Chapter

NEUROTOXIC AND INFLAMMATORY MEDIATORS ELICITED BY ASTROCYTES AND MICROGLIA IN RESPONSE TO MYELIN BASIC PROTEIN (MBP)

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune, neurodegenerative disease that occurs within the central nervous system (CNS). This debilitating disease leads to severe impairments in motor function as well as altered gait, balance difficulty, visual disturbances, and cognitive dysfunction. These symptoms are the result of inflammation, demyelination, and neuronal damage and death. There is evidence which suggests that resident CNS cells may be involved in perpetuating the neuronal injury that occurs during the disease state. As demyelination occurs within the CNS, astrocytes and microglia are able to interact with myelin breakdown products, specifically myelin basic protein (MBP). The binding of MBP by astrocytes and microglia leads to their activation. This results in the secretion of neurotoxic and inflammatory mediators. These mediators elicit further damage to the neurons, thereby propagating neuronal injury and death. This chapter will illustrate how astrocytes and microglia respond to MBP and in turn elicit neurotoxic and inflammatory mediators. The mediators discussed will include CCL-2, tumor necrosis factor-alpha, glutamate, and nitric oxide. The role of these mediators in neurodegeneration will be examined, and possible mechanisms for therapy will also be considered.

INTRODUCTION

In this chapter, we will illustrate how astrocytes and microglia elicit neurotoxic and inflammatory mediators in response to interactions with myelin basic protein (MBP). Once activated by MBP, astrocytes and microglia secrete CC chemokine ligand 2 (CCL-2) and tumor necrosis factor-alpha (TNF-α). This causes microglia to produce and secrete glutamate

and nitric oxide (NO). The role these components have in neuronal damage and/or death will be discussed in the context of multiple sclerosis, as they are common to individuals with this disease. We will also discuss how they contribute to further damage within the CNS. We conclude this chapter by exploring how a further understanding of the mechanisms by which these mediators function can be utilized as possible avenues for therapy.

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a devastating neuroinflammatory autoimmune disease that occurs within the central nervous system (CNS). It is the most common demyelinating disease, resulting in the loss of the protective myelin sheath that encloses axons, leading to chronic inflammation. The etiology of MS is still unknown, however, the accepted belief is that it is the result of an autoimmune response directed by autoreactive T cells specific for myelin antigens. The T cells mediate an inflammatory response that leads to demyelination and neuronal damage [1, 2].

Chronic inflammation is the hallmark of MS pathology, which includes the presence of focal white matter lesions called plaques. The plaques are characterized by primary demyelination and gliosis [3]. Lesions may be distributed throughout the CNS, but there appears to be a preference for the optic nerves, brainstem, periventricular white matter, and the cerebellum. Axonal injury has also been demonstrated early during the course of the disease. Axonal damage is likely due to the release of toxic inflammatory mediators within the lesion by resident brain cells. These factors include cytokines, chemokines, excitotoxins, nitric oxide, and free radicals [4].

The symptoms of MS vary based on severity and progression of the disease, but are all associated with the invasion of inflammatory cells across the blood-brain barrier [5]. The symptoms may include vision problems, muscle weakness, fatigue, moderate to severe pain, movement disorders (lack of coordination and tremors), vertigo, and speech problems, and cognitive impairments (difficulty with concentration, attention, and memory, and poor judgment).

Although MS can occur at any age, the typical age of onset is between 20-40 years and women are twice as likely to become affected as men [6]. It is clear there is a genetic factor involved as the risk of developing MS in the general population is 1 in 750 compared to people with siblings that have MS which have a 1 in 100 chance of developing the disease. However, there must be other risk factors involved as an identical twin has only a 30% chance of developing MS if their twin develops the disease. Other risk factors have also been postulated to be involved with initiating disease. For example, people who were exposed to Epstein-Barr virus have a higher rate of MS than those who have not been exposed [7]. Also, it has been shown that the risk of MS seems to increase as one moves further from the equator in either direction [8, 9]. This has led researchers to the hypothesis that low sunlight and vitamin D insufficiency may play a role in initiating disease [10]. Inhalants may also participate in initiating disease. Smoke, either directly or indirectly through second hand smoke, has been linked to an increase risk of developing MS as has solvent exposure [11-13].

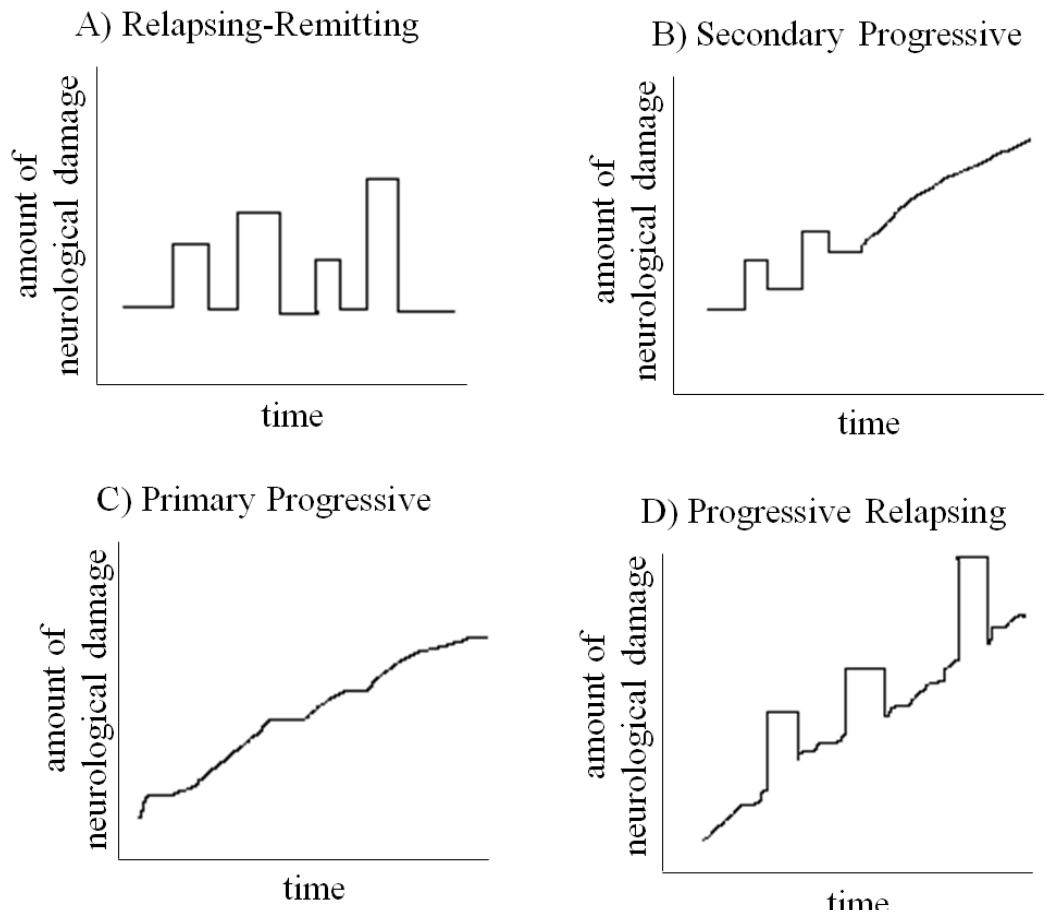

Figure 1. Subtypes of Multiple Sclerosis.
(A) *Relapsing- Remitting Multiple Sclerosis (RRMS*). Individuals diagnosed with RRMS experience a series of attacks followed by complete or partial recovery (remission) of symptoms. The individual may be in remission weeks to years before another attack (relapse) occurs. This is the most common subtype of MS. (B) *Secondary Progressive Multiple Sclerosis (SPMS)*. Individuals with RRMS can convert to SPMS 10-30 years after initial diagnosis. In SPMS neurological symptoms do not subside between relapses, but instead get successively worse until a steady progression of disability replaces cycles of attacks. The shift is from relapses to a continual increase in symptom severity. (C) *Primary Progressive Multiple Sclerosis (PPMS)*. Patients with PPMS experience a slow and steady decline in neurological function. There are no periods of remission and symptoms do not decrease in intensity. (D) *Progressive Relapsing Multiple Sclerosis (PRMS)*. PRMS is characterized by a steady progression of neurological damage accompanied by sporadic periods of relapses. However, unlike RRMS, the neurological damage and symptoms do not subside during the remission periods.

MS is subtyped according to disease progression. Approximately 65-80% are initially diagnosed with relapsing-remitting (RRMS), the most common form. In this form of the disease, there are relapses where new symptoms appear (Figure 1A). These relapses are followed by periods of remission of symptoms where the individual recovers either partly or entirely, until another relapse occurs. Of those with RRMS, 50% convert to secondary progressive (SPMS) within 10 years, with 90% converting to SPMS after 30 years. Patients with SPMS have a steady progression of neurological damage having previously experienced

RRMS for several years (Figure 1B). This is in contrast to primary progressive (PPMS), affecting 10-20% of those diagnosed, where there is a steady decline with no relapses or remissions (Figure 1C). Six to ten percent of individuals are diagnosed with the last form, progressive relapsing (PRMS). In PRMS, there is a steady progression of neurological damage with relapses and remission (Figure 1D). There is recovery that follows the relapses, but there is a worsening of symptoms between attacks.

To gain further insight into disease development, progression and treatment, several animal models have been used. The prototypical animal model is experimental autoimmune encephalomyelitis (EAE). Inflammation and disease initiation is caused by an autoimmune response to various CNS antigens, including myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendroglial protein (MOG). The EAE model is clinically and pathologically similar to MS, although some differences exist depending on the animal and antigen used. For example, induction of EAE in the rat with MBP-autoreactive T cells results in a monophasic disease with little demyelination [14]. On the other hand, the mouse is more susceptible to disease, where induction with PLP-autoreactive T cells results in a relapsing form of EAE, but induction with MOG-autoreactive T cells results in a chronic progressive form of the disease [15, 16]. As previously mentioned, chemokines are important in mediating axonal damage. EAE models have been particularly useful in elucidating the roles chemokines play in disease by identifying their presence and function [17-19]. Also, the use of knockout animals has demonstrated which chemokines are important in disease severity as knockout animals either have reduced severity or are resistant to induction of disease [20, 21]. The significance of chemokines in disease will be discussed later in this chapter.

MYELIN BASIC PROTEIN

As previously mentioned, the axons are ensheathed in myelin which is essential to the function of the nervous system, specifically the speed and efficiency of action potentials which travel down the axon. Myelin is formed by specialized cells, oligodendrocytes in the CNS and Schwann cells in the peripheral nervous system (PNS), and is composed of many different proteins. One major protein is myelin basic protein (MBP), comprising approximately 30% of the total myelin in the CNS. MBP is the second most abundant myelin protein after proteolipid protein (PLP). Alternatively, in the PNS, MBP is not an essential protein, being present in small quantities and comprising less than 18% of total myelin protein. Therefore, within the PNS, MBP is not required for formation of the myelin sheath, but is thought to perform some other specific function. The myelin sheath containing MBP is present in virtually all vertebrates and the sequence of MBP is highly conserved from mammals to birds to amphibians [22].

MBP is expressed on the cytosolic surface of myelin and is responsible for adhesion of compact myelin. This is evident in the *shiverer* mutant mouse. This mouse has an autosomal recessive mutation where much of the MBP gene is deleted. This mutation results in tremors that begin at day 14 and last until the mouse's death at day 50-100 [23]. These mice also suffer from seizures and hypomyelination. The direct link between MBP being responsible for the *shiverer* phenotype came from experiments where MBP transgenic animals were created by injecting wildtype MBP into the pronucleus of fertilized *shiverer* eggs. The

animals homozygous for the transgene expressed approximately 25% of normal MBP, had compact myelin, and no longer had tremors or seizures. Homozygosity also resulted in a normal life span for these animals [23]. These experiments clearly solidified the role of MBP in the formation of compact myelin within the CNS.

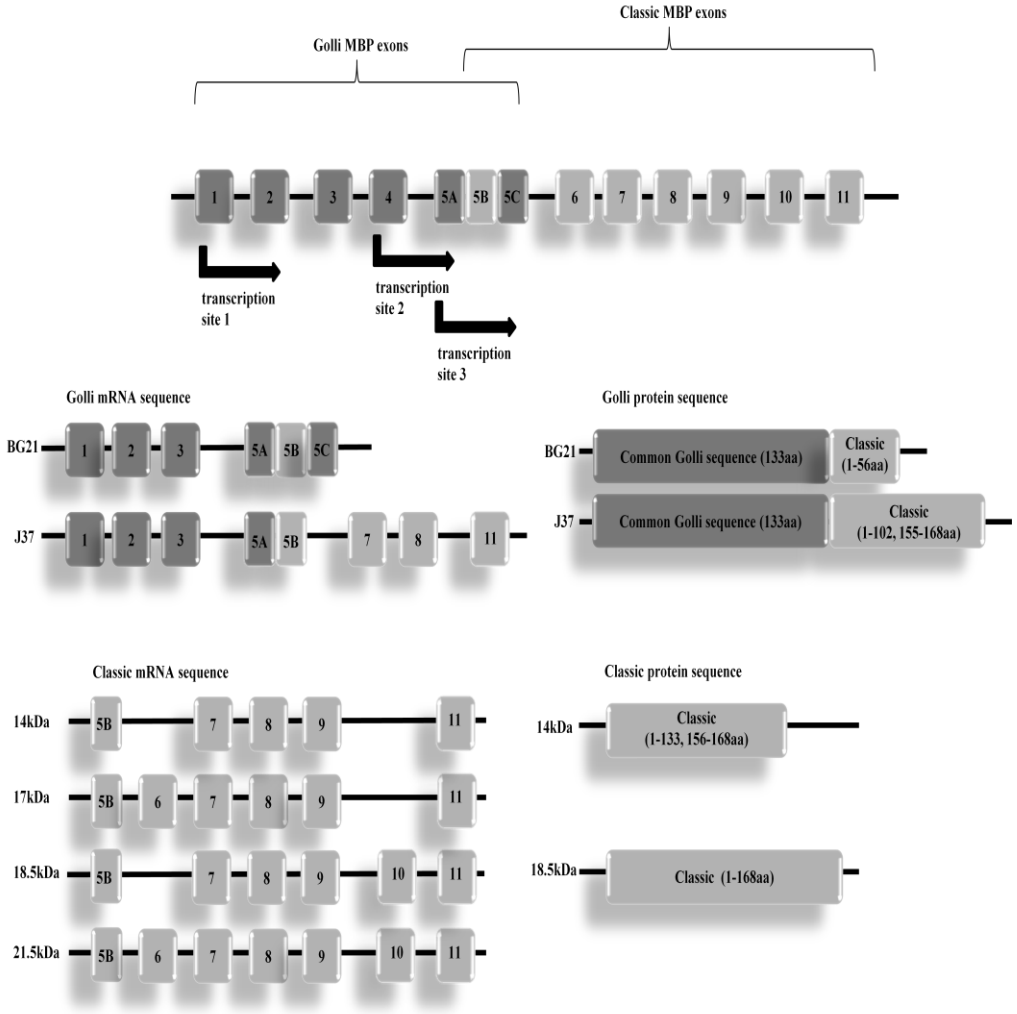

Figure 2. MBP gene and the two families of MBP products.
There are three transcription start sites within the MBP gene. The first transcription start site produces the golli products, with BG21 and J37 being the main isoforms. Each isoform contains the specific golli domain of 133 amino acids (shaded boxes) and variable lengths of classic MBP sequence (light boxes) at the C-terminal portions of the molecules. The common golli domain is encoded by exons 1-3. The classic MBPs are expressed from two transcription start sites downstream of the golli transcription start site. The 14kDa and 18.5KDa forms of the classic MBPs predominate in adult human myelin.

In 1993, it was discovered that the MBP gene contains three transcription start sites; transcription start site 1 encodes the *g*ene of *oli*godendrocyte *li*neage (golli) proteins and transcription start sites 2 and 3 encode the classic or canonical MBPs (Figure 2) [24]. The

golli MBPs contain an additional 133 amino acid sequence derived from exons 1-3 upstream of the classic MBP transcription start sites. This sequence differentiates them from the classic MBP sequence (Figure 2). Golli proteins are expressed more ubiquitously, localizing to the immune system (thymocytes and T cells) and the nervous system [24, 25]. Golli proteins have also been found to be developmentally regulated, in that levels tend to be high during embryonic development and then expression wanes with age [26]. However, in the MS brain, increased golli expression has been seen in activated microglia, macrophages, and oligodendrocyte progenitors around the lesion [27].

There are various splice isoforms of the classic MBPs. The 17kDa and 21.5kDa forms are distributed diffusely through the cytoplasm and accumulate in the nucleus of oligodendrocytes. The 18.5kDa variant is the major adult isoform. Human CNS MBP has extensive post-translational modifications including deimination, deamidation, phosphorylation, methylation, and acylation [28]. Due to these modifications, there are several charged isomers of the 18.5kDa MBP, designated C1-C8. The C1 isomer is the least modified, most positively charged, and most abundant in the normal brain. The remaining isomers lose one positive charge successively due to increased modification [28-30]. It has been shown that MBP from MS tissue is less phosphorylated and less deamidated than normal MBP, and that the deiminated isoform (C8) is highest in children and decreases with age [28]. However this isoform has been found in MS tissue and suggested to play a role in the associated pathogenesis [31].

CELLS OF THE CENTRAL NERVOUS SYSTEM

Neurons

It is estimated that there may be as many as 100 billion neurons in the nervous system. A typical neuron is composed of three parts; a soma or cell body, numerous dendritic extensions, and a long axon ending in synaptic end bulbs. The cell body of the neuron contains the nucleus and the organelles. The dendritic extensions stretch out like branches from the cell body. It is primarily through these extensions that the neuron receives impulses from other neurons. In most neurons the axon is the longest extension. The axon joins the cell body at the axon hillock, a tapered portion of the cell body. Close to the axon hillock is the trigger zone. This is where the action potential is initiated. The action potential is an electrical signal that moves along the length of the axon due to rapid exchange of ions across the axonal membrane. The axons of neurons are covered with a myelin sheath, the function of which is to keep the action potential moving steadily and smoothly down the axon. Degeneration of the axon and injury of the neuron that occur after demyelination contributes significantly to the neurological dysfunction that is seen in people with MS [32-34]. Axonal transection has been shown to contribute to degeneration. This is seen in the MS lesion with frequency of axonal transection increasing with degree of inflammation [35]. Neuronal loss has been estimated to be approximately 18-35% throughout the MS brain [36]. Macrophages/microglia are found adjacent to damaged neurons, and release of toxic and inflammatory mediators from these cells, including reactive nitrogen species, cytokines, and chemokines, can

contribute to and/or enhance demyelination, axonal degeneration, and blood-brain barrier (BBB) disruption (reviewed later in this chapter).

Along with myelin, ion channels also contribute to the propagation of the signal. Oligodendrocyte-soluble factors induce clustering of Na^+ channels at the nodes, which are segments of bare axons between myelin [37, 38]. These ion channels are essential in the saltatory conduction of the action potential that occurs. However, after demyelination occurs, the Na^+ channels are no longer clustered at the nodes, but are now scattered along the axon [39]. This is also seen in the *shiverer* mouse, where the ion channels are distributed along the entire length of the axon [40, 41]. However, proper conduction can be restored in chronic demyelinated axons when Na^+ channels are upregulated and reorganized.

Astrocytes/BBB

Astrocytes are the most abundant cell type in the CNS, comprising roughly 90% of brain mass, and are critical to the function of the brain [42]. Astrocytes have a critical supportive role within the brain. These cells are responsible for promoting neovascular coupling, promoting the influx of cells through the secretion of chemokines, buffering certain ions, the release of glutamate, maintenance of general metabolism, the control of brain pH, and production of antioxidants [43-46]. By providing an adequate chemical environment, astrocytes allow for correct neuronal signaling, as well as neuronal protection [47]. However, when astrocytic damage does occur, there is dysfunction which leads to neuronal damage/death [48]. Reactive astrogliosis is a condition that occurs in astrocytes when they respond to CNS trauma. This results in uptake of excitotoxic glutamate, production of glutathione, release of adenosine, and release of inflammatory cytokines [43].

It was long thought that the brain was an immune privileged organ. However, that is no longer considered accurate. Instead, it is now known that there is a structure which limits entry of blood-borne leukocytes into the brain. This structure is the blood-brain barrier (BBB). The BBB is a selectively permeable barrier that regulates the transport of materials from the blood to the brain through the use of tight junctions (TJ). The BBB is composed of endothelial cells (EC), pericytes, astrocytic endfeet, neurons, and microglia (Figure 3). EC, neuronal, and non-neuronal glia comprise what is often referred to as the "neurovascular unit". The close proximity of all of these cells with each other allows for beneficial processes to occur essential for normal CNS function. Although other cells are present, due to their intimate contact with EC of the vessel, astrocytes are vital in the formation and maintenance of the BBB (Figure 3B, 3C). The BBB is able to limit the entry of substances (red blood cells and leukocytes) from the blood into the brain, however, during inflammatory diseases, the integrity of the BBB can be compromised allowing for substances to now enter the brain. One mechanism by which permeability may be compromised is through the astrocytic endfeet. Cytokines such as TNF-α, CCL-2, and other immune modulators released by astrocytes can alter permeability by directly affecting EC and TJs [49-52]. BBB dysfunction has been implicated in a variety of neurological diseases, and in MS it has been observed both histologically since the early 1960s [53] and clinically through CT and MRI scans since the 1980s [54]. An increased BBB permeability results in enhanced leukocyte infiltration into the brain [55, 56], and BBB dysfunction has also been seen in normal appearing white matter in MS tissue preceding the formation of demyelinating lesions as well as in inflammatory silent

inactive lesions [57-61]. EAE studies have shown that disease severity correlates with degree of BBB dysfunction, demonstrating that TJ alteration is associated with onset of clinical symptoms [62-64], and similar associations have been made in MS tissue [65, 66].

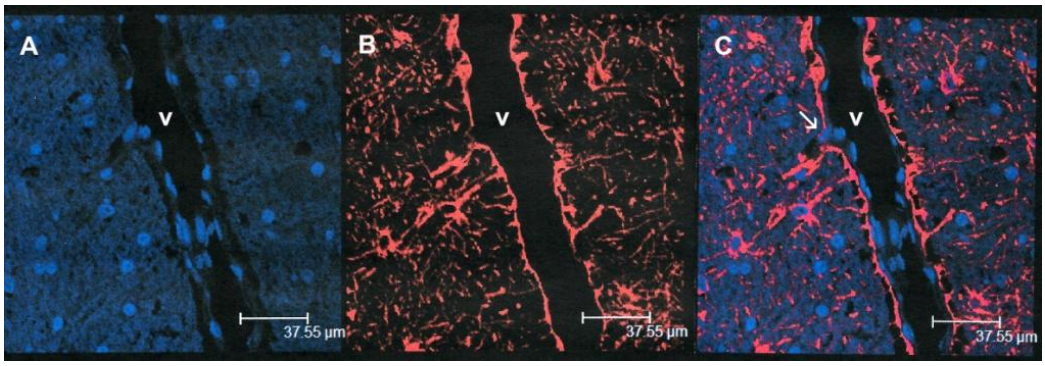

Figure 3. Immunofluorescence of the human blood-brain barrier.
Immunohistochemistry of the blood-brain barrier in brain tissue from a 35 year old individual. (A) Nuclei (DAPI, blue) and (B) astrocytes (anti-GFAP Cy3, red) were visualized immunofluorescently. The astrocytic endfeet are seen along the wall of the vessel (v). (C) Colocalization of nuclei with astrocytes, showing cells in the process of crossing the blood-brain barrier (arrow). Scale bar = 37.55µm. Figure courtesy of Dr. Eliseo Eugenin, The Public Health Research Institute, University of Medicine and Dentistry of New Jersey.

Microglia

Similar to astrocytes, microglia have a role in protecting and supporting neurons. Microglia play a critical role in normal brain maturation and development, including apoptosis, axonal growth and guidance, neuronal differentiation, astrocytic proliferation, and angiogenesis [67]. However, unlike astrocytes, microglia have an immune function as well. They are the resident phagocytes of the brain. They are continuously monitoring the CNS microenvironment and can also generate mediators that can affect surrounding cells, including neurons and astrocytes. Microglia contain MHC class I and II receptors, Fc receptors, CD40 receptor, chemokine/cytokine receptors, as well as many other immunological receptors [68-73]. Along with secreting cytokines, microglia can also serve as antigen presenting cells [74, 75].

Under normal resting conditions, microglia can produce anti-inflammatory and neurotrophic factors. However, microglia may become activated when there is foreign antigen or in the event of tissue damage. When this occurs, they can promote an inflammatory response and initiate tissue repair. As evidenced by the facial nerve axotomy paradigm, peripheral axotomy results in microglial activation within the CNS, and these activated microglia are essential in reinnervating axons and carrying on neuroprotective functions, thereby underscoring the significance of microglial-mediated inflammation in neuronal regeneration. Although microglial activation may facilitate tissue repair, sustained activation can result in neuronal degeneration and death, which is thought to be due to secretion of

cytokines and excitotoxic mediators. When damage and the inflammatory response in MS are short-lived, remyelination and recovery can occur, as seen in RRMS. When the inflammation is chronic, the repair is hindered which eventually leads to neuronal death. This can be seen in the progressive forms of the disease associated with neurodegeneration, where significant levels of activated microglia were found [76, 77]. The importance of microglia in carrying out these neurodegenerative processes is evidenced by selectively ablating microglia in mice with EAE which resulted in a delay in disease onset and repression of clinical signs including decreased inflammatory infiltrates [78]. Also, when animals with EAE were treated with a compound that inhibits glial cell activation (both microglia and astrocytes) the severity of disease was significantly reduced as well as demyelination and inflammation, which was associated with increased survival of oligodendrocytes [79].

CCL-2 AND BBB PERMEABILITY

Recruitment of macrophages and T cells into the CNS is the result of chemokine expression by microglia. As mentioned, the etiology of MS is believed to be the result of a systemic infection or inflammation of the CNS. Either of these two circumstances will lead to the secretion of chemokines which are small, soluble, chemoattractant, immune modulating proteins. BBB permeability found in the MS disease state is most likely due to the increased chemokine production and the resulting augmentation of adhesion molecule expression on endothelial cells.

Studies have shown that CCL-2 has a pathogenic role in both MS and EAE. The sources of CCL-2 in the CNS are the neurons, parenchymal astrocytes, and microglia. CCL-2 stimulates the migration of monocytes from the blood into the CNS, and is also responsible for monocytic maturation into destructive, phagocytic macrophages. Additionally, CCL-2 secretion leads to the migration of autoreactive T cells into the CNS. These macrophages and autoreactive T cells recognize myelin which leads to both the direct and indirect damage of neurons. Studies of MS plaques have revealed that macrophages are a significant source of chemokines. MS tissue samples have elevated CCL-2 levels and i*n vitro* BBB models have shown selective migration of CCR-2 positive T cells and monocytes into the CNS [80]. Treatment of endothelial cells with MBP resulted in significant increase of CCL-2 protein secretion, and treatment of either EC or astrocytes in a BBB model with MBP showed increased permeability possibly due to decreased TJ proteins [81]. CCL-2 knockout mice induced to develop EAE have shown impaired macrophage recruitment. These mice demonstrate a less severe disease condition and suffer fewer relapses compared to wild type animals [82]. Attempts to induce EAE in CCR-2 knockout mice have failed to produce disease. In these mice there was an absence of monocytic infiltrates into the CNS [20, 21]. Treatment with anti-CCL-2 antibodies in mice with EAE resulted in reduced severity of acute EAE as well as a decrease in relapses in chronic relapsing-EAE [83]. In rats, naked DNA vaccination against CCL-2 prevented EAE induction [84]. These studies clearly demonstrate the integral role of CCL-2 in immune cell recruitment into the CNS across a compromised BBB.

TNF-α AND THE INDUCTION OF NEUROTOXICITY

Cytokines are proteins made and secreted by cells. Cytokines can affect the behavior of neighboring cells as well as the cells that secreted them. In CNS inflammation, resident astrocytes of the BBB as well as recruited macrophages and T cells secrete several cytokines, including as IL-1, IL-6, IL-10, TNF-β, and TNF-α.

TNF-α is significantly upregulated in MS and is expressed by macrophages, microglia, and astrocytes in chronic lesions. TNF-α has also been shown to be the most abundant cytokine produced when MBP antigen specific T cells are exposed to glia [85]. TNF-α expression leads to increased local inflammation and activation of macrophages and endothelial cells. This results in increased tissue damage and enhanced infiltration of immune cells through the BBB. TNF-α also increases MHC-I and MHC-II expression on resident brain cells, and this expression further stimulates APCs and T cells. Stimulated macrophages secrete reactive oxygen intermediates, nitric oxide (NO), matrix metalloproteases, and additional TNF-α, all of which cause tissue damage and death. The stimulated T cells are autoreactive and cause further damage to the CNS by inducing an inflammatory cascade resulting in additional TNF-α secretion which stimulates the secretion of other neurotoxic mediators [86]. In addition to TNF-α stimulating the release of NO, NO can also induce the secretion of TNF-α, further perpetuating the inflammatory cascade [87]. *In vivo* studies using antibodies to block TNF-α demonstrate that this cytokine is integral in the development of EAE [88]. Human studies have shown a positive correlation between TNF-α levels and the clinical course of the disease [86].

ROLES OF GLUTAMATE AND NITRIC OXIDE IN CELL DEATH

As previously mentioned, EC and astrocytes secrete CCL-2 when treated with MBP [81] and TNF-α is produced in abundance when MBP antigen specific T cells are exposed to glia [85]. These proinflammatory mediators stimulate microglia to release high amounts of glutamate. When expressed at normal levels, glutamate is a neuro-excitatory amino acid. Glutamate concentrations have been shown to be elevated in acute lesions as well as in normal appearing white matter of MS patients [89]. In excess, glutamate is harmful and triggers a cascade that results in neuronal death. Glutamate-induced neurotoxicity may be augmented by the inability of astrocytes to uptake extracellular glutamate. Glutamate clearance is mediated by the GLAST protein. It has been shown in rats with EAE that secretion of TNF-α from autoreactive T cells leads to the downregulation of gene transcription of GLAST, thereby inhibiting glutamate clearance [90].

The binding of excess glutamate to neuronal ionotropic glutamate receptors leads to the release of large quantities of calcium. The increase of intracellular calcium activates digestive enzymes such as phospholipases, endonucleases, and proteases which damage DNA. This results in mitochondrial damage and the activation of apoptosis [91]. The release of calcium also activates nitric oxide synthase (NOS) and leads to the production of NO, which is chemically reactive with oxygen and oxygen free radicals resulting in the formation of reactive nitric oxide species (RNOS). NO is also secreted by activated macrophages. The

increased levels of NO lead to NO toxicity which is mediated by oxidative and nitrative stress caused by RNOS. All cellular macromolecules are potential targets of these damaging effects. Eventually the oxidative and nitrative damage impairs the cellular function to where the cell will undergo apoptosis or necrosis [92]. NO also inhibits glycolysis by the nitrosylation of GADPH and diminishes mitochondrial respiration by competitively inhibiting cytochrome c oxidase, the terminal member of the electron transport chain [91, 92]. In addition to impeding cellular respiration, NO toxicity results in electron leakage which leads to free radical production. Therefore, toxicity can be an indirect result of glutamate induced NO production in neurons, or it can result directly from NO secreted by activated macrophages.

The pathological significance of NO inducing neurotoxicity is seen in both MS and EAE. Abnormally increased expression of NOS and NO have been identified in the brains of MS patients in addition to EAE brains [93, 94]. The treatment of primary rat cortical cultures with inhibitors of NOS have been shown to selectively prevent toxicity [95]. The significant role of NO in EAE has been further demonstrated by studies of cortical cultures from NOS knockout mice which show resistance to neurotoxicity [96].

MS patients and animal models show increased levels of NOS and nitrotyrosinated proteins in CNS lesions. Moreover, MS patients have increased NO metabolite concentrations in their cerebrospinal fluid as compared to controls [97].

FUTURE THERAPEUTIC DIRECTIONS

Several studies for the treatment of MS have been conducted which block or modulate the pathways of expression of these inflammatory mediators. A major impediment to the treatment of MS is that the mediators of disease are not disease-specific. Cytokines, chemokines, neurotransmitters, NOS and NO are all used by numerous cells for a myriad of physiological outcomes. Drugs that target these chemicals could lead to systemic problems and exacerbate the disease state. The issue of systemic damage has led to the increased development of specifically targeted therapies. For instance, the DNA sequencing of glutamate receptor genes has led to the discovery of single nucleotide polymorphisms (SNPs) in patients with MS [98]. These SNPs may allow for patient-specific therapies based on individual gene sequences. Another avenue of exploration for the treatment of MS is the initiation of antigen specific tolerance. The direct targeting of autoreactive T cells is the ideal treatment strategy for autoimmune diseases such as MS. The goal of this type of immunotherapy is to develop antigen-specific unresponsiveness without systemic effects. Immunotherapies that provoke tolerance in myelin specific T cells have been studied in animals, as well as in clinical trials, but as of yet, the transition of these therapies from bench to bedside has continued to elucidate researchers [99]. Another possible treatment modality is to repair damage in the CNS by the use of stem cells. It has been shown that mesenchymal stem cells (MSC) promote recovery of the CNS in animal models [100]. MSC secrete soluble factors that protect neurons against glutamate neurotoxicity by reducing their sensitivity to glutamate. Further studies towards the identification of these factors are underway and show great promise as therapeutic agents.

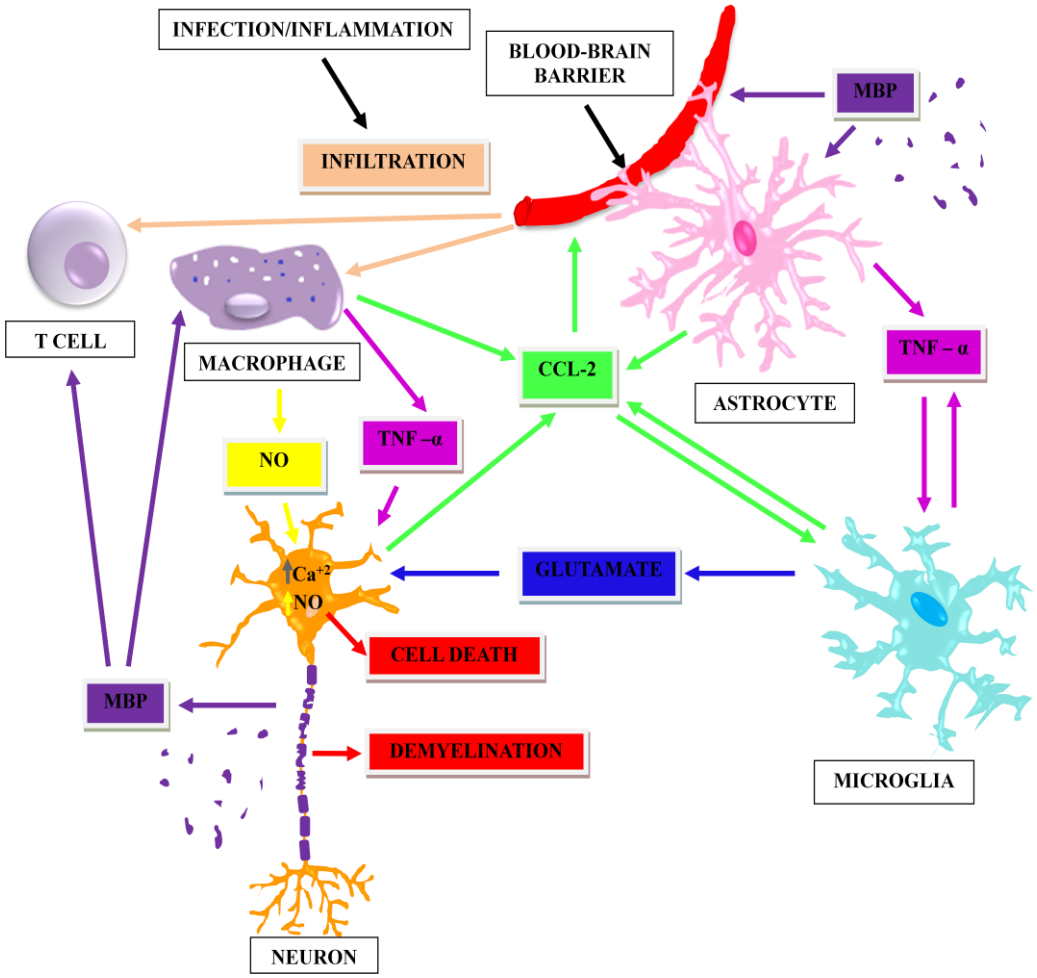

Figure 4. Cells and cytotoxic mediators involved in the neurotoxicity seen in MS. Although the precise cause of MS is still unclear, there does seem to be an initial inflammatory component involved. Once the inflammatory process begins, mediators are secreted from incoming and resident cells. These mediators include CCL-2 and TNF-α. These mediators function in both an autocrine and a paracrine fashion resulting in astrogliosis and microglial activation. The result of this process is secretion of glutamate and NO which, along with TNF- α, are involved in neuronal damage/demyelination and death. For simplicity, the arrows indicating TNF-α secretion from T-cells, macrophages, and astrocytes acting upon the blood brain barrier have been omitted. TNF-α -tumor necrosis factor-alpha, CCL-2 - CC chemokine ligand-2, NO - nitric oxide, MBP - myelin basic protein, Ca^{+2}-calcium.

CONCLUSION

There are numerous factors that participate in the development and exacerbation of MS. The incorporation of all of these components is beyond the focus of this chapter. Our goal is to show a clear depiction of how the immune system itself exacerbates the condition and results not in resolving neuronal damage but causing and enhancing it. One hypothesis is that astrocyte, as well as microglia, which are the first line of defense in the CNS, secrete CCL-2 and TNF-α in response to neuronal injury. One major role in the pathology of MS lies in the

cyclical immunostimulatory pathways of the disease. For example, TNF-α released from astrocytes and microglia stimulates macrophages and autoreactive T cells to secrete additional TNF-α, and it also stimulates the secretion of NO from macrophages and microglia, which result in a further increase of TNF-α secretion. TNF-α and CCL-2 secretion lead to the activation of microglia which results in their release of glutamate. The increased glutamate levels lead to the release of large quantities of intracellular calcium which activates intracellular digestive enzymes and NOS. NOS expression results in the increase of NO which damages the cell by oxidative and nitrative stress, leading to cell death. This neurotoxicity then leads to the physiological manifestations of MS.

Although the initiating cause of the immunological cascade that results in MS is still unknown, several of the intermediate components that lead to its pathology have been identified. Continued research into these factors and their multitude of interactions will bring us closer to finding improved therapies and eventually a cure.

REFERENCES

[1] Hauser, S. L. & Oksenberg, J. R. (2006). The Neurobiology of Multiple Sclerosis: Genes, Inflammation, and Neurodegeneration. *Neuron, 52*, 61-76.

[2] Trapp, B. D. & Nave, K. A. (2008). Multiple Sclerosis: An Immune or Neurodegenerative Disorder? *Annu Rev Neurosci, 31*, 247-269.

[3] Lassmann, H. (2005). Multiple Sclerosis Pathology: Evolution of Pathogenetic Concepts. *Brain Pathol, 15*, 217-222.

[4] Kornek, B. & Storch, M. K. & Weissert, R. & Wallstroem, E. & Stefferl, A. & Olsson, T. & Linington, C. & Schmidbauer, M. & Lassmann, H. (2000). Multiple Sclerosis and Chronic Autoimmune Encephalomyelitis: A Comparative Quantitative Study of Axonal Injury in Active, Inactive, and Remyelinated Lesions. *Am J Pathol, 157*, 267-276.

[5] Hafler, D. A. (2004). Multiple Sclerosis. *J Clin Invest, 113*, 788-794.

[6] Sadovnick, A. D. & Baird, P. A. (1982). Sex Ratio in Offspring of Patients with Multiple Sclerosis. *N Engl J Med, 306*, 1114-1115.

[7] Martyn, C. N. & Cruddas, M. & Compston, D. A. (1993). Symptomatic Epstein-Barr Virus Infection and Multiple Sclerosis. *J Neurol Neurosurg Psychiatry, 56*, 167-168.

[8] Kurtzke, J. F. (1975). A Reassessment of the Distribution of Multiple Sclerosis. Part One. *Acta Neurol Scand, 51*, 110-136.

[9] Kurtzke, J. F. (1993). Epidemiologic Evidence for Multiple Sclerosis as an Infection. *Clin Microbiol Rev, 6*, 382-427.

[10] Cantorna, M. T. (2008). Vitamin D and Multiple Sclerosis: An Update. *Nutr Rev, 66*, S135-138.

[11] Amaducci, L. & Arfaioli, C. & Inzitari, D. & Marchi, M. (1982). Multiple Sclerosis among Shoe and Leather Workers: An Epidemiological Survey in Florence. *Acta Neurol Scand, 65*, 94-103.

[12] Hernan, M. A. & Jick, S. S. & Logroscino, G. & Olek, M. J. & Ascherio, A. & Jick, H. (2005). Cigarette Smoking and the Progression of Multiple Sclerosis. *Brain, 128*, 1461-1465.

[13] Mikaeloff, Y. & Caridade, G. & Tardieu, M. & Suissa, S. & Group, K. S. (2007). Parental Smoking at Home and the Risk of Childhood-Onset Multiple Sclerosis in Children. *Brain, 130*, 2589-2595.

[14] Pender, M. P. & Stanley, G. P. & Yoong, G. & Nguyen, K. B. (1990). The Neuropathology of Chronic Relapsing Experimental Allergic Encephalomyelitis Induced in the Lewis Rat by Inoculation with Whole Spinal Cord and Treatment with Cyclosporin A. *Acta Neuropathol, 80*, 172-183.

[15] Iglesias, A. & Bauer, J. & Litzenburger, T. & Schubart, A. & Linington, C. (2001). T- and B-Cell Responses to Myelin Oligodendrocyte Glycoprotein in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. *Glia, 36*, 220-234.

[16] Morris-Downes, M. M. & Smith, P. A. & Rundle, J. L. & Piddlesden, S. J. & Baker, D. & Pham-Dinh, D. & Heijmans, N. & Amor, S. (2002). Pathological and Regulatory Effects of Anti-Myelin Antibodies in Experimental Allergic Encephalomyelitis in Mice. *J Neuroimmunol, 125*, 114-124.

[17] Berman, J. W. & Guida, M. P. & Warren, J. & Amat, J. & Brosnan, C. F. (1996). Localization of Monocyte Chemoattractant Peptide-1 Expression in the Central Nervous System in Experimental Autoimmune Encephalomyelitis and Trauma in the Rat. *J Immunol, 156*, 3017-3023.

[18] Columba-Cabezas, S. & Serafini, B. & Ambrosini, E. & Aloisi, F. (2003). Lymphoid Chemokines Ccl19 and CCL-21 Are Expressed in the Central Nervous System During Experimental Autoimmune Encephalomyelitis: Implications for the Maintenance of Chronic Neuroinflammation. *Brain Pathol, 13*, 38-51.

[19] Miyagishi, R. & Kikuchi, S. & Takayama, C. & Inoue, Y. & Tashiro, K. (1997). Identification of Cell Types Producing Rantes, Mip-1 Alpha and Mip-1 Beta in Rat Experimental Autoimmune Encephalomyelitis by in Situ Hybridization. *J Neuroimmunol, 77*, 17-26.

[20] Fife, B. T. & Huffnagle, G. B. & Kuziel, W. A. & Karpus, W. J. (2000). Cc Chemokine Receptor 2 Is Critical for Induction of Experimental Autoimmune Encephalomyelitis. *J Exp Med, 192*, 899-905.

[21] Izikson, L. & Klein, R. S. & Charo, I. F. & Weiner, H. L. & Luster, A. D. (2000). Resistance to Experimental Autoimmune Encephalomyelitis in Mice Lacking the Cc Chemokine Receptor (Ccr)2. *J Exp Med, 192*, 1075-1080.

[22] Harauz, G. & Ishiyama, N. & Hill, C. M. & Bates, I. R. & Libich, D. S. & Fares, C. (2004). Myelin Basic Protein-Diverse Conformational States of an Intrinsically Unstructured Protein and Its Roles in Myelin Assembly and Multiple Sclerosis. *Micron, 35*, 503-542.

[23] Readhead, C. & Takasashi, N. & Shine, H. D. & Saavedra, R. & Sidman, R. & Hood, L. (1990). Role of Myelin Basic Protein in the Formation of Central Nervous System Myelin. *Ann N Y Acad Sci, 605*, 280-285.

[24] Campagnoni, A. T. & Pribyl, T. M. & Campagnoni, C. W. & Kampf, K. & Amur-Umarjee, S. & Landry, C. F. & Handley, V. W. & Newman, S. L. & Garbay, B. & Kitamura, K. (1993). Structure and Developmental Regulation of Golli-Mbp, a 105-Kilobase Gene That Encompasses the Myelin Basic Protein Gene and Is Expressed in Cells in the Oligodendrocyte Lineage in the Brain. *J Biol Chem, 268*, 4930-4938.

[25] Pribyl, T. M. & Campagnoni, C. W. & Kampf, K. & Kashima, T. & Handley, V. W. & Mcmahon, J. & Campagnoni, A. T. (1993). The Human Myelin Basic Protein Gene Is

Included within a 179-Kilobase Transcription Unit: Expression in the Immune and Central Nervous Systems. *Proc Natl Acad Sci U S A, 90*, 10695-10699.

[26] Landry, C. F. & Ellison, J. A. & Pribyl, T. M. & Campagnoni, C. & Kampf, K. & Campagnoni, A. T. (1996). Myelin Basic Protein Gene Expression in Neurons: Developmental and Regional Changes in Protein Targeting within Neuronal Nuclei, Cell Bodies, and Processes. *J Neurosci, 16*, 2452-2462.

[27] Filipovic, R. & Rakic, S. & Zecevic, N. (2002). Expression of Golli Proteins in Adult Human Brain and Multiple Sclerosis Lesions. *J Neuroimmunol, 127*, 1-12.

[28] Kim, J. K. & Mastronardi, F. G. & Wood, D. D. & Lubman, D. M. & Zand, R. & Moscarello, M. A. (2003). Multiple Sclerosis: An Important Role for Post-Translational Modifications of Myelin Basic Protein in Pathogenesis. *Mol Cell Proteomics, 2*, 453-462.

[29] Chou, F. C. & Chou, C. H. & Shapira, R. & Kibler, R. F. (1976). Basis of Microheterogeneity of Myelin Basic Protein. *J Biol Chem, 251*, 2671-2679.

[30] Zand, R. & Li, M. X. & Jin, X. & Lubman, D. (1998). Determination of the Sites of Posttranslational Modifications in the Charge Isomers of Bovine Myelin Basic Protein by Capillary Electrophoresis-Mass Spectroscopy. *Biochemistry, 37*, 2441-2449.

[31] Mastronardi, F. G. & Moscarello, M. A. (2005). Molecules Affecting Myelin Stability: A Novel Hypothesis Regarding the Pathogenesis of Multiple Sclerosis. *J Neurosci Res, 80*, 301-308.

[32] Dutta, R. & Trapp, B. D. (2007). Pathogenesis of Axonal and Neuronal Damage in Multiple Sclerosis. *Neurology, 68*, S22-31; discussion S43-54.

[33] Ramagopalan, S. V. & Dobson, R. & Meier, U. C. & Giovannoni, G. (2010). Multiple Sclerosis: Risk Factors, Prodromes, and Potential Causal Pathways. *Lancet Neurol, 9*, 727-739.

[34] Reynolds, R. & Roncaroli, F. & Nicholas, R. & Radotra, B. & Gveric, D. & Howell, O. (2011). The Neuropathological Basis of Clinical Progression in Multiple Sclerosis. *Acta Neuropathol, 122*, 155-170.

[35] Trapp, B. D. & Peterson, J. & Ransohoff, R. M. & Rudick, R. & Mork, S. & Bo, L. (1998). Axonal Transection in the Lesions of Multiple Sclerosis. *N Engl J Med, 338*, 278-285.

[36] Bo, L. (2009). The Histopathology of Grey Matter Demyelination in Multiple Sclerosis. *Acta Neurol Scand Suppl*, 51-57.

[37] Baumann, N. & Pham-Dinh, D. (2001). Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System. *Physiol Rev, 81*, 871-927.

[38] Bradl, M. & Lassmann, H. (2010). Oligodendrocytes: Biology and Pathology. *Acta Neuropathol, 119*, 37-53.

[39] Craner, M. J. & Newcombe, J. & Black, J. A. & Hartle, C. & Cuzner, M. L. & Waxman, S. G. (2004). Molecular Changes in Neurons in Multiple Sclerosis: Altered Axonal Expression of Nav1.2 and Nav1.6 Sodium Channels and Na+/Ca2+ Exchanger. *Proc Natl Acad Sci U S A, 101*, 8168-8173.

[40] Andrews, H. & White, K. & Thomson, C. & Edgar, J. & Bates, D. & Griffiths, I. & Turnbull, D. & Nichols, P. (2006). Increased Axonal Mitochondrial Activity as an Adaptation to Myelin Deficiency in the Shiverer Mouse. *J Neurosci Res, 83*, 1533-1539.

[41] Waxman, S. G. & Ritchie, J. M. (1993). Molecular Dissection of the Myelinated Axon. *Ann Neurol, 33*, 121-136.

[42] He, F. & Sun, Y. E. (2007). Glial Cells More Than Support Cells? *Int J Biochem Cell Biol, 39*, 661-665.

[43] Hamby, M. E. & Sofroniew, M. V. (2010). Reactive Astrocytes as Therapeutic Targets for Cns Disorders. *Neurotherapeutics, 7*, 494-506.

[44] Kimelberg, H. K. & Nedergaard, M. (2010). Functions of Astrocytes and Their Potential as Therapeutic Targets. *Neurotherapeutics, 7*, 338-353.

[45] Parpura, V. & Grubisic, V. & Verkhratsky, A. (2011). Ca(2+) Sources for the Exocytotic Release of Glutamate from Astrocytes. *Biochim Biophys Acta, 1813*, 984-991.

[46] Volterra, A. & Meldolesi, J. (2005). Astrocytes, from Brain Glue to Communication Elements: The Revolution Continues. *Nat Rev Neurosci, 6*, 626-640.

[47] Newman, E. A. (2003). New Roles for Astrocytes: Regulation of Synaptic Transmission. *Trends Neurosci, 26*, 536-542.

[48] Greve, M. W. & Zink, B. J. (2009). Pathophysiology of Traumatic Brain Injury. *Mt Sinai J Med, 76*, 97-104.

[49] Abbott, N. J. & Ronnback, L. & Hansson, E. (2006). Astrocyte-Endothelial Interactions at the Blood-Brain Barrier. *Nat Rev Neurosci, 7*, 41-53.

[50] Didier, N. & Romero, I. A. & Creminon, C. & Wijkhuisen, A. & Grassi, J. & Mabondzo, A. (2003). Secretion of Interleukin-1beta by Astrocytes Mediates Endothelin-1 and Tumour Necrosis Factor-Alpha Effects on Human Brain Microvascular Endothelial Cell Permeability. *J Neurochem, 86*, 246-254.

[51] Kim, J. H. & Kim, J. H. & Park, J. A. & Lee, S. W. & Kim, W. J. & Yu, Y. S. & Kim, K. W. (2006). Blood-Neural Barrier: Intercellular Communication at Glio-Vascular Interface. *J Biochem Mol Biol, 39*, 339-345.

[52] Schwaninger, M. & Sallmann, S. & Petersen, N. & Schneider, A. & Prinz, S. & Libermann, T. A. & Spranger, M. (1999). Bradykinin Induces Interleukin-6 Expression in Astrocytes through Activation of Nuclear Factor-Kappab. *J Neurochem, 73*, 1461-1466.

[53] Broman, T. (1964). Blood-Brain Barrier Damage in Multiple Sclerosis Supravital Test-Observations. *Acta Neurol Scand Suppl, 40*, SUPPL 10:21-14.

[54] Ebers, G. C. & Vinuela, F. V. & Feasby, T. & Bass, B. (1984). Multifocal Ct Enhancement in Ms. *Neurology, 34*, 341-346.

[55] Engelhardt, B. & Vestweber, D. & Hallmann, R. & Schulz, M. (1997). E- and P-Selectin Are Not Involved in the Recruitment of Inflammatory Cells across the Blood-Brain Barrier in Experimental Autoimmune Encephalomyelitis. *Blood, 90*, 4459-4472.

[56] Lassmann, H. & Schwerer, B. & Kitz, K. & Egghart, M. & Bernheimer, H. (1983). Pathogenetic Aspects of Demyelinating Lesions in Chronic Relapsing Experimental Allergic Encephalomyelitis: Possible Interaction of Cellular and Humoral Immune Mechanisms. *Prog Brain Res, 59*, 305-315.

[57] Claudio, L. & Raine, C. S. & Brosnan, C. F. (1995). Evidence of Persistent Blood-Brain Barrier Abnormalities in Chronic-Progressive Multiple Sclerosis. *Acta Neuropathol, 90*, 228-238.

[58] Filippi, M. & Rocca, M. A. & Martino, G. & Horsfield, M. A. & Comi, G. (1998). Magnetization Transfer Changes in the Normal Appearing White Matter Precede the

Appearance of Enhancing Lesions in Patients with Multiple Sclerosis. *Ann Neurol, 43*, 809-814.

[59] Kwon, E. E. & Prineas, J. W. (1994). Blood-Brain Barrier Abnormalities in Longstanding Multiple Sclerosis Lesions. An Immunohistochemical Study. *J Neuropathol Exp Neurol, 53*, 625-636.

[60] Matthews, P. M. & Arnold, D. L. (2001). Magnetic Resonance Imaging of Multiple Sclerosis: New Insights Linking Pathology to Clinical Evolution. *Curr Opin Neurol, 14*, 279-287.

[61] Soon, D. & Tozer, D. J. & Altmann, D. R. & Tofts, P. S. & Miller, D. H. (2007). Quantification of Subtle Blood-Brain Barrier Disruption in Non-Enhancing Lesions in Multiple Sclerosis: A Study of Disease and Lesion Subtypes. *Mult Scler, 13*, 884-894.

[62] Fabis, M. J. & Scott, G. S. & Kean, R. B. & Koprowski, H. & Hooper, D. C. (2007). Loss of Blood-Brain Barrier Integrity in the Spinal Cord Is Common to Experimental Allergic Encephalomyelitis in Knockout Mouse Models. *Proc Natl Acad Sci U S A, 104*, 5656-5661.

[63] Kean, R. B. & Spitsin, S. V. & Mikheeva, T. & Scott, G. S. & Hooper, D. C. (2000). The Peroxynitrite Scavenger Uric Acid Prevents Inflammatory Cell Invasion into the Central Nervous System in Experimental Allergic Encephalomyelitis through Maintenance of Blood-Central Nervous System Barrier Integrity. *J Immunol, 165*, 6511-6518.

[64] Morgan, L. & Shah, B. & Rivers, L. E. & Barden, L. & Groom, A. J. & Chung, R. & Higazi, D. & Desmond, H. & Smith, T. & Staddon, J. M. (2007). Inflammation and Dephosphorylation of the Tight Junction Protein Occludin in an Experimental Model of Multiple Sclerosis. *Neuroscience, 147*, 664-673.

[65] Kirk, J. & Plumb, J. & Mirakhur, M. & Mcquaid, S. (2003). Tight Junctional Abnormality in Multiple Sclerosis White Matter Affects All Calibres of Vessel and Is Associated with Blood-Brain Barrier Leakage and Active Demyelination. *J Pathol, 201*, 319-327.

[66] Minagar, A. & Ostanin, D. & Long, A. C. & Jennings, M. & Kelley, R. E. & Sasaki, M. & Alexander, J. S. (2003). Serum from Patients with Multiple Sclerosis Downregulates Occludin and Ve-Cadherin Expression in Cultured Endothelial Cells. *Mult Scler, 9*, 235-238.

[67] Czeh, M. & Gressens, P. & Kaindl, A. M. (2011). The Yin and Yang of Microglia. *Dev Neurosci, 33*, 199-209.

[68] D'aversa, T. G. & Weidenheim, K. M. & Berman, J. W. (2002). Cd40-Cd40l Interactions Induce Chemokine Expression by Human Microglia: Implications for Human Immunodeficiency Virus Encephalitis and Multiple Sclerosis. *Am J Pathol, 160*, 559-567.

[69] Hayes, G. M. & Woodroofe, M. N. & Cuzner, M. L. (1988). Characterisation of Microglia Isolated from Adult Human and Rat Brain. *J Neuroimmunol, 19*, 177-189.

[70] Morioka, T. & Baba, T. & Black, K. L. & Streit, W. J. (1992). Immunophenotypic Analysis of Infiltrating Leukocytes and Microglia in an Experimental Rat Glioma. *Acta Neuropathol, 83*, 590-597.

[71] Perry, V. H. & Gordon, S. (1987). Modulation of Cd4 Antigen on Macrophages and Microglia in Rat Brain. *J Exp Med, 166*, 1138-1143.

[72] Prinz, M. & Hanisch, U. K. (1999). Murine Microglial Cells Produce and Respond to Interleukin-18. *J Neurochem, 72*, 2215-2218.

[73] Streit, W. J. & Hurley, S. D. & Mcgraw, T. S. & Semple-Rowland, S. L. (2000). Comparative Evaluation of Cytokine Profiles and Reactive Gliosis Supports a Critical Role for Interleukin-6 in Neuron-Glia Signaling During Regeneration. *J Neurosci Res, 61*, 10-20.

[74] Frei, K. & Siepl, C. & Groscurth, P. & Bodmer, S. & Schwerdel, C. & Fontana, A. (1987). Antigen Presentation and Tumor Cytotoxicity by Interferon-Gamma-Treated Microglial Cells. *Eur J Immunol, 17*, 1271-1278.

[75] Hickey, W. F. & Kimura, H. (1988). Perivascular Microglial Cells of the Cns Are Bone Marrow-Derived and Present Antigen in Vivo. *Science, 239*, 290-292.

[76] Kutzelnigg, A. & Lucchinetti, C. F. & Stadelmann, C. & Bruck, W. & Rauschka, H. & Bergmann, M. & Schmidbauer, M. & Parisi, J. E. & Lassmann, H. (2005). Cortical Demyelination and Diffuse White Matter Injury in Multiple Sclerosis. *Brain, 128*, 2705-2712.

[77] Magliozzi, R. & Howell, O. W. & Reeves, C. & Roncaroli, F. & Nicholas, R. & Serafini, B. & Aloisi, F. & Reynolds, R. (2010). A Gradient of Neuronal Loss and Meningeal Inflammation in Multiple Sclerosis. *Ann Neurol, 68*, 477-493.

[78] Heppner, F. L. & Greter, M. & Marino, D. & Falsig, J. & Raivich, G. & Hovelmeyer, N. & Waisman, A. & Rulicke, T. & Prinz, M. & Priller, J. & Becher, B. & Aguzzi, A. (2005). Experimental Autoimmune Encephalomyelitis Repressed by Microglial Paralysis. *Nat Med, 11*, 146-152.

[79] Guo, X. & Nakamura, K. & Kohyama, K. & Harada, C. & Behanna, H. A. & Watterson, D. M. & Matsumoto, Y. & Harada, T. (2007). Inhibition of Glial Cell Activation Ameliorates the Severity of Experimental Autoimmune Encephalomyelitis. *Neurosci Res, 59*, 457-466.

[80] Mahad, D. & Callahan, M. K. & Williams, K. A. & Ubogu, E. E. & Kivisakk, P. & Tucky, B. & Kidd, G. & Kingsbury, G. A. & Chang, A. & Fox, R. J. & Mack, M. & Sniderman, M. B. & Ravid, R. & Staugaitis, S. M. & Stins, M. F. & Ransohoff, R. M. (2006). Modulating CCR-2 and CCL-2 at the Blood-Brain Barrier: Relevance for Multiple Sclerosis Pathogenesis. *Brain, 129*, 212-223.

[81] D'aversa, T. G. & Eugenin, E. A. & Lopez, L. & Berman, J. W. (2012). Myelin Basic Protein Induces Inflammatory Mediators from Primary Human Endothelial Cells and Blood-Brain-Barrier Disruption: Implications for the Pathogenesis of Multiple Sclerosis. *Neuropathol Appl Neurobiol*,

[82] Huang, D. R. & Wang, J. & Kivisakk, P. & Rollins, B. J. & Ransohoff, R. M. (2001). Absence of Monocyte Chemoattractant Protein 1 in Mice Leads to Decreased Local Macrophage Recruitment and Antigen-Specific T Helper Cell Type 1 Immune Response in Experimental Autoimmune Encephalomyelitis. *J Exp Med, 193*, 713-726.

[83] Karpus, W. J. & Fife, B. T. & Kennedy, K. J. (2003). Immunoneutralization of Chemokines for the Prevention and Treatment of Central Nervous System Autoimmune Disease. *Methods, 29*, 362-368.

[84] Youssef, S. & Wildbaum, G. & Karin, N. (1999). Prevention of Experimental Autoimmune Encephalomyelitis by Mip-1alpha and Mcp-1 Naked DNA Vaccines. *J Autoimmun, 13*, 21-29.

[85] Sun, D. & Hu, X. & Shah, R. & Zhang, L. & Coleclough, C. (1996). Production of Tumor Necrosis Factor-Alpha as a Result of Glia-T-Cell Interaction Correlates with the Pathogenic Activity of Myelin Basic Protein-Reactive T Cells in Experimental Autoimmune Encephalomyelitis. *J Neurosci Res, 45*, 400-409.

[86] Schmitz, T. & Chew, L. J. (2008). Cytokines and Myelination in the Central Nervous System. *ScientificWorldJournal, 8*, 1119-1147.

[87] Sun, D. & Coleclough, C. & Cao, L. & Hu, X. & Sun, S. & Whitaker, J. N. (1998). Reciprocal Stimulation between Tnf-Alpha and Nitric Oxide May Exacerbate Cns Inflammation in Experimental Autoimmune Encephalomyelitis. *J Neuroimmunol, 89*, 122-130.

[88] Ruddle, N. H. & Bergman, C. M. & Mcgrath, K. M. & Lingenheld, E. G. & Grunnet, M. L. & Padula, S. J. & Clark, R. B. (1990). An Antibody to Lymphotoxin and Tumor Necrosis Factor Prevents Transfer of Experimental Allergic Encephalomyelitis. *J Exp Med, 172*, 1193-1200.

[89] Srinivasan, R. & Sailasuta, N. & Hurd, R. & Nelson, S. & Pelletier, D. (2005). Evidence of Elevated Glutamate in Multiple Sclerosis Using Magnetic Resonance Spectroscopy at 3 T. *Brain, 128*, 1016-1025.

[90] Korn, T. & Magnus, T. & Jung, S. (2005). Autoantigen Specific T Cells Inhibit Glutamate Uptake in Astrocytes by Decreasing Expression of Astrocytic Glutamate Transporter Glast: A Mechanism Mediated by Tumor Necrosis Factor-Alpha. *FASEB J, 19*, 1878-1880.

[91] Su, K. G. & Banker, G. & Bourdette, D. & Forte, M. (2009). Axonal Degeneration in Multiple Sclerosis: The Mitochondrial Hypothesis. *Curr Neurol Neurosci Rep, 9*, 411-417.

[92] Boje, K. M. (2004). Nitric Oxide Neurotoxicity in Neurodegenerative Diseases. *Front Biosci, 9*, 763-776.

[93] De Groot, C. J. & Ruuls, S. R. & Theeuwes, J. W. & Dijkstra, C. D. & Van Der Valk, P. (1997). Immunocytochemical Characterization of the Expression of Inducible and Constitutive Isoforms of Nitric Oxide Synthase in Demyelinating Multiple Sclerosis Lesions. *J Neuropathol Exp Neurol, 56*, 10-20.

[94] Lin, R. F. & Lin, T. S. & Tilton, R. G. & Cross, A. H. (1993). Nitric Oxide Localized to Spinal Cords of Mice with Experimental Allergic Encephalomyelitis: An Electron Paramagnetic Resonance Study. *J Exp Med, 178*, 643-648.

[95] Dawson, V. L. & Dawson, T. M. & London, E. D. & Bredt, D. S. & Snyder, S. H. (1991). Nitric Oxide Mediates Glutamate Neurotoxicity in Primary Cortical Cultures. *Proc Natl Acad Sci U S A, 88*, 6368-6371.

[96] Dawson, V. L. & Kizushi, V. M. & Huang, P. L. & Snyder, S. H. & Dawson, T. M. (1996). Resistance to Neurotoxicity in Cortical Cultures from Neuronal Nitric Oxide Synthase-Deficient Mice. *J Neurosci, 16*, 2479-2487.

[97] Svenningsson, A. & Petersson, A. S. & Andersen, O. & Hansson, G. K. (1999). Nitric Oxide Metabolites in Csf of Patients with Ms Are Related to Clinical Disease Course. *Neurology, 53*, 1880-1882.

[98] Baranzini, S. E. & Srinivasan, R. & Khankhanian, P. & Okuda, D. T. & Nelson, S. J. & Matthews, P. M. & Hauser, S. L. & Oksenberg, J. R. & Pelletier, D. (2010). Genetic Variation Influences Glutamate Concentrations in Brains of Patients with Multiple Sclerosis. *Brain, 133*, 2603-2611.

[99] Turley, D. M. & Miller, S. D. (2010). Prospects for Antigen-Specific Tolerance Based Therapies for the Treatment of Multiple Sclerosis. *Results Probl Cell Differ, 51*, 217-235.

[100] Voulgari-Kokota, A. & Fairless, R. & Karamita, M. & Kyrargyri, V. & Tseveleki, V. & Evangelidou, M. & Delorme, B. & Charbord, P. & Diem, R. & Probert, L. (2012). Mesenchymal Stem Cells Protect Cns Neurons against Glutamate Excitotoxicity by Inhibiting Glutamate Receptor Expression and Function. *Exp Neurol, 236*, 161-170.

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