8
Astrocytes, Oligodendrocytes, and Schwann Cells

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8.1. Introduction

Central nervous system (CNS) is composed of two major cell types: neuron and glia. Astrocytes and oligodendrocytes belong to the latter category. Astrocytes, through an intricate network surrounding blood vessels, play an important role in supplying food, water and ions from periphery to the CNS and maintain CNS homeostasis. Astrocytes also play an active role in neurogenesis. However, under inflammatory or neurodegenerative conditions, astrocytes produce proinflammatory mediators and take active part in the ongoing events. Neurons in the CNS are covered by myelin sheath that maintains conduction of nerve impulse. Consistently, the CNS houses oligodendrocytes for myelin synthesis. On the other hand, Schwann cells are the myelinating cells in the peripheral nervous system (PNS). Balanced expression of several genes and activation of transcription factors critically regulate the entire complicated functional network of astrocytes, oligodendrocytes and Schwann cells. Keeping a birds' eye view, this chapter delineates genesis and functional aspects of astrocytes, oligodendrocytes and Schwann cells.

8.2. Historical View

For decades, astrocytes and oligodendrocytes were considered as silent partners of neurons in the CNS. It was known that astrocytes, like neurons, were unable to transmit messages as they did not possess voltage and ion gated channels. With the advancement of science, it is now well accepted that astrocytes possess ion channels as well as G-protein coupled receptors necessary to sense and respond to neuronal activities. Recent advancements also reveal that oligodendrocytes, apart from myelinating neurons in the CNS, secrete some growth factors to help neuronal growth and development. On the other hand, under disease conditions, astroglia undergo proliferation and gliosis. Activated astroglia also secrete neurotoxic molecules that may be involved in the loss of neurons in neurodegenerative disorders and the damage of oligodendroglia in neuroinflammatory demyelinating disorders.

The present chapter focuses on biology and functional aspects of astrocytes and oligodendrocytes ranging from their genesis to their enormous role in maintaining CNS homeostasis along with their role in CNS pathology. The biology and function of PNS myelinating Schwann cells has been discussed later as a separate section (Section 6).

8.3. Development of Astrocytes and Oligodendrocytes in the CNS

The vertebrate nervous system including neurons, astrocytes, oligodendrocytes, and other cells originates from a flat sheet of neuroepithelial cells, constituent of the inner lining of neural plate along the dorsal surface of embryo (Fujita, 2003). These neuroepithelial cells are the earliest precursors in the developing CNS.

8.3.1. Generation of Glial Precursor Cells

During neurogenesis, neuroblasts are first derived from stem cells and then migrate peripherally to the mantle and marginal layers in the developing brain. After that, DNA synthesis in neurons is completely ceased and the progenitor cells enter into the phase of gliogenesis in the neural tube. These glioblasts are functionally different but morphologically indistinguishable from the multipotent stem cells and eventually differentiate first into functional astrocytes and then

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oligodendrocytes. The quiescent form of the glioblasts called microglia comes after these events.

Differentiation of cortical progenitor cells is being controlled by some transcription factors having basic-helix-loop-helix (bHLH) motifs. These are NeuroD, Neurogenin, Mash, Olig, Id, and Hes families of protein. The restricted and time-dependent binding of these transcription factors with corresponding DNA sequences present in the promoter of different developmental genes determines the outcome of final cell types. Recent developments (Gotz and Barde, 2005; Alvarez-Buylla et al., 2001) show that neuron and glia are generated from same progenitors/precursors.

8.3.2. Signaling Events Driving the Precursors to Functional Cells: Astrocytes and Oligodendrocytes

The signaling events like hedgehog and notch regulate genesis of functionally distinguished glia and neurons from multipotent stem cells. Hedgehog (Hh) family of signaling molecules are the key organizers of tissue patterning during embryogenesis (Altaba et al., 2002). In mouse, three Hh genes have been identified. These are Desert hedgehog (Dhh), Indian hedgehog (Ihh) and Sonic hedgehog (Shh). The Shh plays a vital role in the development of CNS. In mammals and birds, Shh is the only hedgehog family member that is reported to be expressed in normal CNS.

The oligodendrocyte progenitors (OPs) in caudal as well as ventral neural tube originate under the influence of Shh protein secreted from ventral midline. At this initial stage, Shh patterns the ventral neuroepithelium by controlling the expression of a set of transcription factors PAX6, NKX 2.2, high mobility group protein SOX10, and basic helix-loop-helix proteins Olig1 and Olig2. These Olig genes and SOX10 are co-expressed in cells before the appearance of PDGF-α on OPs. These PDGF-positive OPs then proliferate and migrate away from the ventricular surface to all parts of the CNS before differentiating into functional myelin forming mature cells.

Notch signaling (Yoon and Gaiano, 2005) first specifies glial progenitors and then functions in those cells to promote astrocytes versus oligodendrocytes fate. The ligands of the Notch signaling pathway are expressed in differentiating neurons. The receptors Notch are transmembrane proteins and are found on neural stem cells. Upon ligand binding, intracellular domain (NICD) of Notch is cleaved by γ-secretase which then enters into the nucleus to form a complex with C promoter binding factor (CBF1) and mastermind-like (MAML). Then the complex (NICD:CBF1:MAML) binds to promoter regions of target genes Hes and Herp and up-regulates corresponding HES/HERP proteins. These proteins are bHLH transcriptional regulators that antagonize proneural genes like Mash 1 and neurogenins. As a result, it blocks neuronal differentiation.

8.4. Astrocytes: Biology and Function

In the middle of the nineteenth century, German anatomist and pathologist Rudolph Virchow was wondering about the group of cells in the brain that surround the neurons and fill the spaces between them. Dr. Virchow named these cells as "neuroglia" means "neural glue." He used the term "glue" to represent the gluing function of these cells to hold the neurons in place. Nowadays "neuroglia" is collectively used for all glial cells in the CNS. Later on, due to "star-shaped" appearance, the major neuroglial cells were named as "Astrocytes" (Astra: star; cyte: cells).

8.4.1. Morphology and Markers

Morphologically, astrocytes can be classified into two types: fibrous astrocytes and protoplasmic astrocytes (Brightman and Cheng, 1988). Fibrous astrocytes are located predominantly in white matter and possess fewer but longer processes. These processes form cytoplasmic bundles of intermediate filaments (IFs). The major constituent of these filaments are glial fibrillary acidic protein (GFAP). Under light microscope, the fibrous astrocytes look like a star-shaped cell body with finer processes. These processes are extended for long distances and contain abundant IFs.

The protoplasmic astrocytes, on the other hand, have more complex morphology. They contain highly branched processes that form membranous sheets surrounding the neuronal processes, cell bodies and end-feet on capillaries. In contrast to fibrous astrocytes, these cells have fewer IFs and a greater density of organelles.

Apart from the ultrastructural study, astrocytes can also be identified on the basis of marker proteins (Table 8.1).

8.4.2. Heterogeneous Population of Astrocytes in the CNS

In the CNS, many cells share some characteristics with astrocytes. These "*astrocytes-like*" cells are pituicytes, tanycytes,

TABLE 8.1. Markers of astrocytes.

Marker	Function	Cellular localization	Molecular weight
GFAP	Major constituent of intermediate filament found mostly in adult astrocytes	Cytoplasm	50 kDa (predicted)
EAAT1	Transport of amino acids	Cytoplasm	59.5 kDa
Glutamine synthase	In CNS, the enzyme found only in astrocytes; it catalyzes conversion of glutamate to glutamine	Cytoplasm	43 kDa
S-100	Ca-binding proteins	Cytoplasm/ Nucleus	21–24 kDa

GFAP, glial fibrillary acidic protein; EAAT1, excitatory amino acid transporter

ependymal cells, and Müller glia (Brightman and Cheng, 1988). "Bergmann glia" or Golgi epithelial cell, one of the astrocyte subtypes, is found mainly in cortical region of the cerebellum. The soma of such cell type is located in the Purkinje cell layer; they extend very long processes that end at the glia limitans of pia mater and large blood vessels. Also, there are astrocytes in white matter with more protoplasmic topology and with mixed fibrous and protoplasmic features.

On the basis of morphology and antigenicity, astrocytes from optic nerve cultures were designated as type 1 and type 2 (Raff et al., 1984). The type 1 astrocytes were originally defined as flat, polygonal cells that expressed GFAP but did not bind anti-ganglioside monoclonal antibodies A2B5 or R24 and LB1 except rat neural antigen 2 (Ran 2). These type 1 astrocytes proliferate well in the presence of epidermal growth factor and are found during gliogenesis in early developmental stage. On the other hand, type 2 astrocytes are found as $GFAP^{+}A2B5^{+}$ cells in rat optic nerve culture.

However, it is yet to know whether these morphologically distinct heterogeneous populations of astrocytes are also different in their function or such morphological differences are merely intrinsic.

8.4.3. Physiological Role of Astrocytes in the CNS

8.4.3.1. Maintaining CNS Homeostasis

8.4.3.1.1. Providing Structural Support

Astrocytes have long been considered as structural support cells of the brain. The anatomy of brain microvasculature shows that astrocytic end feet constitute an envelope around blood vessels (Kacem et al., 1998). Astrocytic processes are positioned beneath the pial membrane and the ependymal surface and thereby segregate the CNS parenchyma from external environment (Figure 8.1). The cytoplasmic processes of astrocytes form a close network around the synaptic complex and maintain synaptic integrity (Newman, 2003).

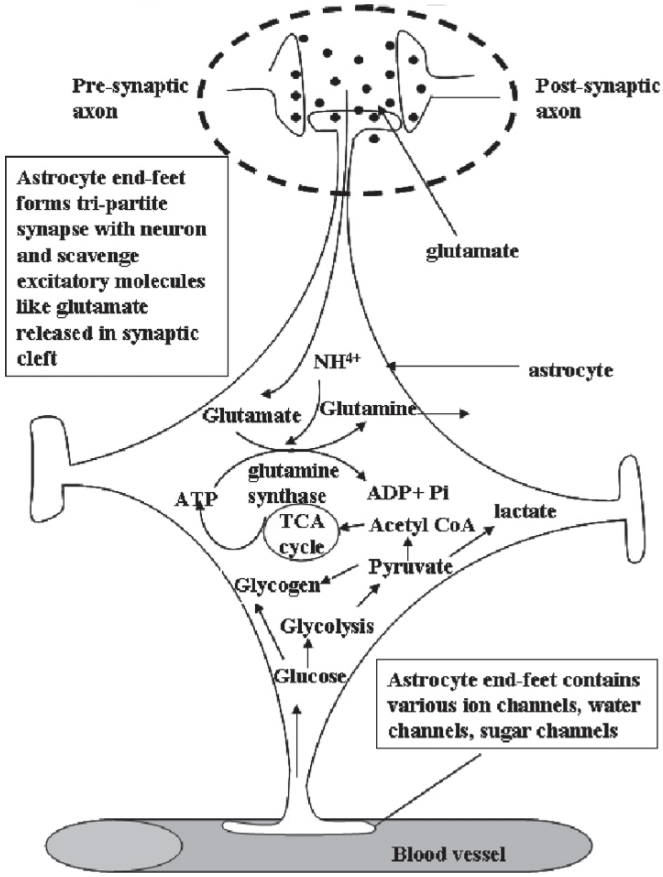

FIGURE 8.1. Maintenance of CNS integrity by astrocytes. Astrocytes endfeet forms a network on blood capillary and regulate transfer of water, ions and sugars. The pre-synaptic position of astrocytes is critical for the uptake of excitotoxic glutamate from neuronal synapse.

8.4.3.1.2. Maintaining Water Balance

Water is essential in the CNS for formation and maintenance of cerebrospinal fluid. Water enters into the CNS either through diffusion due to difference in osmotic pressure or through some specified channels. Astrocytes, through membrane-bound transporter system, maintain water and ionic homeostasis in the brain. The co-transporter system like "sodium-glutamate co-transporter" (Na^{+}-glutamate, EAAT1) and sodium-potassium-chloride ion co-transporter (Na^{+}-K^{+}-Cl^{-} co-transporter, NKCC) are located on astroglial membrane and regulate astrocytic water transport into the CNS (Figure 8.1).

Apart from the co-transporters, astrocytic perivascular system in the brain involves membrane-bound water channels, called aquaporins (Nagelhus et al., 2004). These water channels specifically mediate water fluxes within the brain. Among several members of the aquaporin family, aquaporin 4 and 9 are expressed in astrocytes. The activity of aquaporins is regulated by transmembrane G-protein coupled receptor (GPCR) family of hormonal receptors.

8.4.3.1.3. Maintaining IonHomeostasis

Astrocytes are responsible in maintaining extracellular K^{+} ion concentration at a level compatible with neuronal function. Astrocytes form a syncytium through which it efficiently redistributes K^{+} from perineuronal to perivascular space. Such redistribution of K^{+} is mediated by inwardly rectifying K^{+} ion channels. One such K^{+} ion channel Kir 4.1 is expressed in astrocytes surrounding neuronal synapses as well as blood vessels in the brain (Nagelhus et al., 2004).

In addition to having K^{+} channels, astrocytes bear plenty of other ion channels; function of many of them is still under research. Astrocyte cell surface bears an atypical sodium channel Nax that is assumed to be under voltage-gated sodium

channel family. Nax is exclusively localized to perineuronal lamellate processes extended from ependymal cells and astrocytes. It has been suggested that glial cells bearing Nax channel are the first to sense a physiological increase in sodium level in body fluids (Watanabe et al., 2002).

8.4.3.1.4. Regulating Neurotransmitter and Amino Acid Levels

Astrocytes are active participants in the formation of tri-partite synapse and modulate synaptic activity of neurons. Glutamate plays a central role in astrocytic-neuronal interactions. This excitatory amino acid released by neurons, is taken up by astrocytes from the neuronal synapses via their glutamate transporters. Astrocytes convert glutamate into glutamine and release into the synaptic cleft for being taken up by neurons (Hertz and Zielke, 2004). Astrocytes express several receptors linked to ion channels and second messenger pathways. Activation of receptors e.g. metabotropic glutamate receptor, in turn, elevates intracellular level of Ca^{2+}. Calcium-dependent glutamate release from astrocytes modulates the activity of both excitatory and inhibitory synapses (Figure 8.1).

Apart from glutamate, astrocytes also uptake neurotransmitters like gamma amino butyric acid (GABA), aspartate, taurine, β-alanine, serotonin, and catecholamines. The fate of all these neurotransmitters is to be metabolized within astrocytes.

8.4.3.1.5. Detoxifying Ammonia

Ammonia is toxic for the CNS. Ammonia toxicity may result in neurological abnormalities leading to seizures, mental retardation, brain edema, convulsion, and coma. One of the most important enzymes that catalyze the formation of ammonia in the brain is glutamate dehydrogenase. This enzyme catalyzes reversible oxidative deamination of glutamate and produces ammonia particularly in astrocytes, thereby provides a mechanism for the removal of excess nitrogen from certain amino acids. Brain lacks carbamoyl phosphate synthase 1 and ornithine transcarbamylase, essential enzymes for the urea cycle, and thereby unable to remove accumulated ammonia (Cooper and Plum, 1987). However, astrocytes convert excess ammonia to glutamine via glutamine synthase (Figure 8.1). The excreted glutamine from astrocytes is taken up by neurons. In fact, either in physiological condition or even in hyperammonemic condition, rapid conversion of ammonia to glutamine in astrocytes is the predominant detoxification event in the CNS (Bak et al., 2006).

8.4.3.2. Supplying Energy

Glucose and ketone bodies are the primary source of energy in mammalian brain under normal physiological conditions. In comparison to its weight, which is only 2–3% of total body weight, brain consumes up to one fourth of body's total glucose supply.

8.4.3.2.1. Glycolysis

Astrocytes are the major food depot in the CNS. The food is stored in the forms of glycogen. Several studies suggest that glycogen phosphorylase and synthase are predominantly localized in astrocytes. Glucose is utilized in astrocytes mainly via glycolysis (Wiesinger et al., 1997). Deprivation of glucose in cultured astrocytes results in reduction in ATP/ADP ratio and membrane depolarization. Sugars enter into the metabolic pathway through phosphorylation which is considered as rate determining step. Astrocytes express hexokinase 1, the primary isoform of hexokinase in the CNS. This enzyme is mostly localized in mitochondria, only about 30% of it is found in cytosol.

8.4.3.2.2. Oxidative Metabolism

In order to generate energy in the form of ATP, sugars are bound to enter into oxidative metabolic pathway, the tri carboxylic acid cycle (TCA cycle) (Figure 8.1). Glycolysis generates 2 molecules of ATP and the TCA cycle generates 30 more ATP molecules from one molecule of glucose. The formation of energy in astrocytes is either through utilization of glucose under normal physiological condition or from reserve food storage glycogen via gluconeogenesis.

8.4.3.3. Organizing the Information Network in the CNS

As has been discussed earlier, astrocytes outnumber neurons by about ten to one in the CNS; and yet, historically they were considered to be a sort of glue (γλια) or connective tissues of the CNS. In the recent years though, it is increasingly clear that astrocytes form an integral and active component of the information network in the CNS and have received the "stardom" reflecting their morphology (Haydon, 2001; Nedergaard et al., 2003; Ransom et al., 2003). Astrocytes thus are a critical participant of "the tripartite synapse" (Araque et al., 1999; Perea and Araque, 2002). Indeed, not too long ago an entire book dedicated to "The Tripartite Synapse" that discussed in excellent detail, the anatomical and functional basis of neuroglial interactions, astrocyte calcium excitability, and the role of astrocyte in regulation of synaptic function (Volterra et al., 2002). Any discussion on astrocyte biology is thus incomplete without a consideration of their role in information transfer and intercellular communications in the brain. The details of the molecular mechanisms of this process via specific signaling events in health and disease will be discussed later in this book (see chapter by Pahan and Bidasee). This section highlights the current information on the glial communication networks of metabolite transport through gap junctions and the importance of calcium as a hallmark regulator of glial function.

8.4.3.3.1. Role of GAP Junctions

In the brain, astrocytes form a syncytium, or a network of integrated cells (Scemes, 2000). Such a syncytium consists of astrocytes that have cytoplasmic continuity in adjacent cells

through gap junctions (Bennett et al., 2003). Gap junctions serve as a conduit between two astrocytes and consist of two hemichannels, also called connexones. These hemichannels or connexones are contributed by the juxtaposed cells and together form the gap junction (Figure 8.2). Hemichannels assembled in the endoplasmic reticulum consist of junctional proteins belonging to the family of connexins (Contreras et al., 2003; Saez et al., 2003). Although a variety of connexins are expressed by astrocytes, connexin (Cx) 43 is the predominant astrocyte connexin (Theis et al., 2005). These gap junctions form channels or pores about 1–1.5 nm in diameter and permit transfer of small metabolites including, but not limited to, NAD, ATP, glutamate and Ca2+ (Saez et al., 2005). Gap junctions formed with Cx43 are also permeable to dyes, such as Lucifer yellow or propidium, which serve as experimental tools for studies on gap junction function. The presence of gap junctions between astrocytes and the tripartite synapse consisting of the conventional synapse ensheathed by astrocyte processes together serve as models for neuroglial interactions. Indeed, evidence from astrocyte-neuron co-cultures has demonstrated that the presence of astrocytes in neuronal cultures increases the number of synapses and their efficiency. On the other hand, gap junctional communication and function can be regulated by neurons (Rouach et al., 2004). It is now generally accepted that astrocytes are likely involved in a variety of neurodegenerative diseases; however, alterations in the gap junction communication in pathological conditions, regulation of hemichannels and connexin expression and function is largely unresolved (Nakase and Naus, 2004).

8.4.3.3.2. Role of Calcium

While neurons are most prominently identified with their electrical excitability and astrocytes lack such electrical impulses, calcium waves that propagate through gap junctions have emerged as the parallel mechanism to that of the transfer of electrical impulse from one neuron to another. This of course by no means suggests that calcium communication is unique to astrocytes; indeed, such signals are commonly used by a variety of cells and neurons are no exception to this. In neurons, calcium signals lead to an instant integrated elecrical and chemical communication in synaptic cells. In both cultured astrocytes and astrocytes in intact brain slices, excitation of one cell can form a calcium wave transferred to several neighboring cells in multiple directions. This involves elevated calcium in a single cell followed by elevated intra-

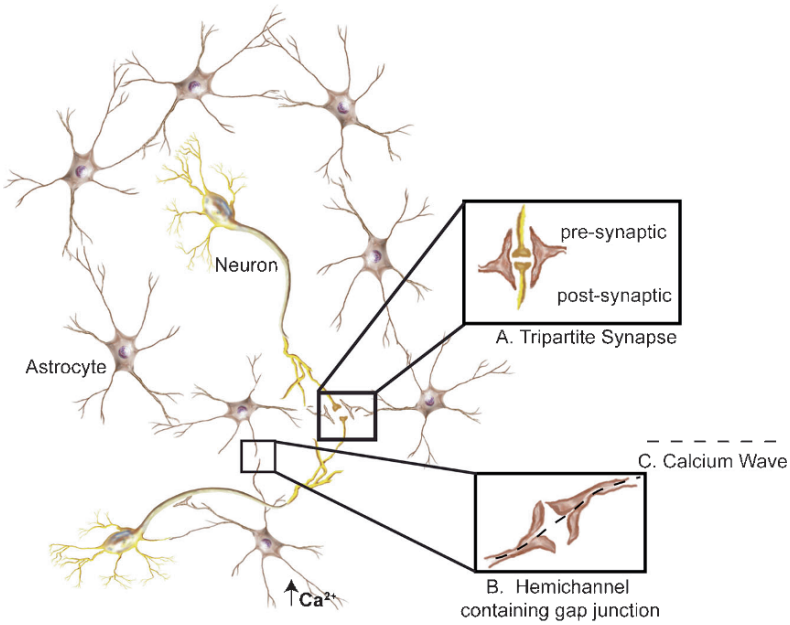

FIGURE 8.2. The astrocyte nexus: The astrocyte processes engulf the synapse forming what is now known as the tripartite synapse, consisting of pre- and post-synaptic elements as the two components along with the astrocyte ensheathment as the third. The brain astrocytes communicate with each other through intercellular connections forming a large network on interconnected cells. These cells join through gap junctions that form a channel through which small molecules can pass from one cell to the next. Calcium, one of the molecules that can pass through these gap junctions can lead to exponential transfers within the astrocyte nexus forming a calcium wave. Such connexin 43 containing functional gap junctions are primarily observed in the astrocytes in the brain.

cellular calcium in other cells. Such transfer of the calcium wave has been related to the Cx43 gap junction coupling of astrocytes both in vitro and in situ (Schipke and Kettenmann, 2004). Mobilization of intracellular calcium is also widely used by astrocytes as a prominent cell signaling mechanism in response to a variety of stimuli both in physiological and pathological conditions (Verkhratsky and Kettenmann, 1996). Calcium ions form an important cellular messenger and can provide an exquisitely sensitive mechanism for signaling, depending on their amount, distribution, amplitudes, and time course.

Even more exciting is that neuronal activity can stimulate such calcium communication in astrocytes and vice versa (Perea and Araque, 2005a). Thus, the role of calcium in the function of the tripartite synapse has received significant recent attention (Araque and Perea, 2004; Hirrlinger et al., 2004; Perea and Araque, 2005b). Ca^{2+} excitability of glia is observed in response to a variety of stimuli (Verkhratsky and Kettenmann, 1996). Such an elevation in intracellular calcium in a single astrocyte thus leads to the elevated calcium in the neighboring cells or the calcium wave described above. The complexity of calcium regulation in astrocytes is even greater, as has been revealed by recent studies showing that the calcium oscillations in a single cell may not even encompass the entire cell volume, but remain restricted to certain microdomains or certain processes within the astrocyte. Thus, we see the beauty of the autonomous functioning of a specific part of the cell that may have encompassed a syncytium of other cells or may ensheathe certain synapses, and provide at the same time the possibility of chemical coupling of the entire cell when given the appropriate stimulus (Carmignoto and Pozzan, 2002; Kettenmann and Filippov, 2002; Nett and McCarthy, 2002). The question as to what such calcium excitability of glia does in the neuroglial interactions is evolving. Certainly, from the example set by neurons where calcium signaling is tightly integrated with chemical release, the possibility that calcium excitability of astrocytes leads to the release of metabolites, which may in turn act in an autocrine or a paracrine manner, has also been investigated. The arena of calcium excitability of glia, their functional syncytium in the brain and their role in the tripartite synapse, all are subjects of intense investigation and will prove valuable in the complete understanding of neuroglial function.

8.4.3.4. Releasing Neuropeptides and Neurotrophins

Apart from its function as "support" cells by maintaining integrity of the CNS, astrocytes release several neuropeptides and neurotrophins. So far, four families of neuropeptides have been demonstrated to be expressed in astrocytes:

A. *Renin-angiotensin family*: The major function of renin-angiotensin system (RAS) in periphery is to maintain body-fluid homeostasis and regulate blood pressure.
B. *Endothelins*: Endothelins (ETs), a group of vasoactive peptides, acts as growth factor, exerting different functions like induction of proliferation, protein synthesis or changes in morphology. The ET1 also increases the rate of glucose 6-phosphate utilization via pentose phosphate pathway.
C. *Enkephalins*: The pentapeptide enkephalins are the ligand of orphan receptors in brain and are present mostly as precursor form in astrocytes.
D. *Neurotrophins*: Astrocytes are capable of releasing several growth factors and neurotrophic factors including epidermal growth factor (EGF), transforming growth factor (TGF), insulin like growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2), brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), and neurotrophin-3 (NT-3) (Wu et al., 2004).

8.4.3.5. Facilitating Neurogenesis

One of the key advances in the field of neurobiology is the discovery that astroglial cells can generate neurons not only during development, but also throughout adult life and potentially even after brain lesion. It has been shown that in neurogenic regions of adult brain (ventricular zone, hippocampus, and olfactory subependyma), astrocytes secrete factors like FGF-2, IGF-1, Shh, BDNF, GDNF, and NT-3 that induce neurogenesis (Altaba et al., 2002) and support the growth of neurons and neural progenitor cells (Wu et al., 2004).

8.4.4. Role of Astrocytes in CNS Disorders

8.4.4.1. Activation of Astrocytes and Gliosis

Recent evidence suggests that astrocytes might act as immunocompetent cells within the brain (Shrikant and Benveniste, 1996). Astrocytes react to various neurodegenerative insults rapidly, leading to vigorous astrogliosis. This reactive gliosis is associated with alteration in morphology and structure of activated astrocytes along with its functional characteristics (Eddleston and Mucke, 1993). The astrocytic processes construct a bushy network surrounding the injury site, thus secluding the affected part from the rest of the CNS area. Subsequently, astrogliosis has been implicated in the pathogenesis of a variety of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease, inflammatory demyelinating diseases, HIV-associated dementia (HAD), acute traumatic brain injury, and prion-associated spongiform encephalopathies (Eng and Ghirnikar, 1994). Although activated astrocytes secret different neurotrophic factors for neuronal survival, it is believed that rapid and severe activation augments/initiates inflammatory response leading to neuronal death and brain injury (Tani et al., 1996; Yu et al., 1993). Enhanced up-regulation of GFAP is considered as a marker for astrogliosis (Eng et al., 1994). GFAP increases at the periphery of ischemic lesion following neurodegenerative insults (Chen et al., 1993). Senile plaques, a pathologic hallmark of Alzheimer's disease, are associated with GFAP-positive activated astrocytes (Nagele et al., 2004). It is reported that in various neuroinflammatory diseases, the increased GFAP expression corresponds to the severity of astroglial activation (Eng et al., 1992; Eng and Ghirnikar, 1994).

Recently our lab showed that various neurotoxins increase the expression of GFAP in astrocytes via nitric oxide (NO) (Brahmachari et al., 2006) suggesting that scavenging of NO may be an important mechanism in attenuating astrogliosis. Although the activation of NF-kB is involved in neurotoxin-induced production of NO in astrocytes, once NO is produced, it does not require the activation of NF-κB to induce the expression of GFAP (Figure 8.3). However, NO induces/increases the expression of GFAP in astrocytes via guanylate cyclase (GUCY)—cyclic GMP (cGMP)—protein kinase G (PKG) pathway (Figure 8.3).

8.4.4.2. Release of Pro-inflammatory Molecules

Upon severe activation in response to various neurodegenerative and neuroinflammatory challenges, astrocytes secrete various pro-inflammatory molecules including pro-inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6, and lymphotoxin), chemokines, reactive oxygen species, reactive nitrogen species, and eicosanoids (Brosnan et al., 1994; Gendelman et al., 1994; Meeuwsen et al., 2003; Van Wagoner et al., 1999). These secreted pro-inflammatory molecules play an important role in the pathogenesis of various neurological disorders (Heales et al., 2004). In cultured murine astrocytes, bacterial lipopolysaccharides (LPS) act as a prototype inducer of various inflammatory responses. LPS is capable of inducing the expression of pro-inflammatory cytokines and inducible nitric oxide synthase (iNOS) in rat primary astrocytes (Pahan et al., 1997) but unable to induce the expression of iNOS in human astrocytes (Jana et al., 2005).

Among different pro-inflammatory cytokines (IL-1β, TNF-α, and IFN-γ) tested, only IL-1β alone is capable of inducing iNOS in human primary astrocytes (Jana et al., 2005). Similarly, among different cytokine combinations, the combinations involving only IL-1β as a partner are capable of inducing iNOS in human astrocytes (Figure 8.4). The combination of IL-1β and IFN-γ induces the expression of iNOS at the highest level in human astrocytes. Different pro-inflammatory transcription factors are involved in the transcription of iNOS in various cell types including astrocytes (Kristof et al., 2001; Liu et al., 2002; Pahan et al., 2002; Xie et al., 1994). All the three cytokines independently induce the activation of AP-1 while IL-1β and TNF-α but not IFN-γ induces the activation of NF-κB. However, among three cytokines, only IL-1β is capable of inducing the activation of CCAAT box/enhancer-binding proteinβ (C/EBPβ) (Figure 8.4) suggesting an essential role of C/EBPβ in the expression of iNOS in human primary astrocytes (Jana et al., 2005). In addition to pro-inflammatory cytokines, viral double-stranded RNA (Auch et al., 2004) and HIV-1 Tat also induce the expression of iNOS and the production of NO in human astrocytes (Liu et al., 2002).

8.4.4.3. Do astrocytes Present Antigen Under Autoimmune Response?

The CNS has long been known as "immunological privileged site" as it is secluded by BBB from peripheral immune system. However, this hypothesis is gradually becoming wrong. Microglia are capable of functioning as antigen-presenting cells (APC) as they express MHC I and II molecules (Carpentier et al.,

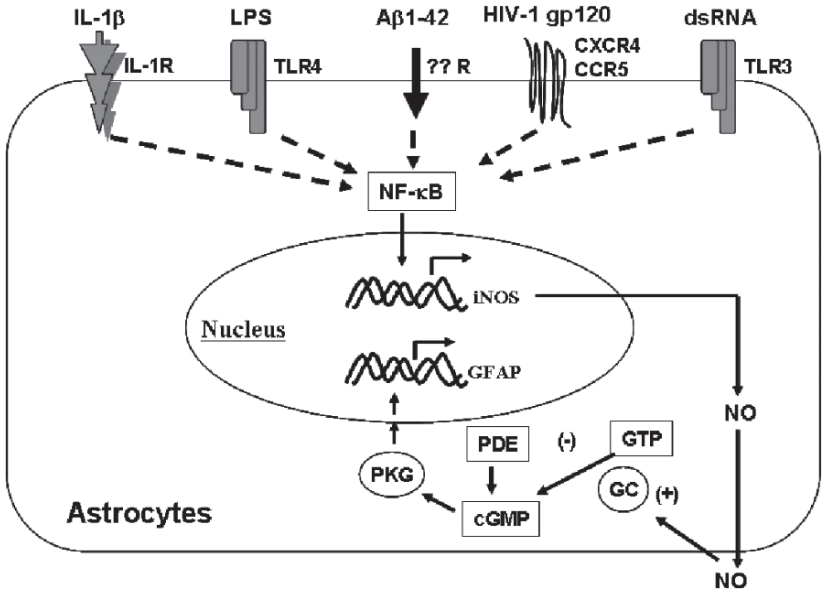

FIGURE 8.3. Various neurotoxins induce the expression of inducible nitric oxide synthase (iNOS) via the activation of NF-κB. Nitric oxide produced from iNOS then induces the activation of guanylate cyclase (GUCY) that catalyzes the production of cGMP. Inhibition of phosphodiesterase may also increase the level of cGMP. Cyclic GMP utilizes protein kinase G (PKG) to increase the expression of GFAP. IL-1R, IL-1 receptor; TLR4, toll-like receptor4; GPCR, G protein-coupled receptor; TLR3, toll-like receptor 3.

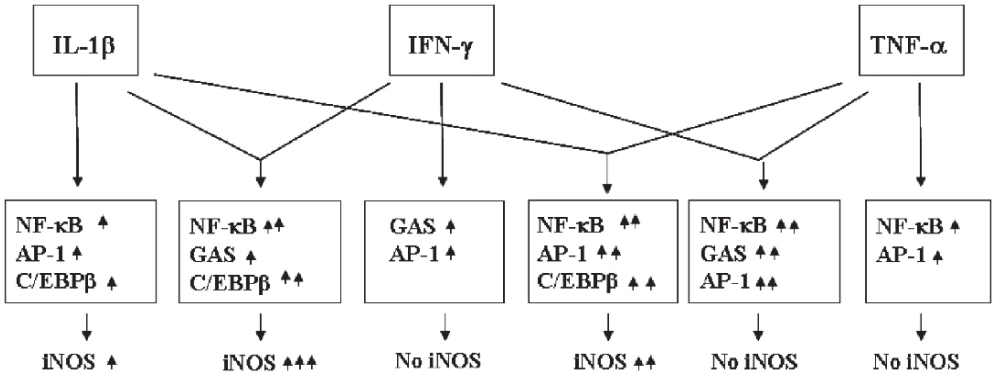

FIGURE 8.4. Expression of iNOS by various pro-inflammatory cytokines in human primary astrocytes. TNF-&bdotalpha;and IFN-γ alone or in combination are unable to induce the expression of iNOS. On the other hand, IL-1B alone or in combination with other cytokines induce iNOS in human astrocytes. Activation of AP-1 and GAS together by IFN-γ is not sufficient for the expression of iNOS. Activation of AP-1 and NF-κB together by TNF-α is also not sufficient for iNOS expression. Activation of AP-1, NF-κB and GAS together even at a higher level by the combination of TNF-α and IFN-γ compared to that induced by individual cytokines is also not sufficient for the expression of iNOS. However, Il-1β capable of activating C/EBPβ, AP-1 and NF-κB induced iNOS in human astrocytes suggesting an important role of C/EBPβ in the expression of iNOS in human astrocytes.

2005; Dong and Benveniste, 2001). In addition, microglia also expresses co-stimulatory molecules B7.1 and B7.2 molecules which play a role during antigen presentation. Another possible candidate as CNS APC is astrocyte. Expression of MHC II in astrocyte upon stimulation with IFN-γ or viruses has been demonstrated both *in vivo* and *in vitro*. However, capability of astrocytes as APC is still a controversial point. Examination of CNS tissues in MS, shows expression of B7-1 or B7-2 co-stimulatory molecules on macrophages and microglia but not on astrocytes. Human astrocytes also do not express co-stimulatory molecules B7-1 or B7-2. On the other hand, murine astrocytes express B7-1 or B7-2 either constitutively or in the presence of IFN-γ. Conflicting results are also found in case of CD40 expression. For example, CD40 expression is observed in fetal human astrocytes but not in adult human astrocytes. Therefore, functional ability of astrocytes as APC needs more research.

8.4.4.4. Formation of Glial Scar: A Double-Edged Sword

Astrocytes play a dual role in inflammatory insults. In one hand, activated astrocytes, characterized by cellular hypertrophy, proliferation and increased GFAP expression represent anisomorphic gliosis. This is the consequence of gross tissue damage and results in the formation of tightly compacted limiting glial margin termed as astrogliotic scar or glial scar. The pro-inflammatory molecules released by reactive astrocytes in the scar cause tissue damage and inhibit neurite outgrowth as well as induce oligodendrocytes death. Chondroitin and keratin sulphate proteoglycans are among the main inhibitory extracellular matrix molecules that are produced by reactive astrocytes in the glial scar and are believed to play a crucial part in failure to regeneration. On the other hand, isomorphic gliosis, formed in response to insult, results in improved recovery and regeneration of the damaged tissue (Eddleston and Mucke, 1993; Silver and Miller, 2004). At the sites distant from injury, activated astrocytes get transformed to a more pronounced stellate shape with increased production of anti-oxidants and soluble growth factors that coordinate tissue remodeling in enhancing the survival of adjacent neurons and glia.

8.4.4.5. Trying to Defend Neurons Against Oxidative Stress and Excitotoxic Damage

One of the hallmarks of various neurodegenerative and neuroinflammatory disorders is oxidative stress-induced CNS damage. Such oxidative stress can damage lipids, proteins and nucleic acids of cells and power-house mitochondria causing cell death in assorted cell types including neurons and oligodendroglia. However, astrocytes having high levels of anti-oxidant enzymes (glutathione peroxidase, catalase, glutathione reductase, and superoxide dismutase) and anti-oxidants (gluthathione and ascorbic acid) try to absorb reactive oxygen species ($O_2^{=}$, O_2^{-}, and $OH^{\cdot}$) and reactive nitrogen species (NO, $ONOO^{-}$), maintain redox homeostasis and defend the insulted CNS (Chen and Swanson 2003; Dringen and Hirrlinger, 2003; Wilson, 1997). In addition, astrocytes also scavenge detrimental molecules such as glutamate, produced during synaptic transmission through neurons (Hertz and Zielke, 2004). Astrocytes convert glutamate to glutamine by glutamine synthetase.

8.4.4.6. Swelling of Astrocytes

Astrocytes undergo rapid swelling in certain acute pathological conditions like ischemia and traumatic brain injury. Different mechanisms are involved in such swelling process of astrocytes. Some of these are, decreasing extracellular fluid osmolarity, intracellular acidosis, formation of ammonia, increase in Na^{+}, K^{+}, $2Cl^{-}$ co-transporter system, and due to drastically

elevated levels of arachidonic acid and its metabolites. Alteration in glutamate metabolism and accumulation of glutamine and its transamination product, alanine is another possible cause of astrocytes swelling. In ischemic condition or in acute brain trauma, proton accumulation in cytoplasm cause astroglial cell swelling predominantly via activation of Na^+/H^+ and Cl^-/HCO_3^- exchangers.

8.4.4.7. Undergoing Apoptosis Under Acute Insults

Although astrocytes usually undergo proliferation and gliosis in various neurodegenerative disorders, under acute insults, astrocytes may undergo apoptosis. *Ex vivo* cell culture studies demonstrate that tumor suppressor protein p53 plays a role in neuronal as well as astrocyte apoptosis (Bonni et al., 2004). HIV-1 infection of the central nervous system (CNS) frequently causes dementia and other neurological disorders in which apoptosis of astrocytes along with neuron has been found. HIV-1 infection of primary brain cultures induces the receptor tyrosine kinase c-kit and causes apoptosis of brain cells including astrocytes (He et al., 1997). The importance of c-Kit in apoptosis of astrocytes has further been confirmed by overexpressing c-Kit in an astrocyte-derived cell line in the absence of HIV-1. The mechanism of c-kit induction by HIV-1 involves transactivation of the c-kit promoter by the HIV-1 Nef protein.

8.5. Oligodendrocytes: Biology and Function

Camillo Golgi was the first to give a good description of glia. A few years later, Cajal, student of Rio Hortega (1921) showed that there are two quite distinct cell types of neuroglia besides astrocytes using silver carbonate impregnation technique which he named oligodendrocyte (OL) and microglia. OLs are specifically the myelin-forming cells of the CNS.

8.5.1. Markers and Morphological Characteristics of Various Developmental Stages of Oligodendrocytes

Among different brain cells, the development of OL has been well characterized. During differentiation, oligodendrocyte lineage cells (early oligodendrocyte progenitors, oligodendrocyte progenitors, pro-oligodendrocytes, immature oligodendrocytes, and mature oligodendrocytes) (Figure 8.5) express stage-specific components that serve as markers of lineage progression (Table 8.2). Morphological characteristics of various developmental stages are shown in Figure 8.5.

8.5.2. Biological Role of Oligodendrocytes in the CNS

The major biological role of OL is myelination. However, OL may also promote neuronal survival, axonal growth and process formation. Neuronal function is also influenced by OL-derived soluble factors that induce sodium channel-clustering along axons. Neurotrophins (NGF, BDNF, and NT3) produced from OL may provide the trophic support for both OL and local neurons.

PSA-NCAM, polysialylated form of neural cell adhesion molecule; PDGFR-α, platelet-derived growth factor receptor α; MBP, myelin basic proteins; PLP, proteolipid protein; DM20, isoform of PLP; MOG, myelin/oligodendrocyte glycoprotein; MAG, myelin-associated glycoprotein; CNPase, 2′,3′-cyclic nucleotide 3′-phosphodihydrolase (Baumann and Pham-Dinh, 2001; Deng and Poretz, 2003).

8.5.2.1. Myelinating CNS Neurons

Myelination is a sequential multi-step process in which a myelinating cell recognizes and adheres to an axon, then ensheathes, wraps and ultimately excludes its cytoplasm from the spiraling process to form compact myelin. An OL is able to myelinate upto 40 axons depending on its localization. Myelin is composed of lipids and proteins, most of which are

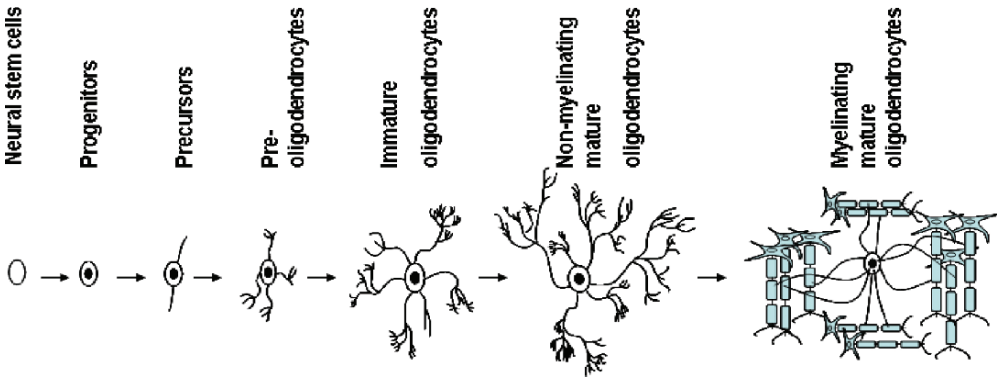

FIGURE 8.5. Different stages of oligodendroglial development.

Table 8.2. Stage-specific markers of oligodendrocytes.

Developmental stages	Markers	Detection	Characterization
Precursor A2B5$^-$	PSA-NCAM, Nestin, PDGFR-α, DM-20	Anti-PDGFR-α antibody	PSA-NCAM$^+$ /Nestin$^+$/
Oligodendrocyte Progenitor cells(OPCs)	NG2/AN2+ proteoglycan, PDGFR-α protein or mRNA, GD3-related gangliosides, DM-20, CNPase	Anti-NG2 antibody, Anti-PDGFR-α antibody, A2B5 antibody	A2B5$^+$ /O4$^-$
Pro/pre-oligodendrocyte	PDGFRα, O4, GD3, NG2/AN2+, PLP/DM20, CNPase	Anti-NG2 antibody, Anti-PDGFR-α antibody, O4 antibody	A2B5$^+$ /O4$^+$
Immature oligodendrocytes	GalC, O4, CNPase, PLP/DM20	O4, O1, CNPase	A2B5-/MBP-/ R-mAb+
Mature oligodendrocytes	GalC, O4, CNPase, MBP, PLP, MAG, MOG	MBP, MOG, PLP, MAG	A2B5-/MBP+

specific for the myelin sheath. The major proteins are MBP, PLP, CNPase, and MAG. In the CNS, axonal factors play a critical role in the myelination process and thickness of the myelin, and that myelination in the CNS depends on a balance between positive and negative axonal signal (Sherman and Brophy, 2005).

8.5.2.1.1. Role of Proteins

Role of CNPase: CNPase was first identified in CNS myelin and it represents about 4% of the total myelin protein in the CNS. It possesses enzymatic activity that catalyses the hydrolysis of 2′, 3′-cyclic nucleotides into their corresponding 2′-neucleotides. However, to date any substrates of this enzyme has not been detected in the brain. Therefore, precise role of this enzyme in brain is unknown. However, it is one of the earliest myelination-specific polypeptides, synthesized by oligodendrocytes prior to the appearance of the myelin structural proteins (MBP and PLP) and its synthesis persists into the adulthood, suggesting a role in the synthesis and maintains of the myelin sheath. Over-expression of CNPase in transgenic mice perturbs myelin formation and creates aberrant OL membrane expansion. Recently, CNP knockout mouse study shows that CNP protein is required for maintaining the integrity of para-nodes and disruption of the axo-glial signaling at this site causes the progressive axonal degeneration (Rasband et al., 2005).

Role of MBP: MBP is one of the major proteins of the CNS, and it constitute about 25–30% of the total protein. The 18.5 kDa isoform of myelin basic protein (MBP) is the exemplar of the family, being most abundant in adult myelin, and thus the most-studied. Shiverer mutation of MBP gene results in the absence of MBP proteins and morphological analysis of the CNS reveals an almost total lack of myelin in the brain, and also the existing myelin is abnormal, presenting no major dense line. Therefore, MBP is necessary for the formation of the major dense line in the CNS myelin (Readhead and Hood, 1990).

Role of MOG: MOG is a member of the immunoglobulin super family, preferentially localized on the outside surface of myelin sheath and on the surface of OL process. Immunocytochemical studies demonstrate that the expression of MOG is late in OL differentiation compared with other major myelin proteins. It is used as a surface marker of oligodendrocyte maturation. This specific CNS protein is a minor component of myelin, constituting 0.01–0.05% of total myelin proteins. It is a 26–28 kDa integral glycoprotein and like other myelin proteins it may exist in multiple forms. Since the location of this protein in outermost surface of the myelin, it is easily accessible to a humoral immune response. MOG not only binds C1q but also may be the protein in myelin responsible for complement activation (Johns and Bernard, 1999).

Role of PLP/DM20: PLP is the most abundant intra-membrane protein and represents about 50–60% of the total protein in the CNS. It is localized predominantly in compact myelin. DM-20 and PLP arise from alternative splicing of a genomic transcript and differ by a hydrophilic peptide segment of 35-amino acids long, the presence of which generates the PLP product. PLP is necessary for normal myelin compaction, but the molecular mechanism for the adhesive function of these proteins is not known. In addition, it has been shown that PLP/DM20 play a metabolic role in maintaining axonal metabolism (Knapp, 1996) and also play an important role in the formation of intraperiod line and in maintaining axonal integrity. In human, mutation of PLP and DM20 gene causes Pelizaeus-Merzbacher disease (PMD), an X-linked dysmyelinating neuropathy, and spastic paraplegia type II (SPG-II) (Duncan, 2005).

Role of other myelin proteins: Besides these four proteins, myelin also contains other proteins that play a critical role in myelin compaction and neuronal function.

Myelin-associated glycoprotein (MAG): MAG is a minor constituent of both the CNS and PNS myelin. MAG found on the myelin membrane adjacent to the axon. MAG is believed to participate in axonal recognition and adhesion, inter-membrane spacing, signal transduction during glial cell differentiation, regulation of neurite out growth, and in the maintenance of myelin integrity.

Myelin associated/oligodendrocyte basic protein (MOBP): MOBP is abundantly expressed in the CNS myelin and shares several characteristics with MBP. MOBP is synthesized by mature OL and localized at the major dense line, suggesting a role in the myelin compaction process.
P2: P2, a basic protein with a molecular weight about 13.5 kDa, is located on the cytoplasmic side of the compact myelin membranes. It may serve as lipid carrier and thus could be involved in the assembly, remodeling and maintenance of myelin (Garbay et al., 2000).

Oligodendrocyte-specific protein (OSP/claudin-11): OSP/claudin-11 and PLP are both tetraspan proteins concentrated in CNS myelin. OSP represents about 7% of total myelin proteins. They possibly play an important role in myelin formation and maintenance due to their localization and concentration in membrane sheaths. Individual OSP/claudin-11- and PLP-null mice have relatively normal-appearing myelin and mild neurological deficits due to their compensatory role. However, when both OSP/claudin-11 and PLP genes are knocked out, mice show severe neurological deficits, markedly abnormal myelin compaction, and smaller axon diameters (Chow et al., 2005).
Cx32: Cx32, an integral membrane protein, is structurally related to PMP22 with four hydrophobic transmembrane domains. Recent studies show that it is also expressed on some areas of the CNS myelin and corresponding myelinating OL. Cx32 is preferentially expressed in oligodendrocytes in the CNS and in Schwann cells in the PNS. In addition to forming gap junctional channels, Cx32 also forms functional hemichannels. Mutation in Cx32 causes a common peripheral demyelinating neuropathy, X-linked Charcot-Marie-Tooth disease (Gomez-Hernandez et al., 2003).

Oligodendrocyte-myelin glycoprotein (OMgp): It is a glycosylated protein with molecular mass 120 kDa. It is located in the para-nodal areas of myelin. During injury, it inhibits the axonal growth by interacting with Nogo-66 receptor (NgR) complex.

Myelin/oligodendrocyte specific protein (MOSP): MOSP is a novel surface protein which is exclusively expressed in CNS myelin. It also plays an important role in membrane/cytoskeleton interactions during the formation and maintenance of CNS myelin.

8.5.2.1.2. Role of Lipids

In the CNS, lipids play an important role in myelin formation along with various protein molecules. One of the major biochemical characteristics that distinguish myelin from other biological membranes is its' high lipid-to-protein ratio. About 70–80% of the dry weight of myelin is comprised of lipid components and 20–30% protein. In every mammalian species, myelin contains cholesterol, phospholipids and glycolipids in molar ratios ranging from 4:3:2 to 4:4:2. In mature brain, cholesterol is the major lipid in myelin (about 20–25%) but generally normal myelin does not contain any cholesterol ester. Cholesterol helps to increase membrane thickness and fluidity as well as ion leakage through membranes which may be relevant to its property of electrical insulation.

Other abundant lipids in myelin are galactosylcerebrosides (Gal-C) and their sulfated derivatives (sulfatides). GalC represent 20% lipid dry weight in mature myelin. Immunological and chemical perturbation studies indicate that these lipids are involved in oligodendrocyte differentiation, myelin formation and myelin stability. These galactolipid-deficient animals exhibit severe tremor, hindlimb paralysis and display electrophysiological deficits in both CNS and PNS (Baumann and Pham-Dinh, 2001).

8.5.2.1.3. Molecules Involved in Positive and Negative Regulation of Myelination

The formation and maintenance of the myelin sheath require the coordination of a number of gene products. While some gene products facilitate myelination, some others try to suppress myelin formation. In the following lines, we describe such positive and negative regulatory mechanisms.

Molecules involved in positive regulation of myelination: OLIG1 and OLIG2 are closely related basic helix-loop-helix transcription factors that are expressed in myelinating OL and their progenitor cells in the developing CNS. Both OLIG1 and OLIG2 are positive regulators of myelination. Specifically OLIG1 has an essential role in oligodendrocyte differentiation and myelination, as it regulates the transcription of major myelin-specific genes MBP, PLP and MAG. On the other hand, OLIG2 is required for the initiation of oligodendrogliogenesis but its role in myelination is controversial (Xin et al., 2005).

Another important molecule that stimulates myelination is GPI-linked neural cell recognition molecule F3/contactin. This is a physiological ligand of Notch that signals via DTX1 to promote the development of OL (Hu et al., 2003; Popko, 2003). Additionally, F3 also transduces signals to glial intracellular Fyn, which then interacts with Tau protein to mediate myelination. As expected, different neurotrophins also favor myelination through the maintenance of oligodendroglial cell health and viability. For example, neurotrophin-3 (NT3) is known to induce both survival and proliferation of oligodendrocyts. NT3 interacts with TrkC to activate CREB that plays a critical role in proliferation and maturation of OPCs, and in the expression of myelin genes (MBP, P2, P0 MOG, PLP, and MAG) and anti-apoptotic gene Bcl-2. In addition, recent studies have identified many other molecules (e.g. PAX3, PPAR-δ, MyT1, SOX, GTX, Sp1, SCIP/Oct6/Tst-1) that may function as positive regulators of myelination (Wegner, 2000).

Molecules involved in negative regulation of myelination: Bone morphogenic proteins (BMP4s) should have a role in regulating bone density! Yes, they do have and in addition, these important molecules also regulate oligodendrocyte development. At early stage, BMPs regulate cell lineage decision and at later stage, they inhibit cell specialization in OL. For example, BMP4

signaling inhibits the generation of OL and enhances the generation of astrocytes from neural progenitor cells both *in vitro* and *in vivo*. BMP4 induces the expression of all four members of the inhibitor of differentiation (ID) family of helix-loop-helix transcriptional inhibitors and blocks oligodendrocyte lineage commitment through the interaction with OLIG1 and OLIG2 (Samanta and Kessler, 2004).

LINGO-1, a transmembrane protein containing a leucine-rich repeat (LRR) and immunoglobin domain, functions as a component of the NgR1/p75 and NgR1/Taj (Troy) signaling complexes. Recent studies show that LINGO1 is also expressed in OL where it negatively regulates oligodendrocyte differentiation and axonal myelination by down-regulating the function of Fyn kinase and up-regulating the activity of RhoA-GTPase. Lack of LINGO-1 expression promotes more axonal myelination due to increased expression of myelin gene such as MBP, CNPase and MOG in OL. LINGO-1 knockout mice also show earlier onset of CNS myelinatin (Mi et al., 2005).

8.5.3. Fate of OL in CNS Pathology

Death of OL and subsequent myelin loss has been reported in a variety of myelin disorders including, multiple sclerosis (MS), X-adrenoleukodystrophy (X-ALD), adrenomyeloneuropathy (AMN), vascular dementia, periventricular leukomalacia (PVL), hypoxia, and ischemia. Several factors that might be associated with OL death in these pathophysiological conditions are discussed below.

8.5.3.1. Role of Autoimmune Trigger in the Death of OL

MS and experimental allergic encephalomyelitis (EAE), an animal model of MS, are autoimmune diseases of the CNS mediated by T cells recognizing self-myelin proteins including MBP, MOG, and PLP. T cells are activated in the periphery by unknown antigens of both myelin and non-myelin origins. After activation, T cells cross the blood-brain barrier and invade into the brain where they accumulate and proliferate in response to antigen re-stimulation. These activated T cells secrete different pro-inflammatory molecules which stimulate not only the resident glial cells (microglia and astroglia) but also other infiltrating cells. $CD4^+$ and γδ T cells express Fas-L which is found to be associated with oligodendroglial death. Furthermore, infiltrating $CD8^+$ T cells interact with MHC class 1 surface receptor of OL and in turn cause oligodendroglial lysis. T cell-derived perforin may also be responsible for oligodendroglial death (Scolding et al., 1990).

8.5.3.2. Role of Cytokines in the Death of OL

Cytokines are important mediators in the inflammatory demyelination observed in MS, EAE, X-adrenoleukodystrophy (X-ALD), and Theiler's virus infection. In these pathologies, pro-inflammatory cytokines and others factors released by endogenous glial cells and/or infiltrated macrophages and $CD4^+$ Th1 cells, accumulate and exert pleiotropic effects on OL. At lower concentrations, these cytokine may be involved in normal development of the nervous system while following brain trauma or inflammatory insults, the overproduction of these cytokines may result in a homeostatic imbalance and may contribute to the outcome of the pathological event. Various cytokines can directly kill OL or it may also affect other signaling pathways that could be involved in the susceptibility of OL. For example, IFN-γ produced by T cells may induce oligodendroglial apoptosis and cell death via JAK-STAT pathway. Another pro-inflammatory cytokine TNF-α induces oligodendroglial death via death signaling pathways (e.g. death inducing signaling complex (DISC), ceramide signaling pathway and stress-activated protein kinase pathways (SAPK) (Buntinx et al., 2004). IL-1 is a strong stimulus for TNF-α release from astrocytes and microglia. Both IL-1 and TNF-α are capable of inhibiting the expression of myelin genes via redox-sensitive mechanism (Jana and Pahan, 2005).

8.5.3.3. Role of Nitric Oxide in the Death of OL

Nitric oxide (NO), a short-lived and highly reactive free radical, is an important physiological messenger in the CNS. However, high level of NO in the CNS has been associated with different type of neurodegenerative diseases such as MS and EAE. During CNS inflammation, activated microglia, astrocytes and infiltrating cells express inducible nitric oxide synthase (iNOS) producing excessive amount of NO. OL at different stages of differentiation are differentially sensitive to NO. For example, OPCs and immature oligodendroctyes are more susceptible than mature OL to NO. However underlying mechanisms are poorly understood. It has been shown that NO reacts with superoxide generated by NADPH oxidase from activated glial cells and infiltrating cells to form peroxynitrite, the most reactive NO derivative. This peroxynitrite plays a critical role in the death of OL (Li et al., 2005).

8.5.3.4. Role of Oxidative Stress in the Death of OL

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) leading to oxidative stress have been implicated as mediators of demyelination and axonal damage in both MS and EAE. Oxidative stress can damage lipids, proteins and nucleic acids of cells and mitochondria potentially causing OL cell death. During oxidative stress-induced oligodendroglial apoptosis, cytochrome c is released from damaged mitochondria, which in turn leads to the activation of the death-related caspases 3 and 9. Another study by Vollgraf et al. (1999) shows that mature OL exposed to oxidative stress undergo chromatin segmentation, condensation via mechanisms involving transcriptional activation of the immediate early stress genes (c-fos and c-jun). An induction of Bax protein has also been reported under oxidative stress condition in OL (Mronga et al., 2004).

8.5.3.5. Role of Ceramide in the Death of OL

Ceramide, the lipid second messenger and a hydrolyzed product of sphingomyelin, is involved in apoptosis of OL. In

pathological conditions, pro-inflammatory cytokines (TNF-α and IL-1β) released specifically from activated microglia and astroglia leads to the activation of sphingomyelinases and production of ceramide in OL via the redox-sensitive mechanism (Singh et al., 1998). Furthermore, a direct role of oxidative stress-induced ceramide production via activation of neutral sphingomyelinase has been recently demonstrated in OL. During Alzheimer's disease, amyloid-β is aggregated in the plaque region that also causes OL death by activating the NSMase-ceramide cascade via redox-sensitive pathways (Lee et al., 2004). In addition, ceramide can inhibit inwardly rectifying K^+ currents and cause depolarization in OL (Hida et al., 1998).

8.5.4. Regeneration of OL

Recent evidences indicate that OPs remain intact both in normal white matter and in demyelinating CNS of patients with MS. OPs can give rise to new OL in experimental conditions and have the ability to repopulate areas from where they are missing (Franklin, 2002).

8.5.4.1. Molecules Involved in the Regeneration of OL

Recent identification of several genes associated with regeneration of OL has become helpful to understand the mechanism of remyelination and formulate a strategy for possible therapeutic intervention in demyelinating disorders. It has been shown that during demyelination, the expression of several genes such as, Nkx2.2, Olig1, Olig2, BMP4, and Fyn are increased in OPs (Lubetzki et al., 2005). Although functions of each of these genes are not clearly understood, some of these genes may lead to differentiation of the quiescent OPs to mature remyelinating OL and help oligodendrocyte regeneration in demyelinated areas (Zhao et al., 2005).

8.5.4.2. Role of Schwann Cells in the Regeneration of OL

During demyelination, after macrophages/microglia remove myelin debris and glial scar, SCs enter into the CNS where they remyelinate axons in the absence of reactive astrocytes. During this period, survival of SCs requires the axon-derived trophic factors (Zhao et al., 2005). Schwann cell can also produce some growth factors like IGF1, FGF2 and PDGF, which promote the migration of OPs and maturation into myelinating OL.

8.5.4.3. Role of Thyroid Hormone in the Regeneration of OL

Thyroid hormone (TH) plays an important role by regulating several stages of oligodendrocyte development and maturation. Oligodendrocytes express TH receptors and during demyelination, TH increases the expression of NGF. This NGF, in turn may lead to an increase in maturation of OPs and remyelination through the activation of Notch-Jagged signaling pathway. TH hormone also up-regulates the expression of PDGFR-α, MBP and CNPase in CNS tissues of animals with MS (Calza et al., 2005).

8.6. Schwann Cells (SCs): Peripheral Glia

The peripheral nervous system contains a number of distinct glial cells, each of which is intimately associated with different parts of the neurons or with specific neuronal cell types. Earlier, these cells were known as the supporting cells of the PNS but recent studies delineate their multifunctional role. These cells are of two types: satellite cells and Schwann cells (SCs). Satellite cells surround the neuronal cell bodies in dorsal root sensory ganglia and in sympathetic and parasympathetic ganglia. These cells help to maintain a controlled microenvironment around the nerve cell body, providing electrical insulation and a pathway for metabolic exchange. The other cells named after German physiologist Schwann are flattened cells with an elongated nucleus oriented longitudinally along the nerve fiber. Surface of all axons in peripheral nerves are ensheathed by non-myelinating or myelinating SCs.

8.6.1. Classification of Schwann Cells (SCs)

In the mature nervous system, SCs can be divided into three classes based on their morphology, biochemistry and function: myelinating Schwann cells (MSCs), non-myelinating Schwann cells (NMSCs) and perisynaptic Schwann cells (PSCs) (Figure 8.6).

MSCs are well characterized and they wrap around axons with a diameter of 1 μm or greater, including all motor neurons and some sensory neurons. This is a mystery why they wrap a specific diameter of the axons. Smaller diameter axons including many sensory and all post-ganglionic sympathetic neurons are myelinated by NMSCs. The NMSCs provide the metabolic and mechanical support to the axon. The NMSCs appear latter than MSCs. They express higher levels of GFAP, p75NTR and cell adhesion molecule L1 compare to MSC. The PSCs located at the neuromuscular junction incompletely wrap around the pre-synaptic terminal of motor axons. They help to maintain a stability of the neuromuscular junction and regulate synaptic transmission (Corfas et al., 2004).

8.6.2. Schwann Cell Development

SCs originate from the neural crest cells, a transient population of cells migrating away from the dorsal part of the neural tube. The signaling pathway and their detailed migratory route are not clearly known. Neural Crest cells are multipotent cells that differentiate to form neurons and glia of the PNS, and also additional cell and tissue types such as melanocytes and connective tissue of the head. Several molecules (e.g. ErbB3, transcription factor SOX10, AP2α and Ets1, the N-Cadherin 6, the low affinity receptor for nerve growth factor p75NTR) have been shown to play important roles during the detachment of neural crest from neural tube (Jessen and Mirsky, 2005).

Markers of lineage progression: Characterization of a number of specific biochemical markers has increased our knowledge on the stages of SC maturation, both *in vivo* and *in vitro*. Some of the biochemical markers have been shown to overlap partially (Table 8.3).

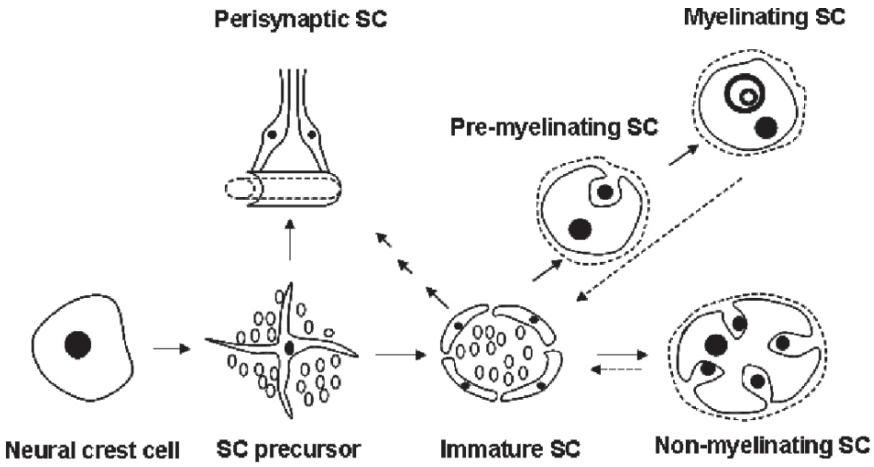

FIGURE 8.6. Different stages of Schwann cell development.

TABLE 8.3. Stage-specific markers of Schwann cells.

Stages	Markers
Neural crest cells	SOX10, AP2α
Schwann cell precursor (SCPs)	Cadherin19, AP2α, low level P0, GAP43, F-spondin, SOX10, BFABP, DHH
Immature Schwann cells	S100β, GFAP, low level of P0, SOX10, O4 antigen, BFABP, DHH
Myelinating Schwann cells	P0, PMP-22, MBP
Non-myelinating Schwann cells	NCAM, GFAP

SOX10, SRY (sex determining region Y) box 10; AP2α, activator protein 2α; DHH, desert hedgehog; GAP43, growth associated protein 43; P0, protein zero; O4, lipid antigen; BFABP, brain fatty acid-binding protein; S100, calcium-binding protein; PMP22, peripheral 22 kDa myelin protein, MBP, myelin basic protein; GFAP, glial fibrillary acidic protein; NCAM, neuronal cell-adhesion molecule.

SOX10, SRY (sex determining region Y) box 10; AP2α, activator protein 2α; DHH, desert hedgehog; GAP43, growth associated protein 43; P0, protein zero; O4, lipid antigen; BFABP, brain fatty acid-binding protein; S100, calcium-binding protein; PMP22, peripheral 22 kDa myelin protein, MBP, myelin basic protein; GFAP, glial fibrillary acidic protein; NCAM, neuronal cell-adhesion molecule.

8.6.3. Signaling Pathways Involved in Survival, Migration and Death of SCs

8.6.3.1. Survival

The survival of immature SCs in late embryonic and pre-natal nerves is probably controlled by a balance between factors that support survival and factors that cause death. Axon-derived neuregulin family (NRG-1, NRG-2, and NRG-3) have been implicated in the biological processes of SCs including fate specification, proliferation, survival, migration, regulating the extent of myelination, and triggering demyelination. It is believed that the interaction between several NRG ligands with different ERB receptors (ErbB2, ErbB3, and ErbB4) on SCs plays a critical role in regulating these steps (Garratt et al., 2000; Michailov et al., 2004). SCs can support their own survival by producing a number of growth factors such as IGF2, NT3, PDGF-β, LIF, and lysophosphatidic acid (LPA) in an autocrine fashion. The autocrine survival circuits are probably important in maintaining the survival of SCs in injured nerves. However, SCPs may need some signal for their survival from neurons.

8.6.3.2. Migration

During development of the PNS, neural crest cells migrate along the outgrowing axons and proliferate in order to produce sufficient number of cells for myelination of axons. Various factors or signaling molecules present on the neighboring cells effect Schwann cell migration in cell culture and it is possible that these signals lead to SCs movements during radial sorting *in vivo*. Integrins, a subgroup of adhesion receptors mediate interaction between cytoplasm and the extracellular environment. This interaction influences the migration of neural crest cells, axonal out growth and SCs differentiation. Integrins can interact with different growth factors, cell adhesion molecules (NCAM and F3) and intracellular cytoskeleton or adaptors proteins. This interaction is crucial for conformational changes and movement of SCs. Several growth factors that regulate the migration of SCs include NRG1, BDNF, GDNF, NT3, and IGF-1. The majority of these molecules are also expressed by SC itself (Yamauchi et al., 2005; Iwase et al., 2005).

8.6.3.3. Death

NGF acting via the p75 neutrophin receptor promotes cell death during the SC injury or infection via activation of c-jun-N-terminal kinase (JNK). It has been found that neonatal p75 neurotrophin receptor mutant mice are less prone to cell death after nerve transaction. Here it is to be noted that the same neurotrophin signaling pathway may also promote survival of

SCs via activation of NF-κB. Although mechanisms behind p75-mediated death of SCs are poorly understood, Yeiser et al. (2004) have shown that NGF signaling through the p75 receptor is deficient in TRAF-6 (–/–) mice and that NGF is unable to kill TRAF-6 (–/–) SCs. In addition, TGFβ is also known to cause apoptosis of SCs via JNK in culture (Jessen and Mirsky, 2005).

8.6.4. Differences Between OL and SC

Although both OL and SC share the common task of synthesizing myelin, there are some differences between the two cell types. See Table 8.4.

8.6.5. Biological Roles

8.6.5.1. Myelinating Peripheral Neurons

SCs cover most part of the PNS neurons by myelin sheath. Although the PNS myelin is mainly formed by the differentiation of the plasma membrane of SCs, myelination of mammalian PNS is a very complex developmental process. It requires intricate timing of several gene expression and cellular interactions between the axon and differentiated SCs (Michailov et al., 2004). In the PNS, mature SCs express Dhh, a family member of the Hh signaling proteins that is involved in the formation of peripheral nerve sheath and is also responsible for the formation of nerve-tissue barrier. Therefore, it has been found that Dhh mutant mice are defective in nerve barrier formation and unable to protect themselves against inflammatory responses. The activity of Dhh is regulated by several molecules such as Notch1, Hes5, MASH-1, and others (Parmantier et al., 1999). Another protein NDRG1 that is abundantly expressed in the cytoplasm of SCs rather than myelin sheath is also essential for maintenance of the myelin sheaths in peripheral nerves.

In addition, some well-known transcription factors such as, KROX20, NF-κB, SOX10, OCT-6, and POU class 3 homeobox 2 (POU3F2) also play an important role in PNS myelination. KROX-20, a master regulator for myelinating SCs, appears to be fundamental in controlling SC differentiation, regulating the expression of a number of genes including periaxin, P0, MBP, and PMP22 by interacting with NAB (NGF1-A binding) proteins. Mutation of this transcription factor Krox-20 is associated with lethal human neuropathy such as congenital hypomyelinating neuropathy (CHN), Dejerine-Sottas syndrome (DSS) and the Charcot-Marie-Tooth (CMT) disease. OCT-6 and POU3F2 have been implicated in the expression of Krox-20 and may therefore positively regulate myelination (Ghislain and Charnay, 2006; Mattson, 2003).

8.6.5.2. Tissue Repair/Regeneration

The SCs play a pivotal role during the event of mechanical damage such as spinal or peripheral nerve injury due to their regenerative properties. SCs in the distal stumps of adult animals can survive for several months in the absence of axons due to injury/insult and these SCs provide both trophic factors and adhesive substrates that promote axonal regeneration and restore the original function. After nerve injury, SCs can transform their phenotype from differentiated myelinating state to the de-differentiating state. During this process, there is also up-regulation of regeneration-associated genes such as the neurotrophin receptor p75 NTR, neuregulin and their receptors (erbB2, erbB3, erbB4), and GAP-43. They also produce different trophic factors (GDNF, TGF-β, IGF-2, NT3, PDGF-β, and LIF), adhesion molecules (L1, NCAM), extra-cellular

Table 8.4. Differences between oligodendrocytes (OL) and Schwann cells (SC).

OL	SC
1. OLs are present only in the CNS.	1. SCs are the major glial cells in the PNS.
2. The sub ventricular zone (SVZ), which is present in late gestational and early post-natal mammalian brain, is a major source of OL.	2. SCs are originated from the neural crest.
3. Oligodendroglial developmental steps are irreversible.	3. Fully differentiated SCs retain an unusual plasticity throughout the life and can readily de-differentiate to form cells similar to immature SCs.
4. One oligodendrocyte extends several processes and can myelinate upto 1 to 40 axons with distinct internodes. node.	4. One SC has an intimate association with axon and each SC forms myelin around a single axon, and lines up along the axon to define a single inter-
5. GM4, one of the most abundant lipids of the CNS, is present in OL.	5. Some glycolipids such as sulfated glucuronyl paragloboside and its derivatives are specific to SC.
6. CNS myelin contains more choline and plasmalogens than PNS myelin (Garbay et al., 2000).	6. PNS myelin contains more ethanol phosphoglycerides than CNS myelin.
7. Basic proteins (MBP and PLP) are major constituents of CNS myelin (about 80% of the total protein) (Baumann and Pham-Dinh, 2001).	7. Glycoproteins (P0 and PMP22) are major constituents of PNS myelin.
8. OLs have phagocytic activity.	8. SCs do not have phagocytic activity.
9. OLs migrate slower and divide and remyelinate at a slower rate than SCs.	9. SCs migrate faster and divide and remyelinate at a faster rate than OLs.
10. OLs are less resistant than SCs to injury.	10. SCs are more resistant than OLs to injury.

matrix molecules (laminin, tenascin), proteoglycans, and collagen type IV in an autocrine/paracrine manner, thereby providing a favorable environment for axonal re-growth and their own survival (Jessen and Mirsky, 1999).

Summary

Astrocytes, OL and SC are not silent partners of others anymore as thought a couple of decades earlier. Recent works have put these cell types in the forefront of neuroscience research. Although astrocytes being the major cell type in the CNS get more attention than the other two cell types, both OL and SC play an equally important role in human health and disease through myelination of neurons in the CNS and PNS respectively. As a result, thousands of cutting-edge research articles are coming out each year describing biological and functional aspects of astrocytes, OL and SC. Therefore, now it is an uphill task to compile everything about these three important cell types in a single chapter. However, here we have made an honest attempt to briefly delineate major biological and functional aspects of these cell types. Although there are vast body of evidence that implicate dysfunction and dysregulation of astrocytes, OL and SC in a number of human neurological diseases, we are still more or less in the dark to draw an unifying picture from these data. An improved understanding of their genesis and function in both healthy and diseased conditions is necessary for better preservation of brain in physiological conditions and for better repairing of this organ in pathophysiological situations.

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Review Questions/Problems

1. During developmental stage which cell types come last:

a. microglia
b. neuron
c. astrocyte
d. oligodendrocyte

2. If there is formation of abnormal Shh protein during embryogenesis:

a. genesis of astrocyte will be affected
b. genesis of oligodendrocytes will be affected
c. genesis of neurons will be affected
d. brain development will be impaired
e. all are true
f. none are true

3. Enzyme glutamine synthase is not found in

a. astrocytes
b. oligodendrocytes
c. none of the above is true
d. all are true

4. Tripartite synapse is formed by

a. neurons
b. microglia
c. astrocytes
d. microglia and astrocytes
e. neuron and astrocyte

5. In the central nervous system, major role of astrocyte is to

a. scavange glutamine
b. scavange cell debris
c. produce ATP
d. all the above
e. none the above

6. In the CNS, glycogen is found only in

a. oligodendrocyte
b. microglia
c. astrocyte
d. neuron
e. all the above

7. In astrogliosis, astrocytes form

a. cluster all around the CNS
b. bushy network surrounding the injury site
c. all are true

8. Schwann cells but not oligodendrocytes have phagocytic activity.

a. True
b. False

9. Unmyelinated axons generally have a smaller diameter than myelinated axons.

a. True
b. False

10. A single Schwann cell forms myelin around one and only one axon while a single oligodendrocyte forms myelin around several separate axons.

a. True
b. False

11. Oligodendrocytes progenitors are identified by A2B5 antibody whereas pre-oligodendrocytes are identified by O4 antibody.

a. True
b. False

12. Which of the sequential stage is correct for oligodendroglial development?

a. Neural stem cells, progenitors, pre-oligodendrocytes, precursors and mature oligodendrocytes.
b. Progenitors, precursors, pre-oligodendrocytes, mature oligodendrocytes and neural stem cells.
c. Neural stem cells, precursors, progenitors, pre-oligodendrocytes and mature oligodendrocytes.
d. Pre-oligodendrocytes, precursors, mature oligodendrocytes, progenitors and neural stem cells.
e. None of the above.

13. Action potentials are conducted rapidly through (choose one)

a. myelinated axons
b. unmyelinated axons
c. large diameter axons
d. small diameter axon
e. both a and c

14. Which of the following molecule is not a part of the peripheral nervous system?

a. LINGO-1
b. BDNF
c. CNTF
d. PDGF
e. EGF

15. Which of the following functions in the nervous system is not provided by the oligodendrocytes?

a. Ensheath axons
b. Supply neurotrophic factors
c. Form the node of Ranvier
d. Phagocytic properties to remove debris.

16. Non-myelinating Schwann cell is characterized by the following properties EXCEPT

a. Wraps axons greater than 1 μm.
b. Appear later than myelinating Schwann cells.
c. Myelinate all post-ganglionic sympathetic neurons.
d. Produce more p75NTR and GFAP.
e. Provide mechanical and metabolic support to the neuron.

17. Gliogenesis and Neurogenesis during development

a. occur simultaneously during development
b. follow this sequence i.e. gliogenesis followed by neurogenesis
c. occur one after the other after the first is completed
d. occur sequentially with overlapping periods

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