Neurodegeneration in multiple sclerosis involves multiple pathogenic mechanisms

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Abstract: Multiple sclerosis (MS) is a complex autoimmune disease that impairs the central nervous system (CNS). The neurological disability and clinical course of the disease is highly variable and unpredictable from one patient to another. The cause of MS is still unknown, but it is thought to occur in genetically susceptible individuals who develop disease due to a nongenetic trigger, such as altered metabolism, a virus, or other environmental factors. MS patients develop progressive, irreversible, neurological disability associated with neuronal and axonal damage, collectively known as neurodegeneration. Neurodegeneration was traditionally considered as a secondary phenomenon to inflammation and demyelination. However, recent data indicate that neurodegeneration develops along with inflammation and demyelination. Thus, MS is increasingly recognized as a neurodegenerative disease triggered by an inflammatory attack of the CNS. While both inflammation and demyelination are well described and understood cellular processes, neurodegeneration might be defined by a diverse pool of any of the following: neuronal cell death, apoptosis, necrosis, and virtual hypoxia. In this review, we present multiple theories and supporting evidence that identify common biological processes that contribute to neurodegeneration in MS.

Keywords: lipid and one-carbon metabolism, hypoxia, oxidative stress, autoantibodies, nuclear receptors

Introduction

Historically, neurodegeneration in multiple sclerosis (MS) was viewed as a secondary process resulting from inflammatory demyelination. While demyelination may play an important role in relapsing remitting stage, it doesn't correlate well with the progressive forms of the disease. Over the past several years, a major shift in thinking about the pathogenesis of progressive forms of MS has occurred.¹⁻¹³ Axonal loss, rather than demyelination, correlates better with clinical disability.^{5,14} A new concept emerging in the MS literature theorizes that axonal loss may occur independently of or may even be the cause of the demyelination in MS.^{5,14} Evidence indicates that neurodegeneration occurs in all stages of the disease.^{9,13,15,16} In addition, the neurodegeneration seen in the progressive forms of MS does not correlate with white matter plaque location but instead, correlates with gray matter and cortical pathology.^{6,13,15,17-21} A post-mortem analysis of spinal cords from MS patients showed that axonal loss in the white matter tracts did not associate with the demyelinated plaques in the region.⁴ This indicates that there might be some pathological mechanisms independent of myelin loss that contribute to the axonal loss and neurodegeneration present in MS. Further evidence has shown that axonal injury can occur before myelin loss,^{4,5,9,22} suggesting that axonal injury and neurodegeneration

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could be independent of demyelination and may occur prior to or in parallel with demyelination. Neurodegeneration is a very complicated mechanism that involves several factors. Perhaps the best way to understand the process of neurodegeneration is to dissect the protein targets and molecular pathways involved. In this review, we will discuss multiple theories of myelin loss and axonal degeneration as the basis of disease pathology, with the goal of shedding light on the common pathways of neuronal destruction.

Hypoxia

Over the years, multiple hypotheses have been proposed to explain the pathogenesis of MS, ranging from viral infection, cytokine-induced apoptosis, and oxidative stress (OS) to molecular mimicry and metabolic disorders.^{23–26} However, none have successfully identified a single pathological mechanism, mainly because MS is a heterogeneous disease, with a multifaceted etiology.^{27,28}

One school of thought suggests MS pathology is due to axonal damage and loss, which occurs when chronically demyelinated neurons reach a state of “virtual hypoxia” associated with reduced adenosine triphosphate (ATP) production, and ion channel and mitochondrial dysfunction. It is believed that the loss of myelin results in an increased energy demand and a relative cellular energy deficit, which eventually leads to neuronal death (Figure 1). In a viable neuron, Na^+/K^+ ATPase is located at the nodes of Ranvier (regions between myelin

sheaths). Evidence suggests that after demyelination, the Na^+ channels undergo redistribution, from localization predominantly on the nodes of Ranvier to a diffuse spread along the axon.^{29,30} Thus, Na^+/K^+ ATPase increases along a demyelinated axon in order to continue saltatory conduction. The increase in Na^+/K^+ ATPase results in an increased energy demand for neuronal firing. In MS patients, this increased energy demand cannot be met because of impaired mitochondrial energy production in the central nervous system (CNS).^{4,22,31} The impaired mitochondrial energy production leaves neurons in a depleted energy state, which has been shown to reduce the ability of Na^+/K^+ ATPase function.³² Depleted mitochondrial energy production and reduced firing ability in the overpopulated Na^+/K^+ ATPase within demyelinated neurons in MS leads to several deleterious downstream effects, among which is impaired neurotransmission. With a lack of efficient Na^+/K^+ ATPase, the cell, in theory, should enter a state of axonal depolarization. This state of axonal depolarization causes the overpopulated Na^+/K^+ ATPase to become leaky, resulting in increased intracellular Na^+ concentrations (Figure 1). It is believed that if axonal Na^+ rises to a concentration greater than 20 mM, the $\text{Na}^+/\text{Ca}^{2+}$ exchanger will operate in reverse, thus acting as a system to dump Ca^{2+} into the axon.³³ The increase in Ca^{2+} within the axon is known as Ca^{2+} loading. Additional sources may contribute to axonal Ca^{2+} loading, including release from intracellular Ca^{2+} stores,³⁴ voltage-gated Ca^{2+} channels,³⁵ and Ca^{2+} -permeable cation channels, such as glutamate-gated receptors.^{36–38} Large quantities of glutamate released by activated immune cells, in turn, activate glutamate receptors, which results in axonal Ca^{2+} -loading and subsequent neuronal death.^{39,40} In hypoxic cells, the reversal of Na^+ -dependent glutamate transporters results in glutamate release.⁴¹ In addition, astrocytes can release glutamate, by exocytosis or hemichannels,^{42,43} and unmyelinated callosal axons release glutamate in a vesicular manner, similar to the normal release at the synapse.⁴⁴ This increase in vesicular release of glutamate within the white matter has implications for the mechanisms of ischemia-induced myelin damage, which can possibly occur through the activation of glial cells.⁴⁴

Hypoxia might also play a role in the formation of MS lesions. Decreased oxygen availability (hypoxia) is often seen in tissues at the sites of chronic inflammation. Inflamed tissue has increased metabolic activity, due to the presence of inflammatory cells and poor perfusion, which is related to blood vessel stenosis and microthrombosis. Therefore, chronically inflamed tissue has an increased demand for and a limited supply of oxygen. This imbalance results in hypoxia at inflammatory sites. Hypoxia also increases the permeability of the blood–brain barrier (BBB) and results in

Figure 1 Schematic representation of the neurodegeneration theories.

Note: Arrows represent increase (upward) and decrease (downward) in cellular processes and metabolites.

Abbreviations: ATP, adenosine triphosphate; B_6 , vitamin B_6 ; B_{12} , vitamin B_{12} ; DNA, deoxyribonucleic acid; Hcy, homocysteine; HDL, high-density lipoprotein; ROS, reactive oxygen species.

the overexpression of proinflammatory genes.^{45–47} Hypoxia is often accompanied by hypoperfusion. In about 50% of MS patients, the blood flow through normal-appearing white matter is reduced.^{48–52} Taken together, both hypoxia and hypoperfusion might be a precipitating factor for MS lesion formation.

Hypoxia-inducible factor (HIF) is an important transcription factor that regulates cellular metabolism and survival under hypoxic stress. HIF is composed of an alpha and beta subunit (HIF- α and HIF- β). Active HIF requires a heterodimeric complex formation of the two subunits, which then translocates to the nucleus, binds to the hypoxia-response

Table I Genes involved in oxidative stress, hypoxia, lipid, and one-carbon metabolism

Gene	Theory	Pathway	References
AMPA/kainate receptor	Oxidative stress	AMP kinase	8, 37, 82, 83
Apo A1	Oxidative stress	Inflammation, immune response,	23, 167, 169, 180
HDL	Inflammation	Lipid metabolism, cholesterol transfer	
CBP/p300	Hypoxia	Apoptosis NF-k β	47, 54
TNF α	Inflammation	Apoptosis	57, 70
TNF β	Hypoxia oxidative stress	Cytokines	
INF γ	Inflammation	Inflammation	70, 167
INF β			
ICAM-1	Autoimmunity	Apoptosis,	40, 64
VCAM-1	Virtual hypoxia	Inflammation	
IL-1 α	Inflammation	Cytokines	168–171
IL-2 α	Virtual hypoxia	T cell survival	
IL-18	Oxidative Stress	Proliferation	
IL-6			
IL-1 β			
HIF	Hypoxia	Inflammation,	47, 53, 55–60, 71, 73, 74
HIF-1		Apoptosis	
HIF α		PI3K/AKT	
HIF β			
NADPH oxidase	Oxidative stress	Apoptosis	62, 78, 149, 172, 180
NOS			
NMDA			
NF-k β	Hypoxia, oxidative stress	Apoptosis	40, 47,
	Autoantibodies	ERK	57–60, 107, 115, 149
	Metabolic disturbances		
HnRNP A1/B2	Autoantibodies	Autoimmune response	10, 11, 85,
NF-155,		Cell survival,	90–92, 94, 100, 106–108,
NF-186		Proliferation,	110–112, 117, 120, 122,
MOG		Apoptosis	123
MAG		Immune response	
PLP			
BHMT	Metabolic disturbances	One-carbon metabolism	23, 138, 144, 148, 151,
MAT			152, 157, 160, 173
GNMT			
CBS			
Methyl transferase			
Methionine synthase			
MTHFR			
PPAR α	Oxidative stress,	Immune response	23, 180,
PPAR β/δ	Hypoxia,	Lipid metabolism	182–201
PPAR γ	Lipid and amino acid metabolism	Cholesterol transfer	
		Apoptosis	
PLA2	Oxidative stress	Immune response	77, 78

Abbreviations: AKT, protein kinase B; AMP, adenosine monophosphate; AMPA, isoxazolepropionic acid; Apo A1, apolipoprotein A1; BHMT, betaine-homocysteine-S-methyl transferase; CBP, CREB-binding protein; CBS, cystathionine beta synthase; CREB, cyclic AMP response element binding protein; ERK, extracellular signal-regulated kinase; GNMT, glycine N-methyltransferase; HDL, high-density lipoprotein; HIF, hypoxia inducible factor; HnRNP, heterogeneous nuclear ribonuclear proteins; ICAM, intracellular adhesion molecule 1; IL, interleukin; INF, interferon; MAG, myelin-associated glycoprotein; MAT, methionine adenosyltransferase; MOG, myelin oligodendrocyte glycoprotein; MTHFR, methylenetetrahydrofolate reductase; NADPH, nicotinamide adenine dinucleotide phosphate; NF, neurofascin; NF-k β , nuclear factor-kappa β ; NMDA, N-methyl-D-aspartate receptor; NOS, nitric oxide synthase; p300, E1A binding protein p300; PI3K, phosphoinositide 3'-kinase; PLA, phospholipase A; PLP, proteolipid protein; PPAR, peroxisome proliferator-activated receptor; TNF, tumor necrosis factor; VCAM, vascular-cell adhesion molecule 1.

element, and associates with coactivators, such as CREB (Cyclic AMP response element binding protein)-binding protein (CBP)/E1A binding protein p300 (p300) (Table 1). The binding results in the activation or suppression of many genes involved in metabolism and cell survival. These changes include an increase in HIF-1 α protein levels, expression of HIF1-inducible genes in the MS brain,⁵³ blood vessel density, and endothelial cell proliferation.^{47,54} HIF-1 α has been shown to play a proinflammatory role in cells of the myeloid lineage and anti-inflammatory role in intestinal epithelial cells.^{55,56}

HIFs regulate cellular stress responses in tandem with nuclear factor-kappa β (NF- κ β) to control hypoxic inflammation through activation of cytokine and hypoxia pathways.⁴⁷ In fact, there is a cross talk between these two transcription factors during hypoxic inflammation. HIF can be activated in response to multiple stimuli, such as bacterial lipopolysaccharide, microtubule disruption, interleukin (IL)-18 and tumor necrosis factor α (TNF- α), hepatocyte growth factor, and reactive oxygen species (ROS). The mechanism of HIF activation involves a NF- κ β -dependent upregulation of HIF-1 α messenger ribonucleic acid (mRNA) levels.⁵⁷ HIF-1 α contains an active binding site for NF- κ β , upstream of the transcription start position.⁵⁸ A recent study demonstrated that NF- κ β controls the HIF pathway in response to TNF- α .⁵⁹ While NF- κ β controls HIF-1 α expression levels, HIF-1 α can regulate NF- κ β signaling. Mice overexpressing HIF-1 α exhibited increase in pro-inflammatory NF- κ β targets.⁶⁰ As it appears from the evidence mentioned, hypoxia alters ATP potential and gene and protein expression, and may contribute to MS lesion formation.

Oxidative stress

Oxidative stress (OS) resulting from the formation of ROS, secreted primarily by macrophages, is believed to play a role in the pathogenesis of MS. ROS are free radicals and related molecules that are defined as any chemical species that contain one or more unpaired electrons.²⁴ The most common ROS are hydroxyl radical (OH $\cdot$), superoxide radical (O $_2^{\cdot-}$), and nitric oxide (NO) as well as other molecules, such as hydrogen peroxide (H $_2$ O $_2$) and peroxynitrite (ONOO $^-$). Unpaired electrons cause ROS to act as electron acceptors, which results in the “stealing” of electrons by ROS (oxidation). ROS occur within a normal cell to a certain extent, and a number of mechanisms are in place to guard against ROS-induced damage; however, it appears that in patients with MS, the ROS exceed the capacity of the cellular defense mechanisms. ROS are known to cause damage to lipids, proteins, and deoxyribonucleic acid (DNA), leading to cellular death by necrosis and apoptosis

(Figure 1). Metals, such as iron, are normally stored within iron-binding proteins. However, injured cells release iron, which is then available to catalyze the free-radical reactions of ROS formation. Other sources of free radical production are the result of oxygen use in mitochondria and enzymatic pathways, such as xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, lipoxygenases, and cyclooxygenase.²⁴ The “respiratory burst” system of activated microglia has also been shown to produce large quantities of ROS. In addition, reactive astrocytes have been shown to produce NO.^{24,61}

The oxygen- and nitrogen-free radicals generated by macrophages have been shown to cause demyelination and axonal injury in experimental autoimmune encephalomyelitis (EAE) and MS.^{62,63} Furthermore, free radicals activate transcription factors, resulting in the upregulation of the expression of many genes that are associated with MS, such as NF- κ β , TNF- α , intracellular adhesion molecule 1 (ICAM-1), and vascular-cell adhesion molecule 1 (VCAM-1)^{40,64} (Table 1). A study by Langemann et al revealed that MS plaques had increased free radical activity as well as decreased levels of important antioxidants, such as glutathione, alpha-tocopherol, and uric acid.⁶⁵ Further evidence has shown that oxidative damage to DNA in MS includes damage to mitochondrial DNA, implicating mitochondria not only in the formation of ROS but possibly as a pathway directly affected by OS.⁶⁶ Studies have also shown that nitric oxide synthase (NOS) is upregulated in inflammatory lesions^{62,67} and that NO and its derivative peroxynitrite inhibit mitochondrial respiration.⁶⁸ NO is both essential for life and toxic. Its immunomodulatory effect helps sustain healthy homeostasis; however, large NO quantities damage axons.⁶⁷ Inflammation induces the production of NO. Excessive generation of NO is an indicator of aging and neurodegeneration. Increased NO concentration raises intracellular Ca $^{2+}$ and Na $^+$ levels and may be responsible for mitochondrial dysfunction.^{67,69} The tissue damage in MS is caused, in part, by elevated levels of NO. In the CNS, NO is produced by macrophages and microglia following the induction of NOS by the proinflammatory cytokines TNF- α and interferon (INF)- γ .⁷⁰ Notably, NO mediates the destabilization of HIF through increased ROS production.^{71,72} Another oxidative agent, H $_2$ O $_2$, has been shown to decrease the HIF-DNA binding capacity and the expression of its target genes.^{73,74} Taken together, these studies suggest that the redox system plays an important role in HIF regulation.

NO can also react with the sulfhydryl groups of proteins, resulting in the S-nitrosylation of target proteins, which

initially can act as a protective mechanism in OS, to defend proteins from degradation. However, increased OS and the overaccumulation of NO results in irreversible cell damage caused by the oxidation of free thiols, nitration of tyrosine residues, and lipid peroxidation.⁷⁵ Increased S-nitrosylation has been detected in the normal-appearing white matter of MS patients' brain compared with that of normal controls, indicating that nitrosative damage is involved in the pathophysiology of MS.⁷⁶

In addition to ROS, glutamate appears to be another major source of OS in the brain, through the activation of ionotropic glutamate receptors. It is possible that damage induced by free radicals can occur via the stimulation of phospholipase A2 and the release of amino acids, which in the presence of free radicals, results in an enhanced release of glutamate.^{77,78} The cerebrospinal fluid (CSF) of MS patients has elevated levels of glutamate.^{79,80} Increased glutamate, via an interaction with alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors, has also shown to be deleterious to oligodendrocytes (which appear to be highly vulnerable to glutamate excitotoxicity).^{81,82} Interestingly, AMPA/kainate receptors are known to have increased permeability to Ca^{2+} , resulting in Ca^{2+} -loading by cells.⁸³ As the OS theory stands, it appears that ROS in the presence of a weakened antioxidant cellular defense results in the damage of cellular components, such as lipids, proteins, nucleic acids, and mitochondrial DNA. These damaged components alter multiple pathways associated with ATP production, upregulation of the genes associated with MS pathology, and increase in glutamate levels (via AMPA/kainate receptors).

Antibodies in neurodegeneration

The hypothesis of molecular mimicry explains the pathogenesis of MS as an autoimmune response to an environmental agent.^{84–89} The antibodies resulting from molecular mimicry have profound effects on neurons and implicate molecular mimicry as a contributor to neurodegeneration and the pathogenesis of neurological disease^{90–96} (Figure 1). The antibodies present in MS patients can be categorized into two major groups: myelin and nonmyelin antibodies. Both types of antibodies have sufficient evidence to support their involvement in the pathogenesis of MS¹¹ (Figure 1). The antimyelin antibody targets include myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), myelin-associated glycoprotein (MAG), and proteolipid protein (PLP).^{97–100} These antibodies have been found in the sera and CSF of MS patients; however, the exact role of such myelin antigens in MS remains contradictory. One study

demonstrated that the development of clinically definite MS could be predicted based on the presence of anti-MOG and anti-MBP antibodies in patients' sera;¹⁰¹ others found no association between anti-MOG and anti-MBP antibodies and MS progression.^{102,103} The immunopathogenic effects of antimyelin antibodies might be epitope-specific⁹⁸ or depend on the antibody confirmation.^{104–106} These earlier studies did not specify the cellular pathways affected by autoantibodies. In a recent study, Ho et al showed that MAG autoantibodies targeted the following natural brain lipids: 1-palmitoyl-2-glutarol-*sn*-glycero-3-phosphocholine (PGPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-(phospho-L-serine), (POPS), 1-hexadecyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (azPC), and 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (azPC ester).¹⁰⁷ Moreover, the authors showed that POPS, PGPC, azPC, and azPC ester affected inflammatory, survival, and apoptotic signaling pathways – specifically, canonical NF- κ B and extracellular signal-regulated kinase (ERK) pathways were activated, in stimulated T-cells isolated from EAE mice. Overall, their data suggest that myelin phospholipids are targeted by autoimmune responses in MS.

A growing number of studies point to the fact that antibody-mediated axonal injury could be initiated by antibodies to nonmyelin antigens. Nonmyelin antibodies present in MS patients have been found to target neuronal surface molecules (axolemma-enriched fractions, neurofascin, and gangliosides), cytoskeletal proteins (neurofilaments [NFs] and NF light chains [NF-Ls]), intracellular enzymes, signaling molecules and chaperones (β -arrestin, retinal arrestin, heat shock proteins, glutamate decarboxylase, and proteasomes), and nuclear antigens (nuclear ribonuclear proteins).¹⁰⁸ These antibodies present a different mechanism of immune-mediated axonal injury. For example, antineurofascin-186 and antineurofascin-155 antibodies were shown to cause an exacerbation of EAE without demyelination, in the spinal cord of rats. When antineurofascin antibodies were cotransfected with MOG-specific T-cells, they selectively targeted the nodes of Ranvier and inhibited neurotransmission in an MS animal model.¹⁰⁹ In other work, chronic progressive MS patients had significantly higher levels of NF-L-specific antibodies in their sera compared with that in patients with other neurological diseases.^{110,111} How the anti-NF and anti-NF-L antibodies arise and their specific effects on the axon are unclear, but their correlation with disease progression appears applicable, in a biomarker-specific manner.^{109–111}

Antibodies to heterogeneous nuclear ribonuclear proteins (hnRNP) A1 and B2 were present in the CSF of MS and

human T-lymphotropic virus 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients but not in normal controls.^{10,85,90–92,94,112} These RNA-binding proteins play a major role in the adjustment of pre-mRNA splicing, through various factors.¹¹³ They also participate in mRNA stability,¹¹⁴ NF- κ B-dependent transcription,¹¹⁵ and telomerase activity.¹¹⁶ HnRNP A1 plays several key roles in neuronal functioning, and its depletion, either due to debilitated cholinergic neurotransmission¹¹⁷ or due to autoimmune reactions, causes drastic changes in RNA metabolism.¹⁰ Recently, RNA-binding proteins have gained attention because a large number of these proteins were mutated in neurodegenerative diseases.^{118,119} RNA-binding proteins use protein aggregation as part of a normal regulated, physiological mechanism that controls protein synthesis. The process of regulated protein aggregation is most evident in the formation of stress granules.¹²⁰ HnRNP A1 has been shown to relocate into cytoplasmic stress granules in the presence of stress stimuli, such as osmotic shock or OS.¹²¹ Recent studies showed that the addition of antibodies to hnRNP A1 affected its distribution, from a primarily nuclear location to a mixed nuclear/cytoplasmic distribution.^{10,122,123} It was known that anti-hnRNP A1 antibodies decreased neuronal firing in vitro, but it was not clear whether the antibodies to this intracellular protein could penetrate neurons and find its target.⁹² Recent studies have revealed that anti-hnRNP A1 antibodies penetrate neuronal cells via clathrin-mediated endocytosis and cause deleterious effects.^{10,92,122,123} Anti-hnRNP A1 antibodies were also shown to increase apoptosis, reduce ATP levels, and cause the redistribution of endogenous hnRNP A1 protein. Thus, anti-hnRNP A1 antibodies altered endogenous protein localization as well as inhibited normal cellular processes in vitro.

In MS patients, the presence of the two types of auto-antibodies may not be mutually exclusive. It is possible that antibodies to myelin antigens may have an impact on the early, relapsing stages of disease, while the nonmyelin antigens play a more dominant role in the progressive stages of MS. More importantly, both types of antibodies may cause neurodegeneration through the activation of apoptotic inflammatory cytokines and immune response pathways.

Role of homocysteine in neurodegeneration

Axonal loss is a key contributor to disability in neurodegenerative diseases. In MS, many studies have suggested that axonal damage is a consequence of demyelination triggered by inflammation.^{124–127} However, substantial axonal loss has also been

detected at the early stages of MS, and several studies suggest that axonal loss is independent of demyelination.^{128–130} Exactly how CNS damage develops is unclear, but it is unlikely to be a direct result of viral infection.¹³¹ Rather a complicated mechanism, involving innate immunity, genetic predisposition, and environmental agents, is at play. The penetration of blood-borne neurotoxins through the compromised blood–brain barrier might play a significant role in axonal degeneration in MS.^{132,133} In addition, microglia cells can produce neurotoxins endogenously and seem to play an important role in neurodegeneration, by acting as an accelerator of neurotoxicity.¹³⁴ One of the neurotoxins is homocysteine (Hcy), a sulfur molecule produced from amino acid methionine. Hcy promotes the activation and proliferation of microglia.¹³⁵ Hcy is a major contributor to oxidative injury and DNA damage¹³⁶ (Figure 1). Elevated Hcy levels are toxic to neurons and might compromise the blood–brain barrier (a hallmark of MS pathology).^{137–142} MS patients have been shown to have elevated Hcy levels, which were associated with cognitive decline.^{143–147} Interestingly, a recent study from our laboratory revealed that patients with primary and secondary progressive MS had significantly higher Hcy levels in their plasma compared with relapsing remitting stage patients and controls.¹⁴⁸ Hcy can be elevated in biological fluids as a result of genetic or metabolic disturbances. Elevated Hcy levels induce adverse effects either directly, through lipid metabolism, or indirectly, via oxidative and endoplasmic reticulum stress (Figure 1). In addition, OS might stimulate the accumulation of Hcy because ROS impair the Hcy conversion to methionine. Hcy modulates substrate levels for various catalytic processes and regulates the expression of genes involved in complex diseases through the activation of NF- κ B.^{149,150} Sharma et al used a literature mining approach to identify the genes and related pathways affected by Hcy. They identified 112 genes modulated by Hcy levels and 23 genes that affected Hcy.¹⁴⁹ Not surprisingly, many of these genes were involved in hypoxia, apoptosis, ROS, inflammation, and lipid metabolism. According to their study, a common link between apoptosis and the inflammatory pathways was endoplasmic reticulum stress, which is closely related to OS. Hcy may induce OS and apoptosis through NADPH oxidase or through the activation of c-Jun N-terminal kinases (JNKs). Hcy is one of the metabolites in the one-carbon cycle (Figure 2), which plays an important role in disorders of the nervous system.

Importance of the one-carbon metabolism in MS

The one-carbon metabolic pathway plays an important role in many biological processes and clinical symptoms, such

Figure 2 Diagram of the one-carbon cycle.

Notes: The major metabolites are presented in the blue frames (methionine, S-adenosylmethionine, S-adenosylhomocysteine, homocysteine); enzymes and cofactors are highlighted in the pink rectangles.

Abbreviations: ATP, adenosine triphosphate; B6, vitamin B6; B12, vitamin B12; BHMT, betaine-homocysteine-S-methyl transferase; CBS, cystathionine beta synthase; DNA, deoxyribonucleic acid; GNMT, glycine N-methyltransferase; Hcy, homocysteine; MAT, methionine adenosyltransferase; MetRS, methionine-tRNA ligase; MS, methionine synthase; MT, methyl transferase; MTHFR, methylenetetrahydrofolate reductase; RNA, ribonucleic acid; SAHH, S-adenosylhomocysteine hydrolase.

as hypomethylation, homocysteinemia, liver dysfunction, and the accumulation of white-matter hyperintensities in the human brain.^{138,148} In addition to Hcy, the one-carbon cycle contains other important molecules, such as S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), and methionine (Figure 2). These metabolites are synthesized within the cycle, through a cascade of biochemical reactions involving vitamins, enzymes, and cofactors. For example, the essential amino acid methionine is converted to SAM in the presence of the enzyme methionine adenosyltransferase, ATP, and magnesium.^{151,152} SAM is further metabolized into SAH, and in the presence of SAH hydrolase, SAH is converted to Hcy. Hcy can be remethylated or recycled back to methionine, in the presence of methionine synthase, vitamins B12, and folate; formed into cystathionine, in the presence of cystathionine beta synthase and vitamin B6; or transformed into Hcy thiolactone by methionyl-tRNA synthetase enzyme (Figure 2). The simultaneous measurements of major metabolites of the one-carbon cycle in MS patients uncovered aberrations in the Hcy conversion back to methionine, and the formation of SAM and SAH.¹⁴⁸

MS patients often have low levels of vitamins B12 (cobalamin), B6 (pyridoxine), and B9 (folate).^{153–157} A severe vitamin B12 deficiency can cause a breakdown of the myelin sheath.¹⁵⁸ B12 deficiency associated either with

poor nutrition, defects in absorption, or disease progression results in neuronal demyelination. The early studies on B12 status in MS patients produced conflicting results; however, as improved techniques became available, the consensus was reached that MS patients have lower levels of B12 in comparison with controls.^{138,153,156,157,159} In addition, MS patients also have reduced B6 levels in their plasma.¹⁴⁸ Vitamin B6 plays a significant role in normal brain development and function, the formation of myelin, and the production of several neurotransmitters (such as serotonin and norepinephrine). A key interaction between vitamin B12 and folate in the one-carbon cycle occurs during the synthesis of methionine from Hcy by methionine synthase, in which both 5-methyltetrahydrofolate (enzyme of the folate cycle) and methyl-vitamin B12 are cofactors. The folate cycle is essential for many genomic and nongenomic methylation reactions via SAM and indirectly, for the synthesis of purines and thymidine, and therefore, of nucleotides, DNA, and RNA.¹⁶⁰ Methylation reactions of DNA and myelin, via SAM, are vitally important in the CNS. Folic acid (B9) deficiency also reduces the activity of methionine synthase.¹⁵⁸ The CNS lacks the alternate betaine pathway of homocysteine remethylation; therefore, if methionine synthase is inactivated, the CNS has greatly reduced methylation capacity.¹⁶¹ Deprivation of folate and B12 increases neurodegeneration, through the activation of ROS and

apoptosis, and increase in cytosolic calcium and intracellular Hcy.¹⁶² In summary, the one-carbon metabolism has profound effects on the CNS, where it plays a central role in DNA synthesis, methylation, gene regulation, synaptic function, and neurotransmission.

Disturbances in lipid metabolism

The human brain has a high lipid content; therefore lipids and their turnover should be considered as good candidate contributors to diseases of the CNS. However, the importance of lipid metabolism in MS has been understudied, mainly due to the central focus on the immune system. Disturbances in lipid metabolism lead to myelin loss, neuronal degeneration, and metabolic distress (Figure 1). Myelin glycolipids received some attention because of their role in autoimmune-mediated demyelination. Apart from the myelin autoantibodies, there is now some evidence for a potential role of cholesterol and lipids in MS. Among the three major pathways of lipid metabolism (exogenous, endogenous, and reverse cholesterol), the cholesterol efflux from peripheral macrophages by microglia deserves special attention. Microglial cells regulate lipid homeostasis in the CNS by maintaining a careful balance between phagocytic and cytotoxic macrophages. A distorted lipid homeostasis results in an imbalance between the cytotoxic and phagocytic microglia.²³ In a comprehensive review of MS, Corthals described the disease as a dysfunction of the metabolism of lipids.²³ The author explained that the immune system relies on lipids for repair and for prevention of inflammation. Therefore, a disequilibrium in lipid metabolism causes deregulation of the immune system. Lipids, and especially oxidized lipoproteins, are the core agents of the immune response during acute inflammation. The oxidation of lipids could increase in hypoxic conditions and cause distortions in lipid metabolism. The importance of controlling dyslipidemia in MS patients has been recently emphasized in several studies.^{163–166} Dyslipidemia was linked to an increased risk for disability progression in a study that analyzed 8,993 MS patients.¹⁶³ Lipid profiles were shown to be associated with magnetic resonance imaging (MRI) outcomes as well as lesion formation in IFN- β -treated patients after the first demyelinating event.^{164–166} Patients treated with intramuscular IFN- β showed an association between higher serum low-density lipoprotein (LDL) cholesterol and total cholesterol, with an increased risk for developing new lesions on T2 weighted scans.¹⁶⁵ Interestingly, a different study showed that patients who had high levels of apolipoprotein A1 (ApoA1) adapted better to IFN- β therapy.¹⁶⁷ ApoA1 is a

major component of high-density lipoprotein (HDL). ApoA1 inhibits contact-mediated activation of monocytes by binding to stimulated T-cells, thereby inhibiting TNF- α and IL-1 β production.^{168,169} Others also found that an increased total cholesterol was associated with increases in the number of contrast-enhancing lesions in clinically isolated syndrome following the first clinical event.¹⁶⁴ The results from these studies suggest the importance of controlling dyslipidemia in MS. Cholesterol-lowering drugs, such as statins, are used to lower cholesterol in humans; therefore it was logical to evaluate these therapies in MS. Animal studies showed that statins inhibited the production of NOS, TNF- α , and IL-6, and lowered disease scores.^{170,171} However, such therapeutic approach in humans resulted in controversial findings.^{172–176} A pilot study using 80 mg simvastatin reported a reduction in the number and volume of gadolinium-enhancing lesions.¹⁷⁶ The next double-blinded clinical trial with atorvastatin (40 or 80 mg) as an add-on to IFN- β treatment showed that patients on statins had either new T2 lesions or more clinical relapses.¹⁷⁷ In 2012, a new randomized clinical trial showed a benefit of simvastatin use in secondary progressive MS patients.¹⁷² Simvastatin reduced brain atrophy by 43% and improved clinical outcomes over the 2-year study period. Overall, statins are well-tolerated and widely used drugs that lower LDL, increase HDL, and reduce inflammation. However, these drugs have been shown to increase ROS generation and suppress the activation of the protein kinase B (PKB)/AKT and extracellular signal-regulated kinase (ERK) pathways, elevate lipid peroxidation, and induce oxidative DNA damage, in human peripheral blood lymphocytes.^{178,179} Increased lipid peroxidation has been shown to be associated with disease exacerbation periods and lesion pathogenesis in MS patients.¹⁸⁰ Statins block the hepatic enzyme 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase responsible for the production of cholesterol in the body. The inhibition of this enzyme also affects the pathways responsible for leukocyte migration and activation, thus providing a beneficial outcome in autoimmune inflammation. Animal studies have shown that Hcy inhibits simvastatin-induced ApoA1 upregulation,¹⁸¹ thus suggesting a link between statins and Hcy metabolism. However, human studies did not provide detailed ApoA1 and Hcy measurements in addition to the MS clinical outcomes in statin trials. At present, it is not entirely clear which statin drug, at what dose, and at which stage of the disease will provide the most benefit to MS patients. Therefore, future research is needed to uncover the protective and/or pathological effects of statins in MS.

Peroxisome proliferator-activated receptors in MS

Perturbations in lipid metabolism negatively affect myelin.^{182,183} Therefore, special consideration should be given to the factors that control lipid turnover in health and disease. Lipid metabolism is regulated by peroxisomes and the peroxisome proliferator-activated receptors (PPARs).^{23,184–186} Remarkably, PPARs also control inflammation.¹⁸⁴ Peroxisomes are responsible for oxygen metabolism, and α - and β -oxidation reactions.¹⁸³ PPARs regulate the function and the number of peroxisomes within the cell as well as numerous biological pathways associated with MS (Figure 3). PPARs form heterodimers with the retinoid X-receptor (RXR). These heterocomplexes regulate the inflammatory response and cytotoxic cell apoptosis, myelin synthesis, neuronal cell proliferation and differentiation, energy and lipid homeostasis, and reactive oxygen species.^{187–190} There are three subtypes of PPARs: PPAR α , PPAR β/δ , and PPAR γ . PPAR α and PPAR γ are present in the lipid core of atherosclerotic lesions, macrophages, foam cells, and smooth muscle cells.^{188,190} PPAR α is one of the several factors that regulate the expression of HDL and ApoA1.¹⁹¹ PPAR α is involved in the acetylcholine metabolism, neurotransmission, and OS defense.^{192,193} PPAR β/δ is involved in the control of brain lipid metabolism, epidermal cell proliferation, fatty acid adipogenesis, and preadipocyte proliferation.¹⁹⁴ All PPAR subtypes have been described in the adult and developing brain and in the spinal cord. Several publications have suggested that PPAR activation might directly affect the viability and differentiation of neuronal cells.^{195–199} Remarkably, the expression of PPAR γ

in the brain has been studied in relation to inflammation and neurodegeneration.¹⁹⁵

For example, PPAR γ was found to be increased in microglia and astrocytes during EAE.²⁰⁰ This knowledge prompted an array of studies utilizing PPAR ligands to modulate the course of the disease in animals.^{198,200–202} These studies have shown that PPAR activation reduced leukocyte infiltration into the brain parenchyma and decreased inflammation and axonal demyelination.

Use of the PPAR γ antagonist GW347845 in peripheral blood mononuclear cells (PBMCs) from MS patients has been shown to result in suppressed T-cell proliferation and reduced secretion of TNF- α and INF- γ .¹⁹⁴ However, these antiproliferative effects were accompanied by reduced cell viability and induced apoptosis in activated lymphocytes. Preincubation of PBMCs with pioglitazone was shown to increase the DNA-binding activity of PPAR γ and decrease NF- κ B DNA-binding activity, in the absence of an acute MS relapse.¹⁹⁴ Interestingly, Hcy is known to downregulate PPAR α expression by competing with its ligands (Figure 4).^{48,149,181,191} These results underscore a cross talk between the two types of transcription factors NF- κ B and PPARs in the regulation of the immune response. The Hcy downregulation of PPAR α suggests that PPAR activation could benefit patients with normal Hcy levels.

Discussion: common pathways of destruction

Hypoxia, OS, ROS, autoantibodies, and disturbances in lipid and one-carbon metabolism affect the health of neurons.

Figure 3 PPARs regulation of cellular processes.

Note: PPARs form complexes with RXR to control multiple cellular processes.

Abbreviations: PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X-receptor.

Figure 4 Common pathways of neurodegeneration.

Note: Metabolic disturbances affect major regulators of cellular processes.

Abbreviations: HIF, hypoxia inducible factor; NF- κ B, nuclear factor kappa β ; PPARs, peroxisome proliferator-activated receptors; ROS, reactive oxygen species.

Hypoxia seems to be secondary to demyelination. Oxidative stress could be caused either by external factors (such as viruses, bacteria, and other environmental agents) or by internal toxins that have accumulated inside neurons due to impaired metabolic processes. Excess ROS generated by macrophages or microglia can lead to inflammation, demyelination, and neuronal degradation (Figure 4). Antimyelin and other autoantibodies could cause significant damage to neurons and activate other destructive pathways.

It is not clear which of the factors precipitate the first signs of neuronal demise. However, from the evidence at hand, it appears that each theory of neuronal degeneration and related pathway overlaps the next (Figures 1 and 4). Autoimmunity (caused by the presence of myelin and nonmyelin autoantibodies), metabolic deregulation in one-carbon and lipid metabolism, hypoxia and OS precipitated by inflammation, ROS, and cytokines can all result in neuronal degradation. Taken together, the studies suggest that all of these processes play important roles in neurodegeneration (Figure 4). Neurotoxins, such as Hcy, released in the vicinity of the CNS promote neuronal injury by inducing the cytokine or OS pathways. OS triggers lipid peroxidation, which in turn, negatively affects myelin. The reactive oxygen and nitrogen species released by invading inflammatory cells can cause demyelination and axonal destruction. Oxygen radicals cause damage by reacting with cellular lipids, proteins, carbohydrates, and DNA.

Impaired one-carbon metabolism adversely affects myelination, DNA methylation, and amino acid and protein conversion reactions, and can trigger inflammation through increased Hcy levels. Compromised Hcy conversion to methionine or cysteine might be a crucial factor responsible for the activation of transcription factors and stimulation of ROS formation (Figure 4). Very few studies have addressed the importance of lipid metabolism in MS; however, the current knowledge points to a possible missing link between the simple lowering of cholesterol and a reduced lesion load. Statins reduce inflammation and lower LDL cholesterol through the inhibition of HMG-CoA reductase. At the same time, statins induce the formation of NO through the induction of endothelial NOS. Therefore, a deeper understanding of the cross talk between inflammation, OS, and cholesterol transport could lead to novel therapeutic strategies.

Neurons have another mechanism of response to stress – through upregulation of transcription factors, such as PPARs. These transcription factors are involved in a plethora of vital cellular processes (Figure 3). PPARs seem to be a common link between ROS, hypoxia, and apoptosis. They are also

involved in the modulation of immune response and lipid metabolism. However, the activation of these transcription factors is deeply influenced by the cellular environment. For example, the presence of large quantities of Hcy and other toxins might result in PPAR inhibition. Hcy appears to play an important role in OS, lipid and one-carbon metabolism, and the regulation of NF- κ B and PPARs (Figure 4). The cofactors affecting the one-carbon cycle metabolites, such as vitamins B6, B12, and folate, should be evaluated in MS patients. PPARs-activation agents are less likely to work in MS patients with high Hcy because of the ongoing production of Hcy in the one-carbon cycle. Hcy levels could be lowered with the increased consumption of vitamins B6 and B12. Therefore, future studies designed to combine PPAR-activation with homocysteine- and cholesterol-lowering strategies could lead to novel therapeutic approaches.

Conclusion

MS is a complex disease, and most progressive MS patients develop a common final pathway of neurodegeneration. The molecules responsible for neurodegeneration remain an ongoing area of investigation. Neurons are very susceptible to OS, hypoxia, autoantibodies, and metabolic disturbances. This review highlighted several targets, mechanisms, and pathways that play important roles in neuronal degeneration. Because of the variability of MS, more than one pathway may contribute to neurodegeneration, and thus, targeted interventions designed to normalize these cellular processes could help delay neuronal degeneration and improve clinical outcomes in MS patients.

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Disclosure

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References

1. Aboul-Enein F, Weiser P, Höftberger R, Lassmann H, Bradl M. Transient axonal injury in the absence of demyelination: a correlate of clinical disease in acute experimental autoimmune encephalomyelitis. *Acta Neuropathol.* 2006;111(6):539–547.
2. Bennett JL, Stüve O. Update on inflammation, neurodegeneration, and immunoregulation in multiple sclerosis: therapeutic implications. *Clin Neuropharmacol.* 2009;32(3):121–132.

3. Bjartmar C, Wujek JR, Trapp BD. Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. *J Neurol Sci.* 2003;206(2):165–171.
4. Dutta R, McDonough J, Yin X, et al. Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Ann Neurol.* 2006;59(3):478–489.
5. Dutta R, Trapp BD. Pathogenesis of axonal and neuronal damage in multiple sclerosis. *Neurology.* 2007;68(22 Suppl 3):S22–S31; discussion S43–S54.
6. Geurts JJG, Barkhof F. Grey matter pathology in multiple sclerosis. *Lancet Neurol.* 2008;7(9):841–851.
7. Kornek B, Storch MK, Weissert R, et al. Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. *Am J Pathol.* 2000;157(1):267–276.
8. Kutzelnigg A, Lassmann H. Cortical lesions and brain atrophy in MS. *J Neurol Sci.* 2005;233(1–2):55–59.
9. Lassmann H, van Horssen J. The molecular basis of neurodegeneration in multiple sclerosis. *FEBS Lett.* 2011;585(23):3715–3723.
10. Lee S, Xu L, Shin Y, et al. A potential link between autoimmunity and neurodegeneration in immune-mediated neurological disease. *J Neuroimmunol.* 2011;235(1–2):56–69.
11. Levin MC, Lee S, Gardner LA, Shin Y, Douglas JN, Cooper C. Autoantibodies to nonmyelin antigens as contributors to the pathogenesis of multiple sclerosis. *J Clin Cell Immunol.* 2013;4:1000148.
12. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. *Nat Immunol.* 2007;8(9):913–919.
13. Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci.* 2008;31:247–269.
14. Silber E, Sharief MK. Axonal degeneration in the pathogenesis of multiple sclerosis. *J Neurol Sci.* 1999;170(1):11–18.
15. Frischer JM, Bramow S, Dal-Bianco A, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain.* 2009;132(Pt 5):1175–1189.
16. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med.* 1998;338(5):278–285.
17. Fisniku LK, Chard DT, Jackson JS, et al. Gray matter atrophy is related to long-term disability in multiple sclerosis. *Ann Neurol.* 2008;64(3):247–254.
18. Geurts JJ, Blezer EL, Vrenken H, et al. Does high-field MR imaging improve cortical lesion detection in multiple sclerosis? *J Neurol.* 2008;255(2):183–191.
19. Lassmann H, Lucchinetti CF. Cortical demyelination in CNS inflammatory demyelinating diseases. *Neurology.* 2008;70(5):332–333.
20. Lucchinetti CF, Gavriloa RH, Metz I, et al. Clinical and radiographic spectrum of pathologically confirmed tumefactive multiple sclerosis. *Brain.* 2008;131(Pt 7):1759–1775.
21. Peterson JW, Trapp BD. Neuropathobiology of multiple sclerosis. *Neurol Clin.* 2005;23(1):107–129, vi–vii.
22. Mahad D, Ziabreva I, Lassmann H, Turnbull D. Mitochondrial defects in acute multiple sclerosis lesions. *Brain.* 2008;131(Pt 7):1722–1735.
23. Corthals AP. Multiple sclerosis is not a disease of the immune system. *Q Rev Biol.* 2011;86(4):287–321.
24. Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. *J Neurol.* 2004;251(3):261–268.
25. Sotelo J. On the viral hypothesis of multiple sclerosis: participation of varicella-zoster virus. *J Neurol Sci.* 2007;262(1–2):113–116.
26. Vartanian T, Li Y, Zhao M, Stefansson K. Interferon-gamma-induced oligodendrocyte cell death: implications for the pathogenesis of multiple sclerosis. *Mol Med.* 1995;1(7):732–743.
27. Ebers GC, Kukay K, Bulman DE, et al. A full genome search in multiple sclerosis. *Nat Genet.* 1996;13(4):472–476.
28. Haines JL, Ter-Minassian M, Bazyk A, et al. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. The Multiple Sclerosis Genetics Group. *Nat Genet.* 1996;13(4):469–471.
29. Black JA, Newcombe J, Trapp BD, Waxman SG. Sodium channel expression within chronic multiple sclerosis plaques. *J Neuropathol Exp Neurol.* 2007;66(9):828–837.
30. Craner MJ, Newcombe J, Black JA, Hartle C, Cuzner ML, Waxman SG. 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.* 2004;101(21):8168–8173.
31. Bechtold DA, Smith KJ. Sodium-mediated axonal degeneration in inflammatory demyelinating disease. *J Neurol Sci.* 2005;233(1–2):27–35.
32. Young EA, Fowler CD, Kidd GJ, et al. Imaging correlates of decreased axonal Na⁺/K⁺ ATPase in chronic multiple sclerosis lesions. *Ann Neurol.* 2008;63(4):428–435.
33. Trapp BD, Stys PK. Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. *Lancet Neurol.* 2009;8(3):280–291.
34. Ouardouz M, Nikolaeva MA, Coderre E, et al. Depolarization-induced Ca²⁺ release in ischemic spinal cord white matter involves L-type Ca²⁺ channel activation of ryanodine receptors. *Neuron.* 2003;40(1):53–63.
35. Fern R, Ransom BR, Waxman SG. Voltage-gated calcium channels in CNS white matter: role in anoxic injury. *J Neurophysiol.* 1995;74(1):369–377.
36. Ouardouz M, Coderre E, Basak A, et al. Glutamate receptors on myelinated spinal cord axons: I. GluR6 kainate receptors. *Ann Neurol.* 2009;65(2):151–159.
37. Ouardouz M, Coderre E, Zamponi GW, et al. Glutamate receptors on myelinated spinal cord axons: II. AMPA and GluR5 receptors. *Ann Neurol.* 2009;65(2):160–166.
38. Ouardouz M, Malek S, Coderre E, Stys PK. Complex interplay between glutamate receptors and intracellular Ca²⁺ stores during ischaemia in rat spinal cord white matter. *J Physiol.* 2006;577(Pt 1):191–204.
39. Matute C, Alberdi E, Domercq M, Pérez-Cerdá F, Pérez-Samartín A, Sánchez-Gómez MV. The link between excitotoxic oligodendroglial death and demyelinating diseases. *Trends Neurosci.* 2001;24(4):224–230.
40. Steinman L. Multiple sclerosis: a two-stage disease. *Nature Immunology.* 2001;2(9):762–764.
41. Lassmann H. Hypoxia-like tissue injury as a component of multiple sclerosis lesions. *J Neurol Sci.* 2003;206(2):187–191.
42. Parpura V, Scemes E, Spray DC. Mechanisms of glutamate release from astrocytes: gap junction “hemichannels”, purinergic receptors and exocytotic release. *Neurochem Int.* 2004;45(2–3):259–264.
43. Ye ZC, Wyeth MS, Baltan-Tekkok S, Ransom BR. Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J Neurosci.* 2003;23(9):3588–3596.
44. Ziskin JL, Nishiyama A, Rubio M, Fukaya M, Bergles DE. Vesicular release of glutamate from unmyelinated axons in white matter. *Nat Neurosci.* 2007;10(3):321–330.
45. Dux E, Temesvári P, Joó F, et al. The blood-brain barrier in hypoxia: ultrastructural aspects and adenylate cyclase activity of brain capillaries. *Neuroscience.* 1984;12(3):951–958.
46. Schmedtje JF, Ji YS, Liu WL, DuBois RN, Runge MS. Hypoxia induces cyclooxygenase-2 via the NF-kappaB p65 transcription factor in human vascular endothelial cells. *J Biol Chem.* 1997;272(1):601–608.
47. Taylor CT. Interdependent roles for hypoxia inducible factor and nuclear factor-kappaB in hypoxic inflammation. *J Physiol.* 2008;586(Pt 17):4055–4059.
48. Varga AW, Johnson G, Babb JS, Herbert J, Grossman RI, Inglesse M. White matter hemodynamic abnormalities precede sub-cortical gray matter changes in multiple sclerosis. *J Neurol Sci.* 2009;282(1–2):28–33.
49. Inglesse M, Adhya S, Johnson G, et al. Perfusion magnetic resonance imaging correlates of neuropsychological impairment in multiple sclerosis. *J Cereb Blood Flow Metab.* 2008;28(1):164–171.

50. Adhya S, Johnson G, Herbert J, et al. Pattern of hemodynamic impairment in multiple sclerosis: dynamic susceptibility contrast perfusion MR imaging at 3.0 T. *Neuroimage*. 2006;33(4):1029–1035.
51. Ge Y, Law M, Johnson G, et al. Dynamic susceptibility contrast perfusion MR imaging of multiple sclerosis lesions: characterizing hemodynamic impairment and inflammatory activity. *AJNR Am J Neuroradiol*. 2005;26(6):1539–1547.
52. Law M, Saindane AM, Ge Y, et al. Microvascular abnormality in relapsing-remitting multiple sclerosis: perfusion MR imaging findings in normal-appearing white matter. *Radiology*. 2004;231(3):645–652.
53. Graumann U, Reynolds R, Steck AJ, Schaeren-Wiemers N. Molecular changes in normal appearing white matter in multiple sclerosis are characteristic of neuroprotective mechanisms against hypoxic insult. *Brain Pathol*. 2003;13(4):554–573.
54. Greer SN, Metcalf JL, Wang Y, Ohh M. The updated biology of hypoxia-inducible factor. *EMBO J*. 2012;31(11):2448–2460.
55. Cramer T, Yamanishi Y, Clausen BE, et al. HIF-1 α is essential for myeloid cell-mediated inflammation. *Cell*. 2003;112(5):645–657.
56. Karhausen J, Furuta GT, Tomaszewski JE, Johnson RS, Colgan SP, Haase VH. Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis. *J Clin Invest*. 2004;114(8):1098–1106.
57. Belaiba RS, Bonello S, Zähringer C, et al. Hypoxia up-regulates hypoxia-inducible factor-1 α transcription by involving phosphatidylinositol 3-kinase and nuclear factor κ B in pulmonary artery smooth muscle cells. *Mol Biol Cell*. 2007;18(12):4691–4697.
58. van Uden P, Kenneth NS, Rocha S. Regulation of hypoxia-inducible factor-1 α by NF- κ B. *Biochemical J*. 2008;412(3):477–484.
59. van Uden P, Kenneth NS, Webster R, Müller HA, Mudie S, Rocha S. Evolutionary conserved regulation of HIF-1 β by NF- κ B. *PLoS Genet*. 2011;7(1):e1001285.
60. Scortegagna M, Cataisson C, Martin RJ, et al. HIF-1 α regulates epithelial inflammation by cell autonomous NF κ B activation and paracrine stromal remodeling. *Blood*. 2008;111(7):3343–3354.
61. Dawson TM, Sasaki M, Gonzalez-Zulueta M, Dawson VL. Regulation of neuronal nitric oxide synthase and identification of novel nitric oxide signaling pathways. *Prog Brain Res*. 1998;118:3–11.
62. Bö L, Dawson TM, Wesselingh S, et al. Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains. *Ann Neurol*. 1994;36(5):778–786.
63. van der Goes A, Brouwer J, Hoekstra K, Roos D, van den Berg TK, Dijkstra CD. Reactive oxygen species are required for the phagocytosis of myelin by macrophages. *J Neuroimmunol*. 1998;92(1–2):67–75.
64. Ukkonen M, Wu K, Reipert B, Dastidar P, Elovaara I. Cell surface adhesion molecules and cytokine profiles in primary progressive multiple sclerosis. *Mult Scler*. 2007;13(6):701–707.
65. Langemann H, Kabiersch A, Newcombe J. Measurement of low-molecular-weight antioxidants, uric acid, tyrosine and tryptophan in plaques and white matter from patients with multiple sclerosis. *Eur Neurol*. 1992;32(5):248–252.
66. Lu F, Selak M, O'Connor J, et al. Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis. *J Neurol Sci*. 2000;177(2):95–103.
67. Smith KJ, Lassmann H. The role of nitric oxide in multiple sclerosis. *Lancet Neurol*. 2002;1(4):232–241.
68. Pachter P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*. 2007;87(1):315–424.
69. Smith KJ, Kapoor R, Hall SM, Davies M. Electrically active axons degenerate when exposed to nitric oxide. *Ann Neurol*. 2001;49(4):470–476.
70. Parkinson JF, Mitrovic B, Merrill JE. The role of nitric oxide in multiple sclerosis. *J Mol Med (Berl)*. 1997;75(3):174–186.
71. Callapina M, Zhou J, Schmid T, Köhl R, Brüne B. NO restores HIF-1 α hydroxylation during hypoxia: role of reactive oxygen species. *Free Radic Biol Med*. 2005;39(7):925–936.
72. Hagen T, Taylor CT, Lam F, Moncada S. Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1 α . *Science*. 2003;302(5652):1975–1978.
73. Liu Q, Berchner-Pfannschmidt U, Möller U, et al. A Fenton reaction at the endoplasmic reticulum is involved in the redox control of hypoxia-inducible gene expression. *Proc Natl Acad Sci U S A*. 2004;101(12):4302–4307.
74. Wang GL, Jiang BH, Semenza GL. Effect of altered redox states on expression and DNA-binding activity of hypoxia-inducible factor 1. *Biochem Biophys Res Commun*. 1995;212(2):550–556.
75. Schopfer FJ, Baker PR, Freeman BA. NO-dependent protein nitration: a cell signaling event or an oxidative inflammatory response? *Trends Biochem Sci*. 2003;28(12):646–654.
76. Bizzozero OA, DeJesus G, Bixler HA, Pastuszyn A. Evidence of nitrosative damage in the brain white matter of patients with multiple sclerosis. *Neurochem Res*. 2005;30(1):139–149.
77. Dumuis A, Sebben M, Haynes L, Pin JP, Bockaert J. NMDA receptors activate the arachidonic acid cascade system in striatal neurons. *Nature*. 1988;336(6194):68–70.
78. Gilgun-Sherki Y, Melamed E, Offen D. Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. *Neuropharmacology*. 2001;40(8):959–975.
79. Barkhatova VP, Zavalishin IA, Askarova LSh, Shavratskii VKh, Demina EG. Changes in neurotransmitters in multiple sclerosis. *Neurosci Behav Physiol*. 1998;28(4):341–344.
80. Stover JF, Pleines UE, Morganti-Kossmann MC, Kossmann T, Lowitzsch K, Kempinski OS. Neurotransmitters in cerebrospinal fluid reflect pathological activity. *Eur J Clin Invest*. 1997;27(12):1038–1043.
81. Matute C, Sánchez-Gómez MV, Martínez-Millán L, Miledi R. Glutamate receptor-mediated toxicity in optic nerve oligodendrocytes. *Proc Natl Acad Sci U S A*. 1997;94(16):8830–8835.
82. McDonald JW, Althomsons SP, Hyrc KL, Choi DW, Goldberg MP. Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. *Nat Med*. 1998;4(3):291–297.
83. Burnashev N. Calcium permeability of glutamate-gated channels in the central nervous system. *Curr Opin Neurobiol*. 1996;6(3):311–317.
84. Höllsberg P, Hafler DA. Seminars in medicine of the Beth Israel Hospital, Boston. Pathogenesis of diseases induced by human lymphotropic virus type I infection. *N Engl J Med*. 1993;328(16):1173–1182.
85. Lee S, Levin MC. Molecular mimicry in neurological disease: what is the evidence? *Cell Mol Life Sci*. 2008;65(7–8):1161–1175.
86. Libbey JE, McCoy LL, Fujinami RS. Molecular mimicry in multiple sclerosis. *Int Rev Neurobiol*. 2007;79:127–147.
87. Oldstone MB. Molecular mimicry and immune-mediated diseases. *FASEB J*. 1998;12(13):1255–1265.
88. Sospedra M, Martin R. Molecular mimicry in multiple sclerosis. *Autoimmunity*. 2006;39(1):3–8.
89. Trowsdale J. Multiple sclerosis: putting two and two together. *Nat Med*. 2006;12(10):1119–1121.
90. Lee SM, Dunnivant FD, Jang H, Zunt J, Levin MC. Autoantibodies that recognize functional domains of hnRNP A1 implicate molecular mimicry in the pathogenesis of neurological disease. *Neurosci Lett*. 2006;401(1–2):188–193.
91. Lee SM, Morcos Y, Jang H, Stuart JM, Levin MC. HTLV-1 induced molecular mimicry in neurological disease. *Curr Top Microbiol Immunol*. 2005;296:125–136.
92. Levin MC, Lee SM, Kalume F, et al. Autoimmunity due to molecular mimicry as a cause of neurological disease. *Nat Med*. 2002;8(5):509–513.
93. Rice JS, Kowal C, Volpe BT, DeGiorgio LA, Diamond B. Molecular mimicry: anti-DNA antibodies bind microbial and nonnucleic acid self-antigens. *Curr Top Microbiol Immunol*. 2005;296:137–151.
94. Sueoka E, Yukitake M, Iwanaga K, Sueoka N, Aihara T, Kuroda Y. Autoantibodies against heterogeneous nuclear ribonucleoprotein B1 in CSF of MS patients. *Ann Neurol*. 2004;56(6):778–786.
95. Yuki N. Ganglioside mimicry and peripheral nerve disease. *Muscle and Nerve*. 2007;35(6):691–711.

96. Kirvan CA, Swedo SE, Heuser JS, Cunningham MW. Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. *Nat Med*. 2003;9(7):914–920.
97. Genain CP, Cannella B, Hauser SL, Raine CS. Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nat Med*. 1999;5(2):170–175.
98. Khalil M, Reindl M, Lutterotti A, et al. Epitope specificity of serum antibodies directed against the extracellular domain of myelin oligodendrocyte glycoprotein: Influence of relapses and immunomodulatory treatments. *J Neuroimmunol*. 2006;174(1–2): 147–156.
99. Moller JR, Johnson D, Brady RO, Tourtellotte WW, Quarles RH. Antibodies to myelin-associated glycoprotein (MAG) in the cerebrospinal fluid of multiple sclerosis patients. *J Neuroimmunol*. 1989;22(1):55–61.
100. Sun JB, Olsson T, Wang WZ, et al. Autoreactive T and B cells responding to myelin proteolipid protein in multiple sclerosis and controls. *Eur J Immunol*. 1991;21(6):1461–1468.
101. Berger T, Rubner P, Schautzer F, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N Engl J Med*. 2003;349(2):139–145.
102. Lim ET, Berger T, Reindl M, et al. Anti-myelin antibodies do not allow earlier diagnosis of multiple sclerosis. *Mult Scler*. 2005;11(4): 492–494.
103. Kuhle J, Pohl C, Mehling M, et al. Lack of association between anti-myelin antibodies and progression to multiple sclerosis. *N Engl J Med*. 2007;356(4):371–378.
104. Bourquin C, Iglesias A, Berger T, Wekerle H, Linington C. Myelin oligodendrocyte glycoprotein-DNA vaccination induces antibody-mediated autoaggression in experimental autoimmune encephalomyelitis. *Eur J Immunol*. 2000;30(12):3663–3671.
105. Bourquin C, Schubart A, Tobollik S, et al. Selective unresponsiveness to conformational B cell epitopes of the myelin oligodendrocyte glycoprotein in H-2b mice. *J Immunol*. 2003;171(1):455–461.
106. Mathey E, Breithaupt C, Schubart AS, Linington C. Commentary: Sorting the wheat from the chaff: identifying demyelinating components of the myelin oligodendrocyte glycoprotein (MOG)-specific autoantibody repertoire. *Eur J Immunol*. 2004;34(8): 2065–2071.
107. Ho PP, Kanter JL, Johnson AM, et al. Identification of naturally occurring fatty acids of the myelin sheath that resolve neuroinflammation. *Sci Transl Med*. 2012;4(137):137ra73.
108. Vyshkina T, Kalman B. Autoantibodies and neurodegeneration in multiple sclerosis. *Lab Invest*. 2008;88(8):796–807.
109. Mathey EK, Derfuss T, Storch MK, et al. Neurofascin as a novel target for autoantibody-mediated axonal injury. *J Exp Med*. 2007;204(10): 2363–2372.
110. Eikelenboom MJ, Petzold A, Lazeron RH, et al. Multiple sclerosis: Neurofilament light chain antibodies are correlated to cerebral atrophy. *Neurology*. 2003;60(2):219–223.
111. Norgren N, Sundström P, Svenningsson A, Rosengren L, Stigbrand T, Gunnarsson M. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. *Neurology*. 2004;63(9):1586–1590.
112. Kalume F, Lee SM, Morcos Y, Callaway JC, Levin MC. Molecular mimicry: cross-reactive antibodies from patients with immune-mediated neurologic disease inhibit neuronal firing. *J Neurosci Res*. 2004;77(1):82–89.
113. Mayeda A, Krainer AR. Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. *Cell*. 1992;68(2):365–375.
114. Hamilton BJ, Burns CM, Nichols RC, Rigby WF. Modulation of AUUUA response element binding by heterogeneous nuclear ribonucleoprotein A1 in human T lymphocytes. The roles of cytoplasmic location, transcription, and phosphorylation. *J Biol Chem*. 1997;272(45):28732–28741.
115. Hay DC, Kemp GD, Dargemont C, Hay RT. Interaction between hnRNP A1 and IkappaBalpha is required for maximal activation of NF-kappaB-dependent transcription. *Mol Cell Biol*. 2001;21(10): 3482–3490.
116. Zhang QS, Manche L, Xu RM, Krainer AR. hnRNP A1 associates with telomere ends and stimulates telomerase activity. *RNA*. 2006;12(6): 1116–1128.
117. Berson A, Barbash S, Shaltiel G, et al. Cholinergic-associated loss of hnRNP-A/B in Alzheimer's disease impairs cortical splicing and cognitive function in mice. *EMBO Mol Med*. 2012;4(8): 730–742.
118. Gao FB, Taylor JP. RNA-binding proteins in neurological disease. *Brain Res*. 2012;1462:1–2.
119. Castello A, Fischer B, Hentze MW, Preiss T. RNA-binding proteins in Mendelian disease. *Trends Genet*. 2013;29(5):318–327.
120. McDonald KK, Aulas A, Destroismaisons L, et al. TAR DNA-binding protein 43 (TDP-43) regulates stress granule dynamics via differential regulation of G3BP and TIA-1. *Hum Mol Genet*. 2011;20(7): 1400–1410.
121. Guil S, Long JC, Cáceres JF. hnRNP A1 relocalization to the stress granules reflects a role in the stress response. *Mol Cell Biol*. 2006;26(15):5744–5758.
122. Douglas JN, Gardner LA, Levin MC. Antibodies to an intracellular antigen penetrate neuronal cells and cause deleterious effects. *J Clin Cell Immunol*. 2013;4(1):1–7.
123. Douglas JN, Gardner LA, Lee S, Shin Y, Groover CJ, Levin MC. Antibody transfection into neurons as a tool to study disease pathogenesis. *J Vis Exp*. 2012;(67):pii 4154.
124. Bjartmar C, Trapp BD. Axonal degeneration and progressive neurologic disability in multiple sclerosis. *Neurotox Res*. 2003;5(1–2): 157–164.
125. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. *Neuron*. 2006;52(1): 61–76.
126. Rieckmann P. Neurodegeneration and clinical relevance for early treatment in multiple sclerosis. *Int MS J*. 2005;12(2):42–51.
127. Rieckmann P, Mäurer M. Anti-inflammatory strategies to prevent axonal injury in multiple sclerosis. *Curr Opin Neurol*. 2002;15(3): 361–370.
128. DeLuca GC, Ebers GC, Esiri MM. Axonal loss in multiple sclerosis: a pathological survey of the corticospinal and sensory tracts. *Brain*. 2004;127(Pt 5):1009–1018.
129. Ganter P, Prince C, Esiri MM. Spinal cord axonal loss in multiple sclerosis: a post-mortem study. *Neuropathol Appl Neurobiol*. 1999;25(6):459–467.
130. Lovas G, Szilágyi N, Majtényi K, Palkovits M, Komoly S. Axonal changes in chronic demyelinated cervical spinal cord plaques. *Brain*. 2000;123(Pt 2):308–317.
131. Levin MC, Lee SM, Morcos Y, Brady J, Stuart J. Cross-reactivity between immunodominant human T lymphotropic virus type I tax and neurons: implications for molecular mimicry. *J Infect Dis*. 2002;186(10):1514–1517.
132. Banks WA, Kastin AJ, Broadwell RD. Passage of cytokines across the blood-brain barrier. *Neuroimmunomodulation*. 1995;2(4):241–248.
133. Umehara F, Abe M, Koreeda Y, Izumo S, Osame M. Axonal damage revealed by accumulation of beta-amyloid precursor protein in HTLV-I-associated myelopathy. *J Neurol Sci*. 2000;176(2):95–101.
134. Centonze D, Muzio L, Rossi S, Furlan R, Bernardi G, Martino G. The link between inflammation, synaptic transmission and neurodegeneration in multiple sclerosis. *Cell Death Differ*. 2010;17(7): 1083–1091.
135. Zou CG, Zhao YS, Gao SY, et al. Homocysteine promotes proliferation and activation of microglia. *Neurobiol Aging*. 2010;31(12): 2069–2079.
136. Kruman II, Kumaravel TS, Lohani A, et al. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. *J Neurosci*. 2002;22(5):1752–1762.
137. Duan W, Ladenheim B, Cutler RG, Kruman II, Cadet JL, Mattson MP. Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. *J Neurochem*. 2002;80(1):101–110.

138. Herrmann W, Obeid R. Biomarkers of folate and vitamin B(12) status in cerebrospinal fluid. *Clin Chem Lab Med*. 2007;45(12):1614–1620.
139. Kamath AF, Chauhan AK, Kisucka J, et al. Elevated levels of homocysteine compromise blood-brain barrier integrity in mice. *Blood*. 2006;107(2):591–593.
140. McCaddon A. Homocysteine and cognitive impairment; a case series in a General Practice setting. *Nutr J*. 2006;5:6.
141. Obeid R, Herrmann W. Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. *FEBS Lett*. 2006;580(13):2994–3005.
142. Oldreive CE, Doherty GH. Neurotoxic effects of homocysteine on cerebellar Purkinje neurons in vitro. *Neurosci Lett*. 2007;413(1):52–57.
143. Sahin S, Aksungar FB, Topkaya AE, et al. Increased plasma homocysteine levels in multiple sclerosis. *Mult Scler*. 2007;13(7):945–946.
144. Teunissen CE, Killestein J, Kragt JJ, Polman CH, Dijkstra CD, Blom HJ. Serum homocysteine levels in relation to clinical progression in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2008;79(12):1349–1353.
145. Triantafyllou N, Evangelopoulos ME, Kimiskidis VK, et al. Increased plasma homocysteine levels in patients with multiple sclerosis and depression. *Ann Gen Psychiatry*. 2008;7:17.
146. Vrethem M, Mattsson E, Hebelka H, et al. Increased plasma homocysteine levels without signs of vitamin B12 deficiency in patients with multiple sclerosis assessed by blood and cerebrospinal fluid homocysteine and methylmalonic acid. *Mult Scler*. 2003;9(3):239–245.
147. Zhu Y, He ZY, Liu HN. Meta-analysis of the relationship between homocysteine, vitamin B12, folate, and multiple sclerosis. *J Clin Neurosci*. 2011;18(7):933–938.
148. Gardner LA, Desiderio DM, Groover CJ, et al. LC-MS/MS identification of the one-carbon cycle metabolites in human plasma. *Electrophoresis*. 2013;34(11):1710–1716.
149. Sharma P, Senthikumar RD, Brahmachari V, et al. Mining literature for a comprehensive pathway analysis: a case study for retrieval of homocysteine related genes for genetic and epigenetic studies. *Lipids Health Dis*. 2006;5:1.
150. McCully KS. Chemical pathology of homocysteine. IV. Excitotoxicity, oxidative stress, endothelial dysfunction, and inflammation. *Ann Clin Lab Sci*. 2009;39(3):219–232.
151. Attia RR, Gardner LA, Mahrous E, et al. Selective targeting of leukemic cell growth in vivo and in vitro using a gene silencing approach to diminish S-adenosylmethionine synthesis. *J Biol Chem*. 2008;283(45):30788–30795.
152. Kotb M, Kredich NM. S-Adenosylmethionine synthetase from human lymphocytes. Purification and characterization. *J Biol Chem*. 1985;260(7):3923–3930.
153. Frequin ST, Wevers RA, Braam M, Barkhof F, Hommes OR. Decreased vitamin B12 and folate levels in cerebrospinal fluid and serum of multiple sclerosis patients after high-dose intravenous methylprednisolone. *J Neurol*. 1993;240(5):305–308.
154. Green R, Miller JW. Vitamin B12 deficiency is the dominant nutritional cause of hyperhomocysteinemia in a folic acid-fortified population. *Clin Chem Lab Med*. 2005;43(10):1048–1051.
155. Miller A, Korem M, Almog R, Galboiz Y. Vitamin B12, demyelination, remyelination and repair in multiple sclerosis. *J Neurol Sci*. 2005;233(1–2):93–97.
156. Miller JW, Garrod MG, Rockwood AL, et al. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. *Clin Chem*. 2006;52(2):278–285.
157. Reynolds EH. Multiple sclerosis and vitamin B12 metabolism. *J Neuroimmunol*. 1992;40(2–3):225–230.
158. Flippo TS, Holder WD. Neurologic degeneration associated with nitrous oxide anesthesia in patients with vitamin B12 deficiency. *Arch Surg*. 1993;128(12):1391–1395.
159. Reynolds EH, Bottiglieri T, Laundry M, Crellin RF, Kirker SG. Vitamin B12 metabolism in multiple sclerosis. *Arch Neurol*. 1992;49(6):649–652.
160. Reynolds E. Vitamin B12, folic acid, and the nervous system. *Lancet Neurol*. 2006;5(11):949–960.
161. Weir DG, Scott JM. The biochemical basis of the neuropathy in cobalamin deficiency. *Baillieres Clin Haematol*. 1995;8(3):479–497.
162. Kifle L, Ortiz D, Shea TB. Deprivation of folate and B12 increases neurodegeneration beyond that accompanying deprivation of either vitamin alone. *J Alzheimers Dis*. 2009;16(3):533–540.
163. Marrie RA, Rudick R, Horwitz R, et al. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. *Neurology*. 2010;74(13):1041–1047.
164. Giubilei F, Antonini G, Di Legge S, et al. Blood cholesterol and MRI activity in first clinical episode suggestive of multiple sclerosis. *Acta Neurol Scand*. 2002;106(2):109–112.
165. Weinstock-Guttman B, Zivadinov R, Horakova D, et al. Lipid profiles are associated with lesion formation over 24 months in interferon- β treated patients following the first demyelinating event. *J Neurol Neurosurg Psychiatry*. 2013;84(11):1186–1191.
166. Weinstock-Guttman B, Zivadinov R, Mahfooz N, et al. Serum lipid profiles are associated with disability and MRI outcomes in multiple sclerosis. *J Neuroinflammation*. 2011;8:127.
167. Gandhi KS, McKay FC, Diefenbach E, et al. Novel approaches to detect serum biomarkers for clinical response to interferon-beta treatment in multiple sclerosis. *PLoS One*. 2010;5(5):e10484.
168. Hyka N, Dayer JM, Modoux C, et al. Apolipoprotein A-I inhibits the production of interleukin-1 β and tumor necrosis factor- α by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood*. 2001;97(8):2381–2389.
169. Burger D, Dayer JM. High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmun Rev*. 2002;1(1–2):111–117.
170. Stanislaus R, Pahan K, Singh AK, Singh I. Amelioration of experimental allergic encephalomyelitis in Lewis rats by lovastatin. *Neurosci Lett*. 1999;269(2):71–74.
171. Pahan K, Sheikh FG, Namboodiri AM, Singh I. Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages. *J Clin Invest*. 1997;100(11):2671–2679.
172. Chataway J, Alsanousi A, Chan D, et al. The MS-STAT trial: high dose simvastatin demonstrates neuroprotection without immune-modulation in secondary progressive multiple sclerosis (SPMS) – a phase II trial. In: Program and abstracts of the 28th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 10–13, 2012; Lyon, France.
173. Lock C. Are “statins” beneficial or harmful in multiple sclerosis? *Neurology*. 2008;71(18):e54–e55.
174. Maier O, De Jonge J, Nomden A, Hoekstra D, Baron W. Lovastatin induces the formation of abnormal myelin-like membrane sheets in primary oligodendrocytes. *Glia*. 2009;57(4):402–413.
175. Markovic-Plese S, Jewells V, Speer D. Combining beta interferon and atorvastatin may increase disease activity in multiple sclerosis. *Neurology*. 2009;72(22):1965; author reply 1965–1966.
176. Vollmer T, Key L, Durkalski V, et al. Oral simvastatin treatment in relapsing-remitting multiple sclerosis. *Lancet*. 2004;363(9421):1607–1608.
177. Birnbaum G, Cree B, Altafullah I, Zinser M, Reder AT. Combining beta interferon and atorvastatin may increase disease activity in multiple sclerosis. *Neurology*. 2008;71(18):1390–1395.
178. Gajski G, Garaj-Vrhovac V, Orescanin V. Cytogenetic status and oxidative DNA-damage induced by atorvastatin in human peripheral blood lymphocytes: standard and Fpg-modified comet assay. *Toxicol Appl Pharmacol*. 2008;231(1):85–93.

179. Qi XF, Zheng L, Lee KJ, et al. HMG-CoA reductase inhibitors induce apoptosis of lymphoma cells by promoting ROS generation and regulating Akt, Erk and p38 signals via suppression of mevalonate pathway. *Cell Death Dis.* 2013;4:e518.
180. Toshniwal PK, Zarling EJ. Evidence for increased lipid peroxidation in multiple sclerosis. *Neurochem Res.* 1992;17(2):205–207.
181. Mikael LG, Rozen R. Homocysteine modulates the effect of simvastatin on expression of ApoA-I and NF-kappaB/iNOS. *Cardiovasc Res.* 2008;80(1):151–158.
182. Chrast R, Saher G, Nave KA, Verheijen MH. Lipid metabolism in myelinating glial cells: lessons from human inherited disorders and mouse models. *J Lipid Res.* 2011;52(3):419–434.
183. Hulshagen L, Krysko O, Bottelbergs A, et al. Absence of functional peroxisomes from mouse CNS causes dysmyelination and axon degeneration. *J Neurosci.* 2008;28(15):4015–4027.
184. Varga T, Czimmerer Z, Nagy L. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim Biophys Acta.* 2011;1812(8):1007–1022.
185. Bocher V, Chinetti G, Fruchart JC, Staels B. [Role of the peroxisome proliferator-activated receptors (PPARs) in the regulation of lipids and inflammation control]. *J Soc Biol.* 2002;196(1):47–52. French.
186. Bocher V, Pineda-Torra I, Fruchart JC, Staels B. PPARs: transcription factors controlling lipid and lipoprotein metabolism. *Ann NY Acad Sci.* 2002;967:7–18.
187. Drew PD, Xu J, Racke MK. PPAR-gamma: therapeutic potential for multiple sclerosis. *PPAR Res.* 2008;2008:627463.
188. Harris SG, Phipps RP. The nuclear receptor PPAR gamma is expressed by mouse T lymphocytes and PPAR gamma agonists induce apoptosis. *Eur J Immunol.* 2001;31(4):1098–1105.
189. Sundaram M, Yao Z. Recent progress in understanding protein and lipid factors affecting hepatic VLDL assembly and secretion. *Nutr Metab (Lond).* 2010;7:35.
190. Yang Y, Gocke AR, Lovett-Racke A, Drew PD, Racke MK. PPAR Alpha Regulation of the Immune Response and Autoimmune Encephalomyelitis. *PPAR Res.* 2008;2008:546753.
191. Mikael LG, Genest J Jr, Rozen R. Elevated homocysteine reduces apolipoprotein A-I expression in hyperhomocysteinemic mice and in males with coronary artery disease. *Circ Res.* 2006;98(4):564–571.
192. Moreno S, Farioli-Vecchioli S, Cerù MP. Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS. *Neuroscience.* 2004;123(1):131–145.
193. Farioli-Vecchioli S, Moreno S, Cerù MP. Immunocytochemical localization of acyl-CoA oxidase in the rat central nervous system. *J Neurocytol.* 2001;30(1):21–33.
194. Schmidt S, Moric E, Schmidt M, Sastre M, Feinstein DL, Heneka MT. Anti-inflammatory and antiproliferative actions of PPAR-gamma agonists on T lymphocytes derived from MS patients. *J Leukoc Biol.* 2004;75(3):478–485.
195. Heneka MT, Klockgether T, Feinstein DL. Peroxisome proliferator-activated receptor-gamma ligands reduce neuronal inducible nitric oxide synthase expression and cell death in vivo. *J Neurosci.* 2000;20(18):6862–6867.
196. Inestrosa NC, Godoy JA, Quintanilla RA, Koenig CS, Bronfman M. Peroxisome proliferator-activated receptor gamma is expressed in hippocampal neurons and its activation prevents beta-amyloid neurodegeneration: role of Wnt signaling. *Exp Cell Res.* 2005;304(1):91–104.
197. Park KS, Lee RD, Kang SK, et al. Neuronal differentiation of embryonic midbrain cells by upregulation of peroxisome proliferator-activated receptor-gamma via the JNK-dependent pathway. *Exp Cell Res.* 2004;297(2):424–433.
198. Smith SA, Monteith GR, Robinson JA, Venkata NG, May FJ, Roberts-Thomson SJ. Effect of the peroxisome proliferator-activated receptor beta activator GW0742 in rat cultured cerebellar granule neurons. *J Neurosci Res.* 2004;77(2):240–249.
199. Heneka MT, Landreth GE. PPARs in the brain. *Biochim Biophys Acta.* 2007;1771(8):1031–1045.
200. Niino M, Iwabuchi K, Kikuchi S, et al. Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-gamma. *J Neuroimmunol.* 2001;116(1):40–48.
201. Feinstein DL, Galea E, Gavrilyuk V, et al. Peroxisome proliferator-activated receptor-gamma agonists prevent experimental autoimmune encephalomyelitis. *Ann Neurol.* 2002;51(6):694–702.
202. Polak PE, Kalinin S, Dello Russo C, et al. Protective effects of a peroxisome proliferator-activated receptor-beta/delta agonist in experimental autoimmune encephalomyelitis. *J Neuroimmunol.* 2005;168(1–2):65–75.

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