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THE CELLULAR PRION PROTEIN IN MULTIPLE SCLEROSIS: A POTENTIAL TARGET FOR NEUROTHERAPEUTICS?

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Abstract

Multiple sclerosis (MS) is a debilitating disease that affects millions. There is no known cure for the disease and neither is the cause of the disease known. Recent studies have indicated that it is a multi-factorial disease with several genes involved. Importantly, sunlight and vitamin D have been implicated in the progression of the disease. The pathogenesis of MS chiefly involves loss of oligodendrocytes, which in addition to being killed by inflammatory mediators in the CNS, also succumbs to loss of trophic support from astrocytes. Neurotrophins play an important role in myelination and the cellular prion protein (PrPC) is a key player in this process. Although the physiological roles of PrP^C remain to be fully understood, increasing evidence suggests multiple roles for PrP^C in regulation of cellular immunity and for its interaction with several neurotrophins that are necessary for homeostasis of the nervous system. This mini-review focuses on the findings establishing a crucial role for PrP^C in the neuropathogenesis of MS, emphasizing its neuroprotective role. Since MS is a multi-factorial disease with unknown etiology and no cure, this review aims to highlight endogenous repair mechanisms mediated by PrP^C that might contribute to functional recovery in MS patients.

Keywords

• Multiple sclerosis • Neuroinflammation • Prions

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The cellular prion protein in MS: a potential target for neurotherapeutics?

Multiple sclerosis (MS) is a medically important disorder that afflicts the central nervous system (CNS) of predominantly young people beyond the age of puberty. Worldwide, there are currently about 2.5 million people afflicted with this disorder. It is a heterogeneous disorder characterized by repeated unpredictable bouts of motor disturbances, partial paralysis, sensory abnormalities and/or visual impairment. These variable signs and symptoms result from inflammatory processes that selectively attack and destroy oligodendrocytes, the cells that form the myelin sheaths around axons in the brain and spinal cord [1]. The diversity of the disease pathology and unknown etiology have made MS among the most damaging of neurological disorders and the least understood in terms of mechanisms of disease progression. Indeed, some investigations have speculated that MS is not a single disease entity but actually represent a spectrum of neuroinflammatory disorders [2]. MS is common among Caucasians, with 0.05-0.15% affected by this chronic and disabling disorder of the CNS, and is less frequently observed in Asians or Africans [3]. Family members of MS patients inherit a higher risk of developing MS, arguing for a strong genetic predisposition to this disease. MS usually begins in early adulthood and affects women more frequently than men. MS usually starts with a relapsing-remitting course (RR-MS) but some 20% of the cases are defined by a primary progressive course (PP-MS) without acute relapses.

Pathophysiology of MS

Clinical and neuropathological features are variable depending on population ethnicity. Lesions in RR-MS patients are usually found in white matter and are characterized by disruption of the blood-brain barrier, local edema and demyelination, typical of inflammatory processes. In PP-MS, inflammatory processes are less dominant but progression to disability and brain atrophy evolves faster [4]. The disease process in MS is predominantly located in the myelinated regions of the brain and spinal cord. In MS affected tissue, inflammation-induced loss of oligodendrocytes and axonal injury are key features of the pathology [5]. The resulting demyelination is predominantly due to inflammation mediated by B cells, T cells, macrophage/microglia and astrocytes [4], signified by plaque formation. Oligodendrocytes undergo damage and perhaps death through the action of several components of the innate and adaptive immune systems in the CNS [6].

Though active plaques from the same patient may be similar, histology reveals evidence for significant heterogeneity in demyelination patterns [7]. Axonal injury in MS is correlated with the extent of inflammation within the CNS. Nevertheless, axonal damage is variable and depends on the severity of inflammation, induction of pathogenic mechanisms as well as diversity in host susceptibility. Demyelination is often accompanied by significant neuronal death in cortical and thalamic MS lesions and also death of retinal ganglion neurons [8].

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Magnetic Resonance Imaging (MRI) studies have revealed that progressive and functional deficits can be associated with axonal loss within lesions and brain atrophy [9]. Axonal injury due to toxic products, including nitric oxide (NO) and proteases, released from macrophages, astrocytes and major histocompatibility complex (MHC) class I-restricted T cells, is found in active lesions during early phases of the disease [9]. Axonal loss is also observed in chronic inactive demyelinated plaques but not in remyelinated lesions, suggesting that compromised trophic support by damaged oligodendrocytes and astrocytes can make axons prone to destruction. Permanent physical and cognitive disability results from cumulative axon loss [10]. Defects in axonal transport can lead to accumulation of axonal amyloid precursor protein (APP). Using APP as a marker of early axonal damage in MS lesions, it was revealed that APP is expressed in areas of acute demyelination and inflammation, but not in chronic areas of the lesions [11]. Earlier studies indicated that in acute MS lesions, APP was detected in T cells, foamy macrophages, activated astrocytes and microglia; also, chronic lesions displayed APP-positive astrocytes and demyelinated axons [12]. Another marker, non-phosphorylated neurofilament (SMI-32) is abundant in neuronal cell bodies and dendrites but is heavily phosphorylated in healthy myelinated axons. An extensive analysis of axonal damage in demyelinated lesions revealed ovoids exhibiting intense SMI-32 immunostaining [13]. Axonal ovoids are transient structures containing axonal debris and surrounding myelin and are characteristic of degenerating axons. Proteolytic enzymes, cytokines, oxidative products and free radicals released by activated immune and glial cells, cause axonal transection. Axonal injury is also correlated with reduced N-acetyl aspartate levels in MRI studies [13]. Thus, axonal injury in MS is a well-established clinical feature.

Prions and MS

The word 'prions' is synonymous to small, proteinaceous infectious particles, distinguished from viruses by the absence of intrinsic nucleic acids. It must be noted that historical experiments in the early 1960s performed on sheep in Iceland showed that when animals were injected with a suspension of the brain of an acute case of MS, the sheep developed a scrapie-like disease, which is now known as transmissible spongiform encephalopathy (TSE) though these results could not be reproduced in animals in the UK. Prion protein is also a component of the normal cells and prion genes have existed prior to speciation of mammals. The cellular prion protein (PrP^{C}) is a highly conserved glycophosphatidylinositol-anchored cell surface sialoglycoprotein concentrated in lipid rafts. Prions cause transmissible neurodegenerative diseases associated with accumulation of PrP^{Sc}, a misfolded and aggregated form of PrP^{C}. While its physiological function remains incompletely understood, much attention has been paid to the role of PrP^{C} as a substrate for the replicative cycle of prions.

PrP^{C} is abundantly expressed in the CNS, particularly in neurons, though expression in astrocytes and oligodendrocytes is also detectable [14]. The neuronal population is established shortly after birth and mature neurons do not divide and are neuroectodermal in origin. They can be excitatory, inhibitory or modulatory in their effect and motor, secretory or sensory in their function [15]. Importantly, axons emerge from the neuron as a slender thread and are frequently myelinated. The dendrites are afferent components of neurons and are arranged in a stellate fashion and lack neurofilaments. The synapse is a specialized junction where axons and dendrites emerging from different neurons intercommunicate [16]. Neurons also play a modulatory role in inflammation, wherein healthy neurons actively suppress immune function of microglia by interaction between CD200 receptor on neurons and its ligand on microglia. CD200 deficient mice showed activated microglia with increased expression of CD45, CD11b and inducible nitric oxide synthase (iNOS) during experimental autoimmune encephalomyelitis (EAE), the mouse model of MS [17]. Restricting the injury within insulated local regions allows robust leukocyte- and microglia -mediated inflammatory reactions to occur. This allows the damaged tissue to recover and also prevent these inflammatory cells from invading adjacent healthy tissue. However, during this process axonal guidance pathways are perturbed and can slow down remyelination [18].

PrP^{C} is widely expressed in peripheral nervous tissue and particularly in cells which constitute the 'hardwired neuroimmune network' which refers to the small calibre afferent nerves in skin and the lamina propria of the aerodigestive tract; in sympathetic ganglia and nerves; in antigen presenting and processing cells (follicular and non-follicular dendritic cells) and activated lymphocytes in the skin, gut- and bronchus-associated lymphoid tissues and secondary lymphoid tissue; in the suprarenal gland and hematopoietic marrow. PrP^{C} is also expressed in the parasympathetic system, enteric and peripheral nervous systems and neuroendocrine system. PrP^{C} may serve as a receptor for a variety of putative ligands including, but not limited to, heparan sulfate, laminin, neural cell adhesion molecule, synaptic proteins and Hsp70/Hsp90 organizing protein/Stressinduced protein 1(Hop/STI-1). Judging from these diverse ligand-receptor interactions, PrP^{C} might have a role in diverse processes including neurodevelopment, synaptic function, neurite outgrowth and neuronal survival. Since deletion of PrP^{C} increased neuronal susceptibility to apoptosis and oxidative stress, and increased seizures and ischemia-induced cerebral damage in mouse, there is strong evidence for a neuroprotective function for PrP^{C}.

Neuroprotection is mediated by neurotrophins. There are several neurotrophins found in the damaged brain. Most are induced by inflammatory cytokines, TNF-α and IL-1β within parenchymal microglia. Microglia-derived IL-1β induces NGF, CNTF and IGF-1, which repair the injured CNS. TNF-α plays a dual role in demyelination (TNF-R-I) and remyelination (TNF-R-II), by interacting with the receptor available [19]. Interestingly, TNF-α binds to TNF-RII (p75) on oligodendrocyte progenitors and promotes their differentiation into mature and functional oligodendrocytes [19]. TGFβ plays a dual role by down-regulating proinflammatory cytokines but also induces

astrocytic activation and glial scar formation that inhibits oligodendrocyte differentiation and remyelination [20]. IL-6, IL-1β and chemokines (CCL2, CCL3 and CCL5) are essential for axonal growth and oligodendrocyte differentiation but also promote inflammatory cell recruitment and survival [20]. IL-1β binds to its receptor on astrocytes leading to the production of growth factors (Neurotrophin [NT], NGF, CNTF) that act on neural progenitors and favor repair of oligodendrocytes and induce remyelination [20]. Similar to IL-6-, CNTF-, oncostatin-M- and IL-11-mediated survival of neurons, leukemia inhibitory factor (LIF)-mediated signaling through its receptor can also potentiate oligodendrocyte survival [21]. Brain repair following injury is mediated by neurotrophins (NGF, Brain-derived neurotrophic factor [BDNF], NT-3, 4/5) and their receptors (Trk A, B & C and the low affinity $p75^{NTR}$) as well as growth factors (platelet-derived growth factor [PDGF], fibroblast growth factor [FGF]-2, IGF-1). These are re-expressed by T and B cells, macrophages, astrocytes and mast cells in the brain lesions. Among growth factors secreted by neural cells, Hop/STI-1 is trophic and secreted by astrocytes [22]. Growth factors binding to their receptors lead to receptor clustering and autophosphorylation of tyrosine residues in the cytoplasmic tail of the receptors. This opens up docking sites for SH2 domain-containing adaptor proteins such as Shc and/or Grb2 and to activation of the Ras/Raf/MEK/ERKs cascade [23]. Expression of extracellular matrix or cell surface proteins, (chondroitin sulphate proteoglycan (CSP), integrin, ephrin, tenascins and semaphorins) and oligodendrocyte proteins (Neurite outgrowth inhibitor [Nogo], myelin-associated glycoprotein [24], MOG, Notch-1) may contribute to neurogenesis, gliogenesis, synaptogenesis, migration etc. Re-expression of Jagged and Notch-1 is important for axonal growth and remyelination. Neurocan, a chondroitin sulphate proteoglycan, can bind to FGF-2 and promote neural-cell-precursor differentiation and bind neural cell adhesion molecule on inflammatory cells, allowing their entry into the brain [20].

Neuroinflammation, which is a feature of MS, is a hot spot of cellular and molecular events involving peripheral immune cells migrating to and attacking nervous tissue. Joining in this revelry are the inflammatory cytokines, chemokines, reactive oxygen species (ROS) and toxins as well as growth promoting cytokines and growth factors. Dangling in the midst of this jamboree are the neural cells that either can survive against all odds, in which case there might be functional recovery, or proceed to die and cause impairments. The spatial and temporal dynamics of various cellular and molecular players in this orchestra are keys to resolution of inflammation. Among the cells involved, T cell-dendritic cell (DC) interaction is a critical event for the initiation of primary immune responses.

Cellular players of neuroinflammation in MS

In MS, infiltrating activated T and B-lymphocytes, macrophages and microglia induce proinflammatory cytokines and a host of other soluble factors leading to inflammation-induced axonal and myelin damage. Current experimental evidence in MS research favors the $CD4^+$ autoreactive T cell as a crucial factor for MS pathogenesis for several reasons. These cells are among the majority infiltrating the CNS and CSF; genetic risk to MS is contributed by human leukocyte antigen (HLA)-DR and -DQ molecules, which when transgenically expressed confer susceptibility to EAE; altered peptide ligands of myelin basic protein $(MBP)_{83-99}$ induce cross-reactive $CD4^+$ T cells exacerbating disease in clinical trials and lastly, $CD4^+$ cells induce antibody production and $CD8^+$ maturation [9]. Tissue damage caused by the initial onslaught of autoreactive T cells release myelin antigens that promote immune responses to additional myelin epitopes, initiating a cascade of events that culminates in chronic disease. Myelin-reactive T cells from MS patients produce T_H1-cytokines (IL-12, IL-23, IL-17, IFN-β) whereas the same cells from healthy individuals are likely to produce T_H2 cytokines (IL-4, IL-10) [25]. In the CNS, only microglia are known to express MHC class II [26]. Though astrocytes express MHC class II by IFN-γ treatment, the constitutive levels of MHC class II are either absent or at low levels on oligodendrocytes, neurons and astrocytes. MHC class I expression on the resident neural cells make them vulnerable to recognition by $CD8^+$ cells. While it has been generally accepted that $CD4^+$ T are the dominant encephalotigenic cells in the brain [27], there are also experimental and clinical data showing that $CD8^+$ T cells outnumber $CD4^+$ T cells in MS lesions [27]. $CD8^+$ cells are also closely apposed to oligodendrocytes and axons suggesting that they may initiate inflammation and tissue injury in MS plaques [27]. Circulating activated memory B cells can serve as antigen presenting cells (APCs) and skew T cell responses towards a pro-inflammatory nature. Certain B cell clones from the CSF of MS patients have revealed receptor editing by which these cells abrogate the ability of the host to induce autoantibodies during B cell development. However, reactivity may be introduced to additional CNS autoantigens [28]. MS patients demonstrate increased immunoglobulins in the CSF, but not in the serum, suggesting that these myelin-specific antibodies which may contribute to destruction of myelin [9] are produced locally in the CNS.

Dendritic cells (DCs) are highly specialized APCs and act as sentinels of the innate immune system and due to their intrinsic ability to produce cytokines and stimulate natural killer (NK) and naïve T cells, DCs link the innate and adaptive arms of the immune system [29]. Under non-inflammatory conditions, the brain parenchyma lacks DCs but high numbers in the brain and CSF are seen following inflammatory conditions. DCs secrete antiviral IFN-γ and also stimulate the adaptive immune system [30]. DCs accumulate in the CNS and CSF in MS and EAE and are detected using the marker DC-SIGN/CD209, a C-type lectin receptor expressed by immature DCs in non-lymphoid tissue, but also expressed by mature DCs in secondary lymphoid tissue [24]. DCs have been shown to engulf myelin components and interact predominantly with $CD8^+$ T lymphocytes in MS lesions. By virtue of chemokines and cytokines released, DCs mature and activate T lymphocytes in the CNS and also migrate to draining lymph nodes where they initiate a new wave of autoreactive T cells to infiltrate the CNS [24].

Since PrP^C is expressed on the surface of cells of the lympho-hematopoietic system, including T cells and DCs, there is perhaps no doubt that PrP^C might regulate the *in vivo* activities of the cells of the immune system during normal or autoimmune T cell-mediated responses. Indeed, mice lacking PrP^C developed earlier onset of EAE. While the disease was more severe, there was a greater involvement of the cerebellum and forebrain, extensive spinal cord damage and persistence of monocytic and T cell infiltrates in the CNS [31]. These results were also corroborated in another study using a PrP-null mouse, where EAE signs were worsened; siRNA against PrP^C also showed a protective effect of PrP^C [32]. In contrast, overexpression of PrP^C in the Tga20 mice resulted in their protection from severe EAE [32]. Examination of the expression pattern in T cells indicates that PrP^C is an activation marker in murine $CD4^+$ cells. PrP^C-null mice showed increased T cell proliferation and higher levels of IFNγ and IL-17A, produced by Th17 $CD4^+$ cells, which provides a likely explanation for disease exacerbation in the PrP^C-null mice. Striking infiltration of perivascular cells into the cerebella and forebrain of PrP^C-null mice indicate that PrP^C has a suppressive effect on inflammation mediated by T cells. While the mechanism of action and the signaling pathways are to be unraveled, engaging PrP^C on T cells by a ligand might be essential to induce apoptosis and thereby prevent disease. On the other hand, the interaction of T cells with DCs at the immunological synapse might lead to alteration in their function during disease. Although, it is likely that the major effects of PrP^C in EAE pathogenesis are due to the decrease in PrP^C expression in peripheral lymphatic tissues and not due to their decrease in the CNS. PrP^C-null mice also demonstrated large numbers of microglia and macrophages in the lesions, which generate ROS leading to oxidative stress-induced damage that these mice are susceptible to. Since PrP^C is highly expressed on DCs [33] and monocytes [34] as well as microglia [35], the notion that only T cells expressing PrP^C regulate EAE pathogenesis might be not easy to explain. Thus, PrP^C is a potential modulator of cellular immunity.

Very importantly, myelin integrity can also be compromised by inflammation and it appears that inflammatory responses are lower in PrP^C null mice compared to wild type mice [36]. Particularly, phagocytosis by macrophages was more active in PrP^C-null mice [37]. Mononuclear phagocytes comprise an essential component of the innate immune system, playing important roles in the defense against infectious agents. Microglia, forming 12% of the CNS cells demonstrate low turn over rate and can be induced to express the surface molecules, CD80, CD86 and MHC class II, necessary for antigen presentation. In the surveillance state, microglia have a 'ramified' morphology, with flattened or angular nuclei, scanty cytoplasm that accumulates at both poles of the cells and are terminally non-dividing cells. Activated microglia assume different shapes and could become bushy with abundant cytoplasm and ramified thick processes lying adjacent to neurons, and are thus called perineuronal microglia. They also appear as rod-shaped microglia with a fusiform, elongated body with small, thin processes. Microglia are postulated to have several roles including sensing and reacting to injury, regulating blood flow and vasculogenesis, phagocytosis, regulating astrogliosis, neurodevelopment by secreting trophic factors and neuroendocrine functions [38]. In MS, microglia may present antigens, including myelin-derived peptides through MHC class II molecules to cytotoxic T cells, leading to disease exacerbation and also recruit further monocytes from the blood stream, enhancing the cytopathic response. However, due to their phagocytic nature, microglia may also rid the brain of damaged cells and thus contribute to resolution of inflammation [38]. Microglia form an important component of the brain stem cell niche [39] (Antony J. M., in press) and can be easily manipulated for therapeutic purposes. The neuroprotective effect of PrP^C allows microglia to increase superoxide production and block proliferation of microglia in response to bacterial endotoxin [35]. Thus, the protective effect of PrP^C might be delivered by lymphoid and myeloid cells.

An interesting feature of EAE in PrP^C-null mice is the disease pattern that was concentrated in the upper CNS structures, which indicates a novel model of neuroinflammation. Regulation of PrP^C is controlled by post-translational mechanisms in the CNS, whereas in the PNS, it is at the level of transcription [40]. In the peripheral nerves, Schwann cells expressing prions are a possible route of transport of the infectious prion particle, though removal of PrP^C expression did not prevent prion neuroinvasion [41]. PrP^C was found to be expressed on Schwann cell surface and in the cytoplasm in sciatic nerves, but not in the myelin sheath of Tga20 PrP^C transgenic animals [42]. The three glycoforms of PrP^C, the non-glycosylated 25-27 kDa and the mono-and diglycosylated forms were found in the mouse Schwann cell line MSC-80 [42], indicating the usefulness of this cell line for investigating peripheral neuropathy. PrP^C might have a role in peripheral neuropathy since PrP^C-deficient Nagasaki ($Prnp^{-/-}$) and Zurich-1 ($Prnp^{o/o}$) mice demonstrate late-onset peripheral neuropathy. $Prnp^{o/o}$ mice are devoid of PrP^C and develop normally but are resistant to prion disease and do not support replication of prions. Indeed, myelin sheaths were primarily affected in these mice, while the axons were spared [43]. However, deletion of PrP^C from Schwann cells did not reveal any overt deficit in myelin integrity or morphology nor did aging of PrP^C null mice reveal any overt phenotype [41]. While these results suggest that the PNS is not the trafficking route of prion infectivity, it begs the question as to the function of PrP^C in Schwann cells.

Transgenic mice expressing a PrP variant lacking the hydrophobic core (aa 111-134) showed reduced life expectancy and chronic demyelinating polyneuropathy (CDP). In addition, these mice also developed CNS vacuolation and astrogliosis in cerebellum, brain-stem and corpus callosum. In fact, B and T cells were not required for CDP pathogenesis. Prion diseases mainly affect the CNS, but in 60 week old $Prnp^{o/o}$-null mice, there was no myelin degeneration in optic nerves, corpus callosum or spinal cords. However, central myelin might be affected in $Prnp^{-/-}$ mice [44]. Restricting expression of PrP^C to neurons and selectively depleting PrP^C from neurons indicate that the expression of PrP^C by neurons is essential for the long-term integrity of peripheral myelin sheaths. Thus, PrP^C might be a critical messenger of transcellular axomyelinic communication

and indicate that regulated proteolysis of axonal PrP^C might expose domains that interact with Schwann cell receptors [43]. There are both PrP^c-dependent and independent pathways for neuroprotection and control of proliferation in the developing retina [45]. In transgenic mice expressing PrP^C under the control of the myelin basic protein (MBP) promoter, PrP^C was found to be strongly and exclusively expressed in oligodendrocytes and Schwann cells, but not in neurons or astrocytes [46].

PrP^C null mice showed significant loss MBP in the spinal cord, particularly in the initial peak of disease and MBP-deficient regions contained increased densities of microglia [31]. Oligodendrocytes and astrocytes are both cells of neuroectodermal origin similar to neurons. Oligodendrocytes are restricted to the CNS and form a layer of lipid-rich and laminated layer called myelin around most axons. Myelin plays an important role in the conduction of action potentials. In MS, myelin degenerates and oligodendrocytes die causing a functional short-circuiting of exposed axonal processes, leading to demyelination [47]. Mice deficient in 2'3' cyclic nucleotide phosphodiesterase (CNPase), a gene expressed in oligodendrocytes, exhibit motor deficits due to axonal damage even though normal-looking myelin is formed [48], suggesting that it could be altered trophic support from oligodendrocytes that contribute to axonal damage rather than demyelination that leads to axonal damage [49]. The NG2 glycoprotein is a membrane protein expressed in the developing and adult CNS by subpopulations of glia including oligodendroglial precursor cells (OPCs). NG2-positive cells are observed in MS lesions and since they are precursors to oligodendrocytes during development, the presence of NG2-positive cells in MS lesions may suggest the possibility of on-going remyelination [50]. Oligodendrocytes may be injured by several means. Oligodendrocytes are vulnerable to perforin [51] through MHC class I effector pathway [52] and $CD4^+$-mediated lytic damage [53] through Fas/FasL or TNF-receptor (TNF-R) via the MHC class II elimination pathway [52]. Importantly T lymphocytes induce considerable damage to oligodendrocytes through a host of soluble secreted factors.

Myelin integrity is maintained by a constitutively active neurotrophic protein complex involving PrP^C, whose effector domain encompasses residues 94-134. It has been proposed that deletion of PrP^C at amino acid residues 111-121 affects signaling mediated by the putative receptor (PrP_R) [54]. Among other factors associated with this complex is Hop/STI-1, a co-chaperone secreted by several tumor cell lines, and primary astrocytes that bind to PrP_R [22,55]. Astrocytic IGF-1 and FGF-2 are essential growth factors for oligodendrocyte precursor proliferation and for maturation into myelin producing oligodendrocytes. High affinity FGF receptors present on oligodendrocytes in demyelinated lesions are stimulated upon ligand binding, leading to growth of oligodendrocytes in lesions. Most importantly, upon activation, astrocyte-derived growth factors and neurotrophic factors promote neuronal survival and regeneration [56]. Astrocytes mediate synaptogenesis wherein high affinity receptors on astrocytes bind to vasoactive intestinal peptides (VIPs) [57]. Also VIP-stimulated astrocytes produce activity-dependent neurotrophic factor that promotes survival of spinal cord and cortical neurons [58]. Thus, astrocytes are thought to be just more than 'brain glue' [59] and contribute to several dynamic processes in the CNS. Astrocytes are the most abundant cells in the CNS and have elaborate star like processes. There are 2 types of astrocytes in the CNS. The fibrous type with many thin processes form a scaffold throughout the grey matter, while the protoplasmic type is short, with thicker processes and form a continuous covering around blood vessels and both respond to injury [60]. Astrocytes connect blood vessels and neurons [59] and maintain the appropriate chemical environment for neuronal signaling. Mice that lack the astrocytic protein, glial fibrillary acidic protein (GFAP) are viable but exhibit morphological and functional alterations and decreased myelination, suggesting a link between astrocyte function and maintenance of myelination [61]. Astrocytes produce soluble factors, particularly growth factors that promote functioning of otherwise defective oligodendrocytes [62], suggesting extensive cross-talk between astrocytes and oligodendrocytes. Activated astrocytes exhibit complement activation in pre-demyelinating lesions from MS brains [63], release glutamate leading to neuronal and oligodendrocyte death [64], express chemokines that lure DCs to sample the antigens in the CNS [65] and a host of other functions that enhance neuropathogenesis of demyelination. Astrocytes can also secrete serine and glutamate [66]. Glutamate produced by active neurons is removed by astrocytes through glia-specific transporters-excitatory amino acid transporter 1 (EAAT1, [rodent, GLAST]) and EAAT2 [rodent, GLT1]. Astrocyte gap junctions are formed by connexins -43 and -30, through which these glial cells dissipate glutamate, in addition to propagation of the intercellular Ca^{2+} waves. In response to CNS injury, astrocytes become activated, involving cellular hypertrophy, changes in gene expression and cellular proliferation forming the classical 'scar tissue'. However the scar-forming reactive astrocytes are essential for spatial and temporal regulation of inflammation post CNS injury. Loss of astrocytes leads to accumulation of extracellular glutamate with subsequent excitotoxicity of neurons and oligodendrocytes [67]. Reactive astrocytes, on the other hand, produce cytotoxins such as NO radicals and ROS that can damage neural cells and contribute to secondary degeneration after CNS insults and can also inhibit axonal regeneration [18]. Thus, astrocytes are able to exert harmful and beneficial effects.

Several populations of PrP^C-positive lymphocytes are sequestered within M cells in the gut wall, or scattered individually or in aggregates within the epithelium and lamina propia of the gut, respiratory epithelium, epididymis, skin, spleen and lymph nodes [40]. The gut associated immune system has been recently implicated in the pathogenesis of MS due to the nature of the microflora that resides in the gut [68]. It could be speculated that MS, which is a multi-factorial disease with unknown etiologies might be regulated by PrP^C-positive lymphocytes and exposure to specific antigenic molecules in the gut might suppress PrP^C on lymphocytes to trigger their migration into the CNS and cause EAE and perhaps MS. Perhaps, the nature of gut microflora in MS patients along with susceptible genes such as PrP^C

might be involved in MS. There is abundant evidence to support the hypothesis that genetics has an important role in an individual's vulnerability to MS, perhaps in conjunction with trigger factors. Epidemiological studies have provided evidence for environmental factors in the disease process. MS relapses are frequently associated with common viral infections and migration from low-to-high risk areas exacerbates the risk of developing MS. A viral infection may initiate MS, presumably an autoimmune disease [69]. Thus, both genetic and environmental factors seem to contribute to development and progression of the disease [70]. MS exhibits several characteristics that are common to autoimmune diseases including polygenic inheritance, evidence of environmental exposure, increased frequency in women, and partial susceptibility conferred by a HLA-associated gene [71]. Despite substantial evidence for polygenic inheritance, the MHC-containing HLA is the only region that has clearly and consistently demonstrated linkage and association in MS genetic studies. Susceptibility to MS involves a significant number of different genes, each with a relatively small contribution. Particularly, MHC genes, namely HLA-DRB1 and HLA-DQB1 showed strong associations with MS in Canadian and Finnish cohorts [72]. The strongest linkage result is on chromosome 1q44 with an increase in the multipoint Logarithm of the Odds (LOD) score and also to other autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis [73]. In addition, there is a potential presence of an MS susceptibility locus on chromosome 7q21-22, delineated by the markers *D7S3126* and *D7S554*. Interestingly, within this region, there are 2 putative genes, protachykinin-1 precursor gene (*TAC1*) and Thymosin-β4, which constitutes a multigene family of IFN-inducible proteins. *TAC1* encodes four products of the tachykinin peptide hormone family, Substance P and neurokinin A, as well as the related peptides, neuropeptide -K and -γ [74]. An etiological role for Substance P in CNS inflammation, such as seen in MS, is likely since Substance P is an inflammatory mediator. Astrocytes express the high-affinity NK-1 Substance P receptor [75]. In astrocytes, Substance P induces secretion of IL-8 through the NFκB activation pathway [76] and of IL-6 through the p38 MAPK pathway [77]. Exposure to viral, bacterial or other pathogens may trigger the disease process, perhaps through a molecular mimicry mechanism where a protein in the pathogen is similar to the host protein, myelin, eliciting an autoimmune response [9]. Interestingly, activation of a host endogenous retroviral protein, HERV-W was found to induce oligodendrocyte death [78].

Indeed, Tsutsui and colleagues speculated on the possibility that human prion gene polymorphisms might impact the clinical course of MS [31]. However, a few studies in this regard have not supported this notion. A single-nucleotide polymorphism (SNP) at codon 129 of the PrP gene (Prnp), located on chromosome 20p12 has been shown to have a significant effect on the clinical course of numerous non-prion neurodegenerative disorders including Alzheimer's Disease (AD), Down syndrome and Wilson disease. While the Prnp129 SNP has a negative effect on long-term memory, methionine/valine heterozygosity is associated with less severe clinical disease in neurodegenerative disorders. However, an examination of PP-MS patients and controls revealed no statistically significant differences in frequency of Prnp129 genotypes between groups [79]. While PP-MS has more neurodegenerative characteristics that other MS phenotypes, the authors also investigated patients with nuclear MS (secondary progressive and relapsing-remitting) and again found no statistically significant differences in frequency of Prnp129 genotypes [80].

Conclusion

PrP^C is a critical axonal protein necessary for myelin maintenance, since its specific deletion from neurons led to adult-onset peripheral neuropathy in mice, whereas its deletion from Schwann cells did not affect myelin maintenance. In contrast, lack of PrP^C leads to CNS demyelination and resulting pathogenesis demonstrated in EAE models. Thus, the pathogenesis resulting from lack of PrP^C is dependent on the cell types expressing PrP^C. One can speculate on how this knowledge can be translated into potential therapies. For example, increasing the level of neuronal PrP^C might be beneficial to prevent or treat axonal degeneration of the PNS while increasing the level of PrP^C in CNS infiltrating innate immune cells and leukocytes may prevent neuropathogenesis of the CNS such as MS. Another important feature of PrP^C is that the proteolytically processed axonal PrP^C might facilitate myelin maintenance indirectly through interactions with an axonal protein or direct interaction with myelin binding partners, such as myelin protein zero, MAG and CNPase. This interaction is essential for mediating the protective effect of PrP^C. There are other binding partners of PrP^C such as HOP/STI-1 that have been demonstrated to bind to PrP^C as well as 96 others in a recent proteomic study [81]. How these partners regulate the biology of prions in MS will be a fascinating study by itself.

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