA]) receptors and voltage-activated calcium channels, resulting in cellular influx of Ca^{2+} and mobilization of other ions (including K^+, Cl^-).[126] An increase in free calcium leads to mitochondrial dysfunction, release of mitochondrial Ca^{2+} and activation of various enzymes (lipases, endonucleases, proteases, catabolic enzymes) including MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) activation.[430] Mitochondrial dysfunction contributes to seizure-mediated neuronal death.[227] Prolonged seizures may ultimately result in neuronal death by apoptotic or necrotic pathways. Based on classic morphological definitions, cell necrosis would appear to be the dominant mechanism.[248] Studies also support involvement of programmed cell death with activation of both the intrinsic and extrinsic pro-apoptotic pathways including altered expression of pro-apoptotic Bcl-2 family genes and increased expression of caspases.[66,194] Continued expression of apoptotic proteins, after the initial injury, may contribute to ongoing pathogenic mechanisms.[139] The early and late cellular effects of seizures on neurons, many of which are linked with the processes that promote

further seizures or 'epileptogenesis' (see section on General Concepts of Epileptogenesis in Focal Epilepsy, p. 723), are summarized in Box 11.1.

Effects of Brief Seizures

One of the most pertinent clinical questions is whether a single brief seizure always causes neuronal damage and how this compares to the cumulative effects of repeated or prolonged seizures. Evidence that prolonged seizures, or status epilepticus, can result in neuronal loss is firmly established.[200] There are experimental data to show that even brief seizures may induce neuronal loss in the hippocampus.[78] In humans, longitudinal or serial MR imaging provide the most compelling evidence that, in some patients, repeated brief seizures can result in progressive hippocampal atrophy.[381,382] However, evidence from quantitative post-mortem neuropathological studies demonstrates that hippocampal or cortical neuronal loss is not inevitable, even with a long history of frequent and prolonged seizures[411] or some epileptic encephalopathies, such as Dravet syndrome.[80]

Influence of Age and Brain Maturity

Infants and children have a higher risk of seizures, and although mostly benign, frequent seizures carry the risk of long-term developmental, cognitive and behavioural problems.[36,201] Although antiepileptic drugs or the underlying condition contribute to these problems, there is accumulating evidence that ongoing seizures play an important role. The immature or developing brain is affected differently by seizures[190] than adult brains, in terms of both the cellular/molecular responses and neuronal injury.[202] In experimental conditions, the developing brain has a lower threshold for seizures, but appears less vulnerable to neuronal loss, axonal sprouting and the toxic effects of glutamate. Seizure susceptibility peaks during the period of rapid brain growth and synaptogenesis. There are several explanations for this: (i) excitation predominates over inhibition in developing neuronal networks; (ii) glutamate receptor subunits, being maturationally regulated, promote excitability and the neurotransmitter γ-aminobutyric acid (GABA) also mediates excitatory rather than inhibitory hyperpolarizing effects; (iii) there are lower synaptic densities and reduced developmental regulation of voltage-gated ion channels, such as potassium (K_v) and sodium channels (Na_v) (dysfunction of the latter being implicated in many early life epilepsy syndromes); and (iv) rates of neurogenesis may be enhanced and astrocytic responses diminished in the developing brain in response to seizures.[338] Experiencing a seizure early in life may increase susceptibility to further seizures and the risk of epilepsy later in life, i.e. '*seizures beget seizures*'.[365]

BOX 11.1. Immediate and longer term cellular effects following a seizure

Immediate/early (minutes to hours)
- Increase $[Ca^{2+}]$ (seconds/minutes)
- Altered kinase activity: phosphorylation or dephosphorylation of enzymes, receptors, ion channels
- MAPK/ERK2 pathway activation
- Immediate early gene expression (including c-fos, c-jun)

Intermediate (hours to days)
- Increased inflammatory mediators (cytokines, TNF, IL-1β, NF-B1)
- Protein synthesis (e.g. endogenous anti-convulsants, somatostatin, NPY)
- Growth factor expression (e.g. BDNF in hippocampal neurons)
- Alteration in subunit expression in glutamate and GABA receptors, with altered function
- Dendritic structural plasticity
- Altered synapse-associated gene expression
- Neuronal cell death

Late to chronic changes (days to months)
- Axonal sprouting e.g. mossy fibres (days, can be persistent)
- Enhanced neurogenesis (weeks)
- Reactive astrocytic gliosis (hypertrophy, proliferation and activation) (weeks/months)
- Altered or reduced capacity for neurogenesis (months)

Acquired (Secondary) Neuropathological Changes as a Consequence of Seizures and Post-Mortem Examination

In examining the brain of a patient with epilepsy, the neuropathologist addresses three main questions: (i) can a cause for the epilepsy be identified; (ii) are secondary changes of previous seizures present; and (iii) how has epilepsy contributed to the underlying cause for death? For the investigation of the first, tissue sampling is influenced by any macroscopic abnormality or by localizing clinical, electrophysiological and/or neuroimaging data. For secondary changes and investigations into cause of death, sampled regions of brain should include those most vulnerable to seizure-related injury, neuronal loss and gliosis: the hippocampus, neocortex, thalamus, amygdala, cerebellum and brain stem.

Secondary Cerebellar Pathology in Epilepsy

Macroscopic atrophy of the cerebellum has long been noted,[107] being present in 25 per cent of cases in one post-mortem epilepsy series.[270] It is generally acknowledged that cerebellar atrophy is likely to be acquired during the course of the epilepsy, rather than a predisposing factor for seizures. MR imaging volumetric studies confirm a correlation between the severity of atrophy and duration of epilepsy.[314] Cerebellar atrophy has been observed in association with generalized and focal seizures syndromes, including temporal lobe epilepsy (TLE).

Neuropathological findings at autopsy may disclose symmetrical atrophy of the anterior lobes or, more commonly, the posterior lobes[111] (Figure 11.1). In mild cases, damage may be restricted to a folium, but in severe cases more generalized atrophy is observed. Crossed cerebellar atrophy (cerebellar diaschisis) is recognized in patients with contralateral destructive cerebral hemispheric lesions associated with seizures, including hemiatrophy. Patients with unilateral TLE, however, typically exhibit bilateral cerebellar atrophy.[314] Regardless of the lobar distribution, the histological findings are Purkinje cell loss and Bergmann gliosis, with relative preservation of basket cells (Figure 11.1). Occasional torpedo-like axonal swellings of Purkinje cells may be observed. The white matter typically appears normal.

membrane peroxidation and disruption, DNA oxidation or protein nitration and cell damage. Normally, more than 80 per cent of the oxygen consumed by the cell is reduced by cytochrome oxidase.[125,175] The remaining 10–20 per cent follows other oxidation-reduction reactions in the cytoplasm and mitochondria that produce the superoxide anion (O_2^-).[125,500] In the brain, the neurotransmitter and cell signalling molecule nitric oxide (NO, produced by nitric oxide synthase-containing microglia and some neurons) reacts avidly with superoxide to generate peroxynitrite ($ONOO^-$), itself a highly reactive species. Interaction of peroxide radical with free ferrous iron (Fe^{++}; present in sites of haemorrhage, as well as in oligodendrocytes about to undergo myelination[89,102] generates the potent hydroxyl radical (OH) *via* the Fenton reaction. Additional sources of free radicals include increased intracellular calcium and mitochondrial injury, activation of proteases leading to conversion of xanthine dehydrogenase to xanthine oxidase, and activation of phospholipase A2 leading to increased generation of oxygen free radicals from cyclooxygenase and lipoxygenase pathways. Free radical injury is the critical component of reperfusion injury: when oxygen is reintroduced into hypoxic-ischaemic tissues, a massive production of oxygen free radicals results, producing reperfusion injury over and above the damage already produced during the hypoxia. The primary antioxidant enzymes required by most cell types to inactivate free radicals are copper- and zinc-containing superoxide dismutase (SOD) (CuZnSOD) and manganese-containing SOD (MnSOD), catalase and glutathione peroxidase. CuZnSOD and MnSOD reduce superoxide anion radical to hydrogen peroxide (H_2O_2), which in turn is reduced to H_2O by catalase and peroxidases. Several clinical and experimental studies have examined the possibility of reducing neurological morbidity in perinatal hypoxic-ischaemic brain injury by two possible means: (i) preventing the formation of free radicals, by inhibition of xanthine oxidase, for example; and (ii) delivering antioxidants or free radical scavengers to sites of increased free radical production.[255,490]

The developing grey matter may be particularly susceptible to free radical injury in hypoxia-ischaemia because of a relative deficiency in the brain's antioxidant enzyme systems.[391] Based upon immunohistochemical detection of the proteins, CuZnSOD and manganese-containing MnSOD appear in the brain as early as 13 gestational weeks, becoming strongly positive after 23 gestational weeks through 2 years of postnatal age.[517] The premature infant also has low plasma levels of glutathione and a relative inability to sequester iron because of low transferrin levels.[491]

Immature oligodendroglia are particularly vulnerable to free radical toxicity. Among the mechanisms underlying this vulnerability is a rise in free radicals in excess of their antioxidant enzyme capacity.[27] The vulnerability of oligodendrocyte precursors to oxidative stress may be due in part to a lack of, or imbalance in the expression of, antioxidant enzymes early in development, before active myelin synthesis. In stage-specific rat oligodendrocyte cultures, O4+/O1+/MBP– negative cells die of free radical toxicity when raised in a medium depleted of cystine (leading to depletion of glutathione and accumulation of reactive oxygen species). In contrast, MBP-positive (mature) oligodendrocytes, as well as astrocytes, survive in such an environment.[28,30] Mature (MBP-positive) cells express higher levels of MnSOD, and introduction of this enzyme *via* an adenoviral vector into pre-myelinating oligodendrocytes confers protection of the mitochondrial membrane potential and thereby cell death from glutathione depletion.[43] Manganese-containing SOD further protects cells from damage by reactive nitrogen species, such as peroxynitrite,[200] which may also play a significant role in white matter damage in PVL.[228]

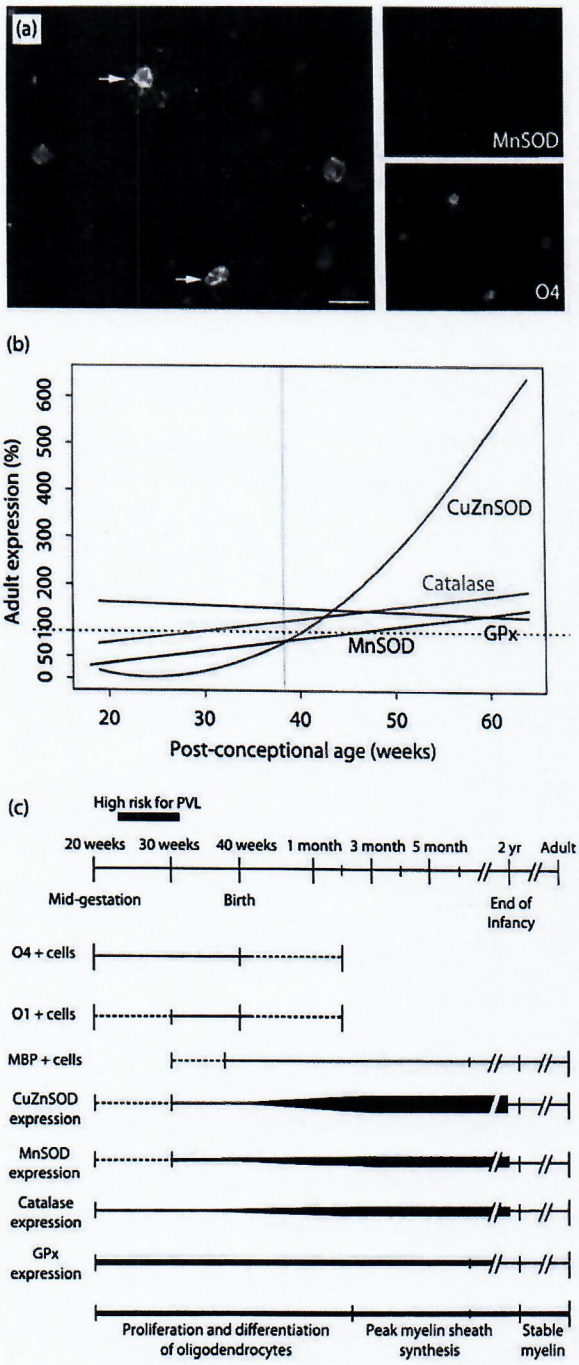
Catalase immunoreactive glial cells are not present in cerebral white matter from mid-gestation until 31–32 gestational weeks, at which time they are first visualized in the deep white matter. By term, all regions of the cerebral white matter (deep, intermediate and superficial) contain catalase-positive glia.[243] CuZn SOD immunoreactive glial cells appear in cerebellar white matter at 25–26 weeks of gestation and in temporal white matter at 31–32 weeks of gestation.[515] An analysis of antioxidant enzyme expression across human cerebral white matter development from 18 to 204 postmenstrual weeks, a developmental lag in the expression of both SODs compared with glutathione peroxidase and catalase was seen (Figure 3.14).[170] This lag suggests a dyssynchrony in tissue capability to sequentially break down superoxides. All antioxidant enzymes have higher-than-adult levels of expression during the peak period of postnatal myelin sheath synthesis in the cerebral white matter (i.e. 2–5 months of age), suggesting a need for maximal antioxidant capacity during high myelin lipid production.

Supporting a role for iron in triggering free-radical-mediated oligodendrocyte injury, cystine deprivation-induced death of oligodendrocyte precursors is prevented by pretreatment with the iron chelator desferrioxamine.[589]

Glutamate Receptors and Excitotoxicity

The excitatory transmitter glutamate may be released from injured neurons or axons, or by reversal of astrocyte glutamate transporters in the setting of hypoxia-ischaemia, to result in an excess of extracellular glutamate, which is toxic to many cell types, in a fashion that is developmentally regulated. Enhanced sensitivity of the immature brain to glutamate-induced toxicity reflects increased receptor density, altered receptor sensitivity (as a result of age-related differences in the molecular constitution of glutamate receptors) and/or differences in modulatory or compensatory mechanisms.[40] Regardless of the cell type, the mechanism is similar, involving calcium influx and subsequent generation of reactive oxygen and nitrogen species, participating in the 'final common pathway' of cellular damage discussed in the preceding section.

A developmental vulnerability to hypoxia-ischaemia in different grey matter regions of the brain is linked by animal data to age-related, transient elevations in glutamate receptors. The neurotoxicity of NMDA in the hippocampus, striatum and neocortex is maximal in the immature rat brain, peaking at postnatal days 6–7.[40] Autoradiographical receptor binding studies in rats indicate that in certain forebrain regions, the immature brain has a higher density of both NMDA and non-NMDA receptors compared with the adult brain.[261,369] Ligand affinities at NMDA receptors depend upon subunit composition, and there are marked developmental changes in the three major subunits – NMDAR1 (NR1), NMDAR2 (NR2A-B) and NMDAR3 – in experimental animals.[312] Non-NMDA glutamate receptors, e.g. AMPA receptor subunits, likewise demonstrate developmental regulation.[518]



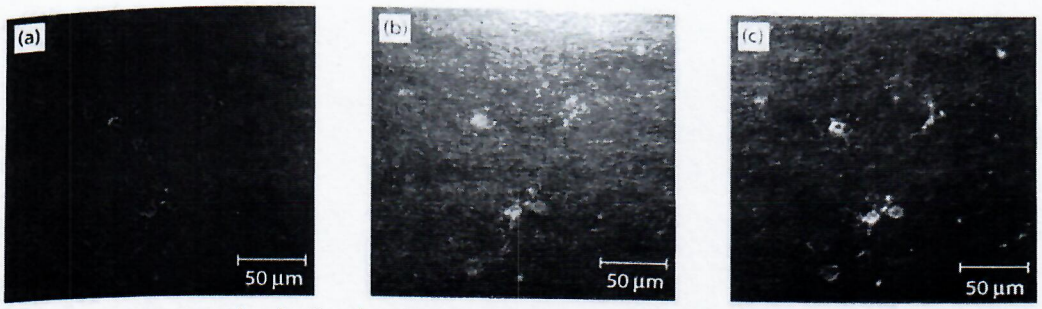
3.14 Dyssynchrony of antioxidant enzyme expression in the developing brain. (a) Expression of manganese superoxide dismutase (MnSOD, green immunofluorescence) by immature oligodendrocytes (O4, red immunofluorescence), demonstrated in the merged image. **(b)** Expression of the superoxide dismutases (MnSOD and CuZnSOD) reaches adult levels after birth. They lag behind the expression of catalase and glutathione peroxidase (GPx), which attain adult levels (or above) before term birth. **(c)** Developmental sequence of oligodendrocyte lineage cells relative to the expression of the major antioxidant enzymes in the developing cerebral (parietal) white matter of the human brain. Bar shows the peak period of vulnerability for periventricular leukomalacia (PVL). MBP, myelin basic protein.

From Folkerth et al.[170] With permission from Lippincott Williams & Wilkins/Wolters Kluwer Health.

In rat cerebral cortical neurons, the subunit GluR2 is relatively deficient, thereby conferring calcium permeability and greater vulnerability to excitotoxicity, in the early postnatal period. This period is a time of known susceptibility to hypoxia-ischaemia and subsequent development of seizures.

In cell culture, it has been shown that oligodendrocytes are vulnerable to micromolar concentrations of glutamate.[401] Moreover, the peak susceptibility to glutamate-induced cell death is in the developmental interval in which immature oligodendrocytes (O1+, MBP–) predominate.[401] The mechanism of glutamate-induced oligodendrocyte cell death involves both non-receptor- and receptor-mediated mechanisms. The receptor-independent mechanism involves glutamate transport into cells *via* glutamate/cystine exchange, resulting in depletion of intracellular cystine and, in turn, of glutathione, a key scavenger of oxygen free radicals, and finally death by intracellular oxygen free radicals.[401,589] Glutamate-induced toxicity to oligodendrocytes is also receptor-mediated.[592] In an oxygen-glucose deprivation system (an *in vitro* model for hypoxia-ischaemia), immature oligodendrocytes are more vulnerable than mature oligodendrocytes to excitotoxicity, an effect mediated by calcium-permeable AMPA receptors and blocked by AMPA antagonists.[126] The likely basis for this susceptibility is a preponderance of GluR2-deficient, and hence calcium permeable, AMPA receptors in premyelinating oligodendrocytes.[518] The lack of GluR2 subunit expression on pre-myelinating oligodendrocytes has been seen in cerebral white matter of the developing human brain during the peak time frame of PVL.[518] In the setting of energy failure, glutamate transporters (Figure 3.15), which are physiologically responsible for clearing extracellular glutamate, operate in reverse and thereby release glutamate into the surrounding tissue, thus contributing to excessive levels of extracellular glutamate.

Immature oligodendroglia are also vulnerable to NMDA as well as non-NMDA receptor-mediated excitotoxicity.[274,368,468] In adult rats, NR1, NR2 and NR3 subunits are detected in myelin, indicating that all necessary subunits are present for the formation of functional NMDA receptors.[368] In perinatal mice, NMDA receptor subunits are expressed on the processes of oligodendrocytes, and NMDA receptor subunit mRNA is present in isolated white matter.[468] In a murine model of perinatal cerebral ischaemia, NMDA receptor activation results in rapid calcium-dependent detachment and disintegration of oligodendrocyte processes. In perinatal models, NMDA receptor subunits are expressed on oligodendrocyte processes, in contrast to the expression of AMPA receptors on somata.[468] Consistent with this observation, injury to oligodendrocyte somata is prevented by blocking AMPA/kainate receptors, whereas injury to processes is prevented by blocking NMDA receptors.[468] The functional significance of NMDA and non-NMDA receptor expression in premyelinating oligodendrocytes is unknown but may involve axon-glial signalling during myelination.[468] Calcium influx through activated NMDA receptors, for example, potentially affects cytoskeletal elements within oligodendrocyte processes and determines stabilization or retraction of the processes as they extend from the somata and make axonal contact.[468] Calcium influx has been shown to activate calpain, which can degrade cytoskeletal proteins and activate Bax, a mediator of apoptosis.[469] Details of this topic are reviewed by Volpe.[568]



3.15 Glutamate transporter expression in the developing brain. Expression of the high affinity glial glutamate transporter GLT1 (also known as solute carrier family SLC1A2, or excitatory amino acid transporter 2; EAAT2) in immature oligodendrocytes with double label immunofluorescence and antibodies to GLT1 **(a,** green) and O4 **(b,** red) in cerebral white matter from a human fetus at 31 gestational weeks. **(c)** Merged image. SLC1A2 is closely related to glutamate/aspartate transporter high affinity sodium-dependent GLAST1 (SLC1A3), as well as EAAC1 (SLC1A1), which is typically thought of as a neuronal transporter. Glutamate transporters on immature oligodendrocytes may serve a critical role in maintaining glutamate homeostasis at a time when unmyelinated callosal axons are engaging in glutamatergic signalling with glial progenitors.

From DeSilva et al.[129] With permission from the Society for Neuroscience.

In human perinatal brain, studies of glutamate receptor subtypes have included a comprehensive cellular localization of AMPA receptor subtypes in the human cerebral cortex and telencephalic white matter.[518] As in the rat, a relative deficiency of GluR2 receptor subunit expression in the human cerebral cortex coincides with the interval of susceptibility (late gestation, term and early neonatal life) to hypoxia-ischaemia and seizures. The developmental profile of glutamate receptor subtypes has been mapped in the human brain stem using tissue autoradiography from mid-gestation to early infancy, and compared with the adult as the index of maturity.[418] There is almost no NMDA receptor binding in the human fetal brain stem at mid-gestation, suggesting that the vulnerability of the fetal brain stem to hypoxia-ischaemia is due to the high concentrations of kainate/AMPA receptors, and not NMDA receptors, at that age. NMDA receptors appear around birth and thereafter in infancy, whereas AMPA receptor binding declines sharply to significantly low levels thereafter. The finding that NMDA receptor/channel binding is almost undetectable in all regions of the human fetal brain stem at mid-gestation is unexpected, given the trophic role for NMDA in early CNS maturation in experimental animals. The brain stem data also suggest a differential development of components of the NMDA receptor/channel complex across early development.[419] Kainate binding is transiently elevated in the fetal and/or infant periods in the basis pontis, the inferior olive, the reticular core and the inferior colliculus, regions all thought to be particularly vulnerable to perinatal but not adult hypoxia-ischaemia.

Cytokine Toxicity

Cytokines are a heterogeneous group of polypeptide mediators that activate the immune response and inflammatory responses.[241] In the CNS, microglia induce reactive astrocytosis *via* release of TNF, IL-1, IL-6 and interferon-γ (IFN-γ).[394,479] Of relevance to perinatal white matter injury, these cytokines are soluble and diffusible, suggesting that they play a role, at least in part, in triggering the diffuse reactive gliosis adjacent to focal PVL, the immediate site of inflammation. Microglia are potently stimulated by endotoxin to produce IL-1β which secondarily stimulates astrocytic expression of both TNF-α and IL-6.[315] *In vitro* studies indicate that TNF-α and IFN-γ have toxic effects upon mature oligodendrocytes.[332,366,458,478,553] TNF exposure results in a cascade leading to oligodendrocyte cell death by apoptosis,[362] but only IFN-γ is directly toxic to immature oligodendrocytes;[553] its expression correlates with free radical adduct formation in these cells.[171] The potentiation of IFN-γ-mediated injury by TNF-α in developing oligodendrocytes may indicate an important role for the latter in human PVL.[7] Furthermore, TNF-α has been identified in hypertrophic astrocytes and microglial cells in PVL.[591] Other cytokines, including IL-2,[272] and IL-6[591] have also been identified in human PVL. It should be emphasized, however, that cytokines may be recruited by ischaemia or other insults, as well as infection.

A causal role for infection and cytokine toxicity in perinatal brain injury (PVL being the most extensively analysed) is indicated by several classic studies in developmental neuropathology: (i) neonates with bacteraemia[323] and neonates born to mothers with chorioamnionitis or premature rupture of membranes[430] are at risk for PVL; (ii) PVL occurs in several animal models of endotoxin-induced injury, with and without systemic hypotension;[19,191,192,594] (iii) cerebral white matter lesions occur in fetal rabbits after the induction of maternal intrauterine infection;[591] (iv) elevation in umbilical cord blood of the pro-inflammatory cytokine interleukin 6 (IL-6) is associated with an increased risk of PVL;[590] and (v) activated microglia are increased in number in PVL[228] and appear to play a central role in the damage related to these mediators.[568] Both the maternal and the fetal inflammatory responses may contribute to the presence of inflammatory mediators in the fetal or infant brain. The transfer to brain of pathogen-associated molecular patterns (PAMPs) in circulating blood, or the transfer of pathogen-activated immune cells into the brain may occur, perhaps facilitated by disruption of the blood–brain barrier by systemic cytokines, or the stimulatory effects of circulating cytokines on brain endothelial cells.[109,195]

As to the specific role of microglia in PVL, upregulation of toll-like receptors (TLRs) on activated microglia indicates a recently recognized capability for innate immunity.[319] These receptors are activated by PAMPs, most significantly