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Alexzander A.A. Asea
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Multiple Sclerosis: Bench to Bedside

Global Perspectives on a Silent Killer

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Preface

Multiple sclerosis (MS) is one of the main causes of disability in young adult population. The estimated burden of the disease worldwide is about three million people. The pathogenic mechanism of MS involves both autoimmune and degenerative processes. These two mechanisms are thought to determine a combination of events leading to several clinical patterns of disease onset and course.

Multiple Sclerosis: Global Perspectives on a Silent Killer provides the most up-to-date and concise reviews on the critical issues of multiple sclerosis from around the world. This book is written by leaders and experts in the field of multiple sclerosis research and is divided into easy-to-read sections. Section I focuses on basic science aspects of multiple sclerosis, including potential biomarkers, molecular biology, heat shock proteins, oxidative stress, genetics, and epigenetics. Section II focuses on clinical and epidemiological aspects of multiple sclerosis, including remyelination therapy and neuroplasticity-based technologies and interventions. This is an important reference book and a must-read for undergraduate and postgraduate medical scholars, basic science researchers, neurology fellows, neurology residents, and neurologists in clinical practice.

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Contents

Extracellular Vesicles in Multiple Sclerosis as Possible Biomarkers: Dream or Reality?

1

Maria Magdalena Barreca, Emanuele Aliotta, and Fabiana Geraci

Abstract

Extracellular vesicles are recently described as specialized structures for intercellular communication. Their role in the central nervous system was diffusely studied in both physiological and pathological condition. In particular, an increased extracellular vesicle number was detected in several autoimmune diseases, including multiple sclerosis, a chronic autoimmune, inflammatory, demyelinating and neurodegenerative disease. This chapter summarizes the available information on the involvement of the extracellular vesicles in multiple sclerosis pathogenesis and their possible use as biomarker of therapy efficacy.

Keywords

Biomarkers • Extracellular vesicles • Multiple sclerosis • Therapy efficacy

Abbreviations

BBB	blood-brain barrier
CNS	central nervous system
CSF	cerebrospinal fluid
EEVs	endothelial derived EV
EVs	extracellular vesicles
IFN	interferon
MMP	matrix metalloproteinases
MRI	magnetic resonance imaging
MS	multiple sclerosis
MV	membrane vesicles
PEVs	Platelet derived EVs

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PPMS primary progressive multiple sclerosis
PS phosphatidylserine
RRMS relapsing-remitting multiple sclerosis
SPMS secondary progressive multiple sclerosis

1.1 Introduction

During the last few years several studies have revealed microvesicles released by the cells as new specialized structures for intercellular communication without direct cell-to cell contact (Ratajczak et al. 2006; Pap et al. 2009; Camussi et al. 2010). The presence of microvesicles in the extracellular space was initially reported by Chargaff and West as a precipitable factor in platelet-free plasma (Chargaff and West 1946). However, microvesicles were considered to be an in vitro artefact or inert cellular debris until De Broe et al. (De Broe et al. 1977) suggested that microvesicles released from human cells result from a specific process. It is now accepted that several cell types release microvesicles (e.g. fibroblast, epithelial, hematopoietic, immune, tumor and stem cells, neurons, microglia, astrocytes, oligodendrocytes and neural progenitors) (Pap et al. 2009; van Poll et al. 2008; Colombo et al. 2012; Marzesco et al. 2005; Bianco et al. 2009; Cossetti et al. 2012; Turola et al. 2012) and recent studies showed that these vesicles may have important roles in both physiological and pathophysiological processes. In fact, many studies demonstrated that these vesicles are involved in intercellular communication, coagulation, cell proliferation, inflammation, tumorigenesis and have an emerging role in the biology of stem cells (Scanu et al. 2008; Kim et al. 2005; Distler et al. 2005; Köppler et al. 2006; Muralidharan-Chari et al. 2010).

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS). MS is characterized by multiple demyelization lesions, axonal degeneration, oligodendrocyte and neuronal loss and glial scar formation, which occur either focally or diffusely through the white and grey matter in the brain and spinal cord (Lassmann et al. 2007). It is established that the pathogenesis of the disease involves genetic, environmental, and immune components (Bernard et al., Bernard and Kerlero de Rosbo 1992; Fox et al. 2006). Currently, the diagnosis of MS is based on the 2010 revised McDonald criteria (Polman et al. 2011), including clinical evaluation supported by CNS magnetic resonance imaging (MRI) and by the presence of oligoclonal bands in the cerebrospinal fluid. However, there are still patients with MS that do not meet this diagnosis criterion. For this reason during the last years many studies have been addressed to the identification of molecular biomarkers. Indeed, they can be used as diagnostic tool to determine the stage of the disease, for prediction and monitoring of therapy efficacy.

The etiology of MS remains unknown, although it is widely held that MS is a Th1/Th17 autoimmune disease where self-reactive effector T cells initiate the inflammatory cascade. In addition to Th1 and Th17, also other cell types, such as $CD8^+$ T cells, B cells, macrophages, and natural killer cells, contribute to MS pathogenesis (Sospedra and Martin 2005; Kasper and Shoemaker 2010; Selmi et al. 2012). Immune activation involved in the onset of the disease causes a release of proinflammatory cytokines (TNF, IL1-β, IFN-γ) plus a proliferation of lymphocytes, monocytes, and platelets (Martino and Hartung 1999). At the same time, endothelial dysfunction of the blood-brain barrier (BBB) affects its permeability, facilitating transendothelial migration of monocytes and T-lymphocytes into CNS (Minagar et al. 2012), which contributes to the formation of demyelinating lesions (Steinman 1996). The sequence of the events in this transendothelial migration includes activation of leukocytes and brain endothelial cells, chemoattraction, leukocyte-endothelium adhesion, proteolysis of the basal membrane surrounding the BBB by matrix metalloproteinases (MMP) (Leppert et al. 2001), and finally extravasation of activated leukocytes.

The clinical course of MS goes from an early inflammatory phase of the disease with relapse and remission, where patients may respond to

immunomodulatory drugs, to a progressive and neurodegenerative phase that is unresponsive to any currently available treatment. According to these clinical courses, MS is classified into relapsing-remitting (RRMS), secondary progressive (SPMS) and primary progressive (PPMS) subtypes (Lublin et al. 2014). All the same pathological features are presented in both RRMS, SPMS, and PPMS, although they vary over time both quantitatively and qualitatively between these three forms of MS and among individuals with the same MS form (Lucchinetti et al. 2000; Peterson et al., Peterson and Fujinami 2007).

1.2 Origins of Extracellular Vesicle

Two different types of microvesicles have been identified on the basis of size, content and mechanism of formation (Morel et al. 2011; Barteneva et al. 2013): exosomes and membrane derived vesicles. Exosomes originate from multivesicular endosomal cell compartment and represent a more homogeneous type of vesicles, enriched in specific components (tetraspanning proteins, CD63, CD19 and alix) (Baglio et al. 2012; Heijnen et al. 1999; Lai et al. 2013; Rozmyslowicz et al. 2003; Théry 2011). On the contrary, membrane vesicles (MV), also named shed vesicles or ectosomes, are larger vesicles, which bud directly from the plasma membrane and are released into the extracellular environment upon cell activation (Ratajczak et al. 2006; Morel et al. 2011; Lai et al. 2013; Boulanger 2010; Cocucci et al. 2009; Dignat-George and Boulanger 2011). Furthermore, another type of membrane vesicles is represented by apoptotic bodies, that are larger than exosomes and membrane vesicles (György et al. 2011). These vesicles are formed exclusively during the late stage of apoptosis and contain nuclear material, DNA and RNA, cellular organelles, and membrane/cytosolic contents (Elmore 2007).

The mechanisms involved in microvesicles budding and release are beginning to emerge and suggest the involvement of ESCRT and/or ARF6 (Cocucci et al. 2009; Muralidharan-Chari et al. 2009; Gan and Gould 2011). The shedding process results in the formation of microvesicles containing cell membrane constituents and cytoplasmic contents (Hugel et al. 2005). Upon release, both types of vesicles, MVs and exosomes, generally referred to as extracellular vesicles (EVs) according to the International Society for Extracellular Vesicles (Katsuda et al. 2013), may either remain in the extracellular space adjacent to the site of origin, or move by diffusion and enter into biological fluids, such as blood, urine, milk, cerebrospinal fluid (CSF) and synovial fluid, modulating biological processes also at remarkable distance from their site of origin. They could have a role as clinically valuable marker of the disease states (Doeuvre et al. 2009), such as endothelial dysfunction, coagulation, and inflammatory state (Burger et al. 2013). EVs contain a variety of cell surface receptors, intracellular signaling proteins and genetic materials derived from the originating cells. Thus, EVsreleased by distinct cell types are molecularly different from each other, reflecting the differential expression of proteins of donor cells. Moreover, EVs also include an array of proteins that differ from those on the cells from which they originate. The functions of extracellular vesicles depend on the phenotype of their parental cells. However, although their cargo reflects the identity of the cells from which they were released, a selective enrichment of specific molecules has been shown to occur (Li et al. 2013). EVs have the same topology of the originating cells but often lose the typical asymmetry of plasma membrane. In fact, in contrast to that membrane, they generally have phosphatidylserine (PS) in the extracellular leaflet (Zwaal et al., Zwaal and Schroit 1997; Sims et al., Sims and Wiedmer 2001; Shet et al. 2003), although a significant number of EVs released from blood cells do not expose PS on the outer leaflet (Shet et al. 2003). Due to this heterogeneous composition no universal markers have been yet identified to define EVs.

1.3 Mechanism of Interaction Between Extracellular Vesicles and Target Cells

Shedding of EVs is considered to be a physiological process that accompanies cell activation and growth. Many stimuli can increase vesicles shedding, such as hypoxia, oxidative stress, and exposure to shear stress (Hugel et al. 2005; Barry and FitzGerald 1999; Beaudoin and Grondin 1991; Février and Raposo 2004; Horstman et al. 2004; VanWijk et al. 2003). Several types of interactions between EVs and target cells have been demonstrated. Indeed, EV interactions with recipient cells can be followed by fusion or endocytosis. Alternatively, EVs can undergo rupture and release their luminal active components, modulating the activity of target cells by protein secretion. EVs influence target-cell behavior in several ways. They can act as a signaling complex, can transfer membrane receptors between cells, deliver proteins to target cells, and also modify the receiving cell phenotype by horizontal transfer of genetic information (Deregibus et al. 2007; Dooner et al. 2008; Yuan et al. 2009). According to the last possibility, in recent years scientist attention has been focused on the capacity of EVs to induce epigenetic changes in target cells. In fact, EVs can transfer not only surface determinants and cytoplasmic proteins but also mRNA and microRNA, which is recognized as a regulatory signal in cell-to-cell communication (Vickers et al., Vickers and Remaley 2012; Zang et al., Zhang et al. 2010). The advantage of nucleic acid release inside vesicles is their protection from extracellular ribonucleases.

1.4 Extracellular Vesicles in the Central Nervous System

In the CNS, EVs have been detected in the cerebrospinal fluid, the only body fluid in direct contact with the brain, and may be involved in several physiological roles, such as neuronal development (Marzesco et al. 2005; Marzesco 2013), synaptic activity (Antonucci et al. 2012), and nerve regeneration (Lai and Breakefield 2012). Furthermore, EVs have been implicated to have pathological roles in many neurodegenerative and neuroinflammatory disease such as stroke (Cherian et al. 2003), vascular dementia, inflammatory (Horstman et al. 2007) and age related neurodegenerative diseases, cerebral malaria (Combes et al. 2005) and multiple sclerosis (Verderio et al. 2012). In MS Verderio and colleagues demonstrated that myeloid EVs increase. These EVs in conjuction with those isolated from plasma can be used as biomarkers allowing monitoring disease onset and progression (Verderio et al. 2012; Huttner et al. 2012; Witwer et al. 2013). The positive aspect of using EVs as biomarker is represented by the possibility to identify their origin as membrane glycoprotein characteristic of the parental cells are present on circulating EVs. Therefore, the detection of distinct EV population could be considered a signal from a specific tissue activation or damage.

1.5 Extracellular Vesicle Role in Multiple Sclerosis

EVs can be isolated both from the plasma and the CSF of patients suffering from MS. Over 20 years ago, Scolding and co-workers detected in CSF EVs released from injured oligodendrocytes (Scolding et al. 1989). Today researcher attention has been focused on the study of EV potential as biomarker and their possible role in immunological pathways involved in multiple sclerosis.

EVs released by endothelial cells stimulated by activated T lymphocytes contained several parental markers (Horstman et al. 2007), such as CD31, CD146, CD54, and they may provide a snapshot of the inflamed endothelium and also provide information on the involvement of platelets and leukocytes in MS. To date, few studies have studied EVs present in CSF during MS onset and progression (Verderio et al. 2012; Sáenz-Cuesta et al. 2014a), meanwhile several studies have been investigated circulating endothelial EVs in blood from MS patients, as biomarkers for BBB damage especially during disease exacerbation (Minagar et al. 2001, 2003,

2012). On the other hand, several studies have showed that EVs released by the endothelial cells of the BBB, by platelets, leukocytes, or myeloid cells can play an active role during MS pathogenesis, and may also have a role in both inflammatory progression and lesion repair Sáenz-Cuesta et al. 2014b). Indeed, it has been shown that EVs released by the BBB endothelial cells are able to activate CD4+ and CD8+ T cells without any stimulatory signal, and this could represent the first step of the autoimmune reaction in the CNS, caused by the transendothelial leukocyte migration (Wheway et al. 2014) Moreover, it has been demonstrated that EVs originated by the endothelial cells contain MMP2/9 that promote the destruction of the BBB (Sbai et al. 2010; Lacroix et al. 2012). MMP are also involved in the cleavage and shedding of surface proteins, including that of TNF (Canault et al. 2007). It has been, also, shown that vesicles released by activated endothelial cells in MS patients are able to promote the migration of monocytes and lymphocytes through the BBB inducing the formation of demyelination areas (Minagar et al. 2001; Jy et al. 2004; Jimenez et al. 2005; Fauré et al. 2006).

Verderio and colleagues demonstrated that myeloid EVs increase in number in the inflamed brain. Moreover, in a recent study on CSF a higher number of EVs derived from IB4+ myeloid cells have been observed in patients with RRMS compared to healthy control patients (Verderio et al. 2012). This data has been associated with the acute phase of the disease and reflect disease severity and the extent microglia activation (Verderio et al. 2012). In particular, EV level peaks during disease onset and during clinical relapses, while there was a decrease in the chronic phase of the disease confirming the involvement of EVs in the inflammatory process a linear correlation between CSF EV level and gadolinium positive lesions in MS patients (Verderio et al. 2012). Today several studies are directed to find a correlation between MV level in the peripheral blood and the clinical of the disease.

Minagar et al. (2001) and Fauré et al. (2006) demonstrated that endothelial derived EV (EEVs) concentration is dependent on disease progression. Moreover, both groups postulated that EVs could be used as biomarkers of BBB damage. In fact, they demonstrated that a high plasma level of endothelial CD31 positive EVs was present during disease exacerbation and it returned to basal level during remission, with positive association with contrast enhancing lesions (Minagar et al. 2001) indicating acute injury to the endothelium. In contrast, CD51 positive EVs were increased during chronic injury to the endothelial cells (Minagar et al. 2001; Fauré et al. 2006). Platelet derived EVs (PEVs) have been also detected in RRMS patients (Sheremata et al. 2008). In recent years, Marcos-Ramiro and colleagues extended the studies on RRMS EVs conducing a comprehensive analysis of circulating platelet- and endothelium- derived EVs in the plasma of all the different clinical forms of MS. They observed a remarkable increase in PEVs ($CD42b^+$) and both EEVs ($CD31^+$ and $CD62E^+$) in all MS clinical forms (Marcos-Ramiro et al. 2014).

1.6 Conclusion

In recent years there has been a dramatic increase in the number of multiple sclerosis patients. As there is no cure for MS, one of the principal objectives of neurologists after diagnosis is to arrest disease progression. To date there is several disease modifying agents (e.g. β-interferon, glatiramer acetate, natalizumab, fingolimod). Therapy choice is mainly based on the risk to benefit ratio but it is complicated by disease heterogeneity confirmed by the presence of different subtype with different disease mechanisms (Lucchinetti et al. 1996). Moreover, therapy efficacy varies individually from patient to patient. At present, therapy efficacy is based on clinical evidences, such as relapses rates, new lesion presence pointed out by MRI, and changes in disability scores. However, the evolution of drug efficacy by using these parameters has limited sensitivity with respect to subclinical disease activity (Barkhof 2002). For this reason, it is essential to identify sensitive and specific

biomarker for assessing therapeutic efficacy. As it has been postulated that EVs may have an active role in demyelination and neurodegeneration in MS and they could be used as biomarker of BBB damage, it is reasonable to consider them as a marker of drug efficacy. Indeed, it has been demonstrated that there was an alteration in endothelial EV level in plasma of patients after IFN-1β1a (Rebif) treatment. In particular, there was a reduction in $CD31^+$ and $CD\ 54^+$ EV level in MS patients following treatment over 12 months with Rebif, and this reduction was similar with volumes of the contrast-enhancing T1-weighted lesions (Lowery-Nordberg et al. 2011; Sheremata et al. 2006). A reduction of $CD31^+$ and $CD54^+$ EVs was also observed after treatment with IFN-1β1b (Betaseron). In addition, CD62E endothelial EV release was affected (Jimenez et al. 2005). A significant reduction in EEVs ($CD105^+$) was observed after fingolimod administration (Zinger et al. 2016). On the contrary, it restored the number of B-cell-derived EVs ($CD19^+$) to healthy control levels. No changes were observed in EV number shedded from T-cell, monocytes or platelets. These results open a new scenario in MS therapy choice and in therapy effectiveness.

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2 Manipulation of Oxygen and Endoplasmic Reticulum Stress Factors as Possible Interventions for Treatment of Multiple Sclerosis: Evidence for and Against

Paul Eggleton, Gary R. Smerdon, Janet E. Holley, and Nicholas J. Gutowski

Abstract

Multiple sclerosis (MS) is normally considered a chronic inflammatory disease of the central nervous system (CNS), where T-cells breaching the blood brain barrier react against proteins of the axonal myelin sheaths, leading to focal plaques and demyelination in the brain and spinal cord. Many current therapies are immunosuppressive in nature and are designed to target the immune system at an early stage of the disease. But there is no cure and MS may evolve into a neurodegenerative disease, where immunomodulatory treatments appear less effective. Neurodegeneration is influenced by oxidative and endoplasmic reticulum (ER) mediated stress which can be induced independently of immune processes. Since 1970, MS patients have been self-managing their long term symptoms using hyperbaric oxygen and reporting improvement in their symptoms, especially bladder control. In contrast, the majority of clinical trial evidence does not support the views of patients. Therefore does oxygen under pressure affect brain tissue by modulating oxidative or ER stress at the cellular level resulting in CNS tissue repair or deterioration? This chapter reviews our understanding and the role of oxidative and ER stress in the context of employing hyperoxia treatments to treat MS and evaluate its effects on neural cells.

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Autoimmunity • Hyperbaric oxygen • Myelin • Neurons • Oligodendrocytes • Unfolded protein response

Abbreviations

ATA	atmospheres absolute
BBB	blood brain barrier
CL	chronic lesion
CNS	central nervous system
EDSS	expanded disability status scale
ER	endoplasmic reticulum
HBOT	hyperbaric oxygen therapy
MS	multiple sclerosis
NAWM	normal appearing white matter
pO_2	partial pressure of oxygen
ROS	Reactive oxygen species
UPR	unfolded protein response

2.1 Introduction

Multiple sclerosis (MS) is a demyelinating autoimmune disease whereby damage to cells of the central nervous system (CNS) results in the generation of lesions that results in loss of neurological function. The disease is categorized to various degrees of severity beginning with preclinical, followed by in most cases relapsing remitting, primary progressive and finally secondary progressive, suggesting a chronic onslaught of inflammation which leads to an increase in neurodegeneration to the CNS. The greatest genetic risk factor comes from carrying the class II HLA-DRB1*1501 allele which can increase susceptibility by 2–4-fold (Odds ratio 3.06; 95 % CI, 2.30–4.08), while Epstein-Barr virus infection has a similar risk association (Odds ratio 2.60; 95 % CI, 1.48–4.59) (Xiao et al. 2015) Geographical latitude (Kinoshita et al. 2015) and ethnic considerations (Langer-Gould et al. 2013) also contribute to the overall chance of developing MS. It is well established that the adaptive immune system plays a role in MS pathology, especially pro-inflammatory T-cells (Cao et al. 2015; Hong et al. 2009). Autoreactive T-cells can be found in the peripheral blood of autoimmune patients and healthy control subjects, but such cells appear to be more resistant to apoptosis and reactive against myelin proteins in MS patients (Mandel et al. 2009; Vergelli et al. 2001). The cause of development of peripheral blood autoreactive T-cells against CNS tissue derived myelin, prior to T-cell exposure to such tissue is largely unknown. In MS, the transmigration of autoreactive T-cells across the blood brain barrier (BBB) can ultimately lead to an escalation of pro-inflammatory damage to myelin-producing oligodendrocytes in close proximity to neuronal axons, leading to major damage and cell death. The oxidative damage and endoplasmic reticulum (ER) stress that ensues (Mhaille et al. 2008), requires the cells of the CNS to either undergo apoptosis or repair, which is controlled to a large extent by the unfolded protein response (UPR) (Stone and Lin 2015). Moreover, the UPR can also influence the ability of various cells to resist apoptosis and influence their cytokine phenotypes (Chan et al. 2011; Kim et al. 2006). Therefore pathways such as the UPR that regulate many aspects of cell survival and repair might be a fruitful area of research in developing therapeutics to alleviate or prevent MS pathology, and are already being investigated for other neurodegenerative diseases (Rozpedek et al. 2015; Torres et al. 2015).

We and others have shown that cells in vitro exposed to 100 % oxygen under hyperbaric pressure (HBO) alter the expression of a wide variety of genes involved in immunity and inflammation (Kendall et al. 2011, 2012, 2013a; Thom 2011). Consequently, HBO might work as a therapy by promoting or suppressing selective genes and

their products in a non-invasive manner. But, how HBO works downstream, at the cellular and biochemical level remains largely unknown and more work is required, but it does not appear to damage DNA in the longer term (Yuan et al. 2011). Hyperbaric oxygen therapy (HBOT), which involves breathing pure oxygen under pressure is used to treat a number of clinical conditions including non-healing wounds (Eggleton et al. 2015) and to ameliorate the side-effects of radiation therapy (Clarke et al. 2008; Glover et al. 2015). However HBOT as a treatment for MS is highly contentious and does not have approval from the USA Food and Drug Administration (US Food and Drug Administration 2013) or The National Institute for Health and Care Excellence (The Guideline Development Group NICE 2014). Despite the non-recommendation by health governance authorities, many patients continue to use HBOT to treat their symptoms and frequently report symptomatic improvement. In the late 1970s and 1980s when HBOT began to be trialled, some clinicians supported the use of HBOT for MS sufferers (Boschetty et al. 1970; Fischer 1983; Fischer et al. 1983; James 1984; James 1983; Neubauer 1978, 1980; Neubauer et al. 2005), while others did not (Barnes et al. 1985b; Neiman et al. 1985; Wiles et al. 1986). This has led to confusion for both patients and clinicians alike. Here we evaluate the pros and cons of HBO treatment in the context of oxidative and ER stress, the unfolded protein response and the changes that occur in cells and their genes under hyperbaric conditions.

2.2 Oxidative and ER Stress in MS Pathology

The cellular damage induced in the CNS of MS patients directly accounts for many of the dysfunctional changes observed in the well-being, mobility and motor processes of individual MS sufferers. Within all nucleated cells, a number of cell repair molecules, the UPR sensor molecules, are sensitive to changes in their environment, and this is particularly so of CNS cells (Giovannoni and Ebers 2007; Hedstrom et al. 2015). Inflammatory cells and the molecules they release can attack oligodendrocytes and the neuronal axons and signal to these cells to shut down and die by apoptotic death. Under the appropriate conditions the UPR can attempt to repair the cell. Whenever the UPR response signal is one of repair, regeneration and remyelination of axons can occur (Gow and Wrabetz 2009). One potential way of driving the decision to repair rather than destroy a cell is to manipulate the ER-mediated UPR stress response. There are several diverse environment factors that can trigger cellular and ultimately ER-stress, namely virus, microbial toxins, oxidative stress and nutrient deficiency (Mkhikian et al. 2011). These stimuli can all trigger additional rapid protein production within the ER to help maintain the status quo of the cell. The rapidity of this process can lead to errors in amino acid biosynthesis, protein folding and glycosylation, inducing degradation factors to deal with the disruption in cellular homeostasis and triggering reactive oxidative (ROS) and nitrosative species (RNS) production. Similarly, activation of ROS and RNS can also activate the UPR, and the UPR has been shown to be elevated in myelin-generating oligodendrocytes of the CNS, as well as other cells of the peripheral nervous system (Lin and Popko 2009).

It is established that oxidative stress plays a role in cellular damage and particularly so in MS neuropathology, where the cerebro spinal fluid (CSF) and plasma are observed to have increased amounts of lipid peroxidation (Calabrese et al. 1998). During lesion formation activated microglia cells release superoxide, which in part can be defended by the antioxidant systems of the brain such as superoxide dismutases (SOD) and reduced glutathione. Free iron can promote CNS damage by catalyzing the production of hydroxyl and peroxyl-based free radicals from hydrogen peroxide and lipid peroxides (Halliwell 2001). The balance between free radical and antioxidant production undoubtedly plays a role in whether inflammation subsides or progresses, leading to lesion development (Gilgun-Sherki et al. 2004; Syburra and Passi 1999), although it has been

questioned whether the formation of ROS in MS is in fact deleterious (Koch et al. 2006). At the cellular level in vitro, the myelin producing oligodendrocytes are thought to be more susceptible to damage by ROS/RNS compared to astrocytes and microglia possibly due to higher iron content and diminished antioxidant defenses (Smith et al. 1999). The molecular events that lead to oligodendrocyte loss and lesion formation are not fully understood, but are known to involve signaling pathways associated with both the ER (Kraus and Michalak 2011) and mitochondria organelles (Aboul-Enein and Lassmann 2005; Dutta et al. 2006; Gilgun-Sherki et al. 2004; Lu et al. 2000). Furthermore, dysfunctional mitochondria are an additional source of ROS production (Mahad et al. 2009; Nickel et al. 2014). Ultimately, inflammation, oxidative stress, demyelination of axons and the lack of remyelination and restoration of axonal function will be partially dependent on the cellular activation of the UPR to these various insults. The response can manifest itself in various ways including accumulation of unfolded or misfolded proteins in the ER. The main UPR sensor pathways are regulated by three proteins inositol requiring kinase 1 (IRE1), activating transcription factor 6 (ATF6), and PKR-like ER kinase (PERK) (Fig. 2.1). The signalling path-

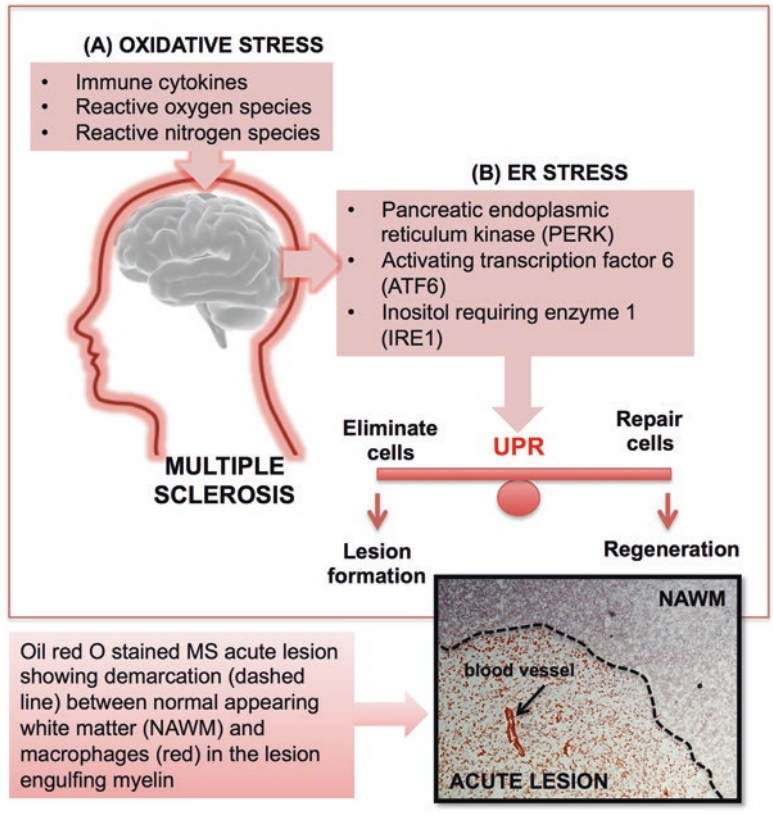


Fig. 2.1 Oxidative stress induces ER stress than can activate the unfolded protein response (UPR) pathways. (**a**) Oxidative stress can arise from localized activated inflammatory cells, secretion of proinflammatory cytokines and induction of ROS and NOS by activated macrophages and microglia in the brain. (**b**) The resulting oxidative stress can lead to damage of lipid, DNA and protein. This in turn can disrupt lipid and protein biosynthesis, resulting in the accumulation of misfolded proteins in the ER and ER stress. In turn, ER stress activates one or more of the three ER-transmembrane transducers of the UPR. Individual stressed cells are then programmed to survive or undergo cell death. Localized regions of the brain where oxidative and ER stress are present can result in the formation of lesions in the CNS white matter

ways that these sensors regulate have been well documented and described in detail with regards to MS (Getts et al. 2008; Stone and Lin 2015), but are triggered initially when B-cell immunoglobulin heavy chain binding protein (BiP) detaches from PERK. Key components downstream of the UPR initiating signal are phosphorylated eukaryotic initiation factor alpha (p-eIF2α) and C/EBP homologous protein (CHOP) which drive cells toward survival (Walter and Ron 2011) or apoptosis (Szegezdi et al. 2006) respectively.

Over a period of time the chronic inflammation, oxidative and ER-stress leads to visible MS pathology in the CNS. Affecting predominantly white matter, demyelinating lesions become clearly distinguishable from the surrounding normal appearing white matter (NAWM) tissue (Fig. 2.2a–c). Evidence of myelin-specific T-cell accumulation leads to the development of lesions which can be acute or sub-acute (sometimes referred to as - chronic active), in which myelin is progressively stripped from the axon sheaths of neurons and is engulfed by macrophages and microglial cells. An additional type of lesion is the chronic lesion (sometimes referred to as chronic silent) in which inflammation has abated and scarred lesions devoid of myelin present within the CNS. Lesions can be seen on MRI scans (Fig. 2.2 d–f). MS lesions defined in terms of inflammatory destruction and neurodegeneration are useful for studies designed to identify differences in gene expression at the DNA and mRNA level of diverse cellular and molecular biomarkers of pathology at distinctive stages of disease progression. As shown in Fig. 2.2a, during the acute phase of lesion formation there is a gradation of infiltrating inflammatory (microglia and macrophages) cells, with more cells close to the lesion border engorged with oil red O stained myelin, providing evidence of demyelination of axons. In the sub-acute stage (Fig. 2.2b) the lesion border appears more distinct, with the central region of the lesion becoming devoid of myelin and oligodendrocytes. The chronic lesions have little evidence of inflammatory cells, typically appear hypocellular and are devoid of a visible inflammatory border with the NAWM and represent a scarred region of irreversible demyelination (Fig. 2.2**c**). However we have recently identified a novel proinflammatory subset of T-cells (CD20+/IL17+) associated with the chronic and acute lesions of MS patients (Holley et al. 2014). The NAWM tissue in MS differs from that of white matter in non-MS brain, in that greater numbers of T-cell infiltrates are detected (Allen et al. 2001; Kutzelnigg et al. 2005), indicative of pre-lesion inflammation and breach of the blood brain barrier (BBB).

Through analysis of significant changes in UPR genes in various MS lesions, a better understanding of the cell response to oxidative and ER stress with respect to MS pathology can be established. A number of microarray studies have identified elevated levels of expression of certain genes including UPR pathway genes in biopsy material obtained from the demyelinating lesions in the CNS of MS patients (Cwiklinska et al. 2003; Lock and Heller 2003; Mycko et al. 2003, 2004; Tajouri et al. 2003). Mycko and colleagues examined differences in gene expression from cell extracts from the border and centres of active lesions, with varying degrees of inflammatory infiltrates. Not surprisingly more genes were upregulated at the DNA level in active lesions compared to inactive lesions both at the lesion borders and centres (87 vs. 69 genes and 65 vs. 22 genes) respectively, which included a number of intracellular signalling and transcription factors (Mycko et al. 2003). The same group went on to look at mRNA gene expression in the same tissue regions and observed a number of ER-stress and heat shock protein genes upregulated in both active and inactive regions of MS lesions including activated transcription factor (ATF4) and heat shock protein 70 (HSP70) (Cwiklinska et al. 2003; Mycko et al. 2004). Tajouri and co-workers also examined NAWM and chronic and acute lesion material from five MS patients with secondary progressive disease and non-MS subjects (Tajouri et al. 2003). The authors observed 139 genes that were differentially regulated >1.5 fold in the five MS lesions compared with NAWM. Several of the genes upregulated were associated with tissue damage and oxidative stress including transferrin (TF), superoxide dismutase 1 (SOD1), glutathione peroxidase

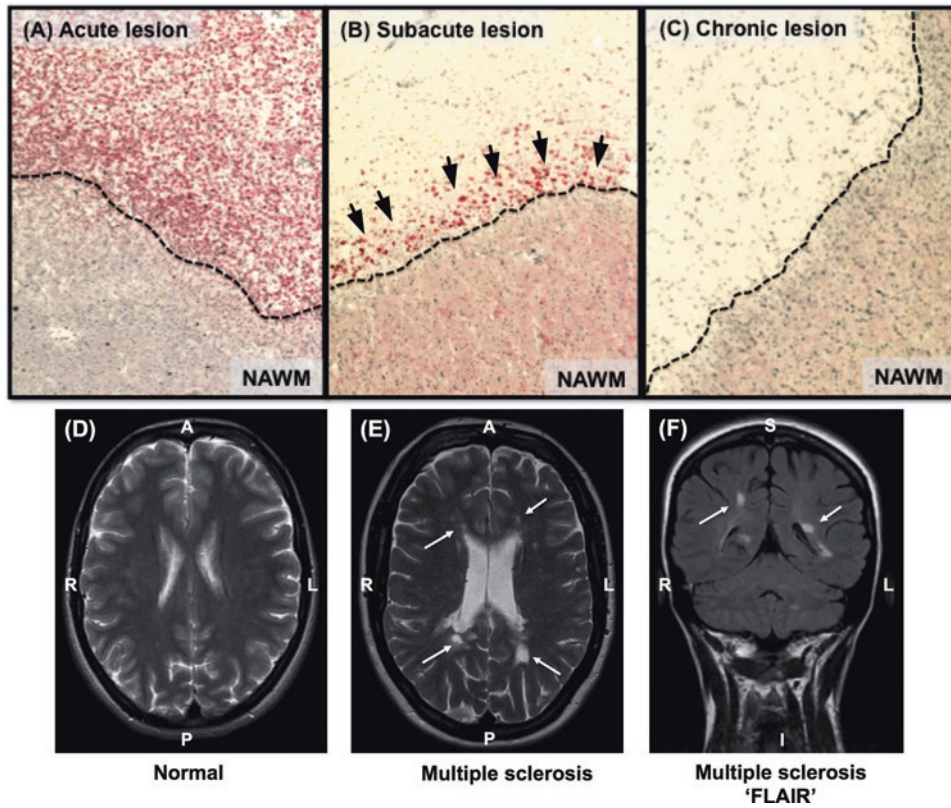


Fig. 2.2 Classification and imaging of MS lesions. Oil red 'O'/hematoxylin staining of 10 μm sections of MS brain tissue, showing lesion areas at the top of each image and NAWM at the bottom, demarcated by a dashed lined. (**a**) Depicts an acute lesion with increasing numbers of oil red 'O' positive macrophages containing myelin and more densely packed towards the lesion border. (**b**) Illustrates oil red 'O' positive macrophages located mainly at the lesion border (black arrow heads) and a demyelinated area of the lesion. (**c**) Shows a chronic lesion, devoid of myelin and oil red 'O' positive macrophages. All images are at 100x magnification. (**d**) Normal brain axial T2 weighted MRI scan. (**e**) Axial T2-weighted MRI in a patient with MS demonstrating several white matter hyper-intense lesions. (**f**) Coronal fluid-attenuated inversion recovery (FLAIR) MRI in a patient with MS demonstrating high-signal intensity lesions in the deep white matter and the periventricular regions. Key: *R* right, *L* left, *A* anterior, *P* posterior, *S* superior, *I* inferior. *White arrows* depict lesions

(GPX1) and glutathione S-transferase (GSTP1) peroxiredoxin I (PRDX1), which are all expressed during free radical formation and in some cases as antioxidants to counteract oxidative stress. More recently, Cunnea and associates have detected elevated expression levels at the mRNA level of a number of ER and hypoxic stress genes in actively demyelinating lesions of MS patients with primary or secondary progressive disease compared to control white matter (Cunnea et al. 2011). Specifically they observed a 2–8 fold elevated expression of BiP, CHOP and ATF4. Interestingly the elevation of these classical ER-stress proteins were not restricted to lesions but also in the NAWM of MS patients, indicative of ER stress occurring prior to lesion formation. Increases in UPR gene products are not restricted to the white matter of MS patients and various grey matter lesions have been shown to have significantly increased levels of CHOP compared to normal grey matter. The increased CHOP appeared to be predominantly associated with microglial cells. Whether increased CHOP in microglial cells predestines such inflammatory cells to undergo apoptosis remains to be elucidated (McMahon et al. 2012). The function and

over expression of CHOP and other UPR genes should be considered on an individual cellular basis, especially in the knowledge that elevated CHOP protects oligodendrocytes from cell death (Gow and Wrabetz 2009).

Oxidative and ER stress appears to have a dynamic affect and differential sensitivity on various UPR response genes and the proteins they encode in human CNS biopsy tissue of specific lesions. Specifically many UPR genes appear to be elevated in MS. However, the underlying mechanisms through which UPR genes act in individual cell types (e.g. oligodendrocytes, neurons, microglial cells) or individual MS patients requires more work. The knowledge gained from such studies might aid the development of therapeutic strategies that protect both oligodendrocytes and neurons in patients with MS. One overall impression is that the UPR appears to be 'over activated' in MS lesions and mechanisms that can suppress the UPR or at least alter it may be of benefit.

2.3 HBOT and MS: Clinical and Patient Perspectives

The data above describes a number of human studies post-mortem, in which evidence of oxidative and ER-stress is clearly implicated in altering CNS tissue cell survival and degeneration. So the logical question is how does environmental oxygen affect MS patients? In 1970, 26 MS patients were treated with 100 % O_2 under hyperbaric pressure (HBOT) at 2 ATA (Boschetty and Cernoch 1970) and fifteen patients symptoms were observed to improve. Over the past 45 years, both clinicians and patients have reported or observed improvements in MS symptoms after HBOT treatment, often as anecdotal reports or in randomized control trials. But the use of HBOT as a treatment for MS remains highly contentious. Indeed HBOT has been regarded by some as no better than other 'alternative' treatments such as oral arsenic, intrathecal injections of tuberculin, oral seaweed and snake venom (Bates 1986). The early history and controversy in using HBOT to treat MS patients has been eloquently described by an advocate pioneer in the field, RA Neubauer (Neubauer et al. 2005). The main conclusion of his and his colleagues report was that HBOT is not a cure, but does stabilize the symptoms in the majority of patients and slows progression in 17–33 % of patients. They also recommend that additional treatments might be required as treatment is transient and the effect of HBOT diminishes over time. The first formal small placebo-controlled, double-blinded study conducted in 1983 produced positive results for HBOT treatment of MS with 'objective' improvement in 12/17 patients compared with 1/20 patients treated with a placebo (Fischer et al. 1983). This was despite using 100 % O_2 at 2 ATA (pure oxygen at 1.5–1.75 ATA has generally been recommended since this initial study) for 90 min. An age-sex placebo group of match MS patients were exposed to 10 % O_2/90 % N_2 for the same time period. To assess MS disability as a whole disease severity must be monitored. A number of clinical scales have been developed to this end, the most well established is the Kurtzke's Expanded Disability Status Score (EDSS) which was originally described in 1955 (Kurtzke 1955) and has been modified through the following decades (Kurtzke 1965, 1970, 1983, 1989, 2000, 2008). Clinical parameters can also be monitored using the Multiple Sclerosis Functional Composite (MSFC), Symbol Digit Modality Test (SDMT) and low contrast visual acuity. In the original Fischer trial in 1983, the successfully treated patients showed improvements in a number of features on the EDSS scale by 1–2 points in mobility, coordination, bladder control and fatigability. Historically, this study was significant as it also provided evidence that MS may be an autoimmune disease whereby oxygen might have immunosuppressive properties. At the time of the study MS was thought by some to consist of venous infarction in the CNS (James 1983) which was disputed by Mertin (Mertin and Mcdonald 1984).

After the Fischer trial of 1983, at least 14 additional double blinded control studies were conducted (Barnes et al. 1985a, 1987; Confavreux et al. 1986; Harpur et al. 1986; Lhermitte et al. 1986; Oriani et al. 1990; Wiles et al. 1986; Wood

et al. 1985), and many of these refuted the original findings. It is fair to say that in hindsight these studies were poorly controlled. In some of these studies all MS patients irrespective of their disease severity as judged by their EDSS were given the same number of treatments, and therefore this was not treating 'like with like'. This has led to inconsistency in results and confusion in the clinical community as to whether HBOT is useful (Adamson 1985; Bates 1986; Jacoby 2001; Kleijnen and Knipschild 1995; Monks 1988; Wynne and Monks 1989). One consistency is that HBOT treatment at pressures below 2.0 ATA for short periods of time are not detrimental to patients, indeed O_2 used at higher ATA have been indicated to improve recovery from brain trauma in patients (Rockswold et al. 2010). To address the controversy two meta-analysis reports have addressed the use of HBOT for MS. In 2004, a Cochrane report evaluated nine randomized control trials comprising of 504 participants. Only two of the nine trials showed a reduction in EDSS score at 12 months (−0.85 compared to sham). The conclusions suggested better well-designed trials would be required to confirm this improvement but overall did not recommend such trials to be performed (Bennett and Heard 2004). The same authors revaluated the use of HBOT in 2010, in trials they had previously analyzed between 1983 and 1987 and came to the same conclusions that HBOT for MS was ineffective. They suggested that 'only staunch advocates would be willing to pursue such investigations', (Bennett and Heard 2010).

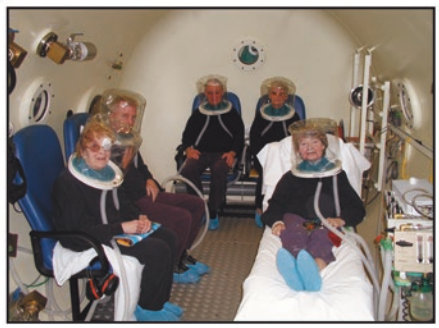
Such 'staunch advocates' come in the form of MS individuals. The internet is full of testimonials from MS subjects (http://www.oxygenunderpressure.com/category/multiple-sclerosis/) and advocate clinicians (Maxfield 2005) who have personally used or employed HBOT, patients have reported feeling better in terms of pain relief, gait, bladder control and overall mobility. The justification for using HBOT for MS is most likely governed by the ability of the treatment to suppress disease symptoms for long periods of time. Despite the resistance and skepticism from many clinicians to prescribe HBOT for MS, several thousand MS individuals use such treatment in the UK and elsewhere every year and report positive outcomes, including decreased fatigue and depression. There are more than 50 hyperbaric centers throughout the UK (Fig. 2.3), where individuals can book a HBOT session, either in a monoplace or multiplace chamber that can accommodate up to 12 people (Fig. 2.3**b**). MS subjects who are more mobile and in the early stages of the disease have anecdotally reported the use of HBOT to be beneficial. Ideally it would be useful to gauge the effectiveness of HBOT on MS individuals with different stages of the disease (e.g. relapsing remitting vs. secondary chronic progressive with and without conventional medication), but no such data exists. The word used frequently by MS individuals is 'stabilize'. Again qualitative testimonials from MS subjects who self-administer HBOT, commonly report a stabilization of their symptoms or mild improvement. One difference between the clinical trials and the practical use of HBO by MS subjects is the frequency of HBOT use by the individuals themselves. Whereas trial protocols in the past, used HBOT on MS patients suffering from differing degrees of severity on ~20 occasions over a period of a month (Fig. 2.4), many of these protocols have not been used over longer periods. In reality MS subjects voluntarily use HBOT more frequently and over a longer period of time. Perrins and James reported on 1384 MS subjects employing long-term treatment of HBOT (Perrins and James 2005). About 9 % were regularly treated with HBOT for 5–15 years and 11 % were treated for 17 years or more. Better stabilization and retardation of MS progression was reported if treatment was used soon after MS was diagnosed and before irreversible lesions developed. As HBOT treatment is not offered by the UK National Health Service (NHS) there are no official numbers of clients or treatments in the public domain. However there are a large number of MS therapy centers located around the UK (Fig. 2.3). One such center in Exeter, Devon, UK opened in 1982. The Exeter Center recommends that 'MS clients' begin with 15 session (5 daily sessions/week for 3 weeks at between 1.5 and 2.0 ATA) and then 'top up' with HBO on a weekly basis, depending on how the

a

Country	Population*	MS population/ 100,000**
England	53.9 million	165
Scotland	5.3 million	212
Wales	3.1 million	140
Northern Ireland	1.8 million	174
UK Total	64.1 million	172.75

* Office of national statistics
** MS Trust statistics

b



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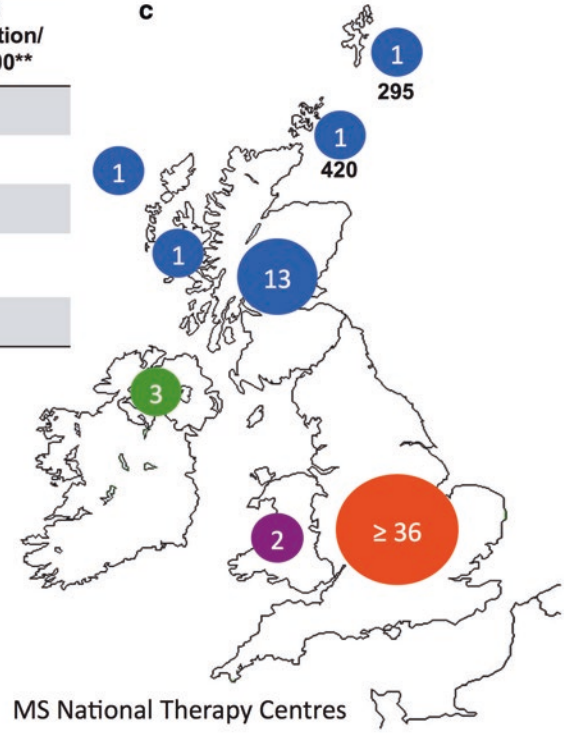

Fig. 2.3 Frequency of multiple sclerosis and quantity and location of HBO chambers for MS patients in the UK. (**a**) The frequency of MS increases with latitude; England & Wales < Northern Island < Scotland. (**b**) Patients frequently use single and multiplace HBO chambers to help alleviate their symptoms. (**c**) There are HBOT chambers located in many major cities and regions around the UK, including the islands of the Scottish coast, where incidences of MS are some of the highest in the world: 420/100,000 in the Orkney Islands and 295/100,000 in the Shetland Islands

client is responding. The client is placed in a three or seven seater chamber and gently placed under pressure at a rate of 1 m/min until the appropriate pressure is reached. Clients breathe 100 % oxygen for 60 min and then the pressure is reversed at 1 m/min until normobaric pressure is reached. Some clients at this center have used the facility for decades and feel it prevents their condition deteriorating (personal communication - Esme Gibbins, Therapies Manager). In 2011, Professor Philip B. James (Emeritus Professor of Medicine, University of Dundee, UK) wrote an open letter (http://www.hjernebarnet.dk/fileadmin/_temp_/Philip_James_-110405.pdf) suggesting over 2.5 million HBOT sessions have been safely provided to over 20,000 individuals in MS National Therapy centers since they began to operate in 1982 (figures up to 2011).

2.4 ER Targeted Therapeutics and MS

2.4.1 Effect of HBOT on Cell Function and Gene Expression

Neurologists may agree or disagree with the merits of using HBOT for MS, but HBOT is used successfully to treat many other conditions, and more information as to the effect of HBOT at the cellular and molecular level is required to aid in the further understanding of the mechanism of action of HBOT. We have investigated the role of hyperoxia on various cells under hyperbaric pressures (HBOT) at 2.4 ATA, including platelets (Shaw et al. 2009), endothelial cells and neutrophils (Almzaiel et al. 2013, 2015; Kendall et al. 2013a; Kendall et al. 2012; Kendall et al. 2013b)

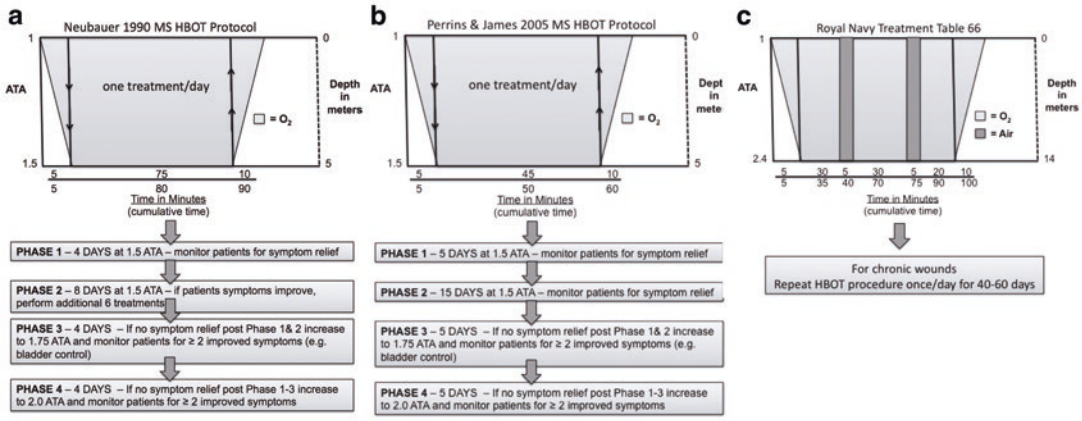

Fig. 2.4 Examples of MS HBOT protocols in comparison to wound treatment HBOT. (**a**) & (**b**) Examples of protocols to treat MS patients with oxygen under pressure. The protocols over the past 25–35 years have evolved but have retained consistently the same treatment time (60 min) and pressure protocols (1.5–2 ATA). (**c**) The HBOT protocols used to successfully treat chronic wounds, commonly employs the use of oxygen at >2 ATA for longer periods ~90 min, with air breaks

and bone tissue (Al Hadi et al. 2015; Al Hadi et al. 2013) We developed a chronic wound model to study neutrophil-endothelial interactions to study the effect of HBOT on individual cell types in chronic wounds (Kendall et al. 2011). The culmination of these and other studies suggested HBO reduces the surface expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells and reduces neutrophil adhesion. Although we did not observe changes in neutrophil adhesion molecule expression CD18, CD11b, CD62L, CD31, we proposed HBOT inhibited neutrophil adhesion to endothelial cells by S-nitrosation (Kendall et al. 2013b). In the context of MS, similar effects of HBOT could possibly inhibit T-cell interaction with brain vascular endothelial cells.

Chronic wounds that are normally exposed to 2 % O_2 are frequently treated with HBO at a pressure of 2.4 ATA. In contrast, MS patients whose brain tissue is normally exposed to 4 % O_2 are normally treated with HBO at 1.5 −2.0 ATA (Fig. 2.4). Recently we examined how oxidative and inflammatory gene expression alters under different pressures. We have cultured human endothelial cells under hypoxic conditions (2 % O_2) as a model because they are important in both wound healing and immune cell interaction in the BBB. We studied the effect of a single 90 min exposure of HBO on a number of categories of genes, including adhesion molecules, apoptosis, angiogenesis and tissue remodeling, inflammation, intracellular signaling and oxygen responses and redox signaling (Kendall et al. 2013a). In these studies a number of genes were sensitive to HBO at both 1.5 and 2.4 ATA compared to cells treated under pressure at 1 ATA and showed reduced levels of expression at the mRNA level that was sustained for at least 22.5 h (the time RNA was extracted from the cells). Notably, 1–4-fold decreases in adhesion molecules; Platelet endothelial cell adhesion molecule1, fibronectin1, angiogenesis factors; angiopoietin 2, connective tissue growth factor, vascular endothelial growth factor receptor 2, endothelial tyrosine kinase, tissue inhibitor of metalloproteinases 3, the chemokine; Interleukin 8, and oxygen response genes; endothelial PAS domain protein 1- HIF-2α and glutathione peroxidase 1. In most cases treatment of endothelial cells with 96.7 % O_2 at 1.5 ATA produced greater reductions in the above genes than when treated with 97.9 % O_2 at 2.4 ATA. When mRNA was quantified from the same endothelial cells, 5 h post HBOT treatment a whole serious of oxygen response genes were downregulated 2–3 fold at both 1.5 and 2.4 ATA, but more so when exposed to O_2 at 1.5 ATA compared with cells treated with O_2 at 1ATA or 2.4 ATA pressure. These included HIF-1α and −2α,

peroxiredoxin 2 and 6, glutathione peroxidase 1, superoxide dismutase 1 and 2, catalase, thioredoxin and glyoxalase 1. We have observed similar increases in antioxidants of the peroxiredoxin family at the protein levels in chronic lesions of MS patients (Holley et al. 2007). Interestingly, HBOT at 1.5 ATA reduced the expression of the ER stress chaperone calreticulin by two fold and in MS, calreticulin levels being known to increase as part of the UPR (Mhaille et al. 2008). This illustrated that several antioxidants and chaperones are sensitive to rapid changes to oxygen levels and adjust their expression accordingly. Perhaps of more interest is that O_2 administered at 1.5 ATA altered the expression of many more genes more effectively than 2.4 ATA. The reason for this is unknown, but raises the possibility that at least for MS, HBOT treatment, oxygen used at 1.5 ATA, that has been adopted over the past 30–40 years by patients, is more effective at altering gene expression in a number of oxidative and ER stress conditions. One question not answered by the above experiments is how long are changes in gene expression retained post HBOT? Our work suggests at least in vitro that the effect is transient and might require multiple and regular exposures to have any long term beneficial effect on oxidative response, ER stress, UPR and inflammatory gene products. This would support the recommendations of Perrins and James, who suggest MS patients should have regular HBOT treatments for it to have a significant effect in improving EDSS scores or preventing further deterioration (Perrins and James 2005).

2.4.2 Neural UPR Sensor Genes

As HBOT can alter gene expression, it would be of great interest to be able to alter gene expression in neural cells in a non-invasive manner. More specifically to arrest or activate UPR and ER-stress regulatory genes that are involved in clearing misfolded proteins, cell repair and death (Fig. 2.5**a**). These processes and their regulation are known to be important in neurodegenerative diseases (Oyadomari and Mori 2004; Soto and Estrada 2008). Oxidative stress in the form of lipid peroxidation (Wang et al. 2014), oxygen consumption to form ROS during myelin sheath attack and mitochondrial injury (Haider 2015) and nitrosative stress (Kallaur et al. 2015) have all been observed to precede the inflammatory response in MS patients.

We therefore examined the effect of HBOT exposure specifically on both differentiated neuron-like SH-SY5Y cells and myelin-producing human oligodendrocytes (HOGs). These neural cells were cultured under the appropriate optimal growth conditions for differentiation and maturation in the presence of 4 % oxygen (Normoxia; 4.0 % O_2/CO_2 at 1.0 ATA) for 4–6 days (neural cells normally exist in a low-oxygen environment) (Ndubuizu and LaManna 2007). Next the cells were treated with HBO (96.7%O_2/CO_2 at 1.5 ATA), or pressure control (2.67 % O_2/CO_2 at 1.5 ATA) treatments for 90 min (Fig. 2.5**b**). All of the gas mixtures contained CO_2 at a level to give a final pCO_2 of 5 kPa, representing the respiration-derived CO_2 at the cellular level. The cells were then placed in their former culture conditions for 5 h or 22.5 h. RNA was isolated from the cells for quantitative real time PCR and analyzed for differences in unfolded protein response (UPR) gene expression pre- and post-exposure to HBO or pressure control (PC) treatment. mRNA expression was analyzed by our previously described methods (Eggleton et al. 2010; Kendall et al. 2011; Kendall et al. 2013a; Kendall et al. 2012). The results revealed that mRNA expression in the major UPR regulatory genes PERK, IRE1α and ATF6α were largely unaffected by HBO or PC treatment 5 h after treatments in SH-SY5Y cells. But 22.5 h post-treatment the PERK and ATF6α mRNA expression levels had reduced by 50 % in PERK in cells treated with HBO or hyperbaric pressure (Fig. 2.5**c**, left panel). Similar reductions in PERK and ATF6α genes were seen in HOG cells after 22.5 h post-treatment with HBO. However, in contrast, to SH-SY5Y cells, IRE1α mRNA levels were reduced by over 50 % following HBO treatment at 1.5 ATA 5 h post-treatment (Fig. 2.5**c**, right panel). In general, hyperbaric pressure and not oxygen accounted for some but not all of the reduced gene expression in the UPR sensor genes, but did appear to act synergistically in down regulating the UPR genes tested. This

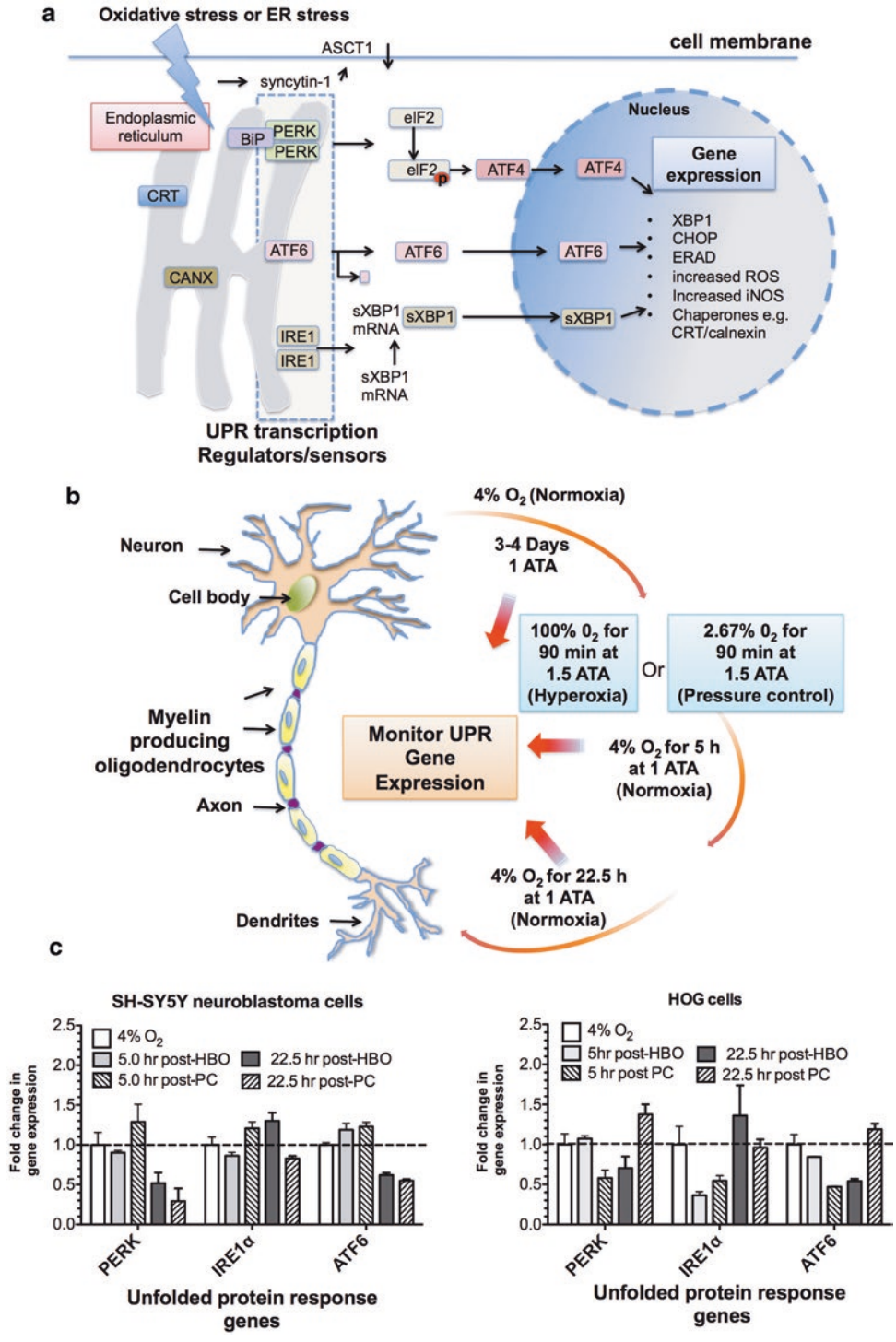

Fig. 2.5 Effect of Oxidative Stress on ER stress regulators in human CNS cells. (**a**) Changes in cellular oxidative status is one condition that can lead to ER stress. The three recognized UPR sensor pathways PERK, IRE1α and ATF6α induce a number of downstream genes that stimulate changes of a number of important enzyme, oxidoreductase and chaperone pathways that aid in the regulated cell death or survival of individual cell types. (**b**) The mechanisms by which changes in oxidative stress induced by hyperoxia effect demyelinating diseases such as MS as induced by HBOT treatment remain unknown. We assessed the effect of hyperoxia under pressure (HBO) and pressure alone (PC) on UPR gene pathways in human oligodendrocytes (HOGs) and neuronal cells (SH-SY5Y). (**c**) The effect of either HBO or PC affected the expression of UPR pathway sensors differently in neuronal or oligodendrocyte cells

might in part explain why in clinical trials of HBOT for MS patients in which a pressure control is used as a placebo, some placebo treated patients report a benefit for the treatment. In the Cochrane analysis of HBO trials conducted in 2004 (Bennett and Heard 2004), all of the trials evaluated administered oxygen to patients at between 1.75 ATA and 2.5 ATA for 90 min. This is despite the recommended protocols suggesting 1.5 ATA should be initially used (Fig. 2.4). In our gene expression study on neural cells we chose 1.5 ATA because this pressure is used to treat brain injury (Stoller 2011, 2015) and we have seen greater reductions in inflammatory gene expression in cells exposed to oxygen at 1.5 ATA compared to 2.4 ATA (Kendall et al. 2013a). These results are encouraging and demonstrate the use of oxygen at a relatively low hyperbaric pressure can markedly reduce the regulatory genes of the UPR pathways responsible for controlling cell death and repair, which are known to be over-expressed in lesions of MS patients as described above.

2.5 Conclusion

There is growing evidence that both ER (Cunnea et al. 2011; Mhaille et al. 2008) and oxidative stress (Guan et al. 2015; Karlik et al. 2015; Lassmann and van Horssen 2015; Ohl et al. 2015; Pasquali et al. 2015) play a role in the pathology of MS. These stress pathways are also the focus of attention to down-regulate inflammation and aid remyelination within the CNS of MS patients. (Getts et al. 2008). A number of pharmacological agents and small molecule therapeutics have or are being trialed in an attempt to reduce ER and/or oxidative stress in MS. (Bahamonde et al. 2014; Khalili et al. 2014; Miller et al. 2013; Naziroglu et al. 2014; Ramirez-Ramirez et al. 2013; Sanoobar et al. 2013; Seven et al. 2013). The problem with all drugs is their ability to target specific cells, and this is made more difficult when attempting to target pharmacological agents across the BBB. Despite this problem, a number of agents are being developed to suppress ROS/RNS and ER stress in the CNS (Chiurchiu 2014). While drug development continues and MS patients await new treatments, many other MS patients continue to seek solace in HBOT treatment. The fact that so many HBOT treatment centers exist worldwide and are used regularly by MS patients is a testament to their usefulness. Despite HBOT treatment not being officially approved for the treatment of MS by the clinical community, the lack of approval is probably of no consequence to individuals who use HBOT and feel they benefit from its effects.

The debate on the pros and cons of using HBOT as a MS therapy will continue ad infinitum until proper regulated trials are conducted, but this may never happen due to lack of patent protection, low financial gains and importantly a lack of understanding as to the precise mechanisms of how oxygen under hyperbaric pressure can reduce the symptoms of MS. For example the paradox that oxidative stress is detrimental to CNS tissue, but brain tissue may benefit from being exposed to 100 % oxygen under pressure warrants a cautious approach. Further studies investigating the effect of hyperoxia under normobaric and hyperbaric conditions at the cellular level may help us understand what some patients with MS already believe and feel - that HBOT is an efficacious therapy.

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Heat Shock Proteins in Multiple Sclerosis

3

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Abstract

Multiple sclerosis (MS) is an immune-mediated and neurodegenerative central nervous system disease, mostly affect myelin sheaths. The MS pathogenesis is still under debate. It is influenced by genetic, environment factors. Heat shock proteins (HSPs) are highly conserved proteins seen in all organisms. Not only heat stress but also under many stress conditions they are overexpressed. Their roles in MS pathogenesis are highly correlated with their location (intracellular or extracellular). In this chapter, we will discuss the role of HSP in MS pathogenesis.

Keywords

Heat shock proteins • Multiple sclerosis • Pathogenesis

Abbreviations

AD	alzheimer's disease
ALS	amyotrophic lateral sclerosis
APAF-1	apoptosis protease activating factor-1
APC	antigen presenting cell
ATP	adenosine three phosphate
CNS	central nervous system
CSF	cerebrospinal fluid
EAE	experimental autoimmune encephalomyelitis
EDSS	expanded disability scale score
HD	huntington disease
HSP	heat shock protein
IL-1β	interleukin 1β
LDL	low density lipoprotein
MAPK-2	mitogen-activated protein kinase 2
MHC	major histocompatibility complex
MS	multiple sclerosis
NAWM	normal-appearing white matter
NBD	nucleotide binding domain
NK	natural killer
OND	other neurologic diseases
PD	parkinson's disease
RRMS	relapsing-remitting multiple sclerosis
TLR	toll like receptor

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TNF-α	tumor necrosis factor-α
WM	white matter

3.1 Introduction

Heat shock proteins (HSP) are highly conserved proteins that found in all organisms; eukaryotic, prokaryotic species and plants. HSPs were first discovered as stress-inducible proteins. They have been characterized as molecular chaperones which prevent aggregation of proteins (Okuno et al. 2016). In the nervous system, HSPs are induced in a variety of pathological states, including cerebral ischemia, neurodegenerative disease, epilepsy, and trauma (Turturici et al. 2011). Their expression has been detected in multiple cell types, including neurons, glia, and endothelial cells. HSPs also exist as extracellular proteins, released both through physiological secretory mechanisms and during necrotic cell death (Brownell et al. 2012).There are several studies of HSPs in neurodegenerative disease like Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), prion disease, Huntington disease (HD), polyglutamine disease have similar pathogenesis associated with protein misfolding and aggregation of proteins within and outside cells. The misfolding and progressive polymerization of otherwise soluble proteins is a common characteristic feature. The chaperone role of HSPs prevents the misfolding of proteins inhibit toxic oligomeric aggregates of the respective disease proteins such as tau and amyloid-β in AD, α-synuclein in PD and huntingtin in HD (Ou et al. 2014).

Gezen et al. demonstrated a systemic downregulation of Hsp90 in early, late onset Alzheimer's disease and minimal cognitive impairment group. Serum Hsp90 levels in all groups were significantly decreased compared with controls. Moreover, administration of Hsp90 inhibitors could prevent Aβ-induced neurotoxicity by increasing levels of HSP70 and Hsp90 (Gezen et al. 2013). The results of this research suggest the decreased serum HSP is a sign of increasing protein aggregation in AD.A similar neuroprotective role for HSPs is observed in PD. α-Synuclein is a 140-amino acid neuronal protein involved in synaptic plasticity and dopaminergic neurotransmission. It has been demonstrated that Hsp70 overexpression reduced α-Syn accumulation and toxicity in both mouse and Drosophila models of PD In vitro studies have also demonstrated that Hsp70 can prevent α-Syn fibrillar assembly (Klucken et al. 2004). In particular, in vitro aggregation experiments have demonstrated that nucleotide-free Hsp70 inhibited amyloid formation, stimulating the formation of amorphous aggregates. In another study, Roodveldt and colleagues demonstrated α-Synuclein mediated Hsp70 depletion in an ATP-dependent manner (Roodveldt et al. 2009).

In amyotic lateral sclerosis known as Lou Gehrig's disease is characterized by progressive loss of motor neurons. HSP27 immunoreactivity was found higher in ALS brains compared with healthy controls (Iwaki et al. 1993). The therapeutic potential of Arimoclomol, a hydroxylamine derivative that induces HSP expression under cellular stress, is currently under investigation in a Phase II clinical trial for ALS patients with SOD1 mutations (Kalmar et al. 2014). MS is an autoimmune demyelinating disease of central nervous system predominantly in white matter. T-cell mediated immune response sheath is the major target. Despite these protein aggregation diseases MS has a different pathogenesis. There many controversial studies about the role of HSP in MS pathogenesis. Still, there is not a consensus whether they are triggering or inhibiting the immune response. In this chapter, we will discuss the effects of HSPs in MS pathogenesis.

3.2 Heat Shock Proteins

İn 1962 Ferruccio RİtossA et al. discovered that temperature shock makes odd puffing pattern and unconventional gene expression in polytene chromosomes of salivary glands in *Drosophila melanogaster* larva (Ritossa 1962). Whereas in 1974 product of these described genes can be identified and termed as heat shock proteins

(Schlesinger 1990). Because of the first description made relation to heat shock; they are termed as heat shock proteins. However, now we also know that HSP expression can be triggered by other stress factors such as cold, UV light, wound healing or tissue remodeling (Matz et al. 1995; Cao et al. 1999). Some of HSP molecules act as chaperones. They help appropriate protein folding /unfolding, assembly of multiprotein complexes, and protection of cells against stress/apoptosis. HSPs also have the function of stress induced denaturation (Hendrick and Hartl 1993; Beere 2004). HSP molecules can be found in intracellular and extracellular compartments. Their function differs according to their placement. Intracellular HSPs have a protective function, on the other hand; extracellular HSP molecules elicit an immune response in adaptive or innate immune system. HSP molecules have a role in antigen presentation on the role of chaperoning. They can transfer antigenic peptides to MHC I and MHC II complexes. Extracellular HSP molecules can stimulate antigen presenting cells.

3.3 Classification

Based on their molecular masses, HSPs are currently classified into seven families. The most known families are small HSPs, HSP70, HSP90, HSP110 (Okuno et al. 2016) (Table 3.1). Each family of HSPs is composed of members expressed either constitutively or regulated inductively. They are targeted to different subcellular compartments. For example, HSP90 is abundantly expressed in the cells; HSP70 and HSP27 are highly induced by various stresses such as heat, oxidative stress, and anticancer drugs. In normal, non-stressed cells HSP70 and HSP27 are either not expressed or at very low levels (Schmitt et al. 2007). In 2009, Kampinga et al. offered a new guideline for the nomenclature of heat shock proteins. This nomenclature comprises HSPH (HSP110), HSPC (HSP90), HSPA (HSP70), DNAJ (HSP40), and HSPB (small HSP) as well as for the human chaperonin families HSPD/E (HSP60/HSP10) and CCT (TRiC) (Kampinga et al. 2009). The high molecular weight groups (HSPA, HSPC, HSPD) are

Table 3.1 Nomenclature of heat shock proteins and their localization

New name	Old name	Moleculer mass Kda	Family members	Localisation
HSPH	Hsp100	100 and over	Hsp100	Endoplasmic reticulum
HSPH	Hsp110	100 and over	Hsp 110	Nucleus/cytoplasm
HSPC family	Hsp90	81–99	HSP90	Cyt/ER
			HSP90(α/β)	Cytoplasm
			Grp94/gp96	ER
HSPA	Hsp70	65–80	HSP70	Cytoplasm/nucleus
			HSP72	
			Hsc70	Cytoplasm/peroxisome
			Grp75	Mitocondria
			Grp78	ER
HSPD1	Hsp60	55–64	Hsp60	Mitocondria
			TCP-1	Cytoplasm
DNAJ	Hsp40	35–54	Hsp40	
HSPB	Small HSP	34 or lower	Hsp27	Cytoplasm/nucleus
			α-βcrystalin	Cytoplasm
			Hsp32	Cytoplasm
			Heme oxyganese	Cytoplasm

ATP-dependent. They can stabilize with binding ATP molecule, so they are called as co-chaperons. Small HSPs are ATP-independent, and their activation modifies by phosphorylation status. In this chapter, we will use the old nomenclature as it is the most well-known one.

Principal HSPs, molecular weight range between ∼15 and 110 kDa, are divided into groups based on both size and function. HSPs found in the cytosol, mitochondria, endoplasmic reticulum, and nucleus. Locations can show variations according to their protein structure. The most well-studied and understood HSPs in mammalians are those with molecular masses of ∼60, 70, 90, and 110 kDa. These HSPs are expressed at euthermic body temperatures (∼37 °C) and in conditions of stress (e.g., heat shock). They have distinct locations and functional properties. Small HSPs, exhibit tissue-specific expression and consist of heme oxygenize, HSP32, HSP27, αB-crystallin, and HSP20.

3.3.1 HSP60

HSP60, acts as a molecular chaperone, like other HSP families. HSP60 identifies proteins that excused to hydrophobic residues and also identifies proteins that form inactive aggregates. HSP- 60 is a mitochondrial molecule, and it has ATPase activity (Brocchieri and Karlin 2000). There are studies that show HSP 60 family has many roles in pathogenesis of autoimmune diseases. Diabetes and arthritis are the best-studied diseases related with HSP −60 family. In the synovial tissue of rheumatoid arthritis patients, HSP-60 expression is detected (Boog et al. 1992). In type-1 diabetic mice models HSP60 mediated T cell activation is established that may resolve insulinitis and hyperglycemia. Furthermore, HSP-60 molecules have a chaperone function in atherosclerosis. The intensity of HSP expression is correlated with the severity of atherosclerosis. It is shown that oxidized LDL molecules make an induction in HSP60 expression. The HSP-60 related T-cell activation occurs and plays a role in inflammatory response of atherosclerosis (Pockley 2001). When HSP60 molecule released to extracellular space, it increases levels of CD4+, CD25+ and suppresses cytotoxic T lymphocyte levels (De Kleer et al. 2010). In addition, HSP60 plays a role in stimulation of dendritic cells and in the induction of T-cell mediated immune response (Feng et al. 2002).

3.3.2 HSP70

The HSP70 is the most studied family of all HSPs. HSP 70 molecules are ATP-dependent and mainly function in proper folding of newly synthesized proteins. İt makes protein complexes and helps the transport of proteins. Stress-inducible HSP 70 molecules help the cell survival (Jaattela 1999). HSP70s are involved in a large variety of cellular processes. Thereby they interact with substrate proteins that are in many different conformations. They have functions during translocation into organelles, disaggregation and refolding of stress denaturated proteins. HSP70s are ATP-dependent chaperones that consist of N-terminal 45 kDa nucleotide binding domain (NBD) and a 25 kDa substrate polypeptide binding domain They do not work alone but interact with co-chaperones of the J-domain protein (DnaJ, HSP40) family, which target HSP70s to substrate proteins and several families of nucleotide exchange factors (Mayer and Kityk 2015). HSP70 levels enhance the sensitivity of sympathetic and parasympathetic arms of the autonomic nervous system to attenuate heat stroke –induced cerebral ischemia and hypotension (Horowitz and Robinson 2007).

HSP 70 molecules have functions in thermotolerance. The accumulation of stress-induced HSP70 makes the cell resistant to stress (hypoxia, ischemia, acidosis, energy depletion, cytokines). Increased HSP 70 levels block caspase activation; nuclear fragmentation and prevents mitochondrial damage. Furthermore, HSP 70 can bind to apoptosis protease activating factor-1 (Apaf-1), so it can prevent apoptotic cascades (Beere et al. 2000). In addition, HSP 70 can prevent caspase-independent apoptosis pathways (Creagh et al. 2000). HSP70 has also been shown

to act at the premitochondrial stage by inhibiting stress-activated kinases. HSP70 has cardio productive beneficial effects (James et al. 1997). Also, HSP 70 molecule has a protective function against light associated damage to retina (Urbak and Vorum 2010). İn human acute leukemia cells HSP70 can act on factors in Bcr-Abl-mediated resistance to apoptosis. HSP70 binds to the death receptors DR4 and DR5, thereby inhibiting TRAIL-induced assembly and activity of death-inducing signaling complex (Guo et al. 2004). Although being the main regulator of the immune system, HSP 70 makes activation in APC cell that makes a release in pro inflammatory cytokines (IL-1β, IL-6, TNF-α). Moreover, HSP 70 molecules are a triggering factor for NK cells (Schmitt et al. 2007).

3.3.3 HSP90

HSP90α and HSP90β proteins are the well-known members of HSP90 family. These isoforms are essential for the viability of eukaryotic cells. The primary chaperone role of HSP90 is to help the maturation, structural adaptation of receptors and signal-transducing kinases. Heat shock protein (HSP) 90 is an ATP-dependent molecular chaperone. HSP 90 is expressed in normal cells and helps regulation of late-stage maturation, activation, and stability of proteins. HSP90 is a dimeric molecular chaperone required for the activation and stabilization of numerous client proteins many of which are involved in essential cellular processes like signal transduction pathways. This activation process is regulated by ATP-induced large conformational changes, co-chaperones and posttranslational modifications (Li et al. 2012).

HSP 90 is involved in signal transduction and other key pathways that are especially important in malignancy it helps to maintain normal protein homeostasis. İn cancer cells HSP90 support the activated or metastable forms of oncoprotein, including many kinases and transcription factors, which are mutated, translocated, amplified or overexpressed (Schmitt et al. 2007). Hsp90 has been the most widely tested target for cancer therapy. Hsp90 inhibition is a promising new treatment strategy showing clinical activity in specific tumor types (non small-cell lung cancer, HER2-amplified breast cancer and multiple myeloma). Inhibition of Hsp90 enhances protein degradation via the ubiquitin-proteasome pathway and causes tumor development.The antitumor effects in preclinical models could potentially prevent the emergence of tumor drug resistance (Ou et al. 2014).

3.3.4 Small HSPs

The molecular weight of small HSPs, ranges between 12–43 kDa. All members of this family contain α-crystallin domain, and this domain considered as a hallmark. This family does not have ATPase activity, so they cannot refold protein themselves. They prevent the aggregation by sequestrating the unfolded proteins (Poulain et al. 2010).

3.3.4.1 Alpha B-Crystallin

Alpha B-crystallin is expressed in several tissues, heart, skeletal muscle and brain. Alpha B-crystallin plays a role in protein degradation, apoptosis, and stabilization of cytoskeletal structures (Boelens 2014).

3.3.4.2 HSP27

HSP27 belongs to the subfamily of small HSPs and plays an important role in inhibition of apoptosis. The function of HSP 27 depends on its phosphorylation status and exposure to stress. (Bruey et al. 2000). HSP27 can be phosphorylated response to different factors (such as mitogens, inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), IL-1β, hydrogen peroxide and other oxidants). HSP27 is expressed in many cell types and tissues, at specific stages of development and differentiation (Garrido 2002). HSP27 is an ATP-independent chaperone; its main chaperone function is protection against protein aggregation. Over expression of HSP 27 molecules protect the cell against apoptotic cell death (Mehlen et al. 1996). Human HSP27 is phosphorylated mainly at three sites (Ser-15,

Ser78, and Ser82). The phosphorylation is catalyzed by various protein kinases like mitogen-activated protein kinase (MAP) activated protein kinase2 (MAPKAPK-2). Unphosphorylated HSP27 forms aggregated to large oligomers while its phosphorylation results in the conformational changes, leading to dissociated small oligomers (Okuno et al. 2016).

3.3.5 Extracellular Role of HSP Molecules

HSPs are known to have both positive and negative effects in macrophage function. Their mission depends on the cellular location of the HSPs. It is proposed that extracellular HSPs might serve as a danger signal to stimulate the immune response, whereas intracellular HSPs could serve as a regulator of the inflammation (Table 3.2). We know that HSP molecules can be found in the extracellular space and on the plasma membrane. After a stress condition, such as inflammation bacterial and viral infections, HSP molecules are released by exomes and reach extracellular compartment (Giuseppina T. et al. 2014). HSP molecules make induction in the innate immune system with interaction with APC cells, and they modulate adaptive immune system. HSP molecules make interaction with APC by Toll-like receptors and scavenger receptors. Extracellular HSP molecules also have peptide carrier function, cytokine inducing effects, immune stimulation of NK cells. They interact with macrophages and APC cells. They can robustly stimulate the release of TNF-α, IL-6, IL-1β, IL-12, NO, as well as chemokines from monocytes/macrophages. After cross-presentation of HSP- chaperoned peptides (antigens or tumor-derived peptides) on MHC molecules, antigen-specific CD8+ T cell response is mediated (Giuseppina T. et al. 2014).

On the other hand, intracellular HSPs have been shown to have anti-inflammatory roles in suppressing macrophage cytokine production and stimulate antiinflammatory cytokines like IL10 (Fig. 3.1). Intracellular HSPs are involved in protecting the organism from a variety of insults by directly interfering with cell death pathway and suppressing the expression of inflammatory genes.

Table 3.2 HSP functions that differ according to their location

	Function
İntra cellular	Chaperon activity, neuroprotection by inhibiting apoptosis
Extracellular	Autoantigen, antigen presentation to MHC class I,II, immune response mediator

3.4 HSPs in MS Pathogenesis

MS is an autoimmune central nervous system disease that affects 2.3 million people worldwide. It damages mostly the myelin around nerves and also axons of the brain and spinal cord. Environmental factors are important in the etiology as there is a tendency of MS in some geographic distributions. High-frequency areas are most of the Europe, Israel, Canada, northern United States, southeastern Australia, New Zealand, and easternmost Russia (Kurtzke 2000). In childhood MS, incidence is low. It makes a peak at 20–40 years, then above 50 years the incidence declines. MS is more common in women (2.3 fold). The mean annual incidence rate is about 4.3:100,000. Life expectancy in MS patients is reduced by 7–10 years (Kamm et al. 2014).

MS plaques are sclerosing areas. Demyelinization, axon loss, inflammation, gliosis is the key pathologic features of them. The MSplaques include blood-brain barrier damage, astrocytic scars, myelin degradation products and inflammatory infiltrates, T lymphocytes, macrophages. Myelin is the main focus on the destruction but in some patients, axons are aggressively affected. The inflammatory cells T-lymphocytes, microglia, macrophages, B-lymphocytes, plasma cells, immunoglobulins, and complements have been identified in MS plaques. The pathology is characterized by multifocal lesions.

In acute stage (active plaque), activated mononuclear cells, including lymphocytes, microglia, and macrophages destroy myelin and oligoden-

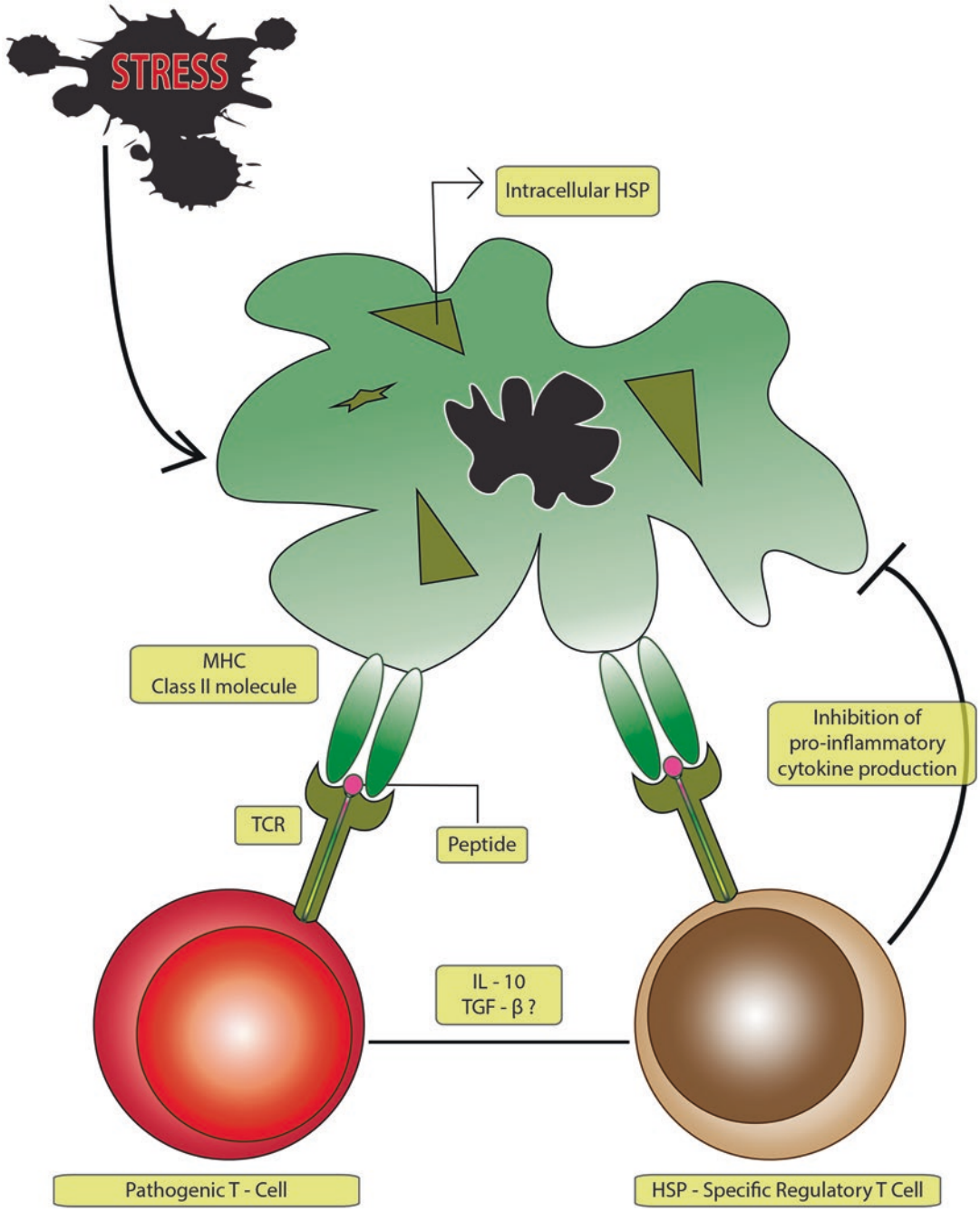

Fig. 3.1 The effects of HSP overexpression in the inflammatory response after exposure of stress. On the *right side* the influence of intracellular HSP overexpression on anti inflammation, on the *left side* the effects of HSP on inflammation are shown

drocytes. Myelin debris is picked up by macrophages. At the early stage, macrophages contain myelin fragments; later, they contain proteins and lipids from chemical degradation of myelin. This evolution takes a few weeks. With time, gliosis develops, and plaques reach a burned-out stage consisting of demyelinated axons traversing glial scar tissue (inactive plaque). Remaining oligo-

dendrocytes attempts to make new myelin. If the inflammatory process is arrested at the early phase, plaques are partially demyelinated (shadow plaque). In more advanced lesions, remyelination is ineffective because gliosis creates a barrier between the myelin producing cells and their axonal targets. The pathological process may be arrested at any time. Demyelination in the cortex and deep gray matter nuclei, diffuse injury of the normal-appearing white matter has also been shown in recent studies (Frischer et al. 2015).

Disease duration, clinical course, age, and gender contribute to the dynamic nature of white matter MS pathology. Active MS plaques predominate in acute and early RRMS and are the likely substrate of clinical attacks. The brain pathology of progressive MS consists of the accumulation of smoldering plaques characterized by microglial activation and slow expansion of pre-existing plaques.

Luccinetti described four fundamentally different patterns of demyelination, based on myelin protein loss, the geography and extension of plaques, the patterns of oligodendrocyte destruction, and the immunopathological evidence of complement activation. Two patterns (I and II) showed close similarities to T-cell-mediated or T-cell plus antibody-mediated autoimmune encephalomyelitis, respectively. The other patterns (III and IV) were highly suggestive of a primary oligodendrocyte dystrophy, reminiscent of virus- or toxin-induced demyelination rather than autoimmunity (Lucchinetti et al. 2000).

The pathophysiology of MS is still under controversies. However, there is a consensus on the role of the immune system. The exact cause of MS is still not known. In genetically susceptible individuals some factors (viruses, low vitamin D levels, other autoantigens) trigger the autoimmune response, especially to myelin and axons of the CNS. Once the autoimmunity is triggered recurrent immune attacks on CNS occurs, the most common form relapsing-remitting disease appears. There are two hypotheses about the beginning of the autoimmunity; outside-in model and inside out model. In the outside-in model, the triggering event begins peripherally. Outside the CNS once the T-cells are activated, they cross the blood-brain barrier, and they are reactivated by local antigen-presenting cells. Secretion of pro-inflammatory cytokines stimulates microglial cells and astrocytes, recruits additional inflammatory cells, and induces antibody production by plasma cells. This inflammation causes tissue damage and demyelinization. In the inside out model as in the other neurodegenerative disease the initial malfunction begins in CNS. Neurodegeneration is the primary event. Secondary event is neuro inflammation and autoimmunity (Fig. 3.1) (Stys et al. 2012).

The role of HSPs in the immunopathogenesis of MS has been studied since 1991. Selmaj et al. firstly, examined the colocalization of T-cell receptor gamma delta cells with HSP65 and HSP70 in MS lesions. HSP65 was expressed on immature oligodendrocytes at the margins of chronic lesions in early postmortem material of 13 MS patients. The unaffected tissue of MS brains did not show immunoreactivity for HSP 65 (Selmaj et al. 1991). Alpha B-crystallin has been said to be an autoantigen in MS since 1991 based on the invivo and in vitro studies. However, there is still debate on this topic since then. In an animal model, crystallin-specific T cells were not able to enhance the clinical symptoms of EAE. Rothbart et al. showed that putative anti-alpha B-crystallin Abs in MS patients. They revealed that these antibodies cross-react with seven other members of the human small HSP family, and they were also found in controls. They ignore B-crystallin is an autoantigen in MS (Rothbard et al. 2011). Small HSPs, α-B-crystallin and HSP27, have been shown in demyelinating plaques of MS brains (Cwiklinska et al. 2003). Van Noort et al. have reported an activated T-cell response against α-Crystalline in the peripheral blood of MS patients (Van Noort et al. 1998). Agius reported that anti-alpha-crystallin autoimmune responses may contribute to pathogenicity in MS and may represent a mechanism of how recurrent attacks of MS develop subsequent to an isolated demyelinating episode. The concentration of anti-alpha-crystallin antibodies in patients with MS is correlated with disease severity and with activity (Agius et al. 1999). In another study,

Alpha B-crystallin was analyzed in chronic active or chronic inactive plaques and normal-appearing white matter (NAWM) in seven MS cases, and white matter (WM) in five control cases. Increased expression of alpha crystallin was detected in all categories of MS tissue compared with control WM. AlphaB-crystallin was expressed on astrocytes, oligodendrocytes and occasionally on demyelinated axons (Sinclair et al. 2005).

Immune responses to HSPs develop in almost all inflammatory diseases. HSPs can prevent or arrest the inflammatory damage, and in initial clinical trials in patients with chronic inflammatory disease, HSP-derived peptides have been shown to promote the production of anti-inflammatory cytokines so that HSPs have an immunoregulatory role in the inflammation (Eden et al. 2005) (Fig. 3.1) In addition to their better-known anti-apoptotic effects, HSPs have significant roles in activation of pro-inflammatory cytokines. Extracellular HSPs may trigger immunological responses while their intracellular chaperone activities appear to prevent apoptosis and stabilize cytoskeletal structures. Extracellular HSPs appear to have functions for intercellular signaling.

Over expression of Hsp70 inside the cell, in such circumstances, appears to be largely anti-inflammatory responses. HSP70 is an important molecular chaperone that has antiapoptotic effects under stress conditions. It promotes innate and adaptive immunity. It has been proposed that HSP70 in MS lesions may protect the cells from inflammation (Mansilla et al. 2012). Thus, over expression of HSP70 may facilitate myelin repair and protect the tissues from demyelination. Boiocchi C et al. studied the role of HSP70b expression and genetic polymorphism in MS inflammation. 159 relapsing remitting and 36 secondary progressive MS patients were compared with 586 healthy controls. GG and GA genotype have shown to have the lower expression of HSP70compared to AA genotype. They hypothesized that over expression of HSP70 as in AA genotype may have less inflammation and better prognosis (Boiocchi et al. 2014). Failure of HSP over expression may lead to MS progression. However, in Japanese studies, they could not find genetic polymorphism of HSP70 in MS patients (Niino et al. 2001).

Experimental autoimmune encephalomyelitis (EAE), which is used in several studies for 30 years, is an animal model of MS. This model shows the major pathologic characteristics of multiple sclerosis. Many currently used drugs are discovered by EAE studies. Myelin basic protein, proteolipid protein myelin oligodendroglial glycoprotein are used as the antigen to destroy myelin sheaths. Large animals, mice, pigs, monkeys are used as a host (Hernandez-Pedro et al. 2013). After the injection of myelin protein, a cell-mediated immune reaction against myelin develops and causes paralysis. The complex of myelin protein-specific T-cell, traverse the brain capillaries. Interaction of these cells with myelin protein causes secretion of pro-inflammatory cytokines, damages the blood-brain barrier. Microscopic examination shows perivenular lymphocytes, similar to acute MS.

In MOG-induced mice model, HSP70 over expression has been shown to have protective effects. Mansilla et al. found reduced EAE susceptibility in HSP70.1 deficient mice. The severity of disease in HSP70.1 mice was reduced during the chronic phase of EAE. Moreover, clinical scores and milder disease progression were seen in HSP70 group. HSP70 deficient mice increased MO specific proliferative response. The HSP70 KO mice were significantly resistant to EAE development (Mansilla et al. 2014).

HSPs may act in antigen presentation and processing via MHC class I pathway and also they can bind on MHC class II molecules and form antigen complexes. Chaperoning activity of Hsp70 promotes immune recognition of protein/peptide antigens, including myelin autoantigens HSP70 expression was analyzed in peripheral-blood mononuclear cells of 49 MS patients, 40 healthy control, 13 rheumatoid arthritis patients. After heat stress, they showed over expression of HSP in MS patients. They proposed HSP70 over expression was correlated with autoimmunity in MS (Cwiklinska et al. 2010).

HSP70 is highly affinitive for hydrophobic peptides. It binds to misfolded proteins and helps

them to refold. In this way, it conserves the cell from apoptosis. On the other hand, if there are excessive amounts of misfolded proteins, the cell cannot accomplish and apoptosis begins. Over expression of HSP70 in neuronal cell cultures and EAE demonstrated efficacy on recovery and apoptosis. HSPs that are located in the intracellular compartment are neuroprotective. However, if they are released to extracellular compartment, they may act like autoantigen and trigger the immune response. Intracellular HSP70 is rapidly absorbed by neurons so glial to axon transfer of HSP70 protects the cell from death. Exogenous supply of HSP70 as a drug may be beneficial to inhibit the neuronal death.

Not only in MS but also in various autoimmune and neurodegenerative diseases, over expression of HSP70 has beneficial effects. In a recent study, it is clarified that HSPs are selectively induced in astrocytes as a secondary response to the development of MS lesions in white matter (WM) but not gray matter (GM) areas of the CNS. Perfereon and colleagues analyzed the transcript levels and the protein distribution profiles for HSPs in MS lesions at different stages. They made a comparison of normal-appearing brain tissue of MS patients with normal brain tissue of non-neurological controls. During active stages of demyelination in WM, they found significantly increased expression of HSP27, α-crystallins in the center of chronic active MS lesions. When they are induced, small HSPs were exclusively expressed in astrocytes. Surprisingly, while the numbers of astrocytes displaying high expression of small HSPs were markedly increased in actively demyelinating lesions in WM, no such induction was observed in GM lesions (Peferoen et al. 2015).

In an EAE model HSP70/ histidine triad nucleotide-binding protein 1 (HINT1) complex could prevent the disease. Proteolipid protein sensitized mouse was treated with HSP70/HINT complex. It induced T cell proliferation and inhibited IL17 secretion. HSP70/HINT complex affected natural killer functions by enhancing NK-dependent immunoregulation in EAE model. In another study, thymic peptides were injected intraperitoneally for 30 days after clinical signs of EAE started. Thymic proteins reduced cytokines, reduced NK kappaB signaling, and the production of HSP72. Conversely, HSP65 gene therapy was not able to prevent EAE neither clinical signs nor immune parameters (Zorzella-Pezavento et al. 2014).

In a Sardinian study where the MS prevalence is highest worldwide, 268 MS, and 231 healthy controls were searched for HSP70in the peripheral blood samples. Mycobacterium avium paratuberculosis HSP70, which is highly homolog with HSP70 was used for detection. Humoral response to MAPHSP70 was significantly high in MS patients. This data provides an evidence of the role of MAPHSP70 in MS (Cassu et al. 2013) immune responses against nonmyelin antigens, like HSPs that are overexpressed in the damaged regions of MS brains to protect the neurons may lead to demyelination. In this situation, HSPs are considered to serve as a target of the immune response.

Antibodies against HSPs (HSP60, HSP70, HSP90, and HSP105) have been detected in the cerebrospinal fluid of MS patients. Chiba et al. analyzed the HSP27, alphaA and alphaB crystallins, HSP60, CCT, Mycobacterium bovis HSP65, *Escherichia coli* GroEL, HSP70, HSC70 and HSP90 antibodies in MS patients by ELISA. Significantly high antibody titers against HSP70 and HSC70 proteins were found in CSF obtained from patients with MS. The antibody titers against HSP70 were high in MS (Chiba et al. 2006) Cid et al. investigated the presence of anti-Hsp90 antibodies in patients with MS. CSF anti-Hsp90 antibody levels were significantly higher in MS patients than in control patients. The presence of anti-Hsp90beta antibodies in the CSF of MS patients during remission could suggest a potential pathogenic role for these autoantibodies in MS (Cid et al. 2007).

In our study, we have suggested that HSPs may be elevated during an acute attack. Fifty relapsing–remitting or secondary progressive MS patients with superimposed relapses were consecutively recruited when they presented with a new attack. The HSP27 levels of MS patients were measured during acute attacks and after a minimum of 2 months of each individual attack.

The HSP levels of MS patients in the attack phase were significantly higher than the values obtained in the remission phase. Higher HSP levels were detected in the attack and remission phases of MS patients compared to the values of the control patients. A pronounced increase in HSP27 levels was observed in the remission phase of MS patients when compared to the controls. There was no correlation between the HSP27 levels during the attacks and age, disease duration, or EDSS scores. However, a positive correlation was observed between the HSP27 levels during the attack and the total attack number. The most impressive finding in our study was the striking increase in HSP27 levels during MS relapses. The correlation of this increase with the total attack number was also noticeable. These results revealed a clear relationship between MS relapses and HSP27 levels. The understanding of the exact mechanism of this response awaits further elucidation Ce et al. 2011). We also studied HSP27 during the migraine attack and HSP70 in drug-resistant epilepsy. There was no meaningful alteration of HSP70 in drug-resistant epilepsy patients. As migraine attack may constitute a stressful condition, we aimed to look for whether an alteration of HSP levels of migraine patients during the occurrence of the attack (Coban et al. 2011). The levels of HSP27 during the attack, was high. However the difference was not statistically meaningful. The increase of the HSP27 levels was meaningfully correlated with the Headache Severity Scores. The positive relationship of HSP during headache severity points was defected. Even though, the migraine attack couldn't cause enough stress to increase HSP27 levels. We think that either the migraine attack or the drug resistant epilepsy are not sufficient to cause an alteration of HSP levels as they are not enough to prompt cell damage like MS attack.

To pursue the potential role of a humoral response to the HSP 60/65 kd family in MS, Gao et al. studied serum and CSF by Western blotting using recombinant Mycobacterium bovis HSP 65 and human HSP 60 as antigens and compared the findings with samples from patients with other neurologic diseases (OND). Analysis of the IgG response in CSF from 18 patients with MS indicated moderate reactivity in 10 cases and no reactivity in eight. In the OND group, reactivity was found in the CSF from one of two patients with Parkinson's disease, four of four Alzheimer's disease patients, and two of two patients with amyotrophic lateral sclerosis. CSF samples from seven of seven patients with subacute sclerosing panencephalitis were negative, as sampled from two normal subjects. There was no reactivity in CSF from two Huntington's disease patients. They conclude that antibodies reactive with HSP 60/65 are present in CSF of some MS patients but are also present in a number of chronic neurodegenerative conditions. The findings indicate that a humoral response to HSP 60/65 in the CSF is not specific for MS (Gao et al. 1994).

HSP27 was markedly enhanced 2.5-to 4-fold in plaque regions, especially in fibrous astrocytes and in hyperplastic interfascicular oligodendrocytes at the lesion edge. HSP70 was less abundant than HSC70, and no significant differences in HSP70 levels were noted between MS and normal white matter. Myelin isolated from active plaques contained 3- to 4-fold more HSC70 than normal myelin. Pronounced expression of HSP70 and HSP27 was also found in MS myelin, although neither protein was detected in normal myelin. Thus white matter undergoing immune-mediated destruction in MS was associated with altered distribution and expression of HSC70 and HSP27. These changes may initially serve to protect myelin from further destruction and facilitate repair; however, enhanced expression of HSC70. HSP70 and HSP27 in myelin may subsequently present as additional immune targets involved in the progression of the disease (Aquino et al. 1997).

In a proteomics-based analysis, the antibody against mitochondrial heat shock protein 70 (mtHSP70) in serum from multiple sclerosis (MS) patients was detected. The anti-mtHSP70 antibody was significantly higher in the serum of MS patients than in serum from Parkinson disease patients, multiple cerebral infarction patients, infectious meningoencephalitis patients, and healthy controls (68 % sensitivity; 74 % specificity). Anti-mtHSP70 antibody proposed to be a diagnostic marker of MS (Sakurai et al. 2011).

In vitro studies, it is shown that HSP70 myelin basic protein complexes were highly immunogenic suggestive of a possible role for Hsp70 in the immunopathology associated with MS (Lund et al. 2006). Yokota and colleagues reported the increased levels of Anti-heat shock protein 70 (HSP70) autoantibodies cerebrospinal fluids of patients with MS. This result may show the pathophysiological role of anti HSP70 autoantibodies in the neuroinflammation in MS (Yokota et al. 2010). There are many investigations for developing a drug that inhibits the MS activation by using EAE model. Some of them are vibsanin B, geldamisin, gene therapy. Vibsanin B preferentially targets HSP90β, inhibits interstitial leukocyte migration, and ameliorates EAE (Ye et al. 2015). Gene therapy with mitochondrial heat shock protein 70 suppresses visual loss and optic atrophy in EAE (Talla et al. 2014). The HSP90 inhibitor, 17-allylamino-17-demethoxygeldanamycin, suppresses glial inflammatory responses and ameliorates experimental autoimmune encephalomyelitis (Dello Russo et al. 2006) Induction of intracellular HSPs, immune reactivity can attenuate autoimmunity; they can elicit specific, protective immunity in MS. These responses excited the investigators for a hope of MS therapy. However more studies must be performed. In the future, researches supporting the therapeutic possibilities of HSPs may enlighten our way going to a complete remission for MS.

3.5 Conclusion

There are conflicting reports on the roles of HSP in MS. There is not a consensus whether HSPs are protective or harmful. Their functions differ according to their placement. Extracellular HSPs may trigger the immune response while intracellular HSPs may act as chaperon proteins and prevent the nerve cells from apoptosis. HSPs induce either proinflammatory or anti- inflammatory cytokines. The target of the future will be to elevate the HSPs that increase anti-inflammatory cytokines in MS in order to suppress the disease activity. More studies are needed to clarify their exact roles and to benefit from their protective functions.

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Meaning of Self in Multiple Sclerosis: Implications for Treatment and Rehabilitation

4

Maciej Wilski and Tomasz Tasiemski

Abstract

Low participation of multiple sclerosis (MS) patients in the therapeutic process is considered a primary area in research on the management of this condition. One of the key research directions is the role of self and self-involvement in MS patients. Clinical symptoms of MS and unpredictability of this condition may affect patients' attitude to their self and self-involvement. Self-image and self-appraisal of one's abilities to cope with the disease exert significant effects not only on patient's emotional status but also on their behavior. This assumption is consistent with the cognitive-behavioral paradigm according to which emotions and behaviors of an individual reflect specific self-interpretation, self-assessed situational context and self-perceived ability to cope with a given situation. Enforcement of self-esteem and self-efficacy may promote self-management and thus, increase patients' participation in the therapeutic process. In this paper, we briefly review recent advances in research on the role of self in treatment and rehabilitation of MS patients.

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Keywords
Multiple sclerosis • Self-efficacy • Self-esteem • Self-management • Treatment adherence

Abbreviations

HAPA	health action process approach
HBM	health belief model
KAPA	knowledge and appraisal personality architecture
MS	multiple sclerosis
SCT	social cognitive theory
TPB	theory of planned behavior

4.1 Introduction

Multiple sclerosis (MS) is an incurable chronic disease of the central nervous system, characterized by the occurrence of multiple motor, sensory, affective and cognitive deficits, and diagnosed primarily in young persons. MS is not a mortal disease, but may although not necessarily, result in extremely severe disability. The disease affects physical, psychological and mental functioning. Current evidence is insufficient to predict the outcome of MS in a given patient, the rate of progression and the type of therapeutic response. MS is characterized by heterogeneous symptoms, variable dynamics of progression and unpredictable cyclicality of remittance-regression periods. Such clinical course as well as side effects of implemented therapies makes MS unpredictable and uncontrollable disease, which in turn markedly hinders psychological adjustment of patients to this condition. This makes MS a unique condition, differing from other diseases, and enforces specific approach to the adaptation process (Byra 2012).

The prerequisite of success in MS treatment, the aims of which are often limited to attenuation of symptoms and delaying progression of the disease, is active involvement of patients in the therapeutic process, and most of all, their adherence to medical recommendations. Patients can play an integral role in improving the quality, safety and costs of health care interventions (Rieckmann et al. 2015). Due to the dynamics of MS, its unpredictable character and frequent changes in health status, patients may put into question the relevance of treatment they have been prescribed. This results in medication non-compliance, absence to control visits, non-adherence to prescribed diet, frequent changes of physicians and treatment methods (Martin et al. 2005; Saunders et al. 2010). According to, Steinberg et al. (2010), adherence to interferon beta treatment among patients with MS does not exceed 41 %, which correlates with the loss of efficacy, higher relapse rate and greater utilization of health resources. Due to medication non-compliance, the physician who prescribed a given treatment is unable to verify its true efficacy. Consequently, low participation of MS patients in the therapeutic process was listed among the priorities of research regarding treatment of this condition (Rieckmann et al. 2015). One of the key directions in this area is research on self and self-involvement of MS patients (Knaster et al. 2011; Fraser et al. 2013).

According to all the principal theories on patient activation, constituting the basis for psychological intervention, self plays a key role in the process of behavioral change. The theories mentioned above include Social Cognitive Theory (SCT, Bandura 1997, 2001), Health Action Process Approach, (HAPA; Schwarzer 1992, 2001), Theory of Planned Behavior (TPB, Ajzen and Fishbein 1980) and Health Belief Model (HBM; Rosenstock et al. 1988). Changing patient's behavior, requires improving his/her knowledge about the treatment, as well as modifying his/her beliefs and self-efficacy, which are one of the core determinants of behavioral

change. Knowledge and Appraisal Personality Architecture (KAPA) theory, proposed by Daniel Cervone (2004), constitutes a quite recent breakthrough in research on cognitive-behavioral paradigm; according to this theory, specific behavior results from dynamic mental and affective processes stimulated by a given situation. The aim of this personality-related concept is to explain which traits of an individual contribute to his/her coherent patterns of experience and action, distinguishing one person from another (Cervone 2004). According to this model, one's opinions may include both the form of a stable self-knowledge (e.g. self-esteem) and self-appraisals that are formed during action based on one's abilities shown in a specific situation (self-efficacy) (Cervone et al. 2004). Knowledge and appraisal represent two different levels in the analysis of cognitive contents (Cervone 2004). With no doubt, disease or disability represent a situational context, in which both the self-knowledge and self-appraisals are subject to considerable changes. These changes are determined not only by the new situation, but also by the power of previous appraisals and emotional experiences, standards for self-assessment and assessment of surrounding world, and system of personal objectives. In this context, dynamic interaction person-situation is an outcome of subjectively assessed individually-specific meaning of this situation to self. Both self-knowledge, being a relatively stable structure, and self-appraisal, which is to a greater extent susceptible to situational influences, are reflected by action. In other words, specific behavior of an individual (e.g. self-management) is determined by his/her self-assessed competencies in a specific situation (self-efficacy) that in turn correspond to one's individual predisposition (self-esteem). The way in which patients perceive themselves and their ability to act are crucial for their involvement in the therapeutic process. This is presented schematically on Fig. 4.1.

Relationships between self-esteem, self-efficacy and self-management have not been subject of many previous studies thus far, especially in the context of such unique entity as MS. The aim of this paper is to systematize our knowledge regarding self in MS, especially in terms of its practical application to treatment and rehabilitation. We hope that this review will stimulate further, more extensive, research in this area.

4.2 Self-Esteem in MS

Self-esteem can be defined as one's appraisal of his/her value (Rosenberg 1989), based on self-assessed functioning in physical, psychological and social sphere. This assessment includes many

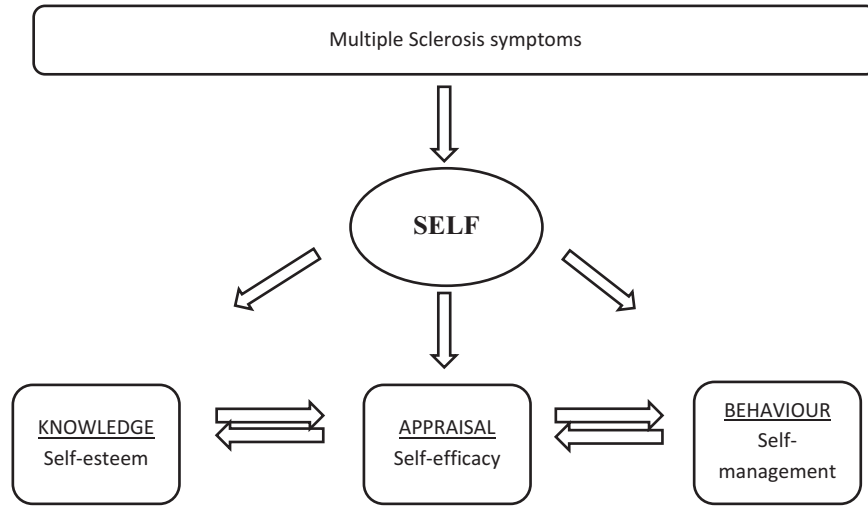

Fig. 4.1 Self in MS

aspects, such as appearance, physical status, mental capabilities, skills, activity, possibility to act, social position, etc. Diagnosis of a disease represents a critical event which considerably alters ones cognitive schemes regarding self. As a consequence of the disease, these schemes are disrupted, which alters one's appraisals of his/her potential ability to cope with the illness. These appraisals, in turn, determine objectives, values and behaviors of an individual, as well as maintain or disrupt his/her emotional balance (Byra 2012). The results of previous studies including patients with various chronic conditions imply that in this group, low self-esteem exerts stronger effect on a negative affectivity than in healthy population (Bisschop et al. 2004). Low self-esteem is also associated with worse social functioning (Nicolson and Anderson 2003), perceived symptom severity (Panides and Ziller 1981; Juth et al. 2008) and greater stress severity (Juth et al. 2008).

Self-esteem has been a subject of research in MS patients as well. Individuals suffering from this condition present with variable symptoms resulting from damage in various parts of their nervous system. Common manifestations of MS include various types of paresthesia, pyramidal and cerebellar syndromes, nystagmus, urological disorders, fatigue and vision impairment. MS may also manifest as various emotional states (depression, euphoria), cognitive deficits, sexual dysfunction and pain syndromes (Neumann 2003). All these ailments with no doubt negatively affect patient's self-image. Each episode of relapse or progression of the disease represents a new challenge, frequently enforcing re-adaptation, since previously developed coping strategies are no longer effective in the new situation. This dramatically decreases one's self-esteem and appraisals of his/her ability to control the disease, which in turn results in rapid unexpected changes in self-image.

These theoretical assumptions were confirmed empirically in patients with MS. One of the first studies dealing with the problem in question was conducted by Walsh and Walsh (1987) who showed that persons suffering from MS present with markedly lower self-esteem. One of the most potent determinants of lower self-esteem turned out to be the degree of physical restriction. These findings were further confirmed by McCabe (2005) who showed that the level of self-esteem in 243 MS patients was markedly lower than in healthy controls. Also the results of another study, conducted by Korwin-Piotrowska et al. (2010) in a group of 63 patients with MS, imply that this disease is associated with low self-esteem and decreased self-acceptance. Moreover, subjects participating in the latter study showed a tendency to withdraw from social contacts, stopped creating new challenges and aims, and focused primarily on observation of their symptoms. Also Jiwa (1995) pointed to important role of these factors in the context of decreased self-esteem (1995). Feelings of uselessness, self-distrust and lack of self-confidence, as well as job abandonment, fatigue, withdrawal from social roles and resignation from active modification of one's destiny were shown to negatively affect self-esteem in MS (Murray 1995; Jiwa 1995; Fragoso et al. 2009). The abovementioned observations were also confirmed by the results of qualitative studies conducted in this group of patients (Boeije et al. 2002; Olsson et al. 2008; Irvine et al. 2009; Mozo-Dutton et al. 2012), in which many salient aspects of self were demonstrated to be lost as a consequence of MS. Patients lack motivation to pursue their life objectives, no longer define new priorities, are less spontaneous and capable of thinking. Perceiving self as an active and independent person who takes care for others and is capable in employment, is replaced by negative self-concepts (Irvine et al. 2009).

Some researchers point to duration of the disease as a factor promoting reinforcement of one's self-esteem. For example, Brooks and Matson (1982) analyzed the adjustment of 103 subjects with MS and concluded that although the disease is chronic and progressive, the majority of their subjects were able to cope with it, as shown by maintenance of positive self-concepts over a 7-year follow-up period. Also according to Walsh and Walsh (1987), the level of self-esteem increases if individuals suffering from MS are able to integrate the reality of the disease with

their self-concept. These findings support the concept of benefit finding and resilience in MS (Mohr et al. 1999; Reynolds and Prior 2003; Pakenham 2007). However, an 18-month study conducted by McCabe (2005) did not document any significant time-related changes in self-esteem of MS patients. According to this author, after an initial dramatic decrease, the level of self-esteem eventually stabilizes at a new, lower level.

Perhaps even more important is the character of MS outcome, which is specific for its type. Patients with relapsing-remitting MS experience periodic resolution of their symptoms or attenuation thereof, which may promote reinforcement of their self-esteem. A study of 108 patients with relapsing-remitting MS, conducted by Rzeszutko (2013), confirmed that both remission and relapse of the condition are associated with low levels of self-acceptance, negative self-appraisal and self-presentation. However, patients with remission and relapse differed in terms of their self-image. Individuals with relapse presented with less favorable self-image, greater emotional liability and impulsiveness, were more prone to aggression and lower self-control. They more often perceived themselves helpless in coping with stress, and showed a tendency to withdraw from activities aimed at achieving long-term objectives. Social contacts stimulated their anxiety, they were more submissive to others and self-limited their sex- and age-specific activities. In contrast, patients in remission were less focused on themselves, showed stronger desire for success and lesser fear of social contacts. Furthermore, they perceived themselves more efficient in dealing with external requirements. Similar results were reported by Papuć and Pawłowska (2005), who conducted a comparative analysis of 26 MS patients with relapse and 16 individuals with remission. The study showed that the subjects with relapse were more often characterized by more negative self-appraisal and self-distrust than the individuals in remission.

Interesting data originate from research on body esteem in MS, which is considered a fundamental component of self-esteem. Many studies involving patients with other conditions showed that physical disability has a negative impact on body esteem and consequently, on overall self-esteem (Keppel and Crowe 2000; Callahan 2004; Sertoz et al. 2009). This is particularly evident in female patients who pay more attention to their appearance and physical attractiveness (Mendelson et al. 2001; Stokes and Frederick-Recascino 2003). Pfaffenberger et al. (2011) examined 40 subjects with MS and showed that women with this condition were more often concerned about their physical deficits and felt less attractive, whereas men typically worried about their sexual problems. However, irrespective of their sex, patients with MS presented with significantly less favorable body image than the controls. In our study (Wilski et al. 2016), including 185 women with MS, positive body esteem turned out to be an important component of self-esteem, and was associated with better psychological and physical status, higher level of received support and belief in personal control over the course of the disease. Surprisingly, however, body esteem did not correlate significantly with clinical factors, such as the time elapsed since the diagnosis, type of MS, and the way of administering anti-MS medication.

Barak et al. (1998) found no significant relationship between body esteem and self-esteem in a group of 35 individuals with MS. Importantly, participants of this study presented with low levels of body esteem, which was to a large degree linked to their concomitant disabilities. However, quite opposite findings were presented by Samonds and Cammermeyer (1989); these authors examined a total of 20 male patients with MS, and found that their body satisfaction/dissatisfaction scores were similar as in sex- and age-matched healthy controls. Moreover, the subjects who were satisfied with their body and self, turned out to be older, had longer history of MS and presented with more severe disability. In another study, including 30 women with relapsing-remitting MS, Kindrat (2007) demonstrated that body image correlated strongly with depression scores; specifically, more favorable body image was associated with lesser depressiveness. However, it should be stressed that no firm conclusions can be drawn on the basis of

research conducted in such small groups of patients as those mentioned above. The evidence from these studies can only be used to identify directions of future research. Another aspect related to body esteem in MS is sexual dysfunction, a common symptom among patients with this condition (Zivadinov et al. 1999; Tepavcevic et al. 2008; Guo et al. 2012). Quite recently, Kolzet et al. (2015) published the results of their study in this matter, conducted in an impressively large group of 4267 subjects with MS. The study showed that patients who reported more severe body image-related sexual dysfunction were typically women with lower educational level, longer history of the disease, greater disability and worse mental status.

In conclusion, this review revealed apparent discrepancies between published studies. Their results are often inconclusive, and a number of different variables were identified as potential determinants of self-esteem and body esteem, which hinders drawing any firm conclusions. These discrepancies may have many reasons, with most likely being differences in research methodology and instruments, small sample size and inappropriate study design. With no doubt, further well-designed studies are needed in this area. Identification of factors determining self-perception is of vital importance, since many previous studies confirmed that positive self-image plays an important role as a determinant of health-related quality of life in physical and psychological sphere (Benito-Leon et al. 2002; Mitchell et al. 2005; Dlugonski and Motl 2012; Wilski and Tasiemski 2015), more effective coping with the disease (O'Brien 1993), lesser perceived stress (Ifantopoulou et al. 2015) and better psychosocial adjustment to MS (Sammonds, Cammermeyer 1989; Jiwa 1995). Owing multifaceted and diverse nature of factors associated with self-esteem, future research should also include interactions between demographic, clinical, and socio-psychological variables.

4.3 Self-Efficacy in MS

In line with the social cognitive theory, health behaviors of an individual are determined by his/her subjective appraisals of self and his/her abilities. A key role in this matter is attributed to the sense of self-efficacy. A person with strong self-efficacy believes that his/her activities may change reality and that he/she is able to undertake such activities (Bandura 2000), which in turn results in initiation thereof. Two directions can be observed in published research dealing with the problem in question: the studies of general and specific self-efficacy.

Most previous studies on the role of self-efficacy and its influence on various aspects of coping with MS dealt with the disease-specific dimension of this parameter. The results of these studies imply than individuals with MS present with relatively lower levels of self-efficacy than persons with disability of other etiology (Shnek et al. 1997, Jongen et al. 2015); these differences were attributed to a unique character of MS, namely its unpredictable and variable outcome. Experiencing disease progression despite doing everything right may have a severe impact on one's confidence in his/her own capabilities. However, the latter may vary depending on MS type. For example, Fraser and Polito (2007) examined a convenience sample of 566 individuals with MS, and showed that patients with relapsing-remitting type of the disease believed in their ability to control MS and to function with this condition to a markedly greater extent than persons with progressive MS. According to the authors of this study, these differences may be related to increasing disability and psychosocial changes taking place over time. In the same study, women with MS presented with markedly stronger self-efficacy than male patients.

The vast majority of researchers postulate the need for intervention aimed at self-efficacy reinforcement. According to Bandura (2004),

improvement of self-efficacy is the most effective way to stimulate health-oriented behaviors and behavioral changes. Self-efficacy reflects how the patient perceives his/her own ability to adopt and maintain health behaviors which are necessary for treatment or rehabilitation; furthermore, this parameter turned out to be a predictor of long-term therapeutic responses (Lau-Walker 2006).

One of the first published studies on self-efficacy in MS was the research conducted by Wassem (1992) in a small sample of 62 patients; in this study, respondents' efficacy expectations predicted 24 % of variance in adjustment to MS. This observation was confirmed by another, longitudinal study conducted by Barnwell and Kavanagh (1997), in which self-efficacy for controlling mood and maintaining social life predicted better adjustment to MS. Another study revealed an association between the MS specific self-efficacy, physical and social functioning (Amtmann et al. 2012). Similar results were also reported by Schmitt et al. (2014), who examined 81 individuals with MS, and concluded that self-efficacy is a significant predictor of self-reported physical, cognitive and social functioning in this condition, also after controlling for the disease-related factors and depressive symptoms. Moreover, the results of many previous studies imply that individuals with MS who present with higher levels of self-efficacy are less prone to depression (Shnek et al. 1997; Thornton et al. 2006), anxiety (Garfield and Lincoln 2012) and fatigue (Trojan et al. 2007); finally, stronger self-efficacy was implicated to positively affect cognitive performance of MS patients (Hughes et al. 2015; Jongen et al. 2015).

Riazi et al. (2004) pointed to self-efficacy as an important modifiable target in clinical practice. Their study, including a total of 89 persons, showed that the level of self-efficacy influences self-reported health status in MS. Specifically, higher pretreatment self-efficacy scores and improvement in this parameter throughout the course of follow-up turned out to be associated with better subjective walking ability, as well as with lesser physical and psychological impact of MS. Important role of self-efficacy was also documented in research on the quality of life, a widely approved measure of treatment and rehabilitation effectiveness. A large body of evidence points to an association between self-efficacy and quality of life in MS (Mitchell et al. 2005). One of the leaders in this area of research is Robert Motl, whose many empirical studies demonstrated that self-efficacy is one of the most important subjective measures determining high quality of life in MS (Motl et al. 2009, 2013). This was also confirmed in our study of 257 patients with MS, in which general self-efficacy was identified as the strongest correlate of health-related quality of life in physical and psychological dimensions (Wilski and Tasiemski 2015).

Also the research on the role of self-efficacy in patient activation is worth emphasizing. As mentioned previously, due to its unique character and the fact of being incurable condition, MS does not promote active involvement of patients in the therapeutic process. This puts greater emphasis on identification of factors that would stimulate participation of MS patients in their treatment and rehabilitation. Analysis of available literature implies that one such factor is self-efficacy. For example, a study of 199 individuals with MS conducted by Goodworth et al. (2016) showed that self-efficacy belongs to the group of variables being associated with patient activation, and as such may promote self-management. These findings were confirmed by our cross-sectional study including 210 patients with MS (Wilski and Tasiemski 2016). The subjects with stronger general self-efficacy tended to present with higher MS self-management levels. Moreover, general self-efficacy was identified as the strongest correlate of self-management in our study sample. These observations are consistent with the data published by Jongen et al. (2014a), according to whom some patients with relapsing-remitting MS are not interested in involvement in self-management programs. The abovementioned authors attributed this observation to the lack of perceived self-efficacy in disease management. Hence, they suggested that self-management programs should focus on improving patients' perception of self-efficacy and self-control. Also other authors pointed to

important role of self-efficacy as a determinant of patients' participation in health-oriented activities (Ennis et al. 2006; Yorkston et al. 2008; Plow et al. 2015). This is particularly important in the case of the disease-modifying therapies, the side effects and modest efficacy of which, as well as painful injection-site reactions, often result in treatment discontinuation. Fraser et al. (2001, 2003, 2004) showed that stronger MS-specific self-efficacy is associated with greater adherence to intramuscular self-injection of medications for relapsing-remitting and progressive MS. A longitudinal study conducted by Mohr et al. (2001) analyzed the influence of self-efficacy expectations on adherence and ability to self-inject interferon beta-1a, an agent recommended for treatment of MS. The study, including a total of 101 patients with relapsing MS, documented a significant association between pretreatment self-efficacy level and 6-month interferon beta-1a adherence.

Many previous studies conducted in this area dealt with physical activity of MS patients. They showed that self-efficacy exerts both direct and indirect effects on physical activity in MS (Vanner et al. 2008; Motl and Snook 2008; Kasser and Kosma 2012; Suh, et al. 2014), and is one of the most important predictors of baseline physical activity levels in individuals with this condition (Ferrier et al. 2010; Kosma et al. 2012). Furthermore, the results of one longitudinal study imply that 18-month changes in self-efficacy may be associated with residual changes in physical activity of persons with relapsing-remitting MS (Suh et al. 2011). Since self-efficacy was also shown to positively affect working hours (Jongen et al. 2014b) and turned out to be one of the strongest predictors of nutritional behaviors in patients with MS (Plow et al. 2012), it with no doubt represents a primary modifiable determinant of adjustment to this condition and as such, should constitute an important intervention target. This hypothesis was already confirmed empirically in interventional studies, in which programs oriented at reinforcement of self-efficacy were shown to produce beneficial effects in MS patients, namely improved their health-related quality of life, reduced pain and promoted health-oriented behaviors (Stuifbergen, et al. 2003; Ng et al. 2013).

4.4 Self-Management in MS

Self-management is generally considered to be a multidimensional construct rooted in cognitive behavioral theory. It is based on an assumption that responsibility for health and management of disease-related behaviors is primarily on the patient's side, rather than in hands of medical personnel. Specifically, self-management may be defined as an active coping with the disease through medication and treatment adherence, participation in therapeutic decisions, self-care, active seeking of information about the illness and emerging treatment options, maintenance of social relationships and emotional balance (Kralik et al. 2004; Bishop et al. 2008). It is self which regulates all the activities mentioned above. According to Bandura (2004), self-management is vital for management of chronic conditions, and due to managing their health habits, people can live longer and healthier lives. Many previous studies demonstrated that treatment outcomes may improve with self-management efficacy (Rae-Grant et al. 2011). Furthermore, programs aimed at reinforcement of self-management in MS were shown to reduce fatigue (Mathiowetz et al. 2005; Navipour et al. 2006; Kos, et al. 2007), improve health related quality of life (O'Hara et al. 2002; Jongen et al. 2015), medication adherence (Berger et al. 2005) and physical functioning (McAuley et al. 2007; Barlow et al. 2009; Bombardier et al. 2008). Moreover, as shown by Bishop et al. (2009), high level of self-management is associated with higher employment rate of MS patients. According to health policymakers, the self-management promoting programs may also contribute to reduction of healthcare costs (Holman and Lorig 2004). Also a systematic review conducted by Rae-Grant et al. (2011) provides some evidence supporting the value of programs aimed at promotion of self-management in MS.

Determinants of self-management in MS constitute another important area of research.

Surprisingly, however, this problem was addressed by only few published studies. Bishop et al. (2008) examined a group of 157 individuals with MS and showed that the disease-specific self-management is associated with perceived control; both perceived control and self-management mediated a relationship between physical and emotional impacts of MS and quality of life. However, a key question arises, which aspects should be particularly emphasized within the framework of programs aimed at improvement of self-management in MS? Are there any modifiable psychological, behavioral or environmental factors that a clinician can address to facilitate self-management in this group? These questions were partially answered by the results of two studies conducted by our group. In the first study (Wilski and Tasiemski 2016), including a group of 210 hospitalized patients with MS of various type and severity, cognitive appraisals, such as general self-efficacy, perception of treatment control and realistic MS timeline perspective, turned out to be more salient correlates of self-management in MS than the objective clinical variables, such as severity, type and duration of MS. Understanding patients' appraisals of self, illness and treatment seems to be essential for their successful activation. The aim of the second study (Wilski et al. 2015), conducted in a group of 283 community-dwelling and hospitalized patients with MS, was to identify demographic, socioeconomic and clinical determinants of self-management in this condition. The study identified a group of MS patients who were at an increased risk of poor self-management and as such, required greater attention from medical staff. The risk group included individuals with low levels of received support and low socioeconomic resources, as well as men and persons with low monthly income. Interestingly, also in this study, clinical variables did not have a considerable impact on self-management level. We found no relationship between self-management, type of the disease and its duration, presence of relapse/remission, general medical and psychological condition and route of administering anti-MS medication. However, both the studies mentioned above were cross-sectional, which precludes any conclusions about causal directions, and both regression models explained a relatively small proportion of variance in self-management in MS (25 % and 11 %, respectively). Our findings are consistent with the results published by Plow et al. (2015), who searched for the determinants of participation in meaningful activities (related to self-management) in a group of 335 patients with MS. The study identified cognitive problems and environmental barriers as the factors that exerted the strongest unfavorable direct effect on the participation rate. According to the same authors, also self-efficacy may indirectly influence participation in meaningful activities due to its involvement in self-management behaviors. However, also in this study, all the variables mentioned above explained a relatively small proportion of variance (19 %) in the dependent variable. This emphasizes the need for further research in this area, including other variables than those mentioned above, and preferably designed as longitudinal and/or interventional studies.

4.5 Conclusion

With current state of knowledge, the principal aim of MS management is to attenuate negative impact of the disease on functioning in physical and psychological sphere, and consequently, to improve quality of life or at least to prevent deterioration thereof (Opara et al. 2005). While the impact on physical consequences of the disease is limited, possible influence on psychological sphere is markedly greater, which raises a possibility to improve functioning of MS patients. Clinical symptoms and unpredictable character of the disease may affect patients' attitude to self and their self-involvement. Self-image and self-ability to cope with the disease exert significant effects not only on the emotional status of patients, but also on their behaviors. This assumption is consistent with the cognitive-behavioral paradigm, according to which emotions and behavior of an individual reflect specific self-interpretation, self-assessed situational context and self-perceived ability to cope with a given situation. Therefore, the most important regulatory function of self-image pertains to undertak-

ing activities aimed at protection, support and development of self (Korwin-Piotrowska and Korwin-Piotrowska 2010).

Consequently, management of one's self with no doubt constitutes the key to solving the problem of low participation of MS patients in the therapeutic process. However, this requires identification of associations between specific areas of self-knowledge, situation-specific self-assessment of one's abilities and behavioral manifestations of coping with the disease. Important role of these relationships was already confirmed in some of the studies mentioned above. Reinforcement of self-esteem and self-efficacy may be a strategy to promote self-management, which in turn may improve patients' participation in the therapeutic process (Jongen et al. 2014a; Plow et al. 2015, Wilski and Tasiemski 2016). Moreover, development of intervention strategies aimed at promotion of self-management may have significant impact on healthcare delivery and costs thereof. Identification of relationships between the individual components of self-knowledge (e.g. self-esteem), value attached to experiencing MS and self-efficacy in coping with the condition, will likely facilitate individualization of treatment programs.

Moreover, the review of available literature implies that perceptions of self should be a routine component of intervention programs, and discussions regarding treatment and rehabilitation efficacy should always consider supporting MS patients in maintaining positive sense of their self. A good way to improve treatment efficacy may be identification of individuals who encounter problems with self-perception; in such cases, psychological intervention may be more effective than a symptomatic treatment of MS. Psychological intervention, based on appropriate cognitive behavioral techniques, should be aimed at changing one's way of thinking about self. Potentially useful techniques include modeling, observational learning, planning alternative strategies, and training for internal self-regulation. These strategies should contribute to more rational and targeted actions, and as a result, increase one's self-management level.

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Multiple Sclerosis and EIF2B5: A Paradox or a Missing Link

5

Insha Zahoor, Ehtishamul Haq, and Ravouf Asimi

Abstract

Multiple sclerosis (MS) is an encumbering inflammatory condition of the central nervous system (CNS) caused by axonal demyelination. There is sufficient evidence suggesting role of eukaryotic translation initiation factor 2B (EIF2B) gene family encoding the five subunits of eIF2B complex-α, β, γ, δ and ε respectively, in causing vanishing white matter (VWM) disease of the brain. Incidentally researchers have proposed overlapping between MS and VWM in terms of clinical, biochemical and genetic aspects, which incited us to write this chapter to explore the association between EIF2B5 and MS. eIF2B plays an essential role in translation initiation and its regulation in eukaryotes. Among EIF2B gene family, EIF2B5 gene encodes the catalytic and a crucial epsilon subunit of the eIF2B protein as most of the alterations have been found in this gene. The recent findings on the association between EIF2B5 and MS susceptibility point towards unfathomable and contentious role of EIF2B5 in MS development. This chapter briefly reviews the insights gleaned from recent studies conducted in understanding the association between EIF2B5 and MS risk. The need of hour is to conduct large scale conclusive studies aimed at expounding the mechanisms behind this relationship.

Keywords

CNS • EIF2B • EIF2B5 • MS • Polymorphism • Susceptibility • VWM

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Abbreviations

CD45	protein tyrosine phosphatase receptor type C
CNS	central nervous system

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CYP27B1	cytochrome P450 family 27 subfamily B member 1
ε	epsilon
eIF2	eukaryotic translation initiation factor 2
eIF2B	eukaryotic translation initiation factor 2B
EIF2B5	gene
eIF2B	protein
GTP	guanosine triphosphate
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSP	heat shock proteins
IL6	interleukin 6
IL-7R	interleukin 7 receptor
MS	multiple sclerosis
MSIF	multiple sclerosis international federation
OMIM	online mendelian inheritance in man
PP	primary progressive
PR	primary relapsing
RR	relapsing-remitting
SNP	single nucleotide polymorphism
SP	secondary progressive
VWM	vanishing white matter
WHO	world health organization

5.1 Introduction

Multiple sclerosis (MS) (Online Mendelian Inheritance in Man, OMIM 126200) also known as disseminated sclerosis or encephalomyelitis disseminata is a convoluted debilitating and long lasting neurological disease caused by demyelination due to an immune attack against the insulating fatty myelin sheath covering the axons of the brain and spinal cord, thereby leading to destruction and dysfunction of nerve cells which results in interruption of the nervous transmission within the brain, and between the brain and spinal cord and other parts of the body (Compston and Coles 2008). It was first described by Jean-Martin Charcot in the year 1868 and the word multiple sclerosis *per se* describes the scars in form of plaques or lesions in the central nervous system (CNS) (Clanet 2008). It is believed that the underlying mechanism behind the disease pathogenesis involves auto-immunological and inflammatory components, thus having an auto-inflammatory nature. The distortion of the nervous transmission leads to a wide continuum of unpredictable signs and symptoms in MS patients affecting different parts of the body depending on the location of the lesion in the brain (Compston and Coles 2008). The symptoms can range from numbness or tingling to blindness and paralysis and these changes can be permanent or temporary (Compston and Coles 2008). With time, the pathological processes underlying it result in gradual neurological deterioration.

The most striking feature about MS prevalence is its regional divergence. Although some studies show a stable incidence worldwide, the number of cases in other regions seems to be increasing. It affects almost all populations across the world, but is more prevalent in Caucasians (Rosati 2001). According to the data presented in 2013 Atlas of MS by the World Health Organization (WHO) and Multiple Sclerosis International Federation (MSIF) (http://www.atlasofms.org), there are approximately 2.3 million people suffering from MS across the globe, reflecting a global increase in its number as compared to the previous data of 2008 Atlas of MS. The reason for this increase is not known. The global median prevalence of MS is about 33 per 100,000 (Atlas of MS, 2013; http://www.atlasofms.org). It occurs more commonly in females than males (Rosati 2001), affecting more often the young to middle-aged people in the age-group of 20–45 years with average age of onset being 30–34 years, however it can also develop occasionally in children and elderly (Compston and Coles 2008; Pugliatti et al. 2006).

There is no single tool for the diagnosis of MS. It often goes misdiagnosed or under diagnosed. It is primarily diagnosed with help of the most commonly used McDonald diagnostic criteria (Polman et al. 2011). By and large MS diagnosis is done through combination of different parameters and is therefore based on the clinical presentation and findings of biochemical and radiological assessment. Clinically, on the basis

of disease course, MS has been categorised into different courses namely relapsing-remitting (RR), secondary-progressive (SP), primary-progressive (PP) and progressive-relapsing (PR) which vary in terms of severity, prevalence and degree of progression (Lublin and Reingold 1996). MS remains a medical mystery and till date there is no cure for it, however for its effective management several therapies have been devised with only partial effect on its progression and course. Its treatment is based on the underlying immunologic aspect and the symptoms manifested over time. The etiology of MS remains poorly understood and studies have shown that it primarily results due to complex interactions between the genetic and environmental components, but genetic risk factors seem to preponderate in its development (Ebers 2008; Goodin 2010; Ramagopalan et al. 2010) (Fig. 5.1). Its susceptibility is governed by different local environmental factors and as such its incidence and prevalence rates vary accordingly (Rosati 2001). The most commonly found risk factors reported to play a role in MS are infectious agents (Compston and Coles 2002), smoking (Ascherio and Munger 2007), vitamin D (Ascherio et al. 2010; Kulie et al. 2009) and geographical location (Rosati 2001). It has been found that MS risk can also be modulated by human immunodeficiency virus (HIV) infection, however it leads to reduced risk of developing MS in infected individuals (Khan et al. 2015).

In the history of human genetics, investigating the genetic aspects of such a convoluted disease remains a biggest challenge till date. It has been seen that alterations in different candidate genes play an important role in MS susceptibility and its genesis (Dyment et al. 2004). It is believed that multiple allele/sequence variants of MS candidate genes may influence the expression of proteins they encode, leading to altered immune response (in case of immune specific gene) within body (Nischwitz et al. 2011). Although it is not believed to be a hereditary disease, however, multifarious variations in form of polymorphisms have been found to play an essential role in its predisposition and it is found to be more prevalent in some ethnic groups (Dyment et al. 2004). Family history plays a crucial role in determining disease risk and poses generally higher risk among relatives as compared to general population (Nielsen et al. 2005).

In the last 20 years, considerable progress has been made in exploring the genetics of MS and

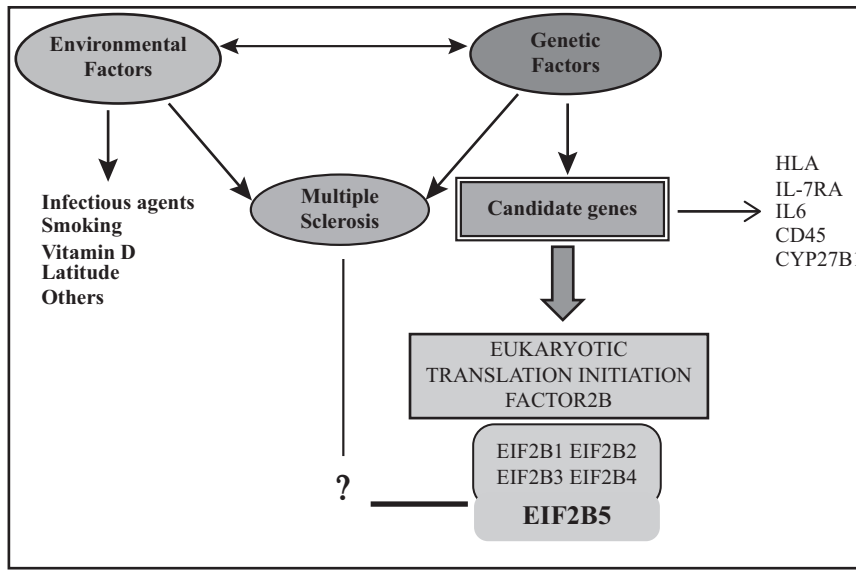

Fig. 5.1 Multiple sclerosis as a multifaceted disease. Complex interactions between different genetic and environmental factors lead to MS

from the findings of some main studies, it has been found that MS susceptibility is linked to numerous genes such as human leukocyte antigen (HLA) (Oksenberg et al. 2004; Olerup and Hillert 1991), interleukin-7 receptor alpha (IL-7RA) (Lundmark et al. 2007), interleukin 6 (IL6) (Mirowska-Guzel et al. 2011), protein tyrosine phosphatase receptor type C (CD45) (Jacobsen et al. 2000), and cytochrome P450 family 27 subfamily B member 1 (CYP27B1) (Ramagopalan et al. 2011). Keeping in view the genetic heterogeneity of MS, even heat shock proteins (HSPs) located within the HLA complex have been reported to play a role in MS predisposition (Brosnan et al. 1996). Incidentally, recent studies have suggested a possible role of eukaryotic translation initiation factor 2B5 (EIF2B5) in MS susceptibility (Ungaro et al. 2011; Haq et al. 2015; Zahoor et al. 2014a), however there is no conclusive report on its role in MS due to conflicting results from different studies carried out on different populations (Fogli et al. 2008; Lucas et al. 2007; Pronk et al. 2008; Zahoor et al. 2014b, 2015). The controversial results regarding the possible association of EIF2B5 gene with MS susceptibility can be attributed to MS heterogeneity, indicating possible influence of different genetic factors together with local environmental factors in governing MS prevalence across different populations. This prompted us to write this chapter to explore the link between MS and EIF2B5. The intensive knowledge of the molecular genetics behind MS can unveil the role played by genes/allelic variants in making it a complex heterogeneous disease as it involves multiple pathways mediated by many genes. At the same time, the identification of different polymorphisms could probably help in effective treatment of the disease during early stages. Consequently, investigating the single nucleotide polymorphisms (SNPs) in EIF2B5 gene in different ethnic groups can further illuminate the linkage between genetics and MS development. This chapter focuses on progress made in unlocking the enigmatic relationship between EIF2B5 and MS.

5.2 MS and EIF2B5

Numerous studies have shown contribution of eukaryotic translation initiation factor 2B (EIF2B) gene family in causing vanishing white matter disease (VWM) (OMIM 603896) of the brain (Leegwater et al. 2001; van der Knaap et al. 2002) and due to similarities of certain features between VWM and MS in terms of involving CNS and chronic disease progression course with gradual episodic worsening of the symptoms (van der Knaap et al. 1998), EIF2B genes have turned out to be the centre of focus for researchers (Haq et al. 2015; Ungaro et al. 2011; Zahoor et al. 2014a; Zahoor et al. 2015). In addition to the above-indicated points, both the conditions get aggravated during fever and elevated temperatures as in febrile infections (Schwid et al. 2003; Sibley et al. 1985; van der Knaap et al. 2006), thus justifying looking for a possible association between EIF2B and MS. It has been reported that both MS and VWM are susceptible to stress caused due to heat, as a result of which variations in EIF2B gene family might be a susceptibility/risk factor increasing the risk of MS development and at the same time influencing disease process underlying it (Pronk et al. 2008; van der Knaap et al. 2006). It is already known that most of the alterations in EIF2B5 not only cause VWM but also other variable phenotypes, making it an active centre for research in similar overlapping diseases. In view of the fact that EIF2B5 among EIF2B genes encodes the catalytic epsilon (ε) subunit of eIF2B heteropentameric complex, it is quite obvious that alterations in this gene may probably have a profound impact on the role played by eIF2B complex (Proud 2001), thus rationalizing looking for association between EIF2B5 and MS susceptibility. Considering this, it is therefore quite possible that variations in EIF2B5 might play a role in MS predisposition by promoting or provoking the underlying disease process. The findings from recent studies have strongly suggested its role in MS predisposition (Haq et al. 2015; Ungaro et al. 2011; Zahoor et al. 2014a).

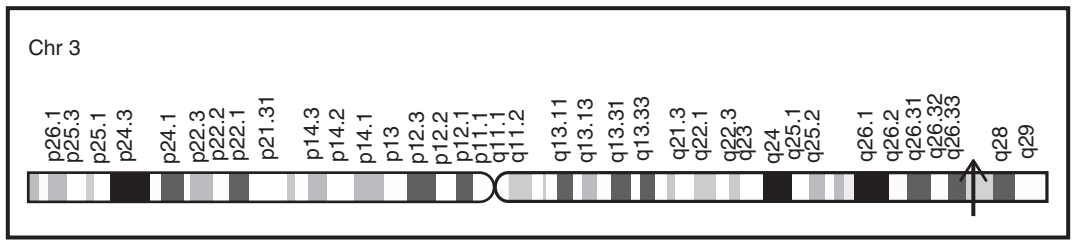

Fig. 5.2 Molecular location of EIF2B5 gene on Chromosome 3 (Source: Gene Cards; http://www.genecards.org)

EIF2B5 (HGNC 3261; UniProtKB Q13144; OMIM 603945) belongs to EIF2B gene family and is located from base pair 184,134,435 to 184,145,311 on the long (q) arm of chromosome 3 (Reference GRCh38.p2 Primary Assembly; RefSeq Chromosome NC_000003.12) at position 27.1 (Fig. 5.2). It spans about 2655 bp region and contains 16 exons which encode a protein of 721 amino acids having molecular mass of 82 kDa. eIF2Bε is a multi-domain protein that is widely found in eukaryotes. It is an essential subunit of the eIF2B complex as it is the largest among other subunits and encloses the minimal catalytic centre (Gomez and Pavitt 2000). Its carboxyl terminal contains the crucial functional domain that binds with its substrate eukaryotic translation initiation factor 2 (eIF2), and facilitates guanosine triphosphate (GTP) exchange on eIF2 in the initiation phase of protein synthesis, resulting in reprocessing of active GTP bound eIF2 for another round of translation (Abbott and Proud 2004). It has become a key player in translation initiation regulation as it contains different phosphorylation sites regulated by diverse signals generated in response to multifarious cellular conditions (Vary et al. 2002; Wang et al. 2001).

Being a ubiquitously expressed protein, its role in neuronal diseases such as MS still remains unclear. It might play a role in MS due to its function in myelination process which requires synthesis of large amounts of lipids and proteins and thus regulation of translation initiation under different stress conditions such as protein misfolding. Consequently, molecular analysis of EIF2B5 in MS could pave way for the purpose of differential diagnosis and improved disease management. In view of the fact that there has been a significant increase in MS prevalence across the globe (Wasay et al. 2006), it becomes obligatory to look for the status of MS related gene SNPs in different populations worldwide. Although a few crucial studies dealing with immunological and genetic aspects of MS have been conducted, yet, there is lack of large scale conclusive epidemiological studies focusing on incidence and prevalence rates of MS across different parts of the world. Considering the present number of persons living with MS across globe, even the rate reported by recent epidemiological findings appears to be underestimated. Furthermore, it becomes obligatory to have a deeper understanding of the role played by racial differences on the selective prevalence of certain sequence variants in a particular population and thereby determine the ultimate impact on MS susceptibility and its proper diagnosis along with global divergence in its geographic prevalence. A variety of potential factors such as methodological and genetic differences related to the diversity of populations are thought to explain the discrepancies found in geographical predominance of certain variants in MS (Bhatia et al. 2015; Pandit and Kundapur 2014).

To the best of our knowledge, there has been no study on MS and EIF2B5 in Asian populations and ours being the first study to evaluate the association of EIF2B5 with MS susceptibility. Analysis of EIF2B5 gene may be helpful for identification of different variations which can significantly influence drug response in MS patients as they are supposed to cause phenotypic differences among individuals. Allelic variants can be used to identify persons at high risk of developing MS and this might enable physicians to use selective approach towards disease management in them. This would entail better under-

standing of the pathophysiological process behind MS development which will certainly aid in establishing newer therapeutic targets. In MS patients, personalized prophylactic regimen can be used which will reduce the disease severity and its progression and it will eventually reduce the likelihood of developing major disability and thus increase the life expectancy. It would be beneficial to look for any adverse effects of drugs in these patients which would eventually enable the development of new drugs at cheaper rates and with fewer side effects in addition to individualized drug therapy.

Our research provides an impetus to explore the association of EIF2B5 with MS susceptibility. It has provided much needed ground for understanding the litigious relationship between the two. Further, there is a need to recognize different MS susceptibility loci in non-HLA genes and look for their relationship in MS development in larger sample number from low prevalence regions. Till date, the studies in this milieu hitherto are not enough, and there is extreme need for an extensive upsurge in large scale conclusive molecular and epidemiological studies in low-risk regions which are unfortunately possible only in high prevalence populations like USA and European regions due to time consuming process of data collection in low prevalence regions.

The variations in EIF2B5 might alter the ability of cells to counter various environmental insults, therefore predisposing individuals to MS. Probably, SNPs especially non-synonymous variants might play a crucial role in the functional diversity of eIF2Bε by destabilizing its structure or altering its solubility, and this may eventually affect its substrate binding ability, nucleotide exchange activity and its sensitivity to regulation as it is known to harbour crucial phosphorylation sites and might be therefore associated with MS susceptibility. Accordingly, evaluation of the EIF2B5 gene in MS could play a role in its differential diagnosis and management. Given the fact that there has been a substantial increase in MS prevalence over the last decade in Asian continent (Wasay et al. 2006), it becomes essential to look for the genetic divergence of MS in different Asian populations as well. This hints towards the incomprehensible role of multifarious unknown environmental risk factors and their possible interactions with relevant genetic components in changing the perspective of MS. All this will certainly aid in knowing the epidemiological status of MS across different parts of the world and also pave the way for research in genetics and drug development for this multifaceted disease.

5.3 Conclusion

In conclusion, our studies have expanded the spectrum of EIF2B5 polymorphisms associated with MS susceptibility, suggesting the potential benefit for more selective and appropriate prophylaxis and personalized treatment for MS. Although, the outcome of our pilot study is novel, suggesting a possible role of EIF2B5 variations in MS predisposition; however a biological corroboration through *in vitro* analysis is required to elucidate the actual biological implications on the function of eIF2Bε vis-a-vis eIF2B complex formation in relation to MS development. At the same time due to limited power of our study, an in-depth larger population based, case control studies, as well as well-designed mechanistic studies are warranted to validate our findings and screening of other genes in EIF2B family needs to be done, to present a cumulative genetic profile influenced by genetics and environment in the genesis of MS. At the same time, studies focussing on incidence and prevalence rates of MS worldwide should be conducted by having active collaborations between a wider group of neurologists and researchers across the globe.

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Molecular Genetic and Epigenetic Basis of Multiple Sclerosis

6

Zohreh Hojati

Abstract

Multiple Sclerosis (MS) is a chronic immune-mediated disease of spinal cord and brain. The initial event in MS occurs when activated $CD4^+$ T cells in periphery exacerbates immune responses by stimulating immune cells such as B cells, $CD8^+$ cells, mast cells, granulocytes and monocytes. These proinflammatory cells pass blood brain barrier by secreting proinflammatory cytokines including TNF-α and INF-$_\gamma$ which activate adhesion factors. APCs (antigen-presenting cells) reactivate $CD4^+$ T cells after infiltrating the CNS and $CD4^+$ T cells produce cytokines and chemokines. These proinflammatory cytokines aggravate inflammation by inducing myelin phagocytosis through microglia and astrocytes activation. MS is believed to have a multifactorial origin that includes a combination of multiple genetic, environmental and stochastic factors. Although the exact component of MS risks that can be explained by these factors is difficult to determine, estimates based on genetic and epidemiological studies suggest that up to 60–70 % of the total risk of MS may be contribute to genetic factors. In continue, firstly we provide an overview of the current understanding of epigenetic mechanisms, and so present evidence of how the epigenetic modifications contribute to increased susceptibility of MS. We also explain how specified epigenetic modifications may influence the pathophysiology and key aspects of disease in MS (demyelination, remyelination, inflammation, and neurodegeneration). Finally, we tend to discuss how environmental factors and epigenetic mechanisms may interact to have an effect on MS risk and clinical outcome and recommend new therapeutic interventions that might modulate patients' epigenetic profiles.

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Keywords

Epigenetic basis • Experimental autoimmune encephalomyelitis • Multiple sclerosis

6.1 Introduction

Multiple Sclerosis (MS) is a chronic immune-mediated disease of spinal cord and brain. Both MS and Experimental Autoimmune Encephalomyelitis (EAE), an animal model of MS, are generally characterized by demyelination, malapropos activation of innate immune cells, aberrant activation of Th1 and Th17 and irregular secretion of cytokines/chemokines (Bhat and Steinman 2009; Ransohoff 2009). The initial event in MS occurs when activated $CD4^+$ T cells in periphery exacerbates immune responses by stimulating immune cells such as B cells, $CD8^+$ cells, mast cells, granulocytes and monocytes. These proinflammatory cells pass blood brain barrier by secreting proinflammatory cytokines including TNF-α and INF-$_\gamma$ which activate adhesion factors. APCs (antigen-presenting cells) reactivate $CD4^+$ T cells after infiltrating the CNS and $CD4^+$ T cells produce cytokines and chemokines. These proinflammatory cytokines aggravate inflammation by inducing myelin phagocytosis through microglia and astrocytes activation. Additional damage to CNS can be occurred by B cells and auto-antibodies (Sospedra and Martin 2005). Since dysregulation of natural signaling pathways can play a critical role in MS pathogenesis, distinguishing their aberration and designing therapeutic drugs are considered as a main goal for scientists. Here we review some of the most important pathways involving in inflammatory responses and mention their association with MS.

Notwithstanding Multiple sclerosis (MS) is not considered to be an inherited disorder, genetic factors have been embroiled in susceptibility to this condition. Genetic linkage studies and genome-wide association studies (GWAS) have recognized genes that may confer susceptible people to develop disease (Patsopoulos and de Bakker 2011; Sawcer et al. 2011). Several genetic loci are regarding an elevated possibility of developing MS. Probably the most studied of such is HLA-DRB1 on chromosome 6 (Sawcer et al. 2011). Cumulatively, the susceptibility loci identified to date by GWAS can account for only a part of this genetic risk and additional investigation are necessary to identify the actual missing heritability of the MS risk (Manolio et al. 2009). MS is believed to have a multifactorial origin that includes a combination of multiple genetic, environmental and stochastic factors (Goodin 2014). Although the exact component of MS risks that can be explained by these factors is difficult to determine, estimates based on genetic and epidemiological studies suggest that up to 60–70 % of the total risk of MS may be contribute to genetic factors (Hawkes and Macgregor 2009; Westerlind et al. 2014). The most persuading evidence originates from twin studies. If the information contained inside the DNA would merely determine disease susceptibility, the concordance rate of MS development in monozygotic twins should be the same. However, the low concordance rate of MS in twins (roughly 30–40 % in monozygotic twins and 3–5 % in dizygotic twins) indicating that genetic factors solely cannot be the main reason behind the condition (Gourraud et al. 2012). DNA sequence modifications of MS-related genes are just a minor factor in the development of the disease and there is growing enthusiasm in the epigenetics of complex conditions such as MS which can be supposed to simplify part of the missing heritability. Epigenetic mechanisms are believed to be involved in the processes that affect the way that environmental risk factors influence disease development and genetic and environmental factors have interaction with each other (Feinberg 2007). Recently, emerging data recommend that epigenetically regulated mechanisms may contribute to the pathophysiology of MS and describe a part of the 'missing heritability' (Fig. 6.1).

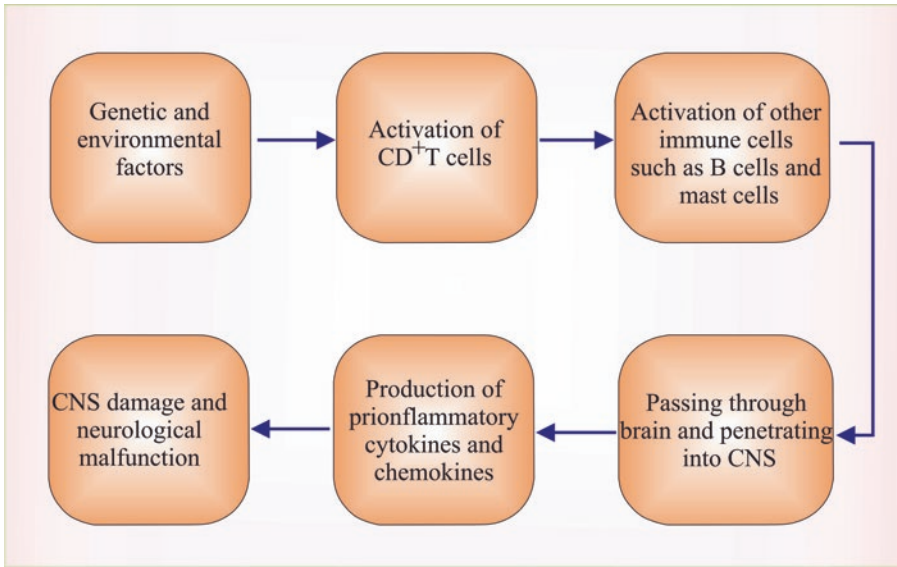

Fig. 6.1 A brief summary of pathophysiology of Multiple Sclerosis

In continue, firstly we provide an overview of the current understanding of epigenetic mechanisms, and so present evidence of how the epigenetic modifications contribute to increased susceptibility of MS. We also explain how specified epigenetic modifications may influence the pathophysiology and key aspects of disease in MS (demyelination, remyelination, inflammation, and neurodegeneration). Finally, we tend to discuss how environmental factors and epigenetic mechanisms may interact to have an effect on MS risk and clinical outcome and recommend new therapeutic interventions that might modulate patients' epigenetic profiles.

6.2 JAK-STAT Pathway

The JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription) pathway is a pivotal signal used by various cytokines to initiate innate immunity, coordinate adaptive immunity and eventually restrict inflammatory responses (O'Shea and Plenge 2012). The JAK/STAT pathway is composed of three main parts: (1) Receptor (2) JAK (Janus Kinase) (3) STAT (Signal Transducer and Activator of Transcription). JAK family consists of 4 members including Jak1, Jak2, Jak3, and Tyk2. All of the members are characterized by seven JAK homology (JH) domains. JH1 and JH2 respectively represent tyrosine kinase domain and pseudokinase domain. Despite of lacking activity, JH2 is vital for regulating kinase activity. Adjacent to the pseudokinase domain is Src homology 2 (SH2) domain. The N- terminal of JAKs is JH5–JH7 also known as FERM domain which mediates interaction with cytokine receptors and also orchestrates catalytic activity (O'Shea et al. 2004) Fig. 6.2a. A total of seven STATs has been reported for mammalian cells: STAT1, -2, -3, -4, -5a, 5b, and -6 (Ihle 2001). STAT proteins have a transactivation domain with one or two crucial amino acids in their C-terminal. These amino acids include a tyrosine (Y) residue which mediates the activation and dimerization of two monomers through its phosphorylation and a serine (S) residue that enhances transcriptional activity whenever it is phosphorylated. Adjacent to transactivation domain, a SH2 domain, a Linker, a DNA binding domain, a coiled –coil domain and an N-terminal domain are located sequentially (Miklossy et al. 2013) Fig. 6.2b. In JAK/STAT signaling, the interaction between cytokines and receptor-associated JAKs leads to phosphorylation of receptor cytoplasmic domain resulting in

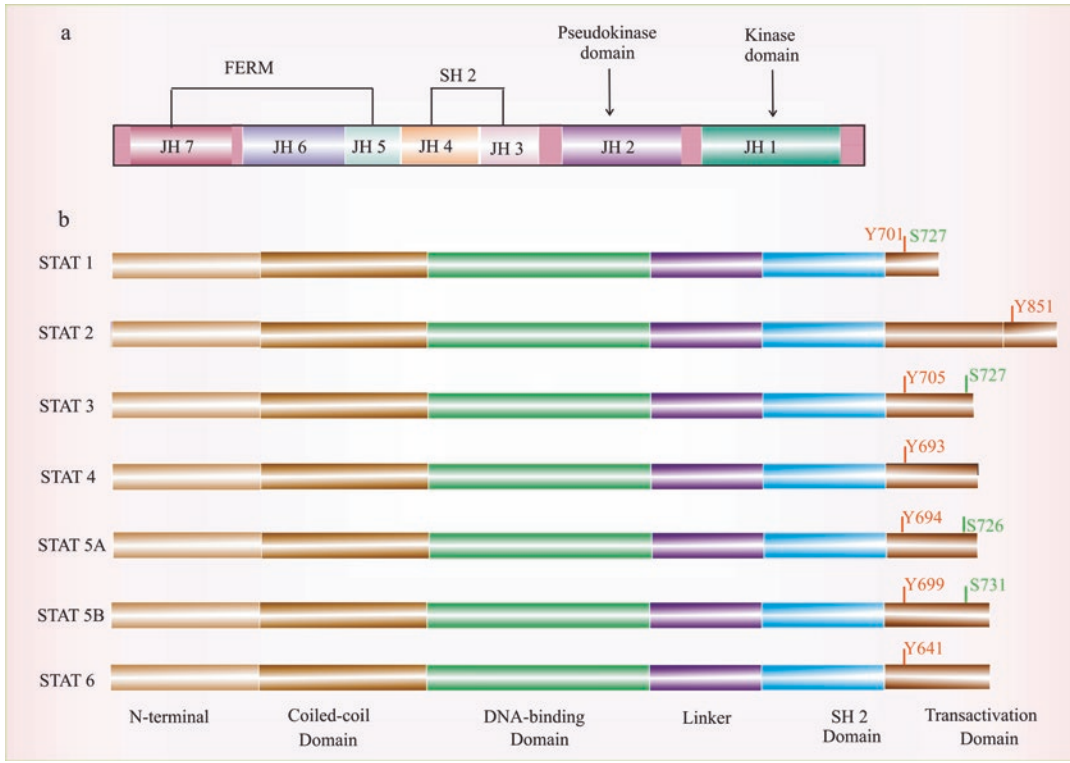

Fig. 6.2 Schematic structure of JAK and STAT proteins. (**a**) Having 7 JAK homology (JH) domains is a common feature in all members of JAK family. (**b**) STAT members have similar N-terminal domain, coiled-coil domain, DNA binding domain, linker and SH2 domain but their transactivation domain is different

recruitment of STATs. Subsequently, the JAKs phosphorylate STATs on the tyrosine residue increasing their activation. Activated STATs in turn dimerize, localize in the nucleus and regulate transcription of target elements Fig. 6.3. Several combinations of JAKs and STATs contribute in different gene expression patterns. As a result, cytokines are the cornerstone in regulating the development and function of myeloid cells and T-cells by means of JAK/STAT pathway (Weaver et al. 2007; Geissmann et al. 2010). For instance, IL-12 induces Th1 differentiation by JAK2/TYK2 and STAT4 activation while IL4 induces Th2 cell differentiation using JAK1/3 and STAT6. IL-6 and IL-23 also lead to Th17 differentiation by JAK1/2 and STAT3 activation (Weaver et al. 2007; Harris et al. 2007; Fig. 6.4). Other functions of JAK/STAT pathway in natural immune cells are regulating the impacts of INF-$_{\gamma}$ on macrophages through JAK1/2 and STAT1, involving in IL-6 family signals by JAK1/2 and STAT3 and mediating GM-CSF signaling by JAK2 and STAT5. Consequently, dysregulation of JAK/STAT pathway is correlated with various immune-mediated diseases such as MS (O'Shea and Plenge 2012).

6.2.1 JAK/STAT Pathway and MS

The entire JAK/STAT pathway components including cytokines and their receptors, JAks, STATs and also SOCs are related to autoimmune diseases. Significant overexpression of cytokines and cytokine receptors have been reported such as IL-2RA, IL-7, IL-7R, IL-12, IL-22R and OSMR. Hyperactivation of STATs has been created by excessive amount of cytokines and downregulation of JAK inhibitors especially SOCS (Suppressor of cytokine signaling).

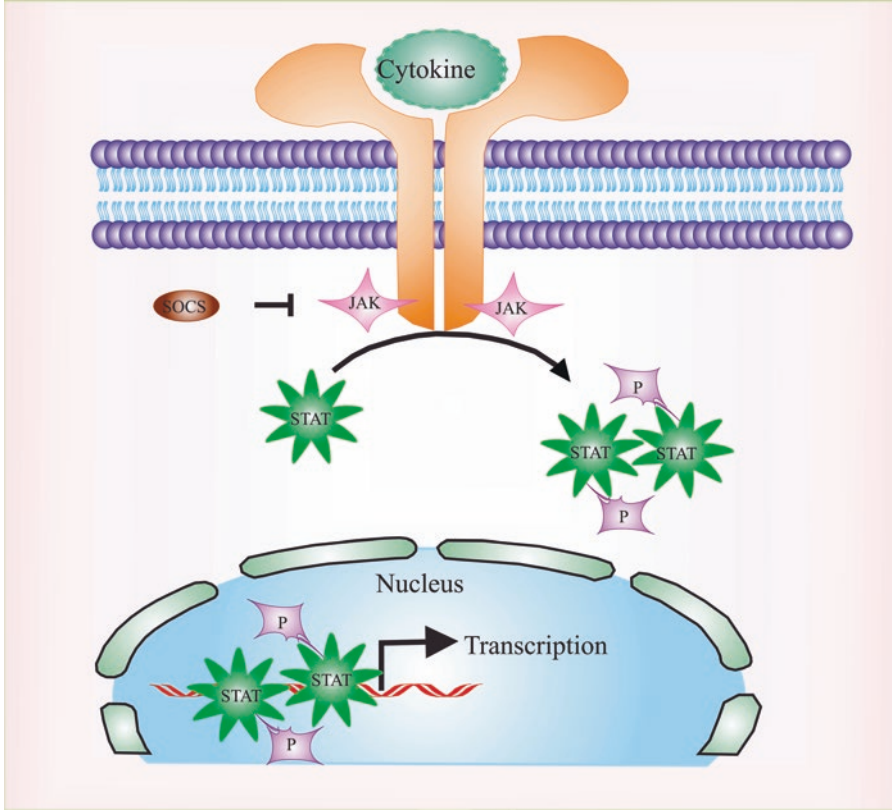

Fig. 6.3 A schematic representation of JAK/STAT pathway. Following the induction of cytokine receptors, activated JAKs result in the STAT phosphorylation, dimerization and translocation to the nucleus regulating target genes expression

Overexpression of some target genes of STAT has been therefore demonstrated in MS and EAE including IL-23R, IL21, IL22, INF-$_{\gamma}$, CXCR3 and HLA-DR Fig. 6.5. STAT3 has been also introduced as a susceptibility gene of MS (Baranzini et al. 2009). Monocytes and T-cells isolated from MS patients in relapsing phase contained exuberant amount of activated STAT3 in comparison of those from patients in remission (Frisullo et al. 2006). A single nucleotide polymorphism of SOCS1 has been approved to be a risk factor of MS (Vandenbroeck et al. 2012). Liu et al. have proposed a promising treatment by AZD1480, a JAK1/JAK2 inhibitor in 2014. This therapeutic procedure precludes immune cells to infiltrate into the CNS, prevents STAT activation, decreases expression of cytokines and chemokines, reduces demyelination and lessens pathogenic responses of Th1 and Th17 (Liu et al. 2014).

6.3 NF-κB Pathway

Although numerous risk factors are known for neurodegenerative diseases such as Multiple Sclerosis, Alzheimer's disease and etc., inflammatory response is the common cause of all neural cell lose. NF-κB is a principle transcription factor which regulates inflammatory responses (Gupta et al. 2010). Unlike most other tissues, this transcription factor is at high levels in neurons showing its critical role in CNS. In more detail, NF-κB significantly controls neural morphology, memory, learning and behavior. A total of 5 members has been detected in NF-κB/Rel family: P50, P52, P65 (Rel-A), Rel-B and c-Rel which are present in both homo- and heterodimers Fig. 6.6a. NF-κB dimers are inhibited by binding to IκB proteins in resting cells (Kaltschmidt et al. 1994). NF-κB can be acti-

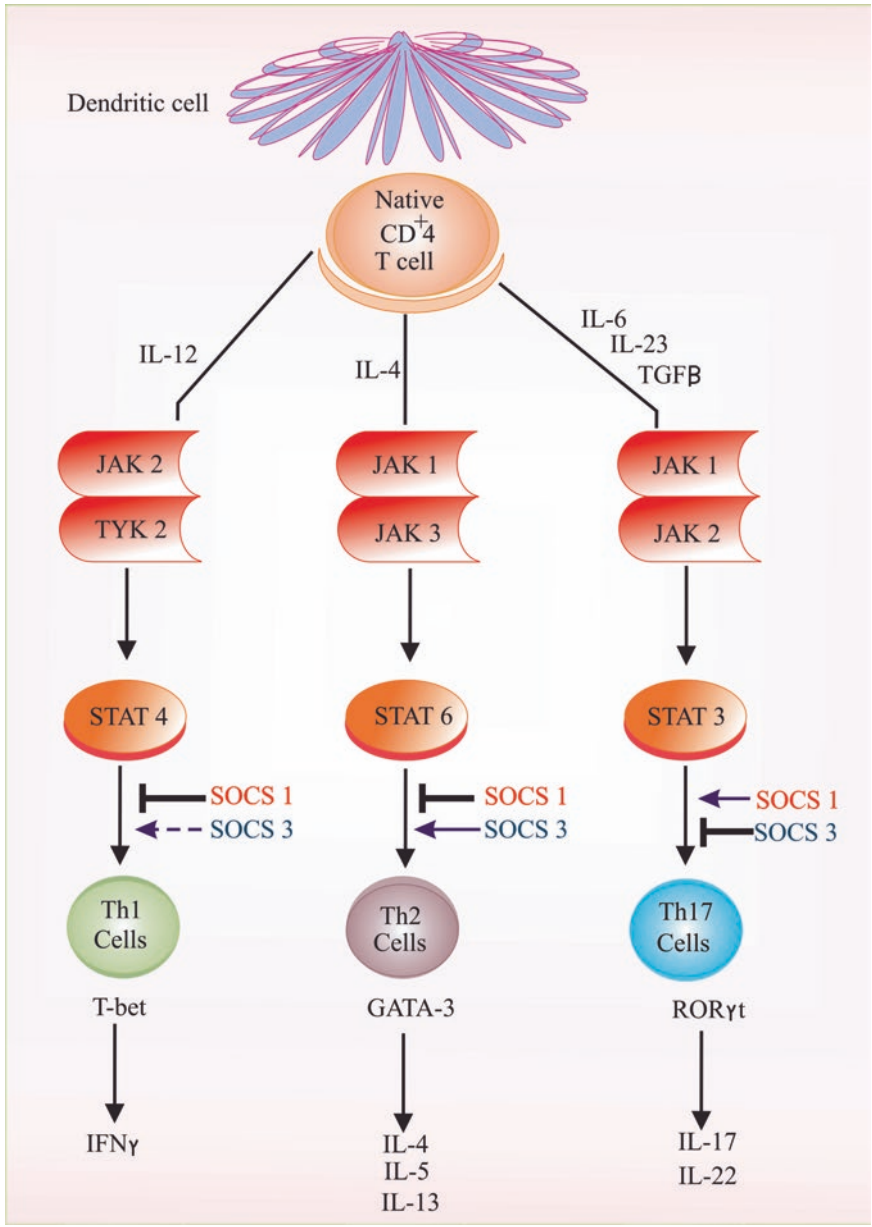

Fig. 6.4 Critical role of JAK/STAT signaling in differentiation of CD4+ T-cells. Various cytokines and JAK/STAT combinations induce distinct differentiation

vated by canonical or non-canonical pathway Fig. 6.6b. A shared step in these two pathways is IκB kinase (IKK) complex activation. IKK1/IKKα and IKK2/IKKβ (catalytic subunits) and NEMO/IKK$_\gamma$ (regulatory subunit) of IKK complex are present in canonical pathway whereas only IKK1/IKKα is active in non-canonical pathway. Activated IKK complex leads to respectively phosphorylation, ubiquitination and degradation of IκB proteins by proteasomes. Subsequently, Released NF-κB dimers can freely localize in the nucleus and regulate expression of target genes (Sarnico et al. 2009; Camandola and Mattson 2007). The canonical signal is triggered

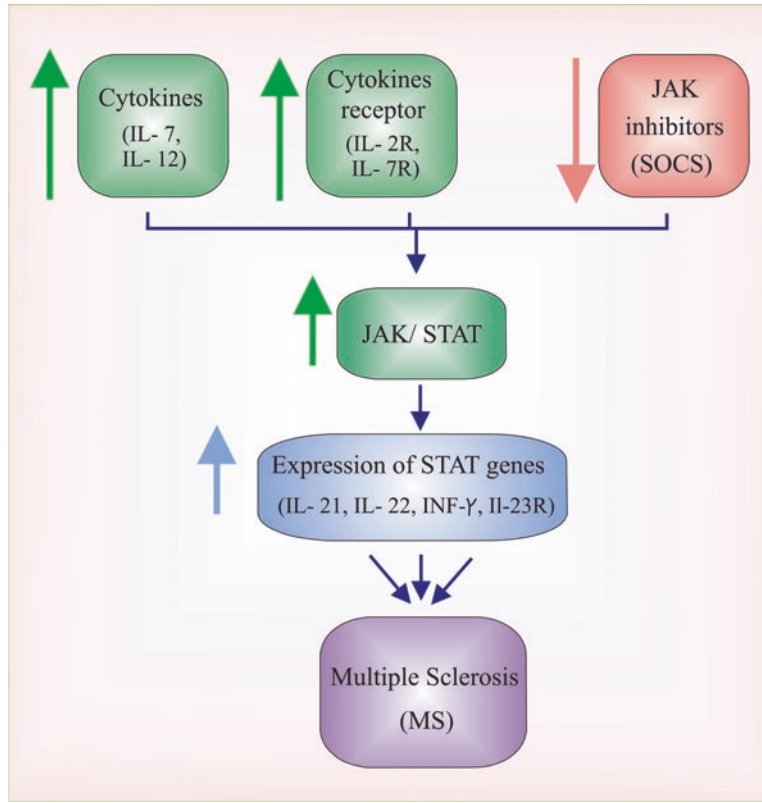

Fig. 6.5 Schematic representation of JAK/STAT role in Multiple Sclerosis

by Toll-like receptors (TLRs), tumor necrosis factor receptor 1 (TNFR1), T cell and B cell receptors (TCR and BCR) and IL-1 receptor (IL-1R). The non-canonical pathway is mediated by particular receptors including CD40, B cell activating factor receptor (BAFF-R) and Lymphotoxin β receptor.

Interestingly, NF-κB plays two opposite roles in neurodegeneration and neuroprotection depending on distinct activation of its subunits (Sarnico et al. 2009). Neuroprotection of NF-κB is stimulated by IL-1β, nerve growth factor (NGF) or metabotropic glutamate receptor-5 (mGluR5) following IKK phosphorylation, IκB degradation and activation of NF-κB dimers containing c-rel. Activated NF-κB dimers then translocate to the nucleus and induce expression of anti-apoptotic, anti-inflammatory and neuroprotective genes (Pizzi et al. 2002). On the other hand, Stimuli such as Aβ peptides increase oxidative stress and Ca^{++} in the neural or glial cells, leading to IKK phosphorylation, IκB ubiquitination and degradation and p50:p65 activation. Activated p50:p65 mediates expression of pro-apoptotic, pro-inflammatory and neurotoxic genes correspondingly include IL-12 and IL-17, caspases and Bax, induced nitric oxide synthase (iNOS). In healthy conditions, there is a balance between c-rel containing heterodimers and p50:p65 dimers whereas in neurodegenerative diseases, activated p50:p65 dimers increase in comparison to c-rel containing heterodimers, causing hyperactivation of pro-apoptotic and pro-inflammatory factors Fig. 6.7 (Srinivasan and Lahiri 2015).

6.3.1 NF-κB and Multiple Sclerosis

There is remarkable correlation between NF-κB and JAK/STAT pathway and they act in a feed-forward loop (McFarland et al. 2013). Significantly, an association between single nucleotide polymorphism in numerous compo-

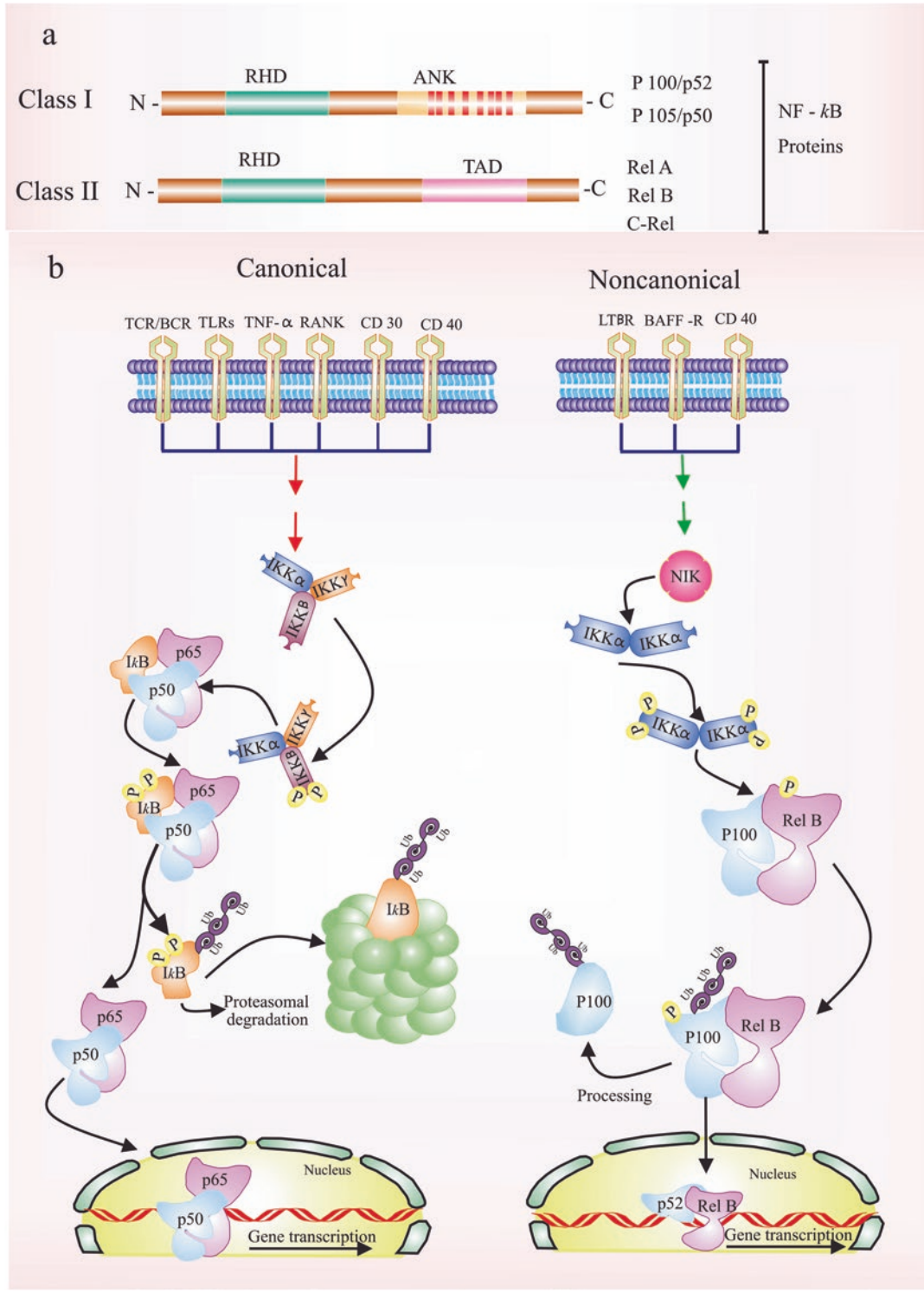

Fig. 6.6 Domain structure of NF-κB subunits and canonical and non-canonical pathways of NF-κB. (**a**) All members of NF-κB/Rel have a conserved domain termed the Rel homology domain (RHD) which is essential for IκB inhibitor binding and nuclear localization. The C-terminal of the Rel subunits has transcriptional activation domain (TAD) while the C-terminal of the NF-κB subunits has ankyrin repeat-containing inhibitory domains (ANK). (**b**) The canonical pathway of NF-κB is triggered by proinflammatory cytokines which bind to the antigen receptors TCR/BC, TLRs, receptor activator of NF-κB (RANK), CD30 and CD40. On the other hand, B cell activating factor receptor (BAFF-R), lymphotoxin β receptor (LT βR) and CD40 engage by non-canonical pathway of NF-κB resulting in production and translocation of p52/RelB into the nucleus

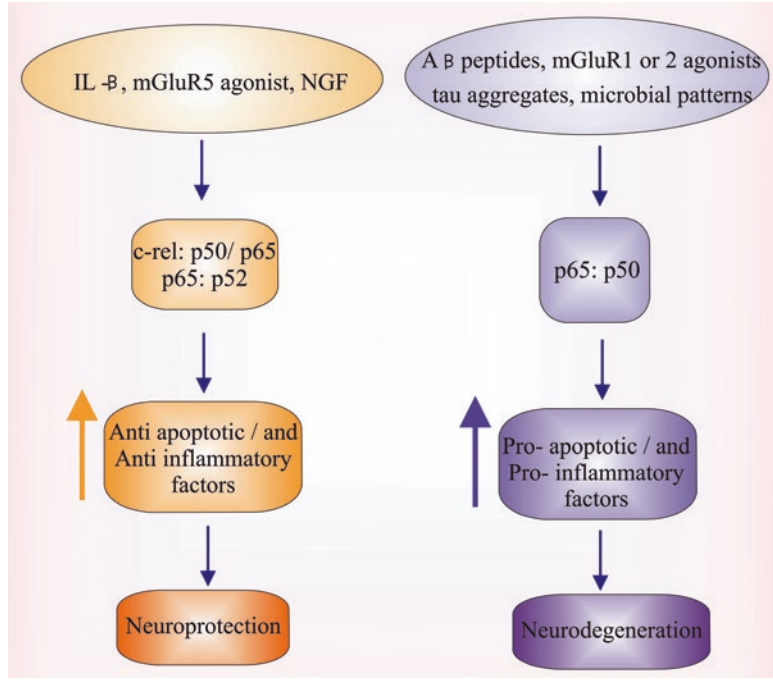

Fig. 6.7 Schematic illustration of NF-κB role in neurodegeneration and neuroprotection. Activation of the non-canonical pathway leads to induction of anti-inflammatory genes and anti-apoptotic factors and finally results in neuroprotection status whereas the canonical pathway plays an opposing role in neurodegeneration by transactivation of pro-inflammatory factors and pro-apoptotic genes (Adapted from Srinivasan and Lahiri 2015)

nents of the NF-κB signal and MS has been displayed suggesting NF-κB hyperactivation. A missense mutation in position 738 of IκBL causes MS susceptibility. An insertion in the promoter of IκBα gene is also associated with primary progressive phase (Miterski et al. 2002) Overexpression of p65, p50 and c-Rel has been reported in macrophages situated in active demyelinating regions (Gveric et al. 1998). NF-κB is the pivotal modulator of demyelination via increasing neuroprotection molecules and inhibiting immune responses. Deletions in IKKβ therefore lessen neuroprotective factors resulting in severe axonal damage (Emmanouil et al. 2011). Different expression of 43 genes regulating NF-κB or regulated by NF-κB has been shown in relapse and remission phase by DNA microanalysis highlighting NF-κB pathway in regulating T cells during relapse (Satoh et al. 2008). The genome –association wide studies (GWASs) have introduced TNFRSF1A (TNF-R1 gene) and MALT1 as MS susceptible loci (De Jager et al. 2009)(Gilli et al. 2011). A general model of the role of NF-κB in MS has been suggested. According to this model, hyperactivation of NF-κB in various cell types induces inflammatory cytokines, adhesion molecules, induced nitric oxide synthase (iNOS) and reactive oxygen species (ROS) (Srinivasan and Lahiri 2015) Fig. 6.8.

6.4 Notch Signaling

The classic Notch signaling is composed of 4 heterodimeric receptors (Notch 1–4) and 5 ligands (Jagged 1, 2 and Delta-like 1, 3 and 4) (Fleming 1998). Ligand-receptor binding in Notch signaling is followed by two proteolytic reactions catalyzed by ADAM metalloproteases and a family of γ-secretases. These consecutive reactions cause the cleavage and releasing of Notch intracellular domain (NICD). Subsequently, NICD translocates to the nucleus and form a transcriptional complex with MAML modulating their target genes (Bray 2006) Fig. 6.9.

6.4.1 Notch Signaling and MS

Several functions have been reported for Notch signaling in MS lesions. Firstly, Notch pathway inhibits the differentiation of oligodendrocytes

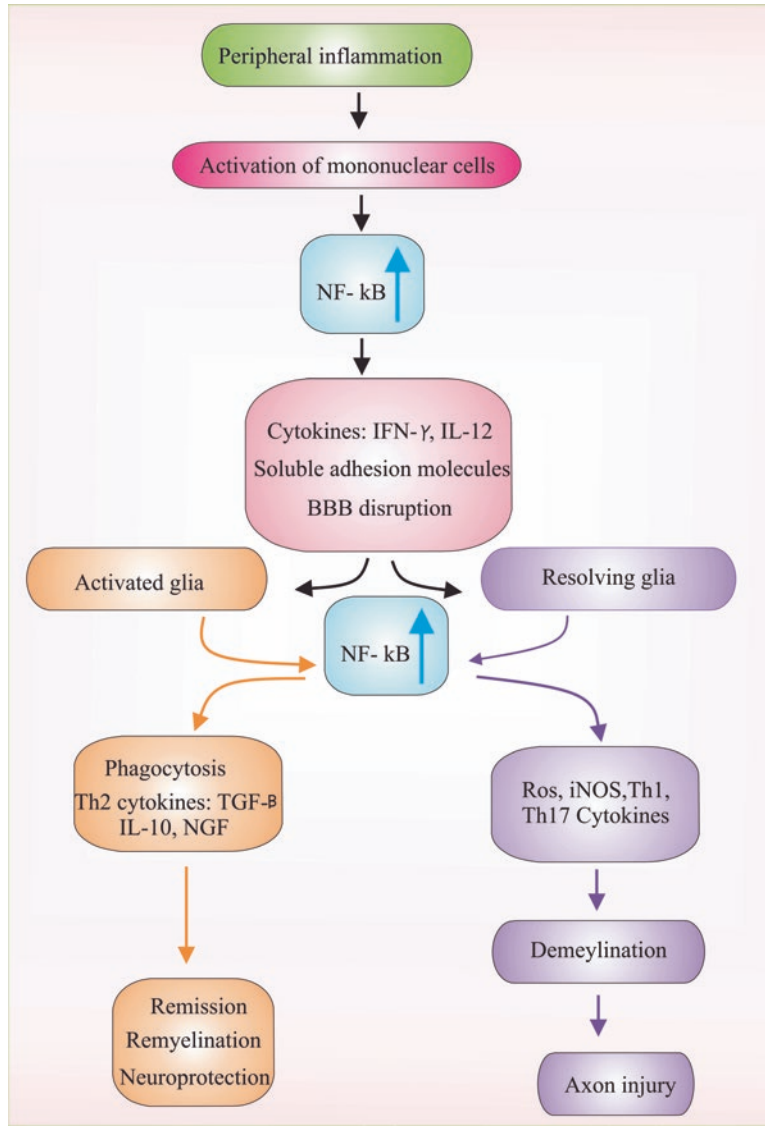

Fig. 6.8 Overview of NF-κB pathway in Multiple Sclerosis (Adapted from Srinivasan and Lahiri 2015)

suggested its possible role in prevention of myelin repair in MS. Oligodendrocytes produce an insulating myelin sheath around neurons optimizing conduction speeds. Generally, Remyelination in MS patients is abortive and its failure leads to MS progression. Abnormal remyelination process is not yet well known but it is proposed that maturation of oligodendrocytes progenitor cells (OPC) is suppressed by an inhibition. Notch as a potential inhibitor is one of the most engaging molecules which can be targeted in MS therapies ameliorating the remyelination process (Juryńczyk and Selmaj 2010). Notch components are expressed in chronic active MS lesions for instance, Notch1 in OPCs and Jagged1 in astrocytes (John et al. 2002). Jagged1 expression is induced in primary human astrocytes presented in MS foci by TGF-β1. Hes5 as a Notch effector is also expressed in OPC suggesting Notch activation in remyelinating oligodendrocytes. Another in vitro study approves the role of Jagged1 in blocking the maturation of oligodendrocytes (Wang et al. 1998). All in all, these findings recommend that Notch reactivation in MS lesions is a critical factor for blocking oligo-

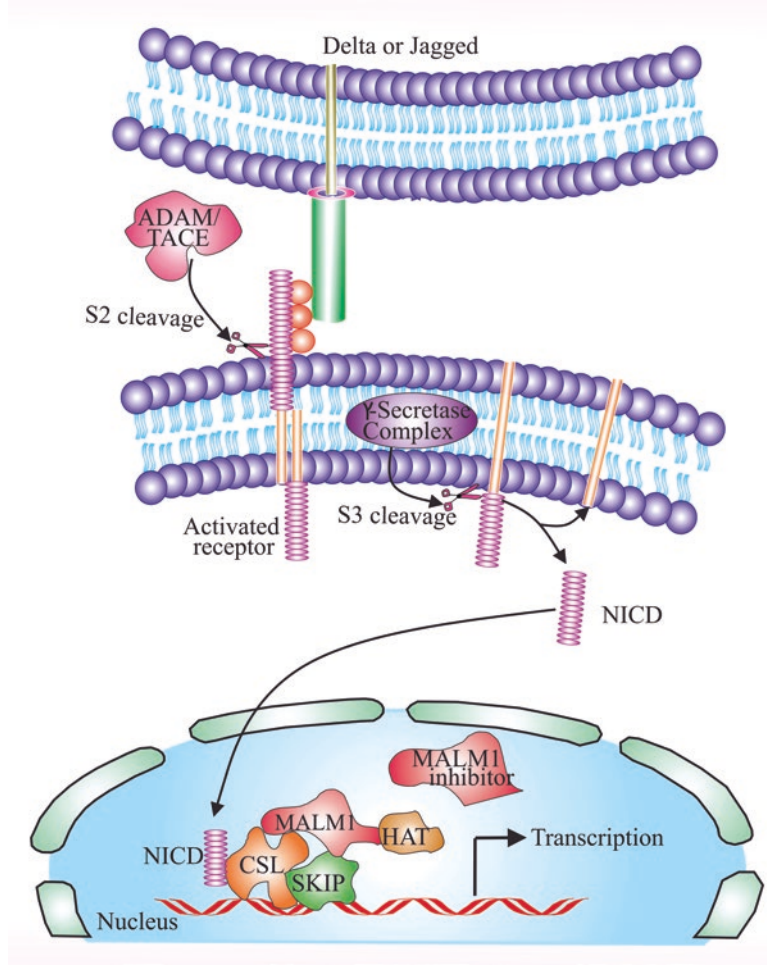

Fig. 6.9 Schematic representation of Notch signaling. Interaction between Notch receptor and ligand leads to two proteolytic reactions. Released Notch intracellular domain (NICD) then translocates into nucleus forming a transcription complex

dendrocytes differentiation and consecutively remyelination failure. On the other hand, contactin-Notch signaling on demyelinated axons naturally stimulates OPC differentiation. In aberrant conditions, NICD does not translocate to the nucleus and aggregates in cytoplasm in consequence of TIP30 overexpression, an importin β inhibitor. Thus, collection of NICDs in cytoplasm results in unsuccessful OPC maturation and neuron remyelination failure within MS silent lesions (Nakahara et al. 2009). Although in both studies Notch 1 is expressed in OPCs of MS patients, Notch pathway plays two contradictory roles based on its ligands. In chronic active lesions, Jagged 1 is expressed highly on astrocytes whereas contactin is the predominant ligand on axons of MS silent lesions. In general, Activated Notch1/Jagged 1 signaling in active lesions and suppressed Notch1/contactin signaling in silent lesions are pathogenic (John et al. 2002; Nakahara et al. 2009). A meta-analysis of 13,896 individuals in Europe introduced Jagged1 as a susceptible gene for Multiple Sclerosis (Ban et al. 2006). Notch pathway also induces IFNγ-producing Th1 cells differentiation (Minter et al. 2005). Interaction between Delta-like ligand (DLL) 1 and 4 of Antigen presenting cells (APCs) and Notch 1 and 2 receptor of IFNγ-producing Th1 cells follows by releasing Notch intracellular domain (NICD) and leads directly and indirectly to interferon-γ (IFNγ) secretion (Radtke et al. 2013). This immune response initiates degeneration of oligodendrocytes, demyelination and remyelination failure in MS lesions (Juryńczyk

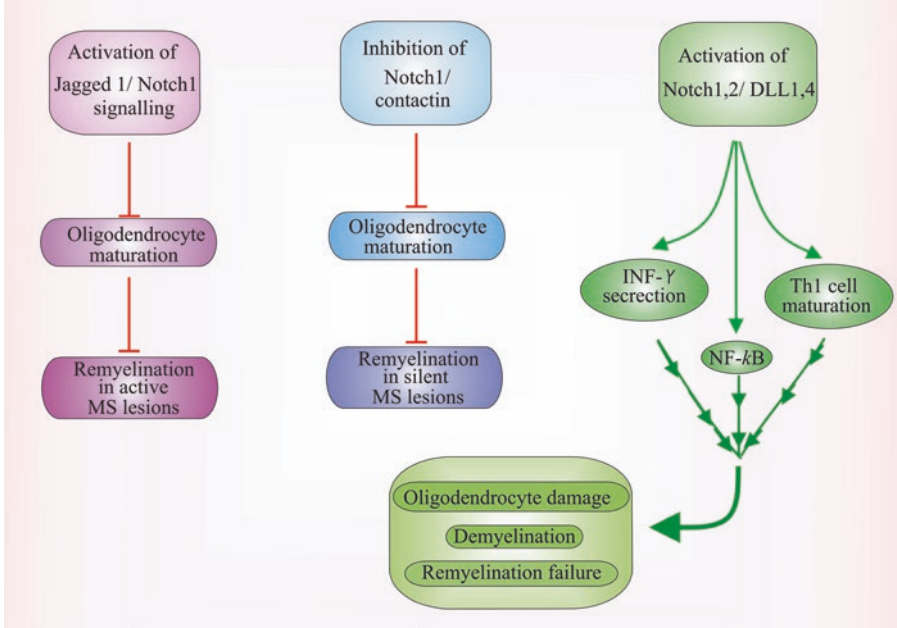

Fig. 6.10 Schematic overview of Notch signaling in MS: In active MS lesions, activation of Jagged1/Notch 1 signaling and in silent MS lesions, inhibition of Notch1/contactin signaling interfere with oligodendrocytes maturation resulting in remyelination failure. Notch1, 2/DLL1, 4 signaling triggers IFNγ-producing Th1 cells maturation, canonical NF-κB activation and IFNγ secretion. The following immune response induces oligodendrocyte damage, demyelination and remyelination failure

and Selmaj 2010). Notch non-canonical signaling has several partners in peripheral T cells such as NF-κB transcription factor (Shin et al. 2006). Activation of NF-κB signaling by Notch can be considered as an indirect impact of Notch in Multiple Sclerosis Fig. 6.10.

6.5 Wnt Signaling

The canonical Wnt pathway also known as Wnt/β-catenin pathway is principally composed of four components: (1) 19 Wnt family members, (2) 10 frizzled membrane receptors (Fz), (3) β-catenin, (4) 4 lymphoid enhancer factors/T cell factors (Lef/Tcf) transcription factors (Bejsovec 2005; Logan and Nusse 2004). In the absence of Wnt molecules, destruction complex consisting of Adenomatosis polyposis coli (APC), the scaffolding protein Axin, protein phosphatase 2A (PP2A), casein kinase 1α (CK1α) and glycogen synthase kinase 3 (GSK3) degrades β-catenin by preparing it for ubiquitination and subsequently proteasome digestion and prevents its accumulation in cytoplasm. In the presence of Signal, Wnt molecules bind to Fz and LRP5/6 recruiting respectively the disheveled (Dvl) protein and Axin to the membrane. This translocation then leads to disruption of the destruction complex and aggregation of β-catenin in cytoplasm (Fiedler et al. 2011). Afterwards, the accumulated β-catenin localizes to the nucleus modulating the transcription of Wnt targeted genes by Lef/Tcf Fig. 6.11. The noncanonical Wnt pathways such as Wnt/planar cell polarity (PCP) pathway and Wnt/calcium pathway do not involve β-catenin and control cell polarity and synapse function.

6.5.1 Canonical Wnt Signaling and MS

Two contradictory roles have been attributed to Canonical Wnt Signaling in myelination and remyelination. Generally, Wnt, β-Catenin, TCF4

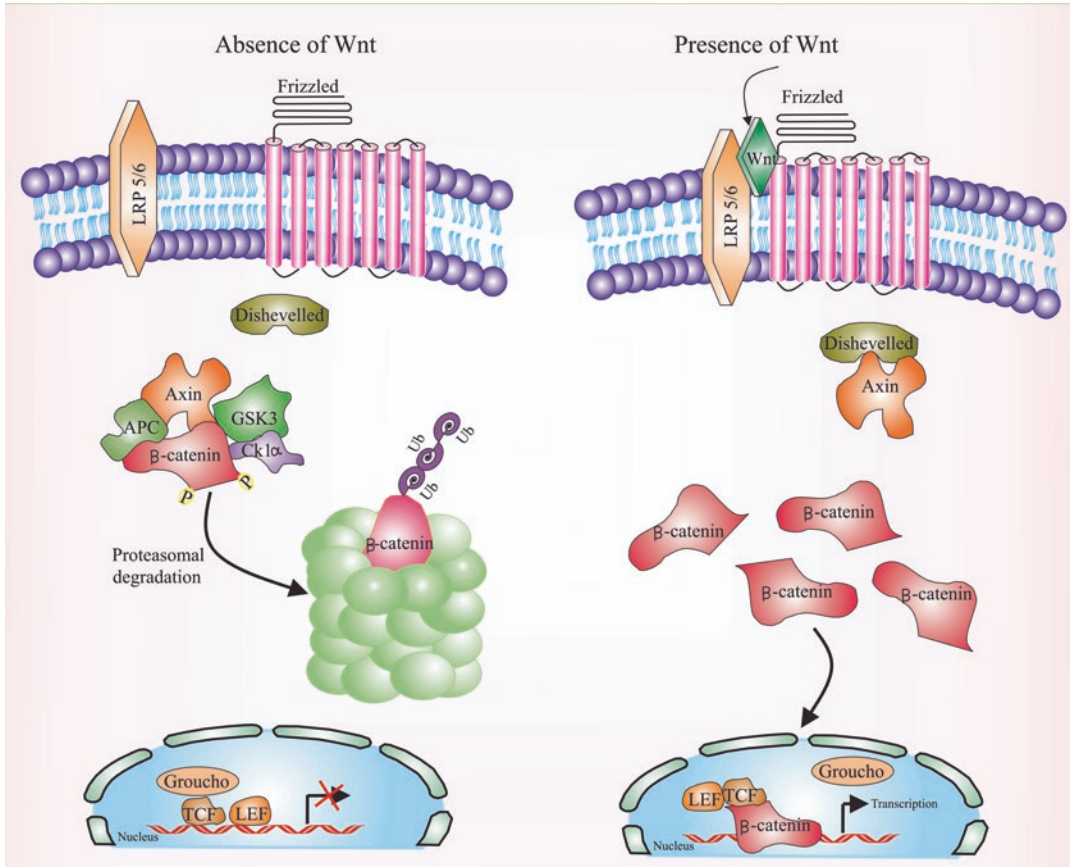

Fig. 6.11 Schematic illustration of canonical Wnt signaling. In the absence of signal, destruction complex conducts β-catenin for ubiquitination and degradation while in the presence of signal, destruction complex is dissociated and released β-catenin enters to the nucleus binding to Lef/Tcf transcription factor (Adapted from Xie et al. 2014)

and Wnt/BMP have been identified as inhibitors of myelination/remyelination while on the contrary Wnt, LRP6, β-Catenin, Lef/Tcf1/3 and 4 can have a promoting effect on this process Fig. 6.12. Regarding inhibitory effect, hyperactivation of Wnt signaling by Wnt3a agonist decreased proteolipid protein (PLP)-positive oligodendrocytes which showed a suppressing effect of Wnt3a on OPCs maturation (Azim and Butt 2011). These findings have been approved when Wnt3a reduced the amount of GalC$^+$ OPCs and myelin basic protein (MBP) (Feigenson et al. 2011). Activation of canonical Wnt signaling results in decreased PLP/MBP expression (Ye et al. 2009) and stabilized β-catenin lessens OPCs differentiation (Lang et al. 2013; Chen et al. 2013). Inhibition of β-catenin/Tcf4 signal induces oligodendrocytes maturation (Ye et al. 2009) and activation of β-catenin/Tcf4 signal blocks oligodendrocytes maturation (Fancy et al. 2009). Moreover, Upregulation of Tcf4 interferes with myelin genes activation (He et al. 2007). Interaction between Wnt signal and bone morphogenetic protein (BMP) signal can synergically inhibits oligodendrocytes maturation (Feigenson et al. 2011). On the other hand, promoting role of Wnt signaling in myelination/remyelination has been reported in other experiments. Wnt3a has a selective impact on adult neural stem cells (aNSCs) promoting their oligodendrogliogenesis (Ortega et al. 2013). Furthermore, Wnt signaling pathway modulates oligodendrocytes, neurons and astrocytes development (Kalani et al. 2008). In 2011, Tawk et al. showed that blocking LRP6,

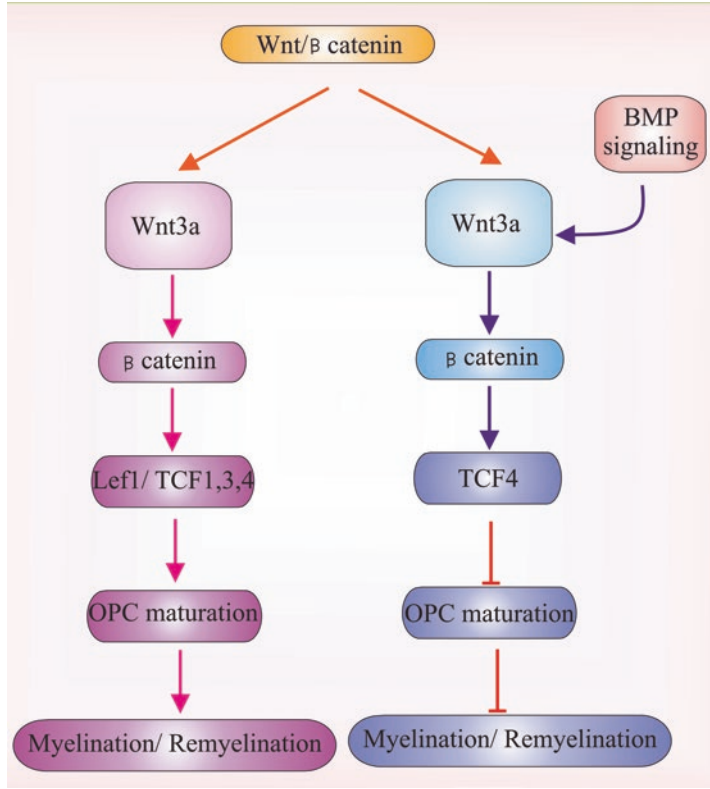

Fig. 6.12 Schematic overview of complicated role of Wnt signaling in myelination and remyelination

Knocking down β-catenin, Knocking down Lef Tcf1/Tcf3 and Knocking down Tcf4 separately inhibit PLP promoter activity (Tawk et al. 2011). Possible mechanisms have been postulated to justify these opposite findings: (1) Wnt signaling pathway has distinct functions in different developmental stages. (2) Interaction of Wnt signaling with various signals can induce or inhibit myelination and remyelination (Xie et al. 2014).

6.5.2 MAPK-p38 and MS

MAPKs (Mitogen-Activated Protein Kinase) contribute to numerous signaling cascades and mediate a variety of cell responses. MAPKs are classified into 4 subgroups: (1) ERKs (2) JNK/SAPK (3) ERK/(BMK1/4) (4) P38. Activation of MAPK-p38 has a great deal of biological consequences including inflammation, apoptosis, cell cycle, cardiomyocyte hypertrophy, development, cell differentiation, senescence and tumor suppression. P38 signaling is triggered by distinct extracellular stimuli such as osmotic shock, ionizing irradiation, growth factors (EGF, CSF-1) and inflammatory cytokines (IL-1, TNF-α) (Zarubin and Jiahuai 2005). Four isoforms have been shown for p38: (1) p38α (2) p38β (3) p38γ (4) P38δ. One of the major roles of the p38 MAPK pathway is the regulation of inflammatory. Following TLR activation in macrophages and dendritic cells (DCs), p38 MAPK induces the secretion of multiple proinflammatory cytokines, e.g., IFN- γ, IL-6, IL-12, IL-23, TNF-α and IL-1β (Liu et al. 2009; Kikuchi et al. 2003; Aicher et al. 1999; Xie et al. 2003; Orabona et al. 2004). p38 MAPK pathway is activated in T cells through antigen stimulation of T cell receptor (TCR), presence of histamine and cytokines such as IL-12 (Rincón and Davis 2009; Noubade et al. 2007; Berenson et al. 2006). This pathway is a requisite for IFN- γ production by Th1effector Tcells (Rincón et al. 1998) and secretion of IL-17 by Th17 cells (Noubade et al. 2011). p38 MAPK

pathway also regulates IFN- γ production through regulating STAT1 activation (Rincón et al. 1998). According to aforementioned evidences, several mechanisms mediated by p38 MAPK have the potential role for MS pathogenesis.

6.5.3 PI3K and MS

Phosphoinositide 3-kinase (PI3K) pathway has been demonstrated to be critical in mediating of the immune responses. PI3Ks are a group of lipid kinases catalyzing phosphorylation of phosphoinositides on their third hydroxyl. Based on their structure and substrate, 3 classes of PI3Ks have been categorized (Hawkins and Stephens 2015). Class I PI3K consists of a regulatory subunit accompanied by a catalytic subunit which recruits PH domain containing effectors such as AKT and activates mTOR through a cascade of signaling (Okkenhaug 2013). All members of Class I PI3K play an essential role in immune mechanisms including PI3Kα, PI3Kβ, PI3kδ, and PI3Kγ. Recent experiments indicate that PI3K increases immune responses by secretion of proinflammatory cytokines (Fortin et al. 2011), which approves the influential effect of PI3K pathway in inflammatory associated diseases such as autoimmune diseases (Soond et al. 2010). PI3K signaling can provoke pro-inflammatory cytokine production such as IL-6 by activation of NF-κB downstream of AKT (Koorella et al. 2014). mTORC1, a downstream effector of AKT, can increase Th1/Th17 differentiation suggesting PI3K signaling involvement in MS (Stark et al. 2015).

6.6 Epigenetic Mechanisms

Epigenetics refers to the investigation of mechanisms that regulate gene expression without altering the DNA sequence and may also modulate the response to several environmental factors, so possibly modifying MS susceptibility (Feinberg 2007; Holliday 2006). Three primary epigenetic mechanisms play key roles in the pathophysiology of MS: DNA methylation, histone modifications, and micro (mi)RNA-associated post-transcriptional gene regulation. The study of epigenetic changes in MS patients could accommodate precious insight into the pathophysiology of this disease, and probably clarify the broad spectrum of clinical phenotypes.

6.6.1 DNA Methylation

DNA methylation is one of the best-identified examples of an epigenetic mechanism which involves the addition of methyl groups (−CH3) to the carbon-5 (C5) position of cytosine residues in CpG dinucleotides context by DNA methyltransferase (DNMT) enzymes (Liu et al. 2010). A number of DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B) catalyze the addition of methyl group to cytosine nucleotides inside DNA. Of the DNMTs, DNMT1 is the major enzyme involved in maintaining (responsible for the maintenance of) DNA methylation patterns during DNA replication and makes sure that the epigenetically modified cytosine residues are preserved after cell division (Weber and Schübeler 2007; Goll and Bestor 2005). Both DNMT3A and DNMT3B are involved in de novo methylation that target unmethylated and hemimethylated sites in nuclear and mitochondrial non-replicating DNA, respectively (Weber and Schübeler 2007; Goll and Bestor 2005; Okano et al. 1999). DNA methylation occurs predominantly at CpG sites in mammals, where a cytosine nucleotide is followed by a guanine (a CpG site). CpG sites can arise singly or in groups of up to many hundred dinucleotide repeats, known as 'CpG islands' which are frequently found in promoter regions of approximately 40 % of mammalian genes. CpG islands are 300–3000 base pairs in length and include greater than 50 % cytosine and guanine nucleotides. The methylation or hypermethylation of cytosine residues in promoter regions usually interferes with sequence recognition by transcription factors and prevents the expression of the associated gene (Klose and Bird 2006; Huynh and Casaccia 2013). DNA

methylation is known to have a crucial role in normal development, cell proliferation, and maintenance of genome stability (Weber and Schübeler 2007).

6.6.2 Post-translational Histone Modifications

Histone modification is another fundamental epigenetic mechanism. In mammalian cells, the packaging of DNA in the nucleus is achieved by its tightly wrapping within chromatin, the packaged form of DNA. The basic unit of chromatin is the nucleosome, composed of 147 bp of double-stranded DNA tightly wrapping around dimers of the histone proteins H2A, H2B, H3, and H4 (histone octamer) and internucleosomal DNA bound to linker histones (Tremethick 2007). Octamers of histone proteins are the basic scaffold for DNA and are crucial for chromatin packaging. The nucleosomal histones tails are rich in lysine and arginine residues that can be modified in response to extracellular signals, thereby allowing regulation of gene expression simply by modifying the interaction between DNA and the other chromatin components. Histone proteins undergo several types of reversible post-translational modifications, including methylation, acetylation, phosphorylation, ubiquitination, citrullination, and ribosylation which can modulate gene expression. The most comprehensively best-understood histone modification is acetylation and/or deacetylation at the lysine residues interceded by histone acetyltransferases (HAT) and histone deacetylases (HDAC). The histone modifications induce changes to the chromatin structure and thereby influence the accessibility of the DNA to transcriptional fators, result in activation or repression of gene expression (Dieker and Muller 2010) For example**,** histone acetylation is often associated with transcriptional upregulation of the associated gene and facilitates the binding of transcriptional factors to DNA, whereas histone deacetylation produces heterochromatin by facilitating histone methylation that contributes to transcriptional silencing (Kouzarides 2007; Urdinguio et al. 2009).

6.6.3 miRNA-Associated Post-transcriptional Gene Regulation

Epigenetic regulation can also be accomplished by small noncoding RNAs particularly microRNAs (miRNAs). miRNAs are a conserved group of small (21–25 nucleotide) doublestranded, noncoding RNAs that play a key role in post-transcriptional gene suppressing by targeting messenger RNA (mRNA), principally at the 3′ untranslated regions (UTR), regulating its translation into protein. miRNAs formed from larger transcripts that fold to create hairpin constructions (Sevignani et al. 2006). After a number of nuclear and cytoplasmic processing steps, mature and functional miRNAs associate with other proteins to produce the RNA-induced silencing complex (RISC) that enables miRNA to regulate gene expression through binding to associated mRNA transcripts (Bartel 2004). Imperfect base pairing triggers the degradation of target mRNA, whereas perfect base pairing causes translational repression of the gene product by the inhibition of translation (Bartel 2004). miRNA-mediated repression of translation play critical roles in a wide variety of cellular processes, such as differentiation, proliferation and apoptosis (Bartel 2004; Chang and Mendell, 2007). Several non-protein-coding RNAs like miRNAs have been also implicated in regulatory functions associated with brain development (Qureshi and Mehler 2012) and neurological disorders (Esteller 2011).

6.7 Role of Epigenetics Changes Associated with MS

Although each of the best-understood epigenetic mechanisms has their distinct effects, an increasing literature shows that all of these processes interact rather than act in isolation. For example, DNA methylation and histone modifications regulate miRNA expression, and themselves are modulated by miRNAs (Han et al. 2007; Koch et al. 2013). Other interactions include those occur between methylated DNA and histone-modifying enzymes, such as recruitment of DNA methyl-

transferases by histone modification (Zhao et al. 2009). These basic epigenetic modifications also exert their effects in concert with particular environmental factors (Koch et al. 2013; Zhou et al. 2014). These interactions propose that the epigenomic alterations are not only regulated by single mechanisms, but also through complex interactions of these basic mechanisms. Epigenetic changes have an effect on various aspects of MS pathophysiology, susceptibility and disease development/progression.

6.7.1 DNA Methylation in Pathogenesis of MS

Methylation of CpG dinucleotides in a number of gene promoter regions that may be responsible for the immune properties of MS have been described. The immunopathology of the disease involves the dominance of T helper cell 1 (TH1) immunity, associated with the cytokine interferon-γ (IFN-γ), over TH2 cell immunity (Kürtüncü and Tüzün 2008). The differentiation of the two T cell types is regulated by epigenetic mechanisms, as is the production of IFN-γ and other cytokines. Aberrant patterns of DNA methylation have been observed in the promoter region for IFN-γ within T helper cells, which may be related to the dominance of TH1 immunity over TH2 immunity in MS (Kürtüncü and Tüzün 2008).

CpG islands Hypomethylation in promoter regions might affect MS development. For example, PAD2 promoter hypomethylation results in overexpression of its product (PAD2 enzyme) (Mastronardi et al. 2007). PAD2 enzyme is responsible for the citrullination of myelin basic protein (MBP), a major component of myelin in the CNS, which is a key player in MS pathophysiology. Modified form of MBP is less stable, and MBP citrullination can makes contribution to less compact myelin that is susceptible to disintegration and eventually a possible autoimmune response to MBP (Musse and Harauz 2007; Mastronardi et al. 2007). Normal-appearing white matter (NAWM) from brain biopsy samples of MS patients demonstrated elevated levels of citrullinated MBP, but contained expanded levels of citrullinated MBP in comparison with normal control level, and individuals with other neurodegenerative diseases, such as Alzheimer's, Huntington's or Parkinson's (Moscarello et al. 1994).

In a genome-wide DNA methylation study, Baranzini and colleagues revealed no reproducible differences in DNA methylation amongst CD4+ lymphocytes of three monozygotic twin pairs discordant for MS (Baranzini et al. 2010). Nevertheless, the result of this study does not exclude the role of DNA methylation in MS, because of the small sample size and differences in sex and ethnicity between the three twin pairs. By analyzing the methylation of 56 differentialy methylated genes, in patients with cancer from DNA, in cell-free plasma of healthy and MS individuals, DNA methylation has been recognized as a potential useful biomarker for disease activity. Remarkable differences between controls and MS patients have been shown in methylation patterns of 15 from 56 genes. The status of promoter methylation in five of these 15 genes could discriminate among sufferes in remission and those in exacerbation (Liggett et al. 2010). Together, these outcomes illustrate that further studies are essential for characterization of DNA methylation role in multiple sclerosis.

To date, no specific studies specifically in the field of MS have been done to explore the epigenetics of neurodegeneration; however with the aid of analyzing the cultured NSC34 cells, Chestnut et al. recognized the association between DNA methylation and neuronal cell death (Chestnut et al. 2011). Overexpression of DNMT3a induced apoptosis and degeneration whithin cultured spinal cord neurons, whereas cultured neurons were protected from apoptosis by inhibition of DNMT3a. When further studies performed, similar effects were found in samples from patients with amyotrophic lateral sclerosis (ALS). These results indicate DNA methylation as a possible contributing factor in neurodegeneration of MS patients. As neurodegeneration develops simultaneously with inflammatory demyelination, the epigenetic changes implicated in inflammation and demyelination may also play

an important role. An important mechanism of CNS damage in MS is inflammation, and suitable transcriptional control of the immune responses is mediated by epigenetic regulation partly. Kumagai et al. found that in comparison with healthy controls, over one-third of MS subjects had significantly higher promoter methylation of SHP-1, a negative regulator of proinflammatory signalling, in leukocytes (Kumagai et al. 2012). So this can be a potential leading cause for decreased SHP-1 expression and increased leukocyte-mediated inflammation.

6.7.2 Histone Modifications in Pathogenesis of MS

Variety of histone modifications have been observed to be associated with the development and manifestation of MS. In a study of patients with progressive MS and healthy controls, elevated level of histone H3 acetylation in oligodendrocytes cells was observed in chronic MS patients, whereas during the early stages of MS, marked histone H3 deacetylation is determined in oligodendrocytes (Pedre et al. 2011). Additionally, the study verified that histone H3 acetylation is elevated in samples from older patients and therefore the extent of acetylation is correlated with the severity of disease (Pedre et al. 2011). When considering epigenetic changes in brain of patients with multiple sclerosis, it is important to clarify the cell specificity of these modifications and the fact that the same modification could have distinct roles in different cell types. For example, studies have demonstrated the association between high levels of histone acetylation in hippocampal neurons and increased transcriptional activity during learning (Levenson et al. 2004), whereas decreased histone acetylation is in correlation with cognitive decline (Peleg et al. 2010). By contrast, histone deacetylation appeared to favour differentiation of oligodendrocytes. Nkx2.2 and Hes5 transcription factors can recruit HDAC1 to the proximal promoter region of MBP genes (Wei et al. 2005). These gene expression changes mediate oligodendrocyte maturation, a process that is often aberrant in MS and which leads to impaired remyelination (Copray et al. 2009). On the other hand, high levels of histone acetylation were characteristic of impaired differentiation of progenitor cells (Shen et al. 2005; Marin-Husstege et al. 2002) and were associated with increased expression of myelin-specific gene repressors (He et al. 2007; Shen et al. 2008). Remyelination destructionin MS patients may derive from reduced oligodendrocyte differentiation (Koch et al. 2013).

During development, reduced expression of transcriptional repressors for myelin genes is required in myelination (Shen et al. 2005). Deacetylation is also important for repair after demyelination. Deacyetylation is a naturally occurring event that is associated with defective repair of myelin. Higher levels of histone acetylation and transcriptional inhibitors were detected in the brains of older compared with younger mice (Shen et al. 2008) and in normal-appearing white matter of patients with MS compared with patients without MS (Pedre et al. 2011). Histone citrullination was increased in animal models of demyelination and in patients with MS compared with patients without MS (Mastronardi et al. 2006). Aberrant citrullination has been detected in myelin proteins (ie, MBP) (Moscarello et al. 1994) and is proposed to contribute to myelin sheath instability and increased proteolysis with release of immunogenic peptides (Pritzker et al. 2000; Harauz et al. 2004) eventually leading to oligodendrocyte apoptosis (Shanshiashvili et al. 2012).

6.7.3 Histone Deacetylase (HDAC) Inhibitors

Cell specificity is important in assessing epigenetic modulators as therapeutic strategies for demyelinating disorders. Due to the reduction of inflammatory infiltrates in animal models, the HDAC inhibitors have been originally proposed as a treatment option for MS and they induce positive influences in animal models of MS. Administration of trichostatin A (TSA) to a MS animal model, attenuated demyelination, spinal

cord inflammation and axonal loss (Camelo et al. 2005). Using HDAC inhibitors can counteract the cognitive decline associated with Alzheimer's disease (Fischer et al. 2007; Kilgore et al. 2010) or traumatic brain injury (Dash et al. 2009; Shin et al. 2009) and protect from axonal damage in impaired axonal transport conditions (Kim et al. 2010). However, systemic use of HDAC inhibitors also negatively affects the generation of new myelin. Histone deacetylation is an important process for developmental myelination (Shen et al. 2005; Marin-Husstege et al. 2002) and adults myelin repair (Shen et al. 2008). Using HDAC inhibitors has a detrimental effect on these processes. This detrimental effect can be applied by preventing myelination of white matter tracts when given during development (Shen et al. 2005) and decreasing the efficiency of endogenous myelin repair if administered to adult mice after demyelination (Shen et al. 2008).

6.8 miRNAs and Multiple Sclerosis

miRNAs mediate various cellular functions and their dysregulation can lead to abnormal conditions such as autoimmunity (Pauley et al. 2009). miRNAs are therefore considered as an ideal biomarker in several diseases. The possible effect of miRNAs in the pathogenesis of MS can be examined using plasma/serum samples (Søndergaard et al. 2013). Distinct studies regarding to miRNAs expression in MS patients are mainly inconsistent even they have done in the same tissue. These heterogeneities among findings can be explained by differences between patient populations, sample type, the amount of miRNAs and methods. A shared result of all studies is that dysregulation of miRNA induces proinflammatory and disease progression (Du et al. 2009; Lindberg et al. 2010; Paraboschi et al. 2011; Guerau-de-Arellano et al. 2011). Moreover, a specific group of miRNAs called NeurimmiRs controls neuronal and immune systems and the crosstalk between them (Soreq and Wolf 2011). For instance, dysregulation of miR-155 in peripheral blood mononuclear cells and aberrant expression of miR-326 in CD4+ T cells have been reported in patients with MS (Paraboschi et al. 2011; Du et al. 2009). miR-155 is also overexpressed in B cells, T cells and macrophages in response to TLR stimulation and cytokine secretion, suggesting its role in inflammatory responses (O'Connell et al. 2010). miR-155 deficiency also prevents EAE progression by disrupting inflammatory T-cells maturation including TH17 cells (O'Connell et al. 2010). These types of cells involve in EAE and MS by secreting proinflammatory cytokine IL-17 (Steinman 2008; Tzartos et al. 2008).

Overexpression of miR-326 in peripheral blood cells has been indicated in MS and EAE especially during relapsing phase whereas lower amounts of miR-326 are expressed in control and remission samples (Du et al. 2009). Upregulation of miR-326 blocks an inhibitor of T cell differentiation and increases certain types of T cells (Du et al. 2009). miR-155 and miR-326 play an essential role in T cell development and silencing them reduces the severity of EAE (Du et al. 2009; Murugaiyan et al. 2011). These findings suggest that miR-155 and miR-326 have different targets in blood and brain and they can modulate distinct gene expression profiles in these two organs of MS patients. Furthermore, miRNA expression profiles have been considered as a possible diagnostic biomarker for Multiple Sclerosis. For instance, a comparison between whole-blood miRNA profiles of relapsing-remitting multiple sclerosis (RRMS) patients with those of healthy controls demonstrated different expression of 165 miRNAs (Keller et al. 2009). miR-145 known as an inhibitor of cell proliferation in cancers (Ban et al. 2011; Cho et al. 2011) has been identified to be the best candidate to distinguish between MS patients and healthy controls (Keller et al. 2009). This miRNA has a reliable sensitivity (%89.5) and specificity (%90) but its correlation to MS is not well-known (Keller et al. 2009). Another investigation on peripheral blood mononuclear cells of RRMS patients and healthy controls indicated overexpression of miR-18b, miR-493, and miR-599 in relapse and overexpression of miR-96 in remission phase (Otaegui et al. 2009).

Active demyelinating lesions have been utilized to evaluate the role of miRNAs in patients' brains. An experiment showed dysregulation of 28 miRNAs and 35 miRNAs respectively in active and inactive MS lesions in comparison to healthy samples (Junker et al. 2009). miR-326, miR-155, and miR-34a have been also overexpressed in active lesions compared with inactive lesions and normal samples. Upregulation of these miRNAs in active lesions blocks the expression of CD47, an inhibitor of macrophage phagocytosis. As a consequence, it will induce macrophage activation and myelin phagocytosis, resulting in production of active lesion (Chari 2007; Junker et al. 2009). Both miR-214 and miR-23a have been exhibited to be upregulated in active and inactive MS lesions. Overexpression of these miRNAs during oligodendrocyte differentiation suggested their contribution in remyelination. miR-219 and miR-338-5p, which target Sox6, Zfp238 and Hes5 genes, inhibit OPC maturation and remyelination in inactive MS lesions (Dugas et al. 2010; Zhao et al. 2010). According to another report, miR-155, miR-338 and miR-491were overexpressed during MS progression. These miRNAs target aldo-keto reductase family members C1 and C2 inhibiting neurosteroid synthesis. Neurosteroids are critical for myelination, axon and dendrite growth (Noorbakhsh et al. 2011). Therefore, dysregulation of miRNA expression can have a significant role in remyelination and demyelination. An influential study indicated that treated MS patients with glatiramer acetate had similar amount of miR-146a and miR-142-3p with healthy controls, suggesting the potential influence of glatiramer acetate in normalizing these miRNA expressions (Waschbisch et al. 2011). Taken together, epigenetic markers including DNA methylation patterns and miRNA expression profiles are proposed as a promising tool for MS diagnosis and treatment.

6.8.1 Environmental Risk Factors and Epigenetic of MS

Three main environmental risk factors have been identified for MS such as vitamin D deficiency, smoking and Epstein-Barr virus (EBV) (Goodin 2014). Although the distinguished correlation between MS and these risk factors is unclear, epigenetic impact of environmental factors is an accepted mechanism in disease progression. Not only MS, but other diseases such as cancer and cardiovascular diseases are affected by vitamin D, smoking, and EBV through epigenetic mechanisms.

6.8.2 Vitamin D Deficiency

Vitamin D deficiency can lead to histone modification and increased risk of MS. Elevated amounts of 1,25-hydroxyvitamin D3, a steroid hormone produced in the skin, decrease the risk of MS progression (Munger et al. 2006) and reduced levels of this vitamin increase disability (Smolders et al. 2008) with more relapses (Smolders et al. 2008; Simpson et al. 2010). Vitamin D can regulate gene expression by recruiting histone acetyltransferases HACTs) or histone deacetylases (HDACs) to its target genes. Expression of enzymes which contribute in histone demethylation can also be controlled by vitamin D (Fetahu et al. 2014). Various studies on cancer cell lines have shown the critical role of vitamin D in histone modification. Furthermore, vitamin D mediates immune responses through epigenetic changes. For example, IL-17A expression has been suppressed by vitamin D which reduces the histone acetylation of IL-17A promoter (Joshi et al. 2011). Some studies have claimed that vitamin D is relevant to DNA methylation but understanding its precise mechanism needs further investigations. Vitamin D can control immune responses in a dose dependent manner (Ascherio et al. 2012) in which higher dose of it reduces T-cell proliferation (Ascherio et al. 2012; Smolders et al. 2010).

6.8.3 Smoking

Development of MS with more frequent relapses and increased lesions is associated with smoking as an environmental risk factor (Ascherio and

Munger 2007). Smoking is demonstrated to affect DNA methylation (Breitling et al. 2011; Allione et al. 2015) resulting in cardiovascular diseases (Breitling 2013) and carcinogenesis (Liu et al. 2006; Ma et al. 2011). In addition, the role of smoking in miRNA expression (Maccani et al. 2010) and histone acetylation (Ito et al. 2001) has been indicated. Maternal smoking has been also shown to stimulate the growth and differentiation of neurons by promoter methylation of BDNF (brain-derived neurotrophic factor) (Toledo-Rodriguez et al. 2010). In another study, miR-16, miR-21 and mi4-146a were reduced as a consequence of maternal smoking during gestation period (Maccani et al. 2010). These results introduce smoking as a potential modulator of DNA methylation and miRNA expression in MS patients.

6.8.4 Epstein–Barr Virus (EBV)

Infected EBV individuals have a higher risk of MS progression in comparison to seronegative individuals which proposes the association between EBV and MS (Ascherio et al. 2001). EBV uses host epigenetic mechanisms to control the expression of its genes in host cells. This effect interferes with host gene expression and is associated with several neoplasms (Niller et al. 2009; Kwong et al. 2002). For instance, latent membrane protein 1 (LMP1), an EBV gene, increases several methyltransferases including DNMT1, DNMT 3a and DNMT 3b that intensify promoter hypermethylation of tumor suppressor genes (Kwong et al. 2002; Tsai et al. 2002). A special type of EBV infection has been indicated to double MS progression regarding to its impact on epigenetic changes (Handel et al. 2010). Moreover, EBV genome encodes several miRNAs that regulate host gene expression. (Riley et al. 2012; Klinke et al. 2014). These evidences support the possible association between EBV and MS.

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Role of Oligodendrocyte Dysfunction in Demyelination, Remyelination and Neurodegeneration in Multiple Sclerosis

7

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Abstract

Oligodendrocytes (OLs) are the myelinating cells of the central nervous system (CNS) during development and throughout adulthood. They result from a complex and well controlled process of activation, proliferation, migration and differentiation of oligodendrocyte progenitor cells (OPCs) from the germinative niches of the CNS. In multiple sclerosis (MS), the complex pathological process produces dysfunction and apoptosis of OLs leading to demyelination and neurodegeneration. This review attempts to describe the patterns of demyelination in MS, the steps involved in oligodendrogenesis and myelination in healthy CNS, the different pathways leading to OLs and myelin loss in MS, as well as principles involved in restoration of myelin sheaths. Environmental factors and their impact on OLs and pathological mechanisms of MS are also discussed. Finally, we will present evidence about the potential therapeutic targets in re-myelination processes that can be accessed in order to develop regenerative therapies for MS.

Keywords

Demyelination • Multiple sclerosis • Oligodendrocyte • Oligodendrocyte progenitor cell • Neurodegeneration • Re-myelination

Abbreviations

BBB	blood-brain barrier
CIS	clinically isolated syndrome
CNS	central nervous system
CSF	cerebrospinal fluid
DIR	double inversion recovery

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DMF	Dimethyl Fumarate
DTI	diffusion tensor imaging
EAE	experimental autoimmune encephalomyelitis
GA	glatiramer acetate
Gd	gadolinium
GM	gray matter
HERV	human endogenous retroviruses
MAG	myelin associated glycoprotein
MBP	myelin basic protein
MMPs	matrix metalloproteinases
LQ	laquinimod
MOG	myelin oligodendrocyte glycoprotein
3D-MPRAGE	3-dimensional magnetization prepared acquisition with gradient-echo
MRI	magnetic resonance imaging
MS	multiple sclerosis
MSRV	multiple sclerosis associated retrovirus
MTR	magnetization transfer ratio
NAWM	normal appearing white matter
OL	oligodendrocyte
omGP	oligodendrocyte myelin glycoprotein
OPC	oligodendrocyte progenitor cell
OSP	oligodendrocyte surface protein
PLP	proteolipid protein
PPMS	primary progressive multiple sclerosis
PSIR	phase sensitive inversion recovery
rHIgM22	human monoclonal IgM antibody 22
RRMS	relapsing remitting multiple sclerosis
SPMS	secondary progressive multiple sclerosis
TNF-α	tumor necrosis factor-alpha
USPIO	ultra-small superparamagnetic particles of iron oxide
WM	white matter

7.1 Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) characterized by inflammatory and degenerative changes in the brain and spinal cord. MS is the most common cause of nontraumatic disability in young adult (Noseworthy et al. 2000) and therefore extensive research efforts has been performed in order to clarify the etiology and pathogenesis of this disease. However the mechanisms involved in tissue injury in the MS brain and spinal cord are incompletely understood mainly because most of the data was obtained from experimental models, while studies on brain lesions of MS patients are sparse. In MS, a multifactorial interplay of genetic and environmental factors leads to a chronic activation of the immune cells and cerebral tissue injury. Numerous studies performing large scale genomic screens identified more than 100 genomic regions in which variants are associated with increased susceptibility (Beecham et al. 2013). The strongest genome-wide susceptibility locus maps to the major histocompatibility complex (MHC)(6p21.3) accounting for approximately 10.5 % of the genetic variance underlying the risk of MS (Isobe et al. 2016). The most specific genetic mutations influencing the risk of MS involve class II alleles: DQA1*01:01-DRB1*15:01 and DQB1*03:01-DQB1*03:02 (Moutsianas et al. 2015). Carrying HLA-DRB1*15:01 have been associated with lower age at the disease onset (Smestad et al. 2007), better response to copolymer 1 immunomodulation (Fusco et al. 2001), increase in the white matter lesion volume and reduction in normalized brain parenchymal volume (Okuda et al. 2009). On the contrary, HLA-B*44:02, a protective allele for MS susceptibility, correlated with better MRI outcomes in terms of brain parenchymal fraction and T2 hyperintense lesion volume (Healy et al. 2010).

Epidemiological studies have identified several viruses as factors that may influence MS risk including Epstein-Barr-Virus, Herpes simplex

virus types 1 and 2, Human herpes virus 6, measles, mumps and rubella. Two recent observational studies (Pohl et al. 2010; Otto et al. 2011) suggests that the humoral immune response that is polyspecific and not exclusively directed against a particular virus may be involved in MS pathogenesis. The envelope protein of MS-associated retrovirus (MSRV) from the human endogenous retroviruses (HERV)-W family currently has the strongest evidence as a potential trigger for MS. An estimated 30 million years ago, exogenous retroviruses are thought to have integrated themselves into human germ line cells, becoming part of human DNA and being transmitted over generations. Usually such HERVs are silenced or expressed at low levels, but in some pathological conditions, such as MS,their expression is higher than that in the healthy population (Morandi et al. 2015). Three HERV families have been associated with MS: HERV-H, HERV-K, and HERV-W. In addition to expression in peripheral immune cells, MSRV is expressed in monocytes and microglia in central nervous system lesions of people with MS and, through the activation of toll-like receptor 4, it has been shown to drive the production of proinflammatory cytokines, reduction of myelin protein expression, and death of oligodendrocyte precursors (Madeira et al. 2016). Available evidence also indicates that HERV-W/MSRV expression is increased in cells infected by exogenous viruses, such as EBV, potentially providing a missing link between environmental triggers, and the immunopathogenic cascades leading to the MS lesions and to disease progression (Morandi et al. 2015).

Another recently recognized environmental risk factor for MS is smoking. Smoking is reported to affect a number of biological mediators of inflammation through its action on immune-inflammatory cells, leading to an immunosuppressant state. (Gonclaves et al. 2011). The Nurses'Health Study showed that the relative incidence rate of MS in current smokers compared to never smokers was 1.6, with a dose-response dependent on pack years smoked. In a recent population based case control study which included 843 MS patients from Sweden, a significant interaction between two genetic risk factors, carriage of human leukocyte antigen DRB1*15 and absence of human leukocyte antigen A*02, was observed among smokers whereas such an interaction was absent among non-smokers (Hedstrom et al. 2011). Among those with both genetic risk factors, smoking increased the risk by a factor of 2.8 in comparison with a factor of 1.4 among those without the genetic risk factors. (Hedström et al. 2015). Other studies showed that smoking is associated with an acceleration of progression from CIS to MS (Di Pauli et al. 2008) and from RRMS to SPMS (Healy et al. 2009; Pittas et al. 2009) and that those who quit fare better (Zivadinov et al. 2009).

Several studies provided evidence for the role of vitamin D deficiency and low sunlight exposure as risk factors for MS. There is evidence for the association between vitamin D and relapses and for the association between lower vitamin D levels and higher levels of disability. Vitamin D has an immunomodulatory role through its anti-inflammatory and anti-autoimmune actions. In the nervous system, vitamin D is involved in the regulation of calcium-mediated neuronal excitotoxicity, in the reduction of oxidative stress, and in the induction of synaptic structural proteins, neurotrophic factors and deficient neurotransmitters (Mpandzou et al. 2016). There is evidence that vitamin D receptor (VDR) – retinoid X receptor heterodimer induces oligodendrocyte progenitor cells (OPCs) differentiation and that VDR agonist vitamin D enhances OPC differentiation. It has been also shown the expression of VDR in OLG lineage cells in MS (de la Fuente et al. 2015). Observational studies exploring the role of vitamin D in relapsing-remitting multiple sclerosis (RRMS) suggest that an elevated serum level is beneficial (Duan et al. 2014; Pierrot-Deseilligny and Souberbielle 2010), as it is associated with reduced disease activity (Løken-Amsrud et al. 2012; Simpson et al. 2010) and severity during early stages of the illness (Ascherio et al. 2014; Smolders et al. 2008).

However a recent double-blind randomized placebo-controlled trial of vitamin D3 supplementation showed that high-dose oral vitamin D3 supplementation prominently increased serum 25(OH)D levels without affecting markers of

systemic inflammation (Røsjø et al. 2015). This result may lead to the hypothesis that the effect of Vitamin D supplementation is related to a certain genetic profile. In order to test this idea, Lin et al. (2014), in a cohort of 141 RRMS patients, examined genes involved in the vitamin D metabolism and vitamin D receptor (VDR)/RXR transcription factor formation and they obtained that the relationship between 25(OH)D and the hazard of relapse was significantly different for different alleles. These data support the hypothesis that gene-vitamin D interactions may influence MS clinical course and protein kinase C family genes may play a role in the pathogenesis of MS relapse through modulating the association between 25(OH)D and relapse. These data may suggest the possible role of vitamin D as regenerative component and identify a new target for remyelination medicines.

The disturbances of myelin structure and metabolism of its components are the most important phenomena in MS and lead to the formation of large confluent plaques of demyelination in the white and gray matter (Lassmann et al. 2007) and also to a diffuse pathology of the white matter. The complex immune mediated attack on oligodendrocytes (OLs) during demyelination is followed by remyelination mainly sustained by oligodendrocyte progenitors cells (OPCs), a population of abundant and widely distributed mutipotent adult central nervous system progenitors. It has been suggested that mature myelin-forming OLs may be able to undergo cell division, implying that they have the potential to generate new cells for remyelination. However recent evidence suggest that this was not the case. Several studies showed that mature OLs from the lesioned area that survive demyelination do not proliferate and those from the margins of the lesion do not migrate into the lesion and make no contribution to subsequent remyelination (Crawford et al. 2016; Keirstead and Blakemore 1997). As a consequence strategies to stimulate endogenous remyelination should focus on enhancement of the OPCs function as opposed to promotion of OLs survival.

7.2 Demyelination in Multiple Sclerosis – White Matter and Grey Matter Pathology and Association Between Pathological, MRI and Clinical Findings

7.2.1 Focal White Matter Demyelination

Focal white matter (WM) MS pathology represented by demyelinating plaques vary with age, time from disease onset and, in early active MS, it is very heterogeneous among patients (Lucchinetti et al. 2000). Active plaques especially early active plaques predominate in early MS and they rarely occur among patients with longstanding disease (Frischer et al. 2015). Active and early active plaques represent the vast majority of plaques found in acute monophasic MS, they appear in about two-thirds of patients with relapsing remitting MS (RRMS) and are also common in secondary progressive MS (SPMS) patients, although in these patients late active plaques predominate over early active plaques. However, among patients with SPMS without attacks, active plaques are rarely found, whereas inactive plaques predominate. The acute active MS plaques in RRMS is heavily infiltrated by macrophages with myelin debris, lymphocytes and large reactive sometimes multinucleated, astrocytes called Creutzfeldt-Peters cells. Oligodendroglia is often present in lesions that show signs of remyelination. There is also axonal injury represented by axonal swellings (Filippi et al. 2012). In progressive MS the majority of active plaques have an inactive lesion core, which is surrounded by a narrow rim of microglia activation and macrophages infiltration (Prineas et al. 2001). The chronic inactive MS plaque is sharply circumscribed and hypocellular, without active demyelination and microglia activation at their edges (Lassmann 2013). Such plaques are characterized by fibrillary gliosis, loss of axons and olygodendrocyte. Inflammation might still be present, especially perivascularly (Filippi et al. 2012). The difference

between active and inactive plaques on conventional MRI is made by the evidence of blood-brain-barrier breakdown as indicated by contrast enhancement. Active plaques typically are associated with gadolinium enhancement that persists for 2–6 weeks on magnetic resonance imaging (MRI) and most likely represent the pathological substrate of attacks (Filippi et al. 2012; Popescu et al. 2013). Slowly expanding lesions called "smoldering plaques" are almost exclusively seen among progressive MS patients especially primary progressive MS (PPMS) as well as secondary progressive MS (SPMS) with and without attacks. Smoldering plaques have been associated with microglial activation, ongoing axonal injury and neurodegeneration (Frischer et al. 2009; Prineas et al. 2001). Few myelin-laden macrophages are present at the plaque edge. This type of slowly active demyelination characterizes progressive MS and it is clinically associated with worsening of the pre-existing symptoms in progressive MS patients (Confavreux and Vukusic 2014; Cottrell et al. 1999). Smoldering plaques provide further pathological evidence that the active demyelinating process seems to decelerate as the disease transforms into the progressive stage and the immune response may shift from adaptive, antigen specific T- and B-cell mediated, to innate (Weiner 2008). On MRI smoldering plaques are identified as enlarging lesions, without gadolinium enhancement, due to the absence of blood-brain-barrier (BBB) leakage.

There are also differences in inflammation in different stages of MS. Inflammation during early stages of MS is associated with profound BBB disruption, allowing the infiltration of the brain by new waves of inflammatory cells from the peripheral immune system that enter the CNS from the circulation whereas, in the progressive stage of MS, inflammation is frequently localized around vessels with intact blood-brain-barrier (Hochmeister et al. 2006). Therefore, with disease progression, the inflammatory process becomes at least in part trapped within the CNS compartment (Lassmann 2013). However, studies with ultra-small superparamagnetic particles of iron oxide (USPIO) showed that USPIO enhancement was seen in areas without signal changes on T2-weighted images, sometimes in absence of gadolinium (Gd) enhancement, suggesting that prelesional accumulation of monocytes preced or is independent of lesion formation and it extends for a long time beyond Gd enhancement. These data suggest that infiltration of macrophages and possibly lymphocytes into the brain occurs through different mechanism than blood-brain-barrier disruption (Filippi et al. 2012). Also, some lesions that enhance with USPIO tend not to develop into black holes showing that the inflammation is diverse and may contribute to tissue repair.

7.2.2 Diffuse White Matter Demyelination

In MS there is also a diffuse pathology of the white matter classified as normal appearing white matter (NAWM) that should be differentiated from diffusely abnormal or dirty appearing white matter. NAWM, originally described in 1979, has been defined pathologically as macroscopically normal white matter that is microscopically normally myelinated and at least 1 cm away from a plaques edge (Allen and McKeown 1979). 72,2 % of specimens of NAWM showed major histological abnormalities such as demyelination, gliosis, small round cells infiltration and the presence of macrophages. Microglial activation, decreased axonal density by 12-42 % and prominent astrocyte activation were also present. These diffuse pathological abnormalities correlate with the extent of cortical lesions but not with white matter lesion load (Kutzelnigg et al. 2005). MRI-defined NAWM assessed from multiple MRI methods (T2-weighted, T1-weighted, MTI, DTI), when analyzed with histological methods, revealed microglia activation. Axonal injury is present, although in lower extent in NAWM (Kutzelnigg et al. 2005; Narayanan et al. 2006; Frischer et al. 2009) being partially explained by Wallerian degeneration leading to nerve fiber degeneration in tracts, traversing focal white matter lesions (Evangelou et al. 2000) and also there is a diffuse axonal injury which is also associated to inflammation but it is present within the whole brain and spinal cord and seems to occur also in non-demyelinated nerve fibers (Lassmann

2010). There is also evidence for the BBB permeability in NAWM in MS patients, most prominent in the periventricular region, intricately linked to the presence of MS relapse activity and attenuated by imunomodulatory treatment (Cramer et al. 2014). Pathologically, dirty-appearing white matter consists of extensive axonal loss, decreased myelin density, and chronic fibrillary gliosis, all of which are abnormal compared with NAWM and different from focal white matter pathology (Seewann et al. 2009). The results of a correlative MRI–pathology study have shown that dirty-appearing white matter has a signal intensity on T2-weighted images that is higher than in the surrounding NAWM, but lower than in the focal white matter lesions (Seewann et al. 2009) and MTI and DTI values showed intermediate abnormalities (Vrenken et al. 2010).

Diffusion tensor imaging (DTI) studies suggested the association between white matter tracts damage and cognitive function in MS patients (Hui Jing YU et al. 2012; Benedict et al. 2007; Chiaravalloti and DeLuca 2008; Kern et al. 2011; Lin et al. 2008). Other DTI studies in MS revealed that widespread white matter injury appears in patients with clinically isolated syndrome (CIS), RRMS and PPMS (Bodini et al. 2009; Raz et al. 2010; Roosendaal et al. 2009). A non-linear pattern of white matter microstructure disruption occurs in RRMS. Alterations are seen early in the disease course within 1 year from onset, reach a plateau within the next 5 years and only later additional white matter changes are detected suggesting the existence of an important period of possible therapeutic window within the early disease stage (Asaf et al. 2015). However studies showed only moderate correlations or inconsistent results for the association between white matter lesions load and rating scales measuring disability and cognitive dysfunction in MS. (Sormani et al. 2010; Meyer-Moock et al. 2014).

7.2.3 Grey Matter Demyelination

In MS patients demyelination was also found in the grey matter (GM), especially in the chronic phase of the disease. At autopsy, cortical lesions are characterized by paucity of cell infiltration and by an intact BBB (Peterson et al. 2001; Bo et al. 2003; Brink et al. 2005; Vercellino et al. 2005; Wegner et al. 2006; van Horssen et al. 2007). Loss of glial cells (−36 %) and synapses (−47 %) were described (Wegner et al. 2006) as well as neuronal loss (18-23 %) (Vercellino et al. 2005). However, in MS, degeneration of cortex was found to be largely unrelated to the presence of cortical lesions (Vogt et al. 2009; Klaver et al. 2015). Three types of cortical lesions can be distinguished. Cortico-subcortical compound lesions, which affect both the grey and white matter, small intra-cortical lesions and subpial lesions, which are reflected by band like superficial demyelination extended into deeper cortical layers (Bo et al. 2003). In progressive MS subpial lesions are the most frequent and are topographycally related to meningeal inflammation (Lassmann 2013). Demyelination and neurodegeneration in cortical MS lesions are mainly driven by oxidative injury (Fischer et al. 2013) and are associated with perivenous inflammation in the early stage of MS and meningeal inflammation in the progressive stage (Lucchinetti et al. 2011; Howell et al. 2011; Choi et al. 2012). The profound oxidative injury observed in MS lesions may be explained by massive expression of NADPH oxidase in microglia, an enzyme important in the induction of oxidative burst (Fischer et al. 2013). These oxidative damage may be amplified by several factors. One is mithocondrial injury which leads to radical production when the mitochondrial respiratory chain is impaired (Murphy 2009). Another factor may be microglia activation due to anterograde or retrograde axonal degeneration secondary to accumulation of disease-related brain damage. In this regard a recent study showed that new cortical lesions are more likely to appear in cortical areas that are connected with sites of previous damage in the white and gray matter (Kolasinski et al. 2012). The third amplification factor may be the liberation of iron from intracellular stores represented mainly by oligodendrocytes and myelin sheaths (Connors and Menzies 1995; Hallgren and Sourander 1958). Iron accumulates in the human brain in an age-dependent process and the

liberation of iron within the lesions is more pronounced in a degenerative process that affects myelin and oligodendrocytes than in a pathological process that affects neurons or other glia (Hametner et al. 2013). Hence, while in the early stage of the disease oxidative injury appear to be mainly driven by inflammation and oxidative bursts, in the progressive stage of the disease, it is amplified by certain factors which are related to aging of the patients and accumulation of brain injury (Lassmann 2012).

The MRI detection of cortical lesions has been improved in the recent years with the advances in MRI. In an early postmortem MRI and histopathology study at 1.5 Tesla (T) only 37 % of cortical lesions were retrospectively detected (Seewann et al. 2012). The lesion enhancement with contrast agent gadolinium-DTPA provided only marginal additional value (Kidd et al. 1999) and the use of fluid-attenuated inversion recovery (FLAIR) increased the detection of (sub)cortical lesions at approximately 60 % in vivo (Boggild et al. 1996; Filippi et al. 1996; Bakshi et al. 2001) and ex-vivo (Geurts et al. 2005a). However most of the lesions, in particular the pure intra-cortical lesions, were still missed (Geurts et al. 2005a). The development of double inversion recovery (DIR) sequences allowed better visualization of the cortex by suppressing the signal of surrounding white matter and cerebrospinal fluid (CSF) in patients (Redpath and Smith 1994; Geurts et al. 2005b), and in post-mortem tissue (Seewann et al. 2012). Also phase sensitive inversion recovery (PSIR) at 1.5 T makes a better gray matter – white matter distinction than T2 sequences. However approximately 82 % of cortical lesions remained undetected with DIR t 1.5 T (Seewann et al. 2012) when controlled with histopathological examination.

The use of high field MRI (between 3 and 7 T) and ultra-high field MRI, higher than 7 T using DIR sequences (Simon et al. 2010), both PSIR and 3-dimensional magnetization prepared acquisition with gradient-echo (3D-MPRAGE) (Nelson et al. 2008) or combination of DIR and PSIR (Nelson et al. 2008) further improved the detection of cortical lesions. Detection varied for different subtypes of cortical lesions: mixed lesions were better detected than subpial lesions (Geurts et al. 2008; Jonkman et al. 2015). Using in vivo 7 T 3D-MPRAGE made possible a better visualization of cortical lesions (Kilsdonk et al. 2013) and a better classification of purely intracortical lesions (Tallantyre et al. 2010). The use of various MRI sequences and of high field MRI allowed a better evaluation of cortical lesions load and showed that cortical lesions correlate strongly with severity and progression of disability and cognitive impairment (Harrison et al. 2015a, b; Calabrese et al. 2007, 2013).

Numerous studies showed that up to 70 % of MS patients present cognitive impairment (Benedict et al. 2006; Chiaravalloti and DeLuca 2008). A recent multicenter study, using voxel based MRI, showed that variable patterns of NAWM and gray matter damage were associated with deficits in selected cognitive domains. Cognitively impaired MS patients had gray matter atrophy of the left thalamus, right hippocampus and parietal regions. They also showed atrophy of several white matter tracts, mainly located in posterior brain regions and widespread white matter diffusivity abnormalities. White matter diffusivity abnormalities in cognitive-relevant white matter tracts followed by atrophy of cognitive-relevant gray matter regions explained global cognitive impairment. (Preziosa et al. 2016). Another study showed that there is a perfusion reduction in the cortical regions in impaired RRMS patients compared with non-impaired patients, in absence of structural differences. (Hojjat et al. 2016).

There is also evidence for a strong correlation between cortical lesions load (including hippocampal lesions) and information processing speed, various memory functions and learning capacity (Roosendaal et al. 2008, 2009; Nelson et al. 2011). Cortical lesions were also found in patients with cognitive impairment as the presenting clinical symptom of MS (Coebergh et al. 2010). Other clinical manifestations that were associated to cortical lesions in MS patients are epileptic seizures. It has been showed that the prevalence of seizures in MS patients is three

times higher than in general population (Uribe-San-Martin et al. 2014). Using DIR sequences, Calabrese et al. showed that cortical lesions were more frequently found in MS patients with seizures, the number of cortical lesions was five times higher in patients with cortical lesions and "worm-like" lesions (lesions that extend through one or more cortical gyri) were seen more frequently in MS patients with seizures (Calabrese et al. 2012). Deep gray matter structures alterations and the clinical significance of these changes have been recently explored. Deep gray matter T2 hypointensities correlates with measures of cognitive dysfunction (Brass et al. 2006; Debernard et al. 2015) and disease duration (Brass et al. 2006) and predicts brain atrophy in patients with MS (Bermel et al. 2005). Several studies showed that deep gray matter atrophy is associated to slowed cognitive processing speed (Bergsland et al. 2015), disease severity (Uddin et al. 2015), disability progression and number of relapses in patients with early RRMS (Horakova et al. 2008).

A recent study used 7 T MRI and showed the presence of thalamic lesions on 24 of 34 patients with MS. The number of lesions was greater in progressive MS compared with RRMS and the lesion burden was correlated with EDSS score and measures of cortical lesions burden but not with white matter lesions burden or white matter volume (Harrison et al. 2015a, b). Several studies showed an association between basal ganglia and frontal/parietal cortical atrophy and fatigue in RRMS (Chalah et al. 2015) suggesting an association between the neurodegenerative process taking place in the striatum—thalamus—frontal cortex pathway and the development of fatigue in relapsing—remitting multiple sclerosis. The inclusion of the posterior parietal cortex as one of the best predictors of the Modified Fatigue Impact Scale cognitive domain suggests the major role of the posterior attentional system in determining cognitive fatigue in RRMS (Calabrese et al. 2010).

7.3 Oligodendrocyte Dysfunction in Multiple Sclerosis

7.3.1 Oligodendrocyte Progenitor Cells and Oligodendrocytes During CNS Development

The central nervous system (CNS) is home for four major classes of glial cells: astrocytes, microglia, oligodencrocytes (OLs) and oligodendrocyte progenitor cells (OPCs). OPCs have stelate morphology and are present in both gray and white matter. OPCs represent the largest dividing population among neural cells and are uniformly distributed, making up, on average, 5 % of total CNS cells (Dawson et al. 2003). They belong to the same population of progenitors that give rise to OLs during CNS development. However, a large fraction of OPCs do not differentiate and remain in a cycling state throughout adulthood (Fernandez-Castaneda and Gaultier 2016). OPCs differentiate into OLs during CNS development (Gensert and Goldman 1997) and also when local CNS injury occurs (Lytle et al. 2009). During development, OPCs are generated in sequential waves from specific germinal regions. In the mouse spinal cord the first wave of OPCs production commences in the ventral neuroepithelium, followed by a second wave of OPCs genesis from more dorsal progenitor domains (Cai et al. 2005) and a third wave that occurs after birth from the progenitor cells around central canal (Rowitch and Kriegstein 2010). The ventrally-derived cells accounts for 85-90 % of adult OLG while dorsaly – derived progenitors only contribute 10-15 %. In the developing forebrain of mice an initial wave of OPCs production commences in the medial ganglionic eminence and enteropeduncular area of the ventral telencephalon, a second and third waves emanate from the lateral and caudal ganglionic eminences and from the cortex after birth giving rise to the majority of adult OLs in mice (Kessaris et al. 2006). Extrinsec factors with opposing effects act on multipotential

neural progenitor cells (NPCs) to specify the oligodendroglial fate both in ventral-dorsal and rostro-caudal orientations such as sonic hedgehog (SHH), autotaxin and fibroblast growth factor (FGF-2) acting as promoters and bone morphogenic protein (BMP) that acts as an inhibitor of OLs specification (Mitew et al. 2014).

After initial glial fate specification has been achieved at the embryonic ventricular zones, OPCs migrate to reach the final sites of myelination. In the spinal cord, most ventrally derived OPCs spread out in a ventro-dorsal and mediolateral trajectory, traversing multiple rostro-caudal levels (Miller and Ono 1998), whereas dorsal-origin OPCs remains mostly in the dorsal and lateral regions of the spinal cord (Fogarty et al. 2005). In the cerebellum and diencephalon, OPCs migrate tangentially and rostro-caudally from the mesencephalic and diencephalic parabasal plates respectively (Garcia-Lopez and Martinez 2010; Mecklenburg et al. 2011). In the developing forebrain, OPCs migrate from the ventral pallidum to populate the lateral and dorsal regions of the telencephalon (Kessaris et al. 2006) or to invade the optic chiasm and spread along the optic nerves (Ono et al. 1997). These complicated migratory routes entail a sophisticated system of molecular guidance including Sonic hedgehog (SHH), platelet-derived growth factor (PDGF-AA), fibroblast growth factor 2 (FGF-2), contact molecules in the extracellular matrix (laminin, fibronectin, merosin, tenascin-C and anosmin-1) (Frost et al. 1996; Garcion et al. 2001; Chun et al. 2003; Bribian et al. 2006, 2008; Relucio et al. 2009, 2012, Leiton et al. 2015, Stoffels et al. 2015) and adhesion molecules such as polysialylated neuronal cell adhesion molecule (PSANCAM) (Decker et al. 2000), Eph/ephrins (Prestoz et al. 2004), avb1integrins (Milner et al. 1996, O'Meara et al. 2016), claudin-11/OSP (oligodendrocyte-specific protein) (Tiwari-Woodruff et al. 2006), AN2/NG2 (neural/ glial antigen 2) (Biname et al. 2013), and N-cadherin (Schnadelbach e al. 2000). OPCs migration is also strongly governed by chemotactic cues such as chemokine (C-X-C motif) ligand 12 (CXCL12) that acts throughout embryonic and postnatal development. Other common regulators of OPCs migration include the large family of pleiotropic factors, the semaphorins, particularly Semaphorin-3A that acts as a repellant, Semaphorin-3F that acts as an attractant and membrane bound Semaphorin-4D/F (Spassky et al. 2002; Armendariz et al. 2012; Wada et al. 2016). Recent studies showed that OPCs require the vasculature as a physical substrate for migration and chemokine receptor 4 (Wnt-Cxcr4) signaling coordinates OPCs-endothelial interactions (Tsai et al. 2016).

When the OPCs are positioned into the final sites, they differentiate into post-mitotic, premyelinating OLs, an event that proceeds in a caudal to rostral direction in the brain but rostro-caudally into the spinal cord (Brody et al. 1987). There is evidence that premyelinating OLs persist up to several months during human development before finally myelinating (Back et al. 2002), giving greater potential for correct axonal selection of the axons that should be myelinated (Almeida and Lyons 2014). OPCs proliferation and differentiation are tightly regulated in order to maintain a fine balance (Hughes et al. 2013). In adults, OPCs are uniformly distributed across the brain and spinal cord but their quantity varied between the white and gray matter (Dawson et al. 2003). Whereas in the white matter, such as the dorsal column of the spinal cord, OPCs account for up to 8 % of all cells, in the dorsal horn of the spinal cord OPCs account for only 3 % of the cellular content (Dawson et al. 2003). There is also a heterogeneity in OPCs population. Fate-mapping studies have shown that OPCs in the white matter differentiate into OLs more frequently than gray matter OPCs (Dimou et al. 2008) and there are also differences in division cycle and proliferative response probable due to factors of environment (Vigano et al. 2013).

7.3.2 Role of Oligodendrocyte in Myelination and Trophic Support of Axons in Humans

The myelination of axons, performed in the central nervous system by oligodendrocytes (OLs), is a very complex cellular interaction specific to

vertebrates. The myelination allows the saltatory conduction, between nodes of Ranvier, of neuronal action potential which increases both speed and energy efficiency of nerve conduction. In addition to myelination, there is increasing evidence that OLs provide trophic support to axons and lactate as energy source (Funfschilling et al. 2012). CNS myelination is a late-occurring aspect of neural development and takes a long time-frame. In humans, most of CNS myelination occurs throughout the first two decades of life, with late-maturing brain structures such as prefrontal cortex myelinating last (Yakovlev and Lecours 1967; Mitew et al. 2014). There is increasing evidence that myelination continues throughout life, either to replace lost OLs and myelin or to myelinate previously unmyelinated axons (Bartzokis et al. 2012; Young et al. 2013). The development of OLs and the myelination process are controlled by an exquisite genetic mechanism since different regions of CNS myelinate at different stages of development and most regions contain a mix of myelinated and non-myelinated axons. There is also increasing evidence about the experience-driven plasticity in the myelination process (Markham and Greenough 2004; Fields 2005). The process of myelination has been extensively studied and several concepts have been formulated in parallel with evolution of examination techniques.

For almost five decades CNS myelin has been described as the sheath generated by wrapping of an OL process around an axon (Hirano and Dembitzer 1967). Recently, a new version of this model has been advanced by Snadeiro et al. (2014) suggesting that "a growing myelin sheath winds around the axon by advancing inner tongue underneath the previously deposited membrane in the center of the myelin segment" and that the assembly of myelin sheath is performed by "membrane transport via the biosynthetic secretory pathway". In contrast, Szuchet et al. (2015) proposed a different model of myelin sheath assembly: on reaching the axon, the "OL process bifurcates with each of its arms embracing it with a slight overlap and probable fusion at the opposite end", myelin membranes are synthesized by OL as independent structural entities and packaged as tubules into specific organelles – myelinophore organelles – within the OL perikaryon, transported and assembled stochastically by homotypic fusion inside the OL process. Numerous studies provided evidence for OL – axon interaction (Aggarwal et al. 2011; Schnaar 2010) that conduce to a modular structure that binds them together (Eshed-Eisenbach and Peles 2013). Also neuronal activity has been studied as regulator of myelination in the CNS (Gibson et al. 2014; Hines et al. 2015; Mensch et al. 2015). Neuronal activity mediates exosome secretion by OLs, which are then endocytosed by neurons and promote survival under oxidative stress or nutrient deprivation conditions (Fruhbeis et al. 2013). Gibson et al. (2014) used optogenetic technology to stimulate mouse premotor cortex and showed that neuronal activity promotes OPCs proliferation, differentiation and myelination but this proliferation did not translate into a proportional increase in myelin thickness, suggesting a potential unconventional fate for the newly generated cells (Gibson et al. 2014).

There is additional evidence for the neuron-OPCs bidirectional crosstalk such the report that OPCs receive excitatory glutamatergic synaptic input via OPC-expressed AMPA receptors (Bergles et al. 2000) and also GABAergic input via OPC-expressed $GABA_A$ receptors (Arellano et al. 2016). At the synapse OPCs have been shown to make contact with pre- and post-synaptic terminals (Bergles et al. 2000) and OPCs contact axons at nodes of Ranvier, suggesting that OPCs could maintain node function (Butt et al. 1999). Also OPCs deletion in the prefrontal cortex compromises glutamatergic signaling in the pyramidal neurons (Birey et al. 2015). Sakry et al., have demonstrated that ectodomain cleavage of NG2, a proteoglycan highly expressed by OPCs, can also modulate neuronal networks (Sakry et al. 2014). During myelination, developing OPCs undergo a 6500-fold increase in membrane area to provide myelin segments to multiple axons (Baron and Hoekstra 2010; Chong et al. 2012), a process which entails high metabolic demands (Harris and Attwell 2012; Nave 2010). Thus, OLs and OPCs require access to a rich vascular supply for nutritive and oxidative substrates.

The mechanisms that coordinate myelination and angiogenesis are unclear although recent studies suggested that OPCs might produce angiogenic factors to encourage revascularization of injured CNS tissue (Cayre et al. 2013; Jiang et al. 2011; Pham et al. 2012). Yuen et al. (2014) showed that brain oxygen tension, mediated by OPC-encoded hypoxia-inducible factor (HIF) function, is an essential regulator of postnatal myelination. Constitutive HIF1/2α stabilization resulted in OPC maturation arrest through autocrine activation of canonical *Wnt7a/7b*. OPCs also showed paracrine activity that induces excessive postnatal white matter angiogenesis *in vivo*, and directly stimulates endothelial cell proliferation *in vitro*. Conversely, OPC-specific *HIF1/2*α loss-of-function leads to insufficient angiogenesis in corpus callosum and catastrophic axon loss. Based on these findings, the authors suggest that OPC intrinsic HIF signaling couples postnatal white matter angiogenesis, axon integrity and the onset of myelination in mammalian forebrain.

7.3.3 Oligodendroglial Dysfunction and Demyelination in Multiple Sclerosis

Oligodendroglial dysfunction and cells death are features of different human CNS diseases such as demyelinating diseases, stroke, traumatic brain, spinal cord injury or neurodegenerative diseases. Persistent demyelination in MS is the result of a disturbed balance between the dysfunction and then loss of OLs that produces demyelination and the impaired/reduced generation of OLs from OPCs inducing insufficient remyelination, evolving in parallel with neuronal loss and axonal damage. In MS two patterns of OLs dysfunction can be distinguished histopathologically: a immune-mediated OLs dysfunction and a primary oligodendrogliopathy. Research on in vitro models and animal models of MS provided evidence for direct cytotoxicity to myelin and OLs by antigen specific cytotoxic T lymphocytes (Na et al. 2008; Lassmann 2014) and specific autoantibodies (Linington et al. 1988), T-cell mediated cytotoxicity independent from antigen recognition (Nitsch et al. 2004) as well as activation of microglia and macrophages by pro-inflammatory molecules (Felts et al. 2005). Other studies identified in serum and CSF of MS patients various antibodies proteins expressed in OL that may be targets for autoimmune response such as myelin basic protein (MBP), proteolipid protein (PLP), myelin associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), transaldolase, oligodendrocyte surface protein (OSP), oligodendrocyte myelin glycoprotein (omGP), NOGO, NG2 and glycolipids (Sun et al. 2015; Ramanathan et al. 2015; Pittock et al. 2007; Hecker et al. 2016). Anti MOG antibodies have been identified in the brain tissue removed from patients with MS at autopsy and were found in higher amount in the brain than in serum or CSF, being also accumulated in the MS lesions (O'Connor et al. 2005). However, the studies that investigated prognostic value of anti-myelin antibodies as predictors for progression from clinical isolated syndrome (CIS) to MS has yielded contradictory results (Tomassini et al. 2007; Kuhle et al. 2007) and the role of anti-MOG antibodies in the pathogenesis of MS remains to be clarified (Ramanathan et al. 2015). Also, antibodies against AN2, a cell surface glycoprotein expressed on OPCs in the developing and adult CNS, have been reported in the CSF from certain patients with multiple sclerosis with active relapses (Niehaus et al. 2000). In vitro, these antibodies block the migration of OPCs, synthesis of myelin and may lead to lysis of OLs. The presence of such antibodies may explain the dysfunction of remyelination processes in MS patients.

Recent studies provided evidence for the important role of microglia and macrophages activation in the pathogenesis of MS and their effects on OLs. Reactive microglia and macrophages are active participants and play a dual role both in the development and expansion of MS lesions and also in the remyelination of lesions (Zhang et al. 2011; Bogie et al. 2014; Peferoen et al. 2015). Clusters of activated microglia are present in the NAWM and in areas of remyelination (Peferoen et al. 2015). Depending on the change in the microenvironment, microglia as

well as macrophages may change the phenotype from the pro-inflammatory (M1) profile to the anti-inflammatory (M2) profile. Also, microglia in preactive MS lesions may express an intermediate phenotype and resemble microglia from reparative remyelinating lesions rather than actively demyelinating lesions (Peferoen et al. 2015). Activated microglia and macrophages synthesize different cytokines, trophic factors, extracellular matrix components and neurotransmitter-like molecules that could exert a protective or a deleterious effect on the adjacent cells depending on the type of macrophage/microglia that produces them (Benjamins 2013). Activated microglia release pro-inflammatory mediators including tumor necrosis factor-alpha (TNF-α), IL-1, proteases and glutamate, among many other molecules with potential deleterious effect on the neural tissue. TNF-α is a pro-inflammatory cytokine that can damage OLs in MS with necrotic pathology by initiating the process of necroptosis (Pasparakis and Vandenabeele 2015). Necroptosis express the cell death by necrosis and it is initiated when caspase-driven apoptosis is blocked. This newly defined programmed necrosis or necroptosis is a well regulated cell death process believed to be involved in inflammatory and neurodegenerative CNS diseases. In a recent report, Ofengeim et al. (2015) provided evidence for the role of necroptosis in MS. The authors demonstrated reduced caspase-8 levels in the microglial cells and activation of RIPK1, RIPK3 and MLKL (characteristic molecules of necroptosis) in cortical lesions from MS brain specimen. They also demonstrated elevated RIPK1 in the OLs, microglia and neurons from corpus callosum both in a cuprizone model as well as in an EAE model of MS. Also, activated microglia produce anti-inflammatory cytokines such as interleukin (IL)-10 that can lead to preactive lesion resolution (Sato et al. 2015).

Another important player in the MS lesions is the astrocyte involved in inflammation as well as the integrity and function of BBB. Astrocytes may play a role in T cell recruitment, activation and differentiation through pro-inflammatory cytokines production such as IL17 and chemokines; several reports show higher CSF levels of CXCL10, CXCL9, CCL5, CCL3, CCL2, CCL12 in MS patients (Xie and Yang 2015; Choi et al. 2014; Sorensen et al. 1999). This finding was further confirmed by the increased expression of these chemokines on astrocytes at the edge of the MS plaques. Astrocytes can also express toll-like receptors (TLRs) that can contribute to MS pathogenesis during progressive stage of the disease (Farez et al. 2009). Astrocyte secrete anti-inflammatory cytokines like IL-10 and contribute to counter immune response regulation (Farez and Correale 2016). The interaction between astrocytes and T cells is bidirectional; T cells also induce biological changes in astrocytes that can either attenuate or exacerbate the disease (Xie and Yang 2015). Astrocytes have also a direct effect on OL injury. Thus, through a direct cell contact-dependent mechanism, astrocytes promotes TNF toxicity to OPCs (Kim et al. 2011). Astrocytes secrete matrix metalloproteinases (MMPs) that increase the permeability and produce the remodeling of the BBB (Williams et al. 2007). In addition, astrocytes may limit remyelination processes through interaction of NOGO with LINGO in MS plaques (Karnezis et al. 2004; Satoh et al. 2007), a novel therapeutic approach currently under investigation in clinical trials.

7.3.4 Oligodendrocytes and Neurodegeneration in MS

Final pathways of pathological mechanism in MS drive to neurodegeneration and may involve activation of death receptors (Lassmann 2014) by cytokines (Cannella et al. 2007), microglia activation, oxidative injury (Lassman and van Horssen 2015), mitochondrial damage (Albanese et al. 2016; Errea et al. 2015; Dutta et al. 2006; Mahad et al. 2008), excitotoxicity (Gilani et al. 2014) or disturbance of the fine balance of ion channel function (Arnold et al. 2015; Waxman 2008). The severe oxidative and mitochondrial injury which produces demyelination within active MS lesions shows a pattern of distal "dying back" oligodendrogliopathy (Aboul-Enein et al. 2003). This is characterized by a primary degen-

eration in the most distal (peri-axonal) oligodendrocyte processes, reflected by a selective loss of myelin associated glycoprotein in initial lesions stages and later followed by OLs apoptosis (Lassmann 2014). The most extensive oxidative injury in active MS lesions is seen within myelin and OLs and it is reflected by accumulation of oxidized lipids in the cytoplasm and the presence of oxidized DNA within nuclei, some of them with features of apoptosis (Haider et al. 2011). Also the high lipid content of myelin sheaths may render it highly vulnerable for lipid peroxidation and its consequences. The major role played by the oxidative stress in MS lesions is also supported by the upregulations of antioxidative defense mechanisms (van Horssen et al. 2010) and the efficiency of antioxidative treatment for RRMS patients (Gold et al. 2012a, b; Strassburger-Krogias et al. 2014). Of particular importance may be also the altered mitochondrial function in axons, leading to chronic cells stress and imbalance of ion homeostasis, resulting in axonal and neuronal death (Mahad et al. 2015). The radical injury is further amplified by transition metals such as iron and copper (Jomova and Valko 2011) and iron toxicity has been suggested to participate in neurodegenerative diseases (Nunez et al. 2012), including MS (Williams et al. 2012).

Iron accumulates within human brain with aging (Hallgren and Sourander 1958) and iron storage, as non-heme iron, mainly take place in OLs and myelin (Connor and Menzies 1995). Iron within the catalytic center of various enzymes is essential for normal brain metabolism such as oxidative phosphorylation and myelination (Todorich et al. 2009). However, in liberated form, ferrous iron ions may generate toxic reactive oxygen species (ROS) which initiate oxidative damage (Kell 2009). Pathological and MRI studies have revealed iron accumulation at the edges of chronic MS lesions (Craelius et al. 1982; Bagnato et al. 2011; Pitt et al. 2010). Iron was present in OLs in the NAWM and accumulated in microglia and macrophages at the lesion edges (Bagnato et al. 2011; Pitt et al. 2010). Several studies found that iron containing OLs and myelin were destroyed in active MS lesions (Marik et al. 2007; Fischer et al. 2013), which presumably led to a wave of iron liberation from the intracellular stores into the extracellular space. They detected extracellular iron, including ferrous iron, especially in active lesions of aged patients with acute MS and short disease duration, where new active lesions form against the background of high tissue iron load. These events may be followed by a shift of cellular iron storage from OLs to microglia and macrophages. The perivascular accumulation of iron containing macrophages suggests that they remove iron from the lesions through perivascular drainage into the cervical lymph nodes, as shown for macrophages containing myelin and neuronal antigens (van Zwam et al. 2009) or USPIO (Oude Engberink et al. 2010). That may explain why inactive demyelinated lesions showed on average a lower iron load than the surrounding NAWM. Hametner et al. (2013) also found that iron decreases in NAWM of MS patients with increasing disease duration. It is likely that all these mechanisms are involved in the pathogenesis of tissue damage at different stages of disease, within or outside from focal lesions and in different stages of plaque formation (Lassmann 2014).

7.3.5 Role of Oligodendrocyte in CNS Remyelination and Regeneration in MS

In adult CNS, the subgranular zone of the dentate gyrus (SGZ) in the hippocampal formation and the subventricular zone of the lateral ventricles (V-SVZ) are the main germinative areas. In these areas reside neural stem/progenitor cells (NSPCs) and OPCs which can give rise to neurons and glial cells (Gonzalez-Perez and Alvarez-Buylla 2011; Ihrie and Alvarez-Buylla 2011; Falcao et al. 2012). In addition, recent studies suggest the presence of nonconventional germinative zones, outside of the V-SVZ and SGZ, such as the cerebral cortex (Nakagomi et al. 2009; Ohira 2011), white matter (Nunes et al. 2003), and pia mater (Nakagomi et al. 2011). Among these germinative areas, the V-SVZ generates the most abundant number of stem cells in the adult brain

that are capable of migrating to a long distance. Under normal conditions in the adult brain, the V-SVZ progenitors generate a large number of neurons with a small number of oligodendrocyte lineage cells. However, after demyelination, the fate of V-SVZ-derived progenitor cells shifts from neurons to OPCs (Maki et al. 2013). Neurons, once fully differentiated, are unable to replicate in response to insult or injury. In contrast to neurons, in response to oligodendrocyte injury and death, local oligodendrocyte precursor cells (OPCs) can proliferate, migrate into the site of damage and differentiate into mature oligodendrocytes capable of replacing the damaged myelin sheath. This process is known as remyelination. Renewal of myelin/oligodendrocyte continues throughout adult life (Dimou et al. 2008; Young et al. 2013) and maintain some plasticity in response to changes in neural activity (Scholz et al. 2009) and brain injury (Nait-Oumesmar et al. 2008).

Remyelination in MS occurs as a spontaneous regenerative process following demyelination (Franklin and Ffrench-Constant 2008; Crawford et al. 2013; Aharoni 2015) and presents greater efficiency in MS lesions appearing early in the disease course (Patani et al. 2007; Patrikios et al. 2006). In MS the efficiency of remyelination declines with age and disease progression. Remyelination of MS lesions is variable and often incomplete. In comparison with very effective remyelination that routinely occurs following demyelination associated with traumatic injury (Lasiene et al. 2008), in many experimental models of MS, and even in many MS lesions (Fancy et al. 2010; Patrikios et al. 2006), histopathological studies show that remyelination may be extensive (Patrikios et al. 2006; Bramow et al. 2010), but often it is insufficient and it diminishes over time, leading to persistent demyelination and axon degeneration (Franklin and Ffrench-Constant 2008; Patrikios et al. 2006; Compston and Coles 2008; Piaton et al. 2009). In general, the extent of remyelination varies from patient to patient and from one lesion to another (Zhang et al. 2016). The remyelination is mostly restricted to the peripheral areas of lesions, starts early during the formation of lesions, and is present in lesions with active demyelination (Bø et al. 2013; Goldschmidt et al. 2009; Lucchinetti et al. 1999). In average, about 10–20 % of chronic lesions are completely remyelinated (so-called shadow plaques) (Barkhof et al. 2003; Patani et al. 2007). However, remyelinated lesion areas may be more vulnerable to repeated demyelinating activity compared to NAWM (Bramow et al. 2010). Observations from a recent magnetisation transfer ratio (MTR) study support the hypothesis that entirely demyelinated lesions found on histopathology are the result of multiple episodes of demyelination and incomplete remyelination (Brown et al. 2014). Unraveling the remyelination process in MS and discovering the reasons for remyelination failure is particularly important as drug discovery efforts have expanded to include neuroprotective and remyelination promoting strategies (Franklin 2002).

Numerous studies on animal models of MS provided evidence that the restoration of new myelin sheaths for demyelinated axons in MS occurs in several steps. Following demyelination, factors produced by microglial cells and astrocytes activate OPCs, which shift from a quiescent to a regenerative phenotype. The activation process involves changes in OPC morphology (Levine et al. 2001), as well as the up-regulation of several genes. Many of the up-regulated genes are those active during developmental OPC generation, such as the transcription factors Olig2, Sox2 and Nkx2.2 (Talbott et al. 2005; Fancy et al. 2004). The OPCs activation response is proportional to the inflammatory reaction that succeeds demyelination and it is required for successful remyelination to occur in animal model systems (Glezer et al. 2006; Miron et al. 2011). The activated OPCs then respond to mitogens and promigratory factors released by the microglia and astrocytes. The directed migration of these cells to white matter lesions has been suggested to be mediated by the chemo-attractant PDGF and semaphorin 3F, the chemo-repellents netrin-1, semaphorin 3A, and the ephrins, as well as the stop-signals CXCL1 and tenascin C (Dubois-Dalcq and Murray 2000; Boyd et al. 2013; Williams et al. 2007; Bin et al. 2013; Sobel 2006;

Kakinuma et al. 2004; Kerstetter et al. 2009; Miron et al. 2011).

To populate demyelinated areas, the recruited OPCs start to differentiate into remyelinating oligodendrocytes (Franklin and Ffrench-Constant 2008; Bradl and Lassmann 2010). The differentiation of OPCs is promoted by insulin-like growth factor (IGF0-1, ciliary neurotrophic factor (CNTF) and thyroid hormone (Zhang et al. 2015a, b). Differentiation requires the function of Olig1, Olig2, Nkx2.6, Myt1 and sex determining region Y box (SOX)-10 (Nunes et al. 2003; Fancy et al. 2004; Nicolay et al. 2007), probably due to interaction of these transcription factors with promoters of myelin genes (Sohn et al. 2006; Miron et al. 2011). The OLs must establish the contact with the axon to be remyelinated before generating the myelin protein membrane. Axon-glia interaction and myelin membrane trafficking are essential for remyelination. In a recent review, White and Krämer-Albers (2014) describe the mechanism of axonal signal integration by OLs, emphasizing the central role of Src-family kinase Fyn during CNS myelination. The authors also discuss myelin membrane trafficking with particular focus on endocytic recycling and the control of proteolipid protein (PLP) transport by soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins.

The development of myelin sheaths follows a similar pattern during developmental myelination and remyelination, although the rate of adult OPC migration is slower (Bradl and Lassmann 2010; Fancy et al. 2010). Also, the myelin sheath in the remyelinated lesions is typically thinner and shorter than in the pre-lesion sheath but, despite its smaller dimensions, it appears sufficient to ensure full functional recovery of the axon (Crawford et al. 2013). Regions of remyelination are often extensive in MS lesions and are referred to as shadow plaques due to the paler staining of the thinner myelin sheaths (Patani et al. 2007).

One of the reasons for remyelination failure in MS might be the impaired migration of OPCs. Thus, despite detection of OPCs in NAWM, oligodendroglial depopulation may be the consequence of the impaired recruitment of OPCs to demyelinating areas (Franklin and Ffrench-Constant 2008; Piaton et al. 2009). OPCs were identified within active MS lesions in humans, but their number and capacity to differentiate decreases with disease duration (Kuhlmann et al. 2008). Another reason for remyelination failure may be the low differentiation rate of OPCs in MS lesions. Cui et al. (2013) showed that the percentage of mature OLs was reduced in all the actively demyelinating lesions and that OPCs were more vulnerable to injury mediators than OLs. Also, recent animal experiments using genetic fate mapping techniques (Tripathi et al. 2010; Zawadzka et al. 2010) implicate OPCs and not mature previously myelinating OLs as the cells responsible for remyelination (Gensert and Goldman 1997; Carroll et al. 1998; Franklin and Ffrench-Constant 2008).

7.4 Oligodendrocyte Progenitor Cells (OPCs) Dysfunction and Potential Therapeutic Targets for remyelinating Therapies in MS

7.4.1 Depletion of OPCs

The traditional view about the progressive failure of remyelination in MS was that it is due to depletion of OPCs after successive episodes of demyelination at the same lesions site, finally exhausting the capacity of system to adequately remyelinate (Franklin 2002; Ludwin 1980). This theory was suggested by the observation that repeated transfer of encephalitogenic T cells and the demyelinating antibody anti-MOG (myelin oligodendrocyte glycoprotein) induced persistently demyelinated lesions (Linington et al. 1992). However, it is not supported by the presence of OPCs and immature oligodendrocytes contacting axons but failing to myelinate, detected in autopsy samples from MS patients (Camara and ffrench-Constant 2007; Chang et al. 2002, Scolding et al. 1998). Additional evidence against this theory is provided by analysis of focal demyelinated lesions showed that areas subjected to successive demyelination-remyelination as well as first time demyelinated

areas showed no evidence of progressive depletion of OPCs (Penderis et al. 2003). Furthermore it has been shown that, despite the presence of endogenous OPCs in chronic lesions of MS (Chang et al. 2002), they often fail to remyelinate axons, suggesting a differentiation failure (Kuhlmann et al. 2008; Hartley et al. 2014).

7.4.2 Impaired Migration

Several studies have identified inhibitors of OPCs migration during remyelination; however, the influence of inhibitors of OPC migration on remyelination is not well understood (Piaton et al. 2011). Group 3 semaphorins are secreted factors that attach to extracellular matrix forming gradients for cells to use as migration cues. Sema3A binds to the receptor Neuropilin (NP)1 and Sema3F to the receptor NP2, and in development, Sema3A is an inhibitory and Sema3F an attractive migratory signal for OPCs (Sugimoto et al. 2001). In adulthood, Sema3A and 3F mRNA expression is absent in white matter, but re-expressed in MS lesions in a differential way in different lesions, with active lesions (more inflammatory and more likely to remyelinate) containing higher mRNA expression of the chemoattractant Sema3F than Sema3A, and chronic active lesions (less inflammatory and less likely to remyelinate) with higher mRNA expression of the chemorepellent Sema3A than Sema3F (Williams et al. 2007). Boyd et al. (2013) assessed the number of OPCs in a series of MS lesions in *postmortem* tissue, and correlated these with pathological classification and Sema3A/Sema3F protein expression. They found a correlation between a lower number of OPCs, chronic active lesion type and a higher expression of the chemorepellent protein Sema3A. In contrast, a low expression of the chemorepellent Sema3A and higher expression of the chemoattractant Sema3F correlates with active lesions and more variable, but generally higher OPC numbers. The authors also tested the hypothesis that the mechanism for these observations is due to the effect of these chemotactic factors on OPCs migration and subsequent remyelination by manipulating levels of Sema3A or 3F in a mouse model of demyelination. They concluded that migration failure is an important cause of remyelination failure and Sema3A/NP1 pathway may be a possible therapeutic target to improve OPCs migration and remyelination in MS.

The role of netrin-1 as a repellent for migrating OPCs during development (Tepavcevic et al. 2014; Bin et al. 2013; Jarjour et al. 2003; Sugimoto et al. 2001) and its expression by neurons and glia in the mature CNS (Manitt et al. 2001) suggest that it might also influence OPC migration and remyelination in MS. Bin et al. (2013) showed that full-length and fragmented netrin-1 are present in adult human white matter, as well as in demyelinated MS lesions, where they are positioned to inhibit OPCs migration. Notably, this inhibitory effect of netrin-1 may negatively affect remyelination in patients with MS because of the accumulation of netrin-1 associated with the extracellular matrix in lesions. Although netrin-1 and netrin-1 fragments may play a positive role in the mature CNS by restricting cell migration, axon growth, and sprouting (Low et al. 2008; Manitt et al. 2006), they inhibit the capacity of OPCs to access and repair demyelinated plaques in pathological circumstances. Thus, the development of strategies to block netrin-1 function holds promise for future treatment of demyelinating diseases, such as MS.

7.4.3 Chemokines

OLs express at least four chemokine receptors, CXCR1, CXCR2, CXCR3 and CXCR4 (Nguyen and Stangel 2001; Omari et al. 2005). CXCL1, one of the ligands to CXCR2 stimulates the proliferation of OLs in vitro (Robinson et al. 1998) and halts the OPCs migration in vivo (Tsai et al. 2002). Histological studies suggest CXCL1 is upregulated around the peripheral areas of demyelination suggesting this receptor/ligand combination modulates responses to injury. Also, localized inhibition of CXCR2 signaling reduces lesion size and enhances remyelination (Kerstetter et al. 2009). The chemokine CXCL12 is a developmental molecule known to orchestrate the

migration, proliferation, and differentiation of neuronal precursor cells within the developing CNS. Patel et al. (2010), using an experimental murine model of demyelination mediated by the copper chelator cuprizone, evaluated the expression of CXCL12 and its receptor, CXCR4, within the demyelinating and remyelinating corpus callosum. CXCL12 was significantly up-regulated within activated astrocytes and microglia in the CC during demyelination, as were numbers of CXCR4+NG2+ oligodendrocyte precursor cells (OPCs). Loss of CXCR4 signaling via either pharmacological blockade or in vivo RNA silencing led to decreased OPCs maturation and failure to remyelinate. These data indicate that CXCR4 activation, by promoting the differentiation of OPCs into oligodendrocytes, is critical for remyelination of the injured adult CNS.

7.4.4 Impaired Differentiation of OPCs in MS

During development, the determination of OPCs is regulated by a complex interplay of intrinsic, extrinsic and epigenetic factors (Rowitch and Kriegstein 2010). Here we describe the role of intrinsic and extrinsic signaling pathways known to regulate OPCs differentiation and how they are modified in MS settings as well as potential therapeutic targets that could promote remyelination.

7.4.5 Olig 2 Factor

Among intrinsic factors, the basic helix-loop-helix (bHLH) transcription factor Olig2 has critical functions in oligodendrocyte determination (Rowitch 2004). Olig2 loss- and gain-of-function studies provided compelling support of its requirement in oligodendrocyte specification (Liu et al. 2007a, b; Maire et al. 2010). Furthermore, Olig2 remains expressed in OPCs (Takebayashi et al. 2000) and overexpression of Olig2 alone triggers OPC differentiation (Liu et al. 2007a, b). Wegener et al. (2015) demonstrate that OLIG2 displays a differential expression pattern in multiple sclerosis lesions that correlates with lesion activity. Strikingly, Olig2 was predominantly detected in NOGO-A$^+$ (now known as RTN4-A) maturing oligodendrocytes, which prevailed in active lesion borders, rather than chronic silent and shadow plaques.

7.4.6 LINGO-1 Signaling

Leucin-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 1 (LINGO-1) is a potent negative regulator of neuron and oligodendrocyte survival, neurite extension, axon regeneration, oligodendrocyte differentiation, axonal myelination and functional recovery (Yin and Hu 2014). LINGO-1 knockout mice exhibit enhanced myelin sheath formation and recovery from EAE (Mi et al. 2007). Also, treatment with LINGO-1 antagonist results in increased OPCs differentiation and enhanced remyelination following EAE and lysolecithin-mediated demyelination (Zhang et al. 2015a, b) suggesting that blocking LINGO-1 may be a useful therapeutic approach (Rudick et al. 2008). The mechanism by which LINGO-1 exerts its regenerative effect in MS is still investigated. In a phase I clinical trial, anti-LINGO-1 monoclonal antibody (BIIB033) showed safety and tolerability in MS patients (Tran et al. 2014). A Phase II trial in people with acute optic neuritis (named RENEW) demonstrated an improvement in the study's primary endpoint, that is, recovery of optic nerve latency (time for a signal to travel from the retina to the visual cortex) relative to placebo. However, secondary endpoints, such as a change in thickness of the retinal layers or visual function were not met in this clinical trial. Results of the SYNERGY study, which combines anti-LINGO-1 therapy with Avonex® for RRMS are anticipated in the year 2016.

7.4.7 Canonical Notch Signaling

The Notch receptors are a family of transmembrane proteins that are cleaved when activated to modulate gene expression (Brosnan and John

2009). Canonical Notch signaling, which occurs through ligands such as Jagged, inhibits OPC differentiation during development (Genoud et al. 2002). However its role in CNS remyelination is still debated. Hammond et al. (2014) recently demonstrated that OPC differentiation following lysolecithin demyelination is inhibited by Jagged1-expressing astrocytes, which directly bind to Notch1 on OPCs (Hammond et al. 2014). Reactive astrocytes express Jagged1 in MS plaques (John et al. 2002) and this expression appears to be regulated by the secreted protein endothelin-1 (ET-1) (Hammond et al. 2014), which inhibits OL differentiation during development (Chamberlain et al. 2015). Notch was found activated in Nestin-expressing neural progenitor cells and in NG2-expressing oligodendroglial precursor cells in the subventricular zone and corpus callosum of lysolecithin-demyelinated rats. Notch activation seemed to be driven by Jagged1, which led to a high expression of downstream gene Hes5 in the subventricular zone of demyelinated rats (Aparicio et al. 2013).

7.4.8 Wnt Signaling

Wnt proteins are secreted ligands that play numerous roles in regulating development, including oligodendrocyte genesis (Ortega et al. 2013). Until recently, most studies demonstrate an inhibitory role for Wnt/β-catenin/Tcf signaling in remyelination. However, this notion has been challenged by recent studies, which showed a pro-myelinating effect of this pathway (Hammond et al. 2015). Several studies suggest that Wnt/β-catenin signaling serves distinct functions in oligodendrocyte specification, differentiation, and myelination depending on timing and dosage (Xie et al. 2014). In support of this hypothesis, Wnt pathway activation was found to affect oligodendrocyte lineage cells in a dose-dependent fashion; low Wnt tone allows OPCs to differentiate and high Wnt tone after injury is associated with permanent white matter injury (Fancy et al. 2014). Also, current studies suggest that Wnt/β-catenin signaling plays distinct roles in oligodendrogenesis, oligodendrocyte differentiation, and myelination in a context-dependent manner (central nervous system regions, developmental stages), and that Wnt/β-catenin signaling interplays with, and is subjected to regulation by, other central nervous system factors and signaling pathways (Guo et al. 2015). Two excellent recent papers (Guo et al. 2015; Xie et al. 2014) review the current contradictory concepts concerning the role of the Wnt pathway in the oligodendrocyte development and remyelination process, attempt to address the potential mechanism underlying this controversy, and recommend caution in targeting the Wnt pathway as a potential demyelinating therapy.

7.4.9 RXR Signaling

Nuclear retinoid X receptor (RXR) pathway plays an important role in cell proliferation and development (Mark et al. 2009). RXRs couple with several other nuclear receptors including retinoic acid receptor, vitamin D receptor, thyroid receptor, and peroxisome proliferator-activated receptor to induce gene transcription (Rastinejad 2001). The RXR-γ isoform is the first identified nuclear receptor to play a role in promoting remyelination. Huang et al. (2011) found that RXR-γ is differentially upregulated during remyelination in rodent and in active and remyelinated MS lesions (Huang et al. 2011). Further, knockdown of RXR-γ receptor by RNA interference/RXR-specific antagonists dramatically reduces OPC differentiation *in vitro* and RXR-γ knockout mice exhibit significantly less mature oligodendrocytes following demyelination (Huang et al. 2011). De la Fuente et al. (2015) showed that RXR-γ binds to several nuclear receptors in OPCs and OLs, one of which is vitamin D receptor (VDR). Using pharmacological and knockdown approaches they showed that RXR-VDR signaling induces OPC differentiation and that VDR agonist vitamin D enhances OPC differentiation. They also showed expression of VDR in OLG lineage cells in multiple sclerosis. These data revealed a role for vitamin D in the regenerative component of demyelinating disease and identified a new target for remyelination. These

studies suggest that RXR-γ signaling may regulate oligodendrocyte differentiation and identify RXR ligands as potential pharmacological targets, many of which are under study or already approved for the treatment of certain cancers (Ballanger et al. 2010).

7.4.10 Endocrine Targets

MS shows a female-to-male gender prevalence and disturbances in sex steroid production (Kipp et al. 2012). Estrogen and progesteron operate in dampening central and brain-intrinsic immune responses and regulating local growth factor supply, oligodendrocyte and astrocyte function (Kipp et al. 2012). Several studies evaluated the correlations between sex hormones levels and disease severity in MS (Triantafyllou et al. 2015). Low estrogen states such as menopause and the postpartum period favor exacerbations of multiple sclerosis in women with the disease. Existing and emerging evidence suggests a role for estrogen in the alleviation of symptoms and reversal of pathology associated with MS in women (Christianson et al. 2015). The cerebrospinal fluid and plasma levels of several neuroactive steroids are modified in relapsing remitting multiple sclerosis male patients. The levels of progesterone and testosterone metabolites are deeply affected in cerebrospinal fluid of these patients (Caruso et al. 2014). A large body of studies has shown that 17-β estradiol (E2), estriol, and other estrogen receptor (ER) ligand treatments has a protective effect on susceptibility to experimental autoimmune encephalomyelitis (EAE) (Crawford et al. 2010). The efficiency of estrogens in ameliorating clinical disease in animal models of MS formed the base for clinical trials in Europe and the United States using estrogen therapy in MS patients. However, synthetic estrogen supplementation is associated with increased risk of breast and uterine cancer, heart disease, and stroke (Prentice et al. 2009). Most of these side effects are thought to be mediated through ERα, not ERβ (Caringella et al. 2011). As a result, ERβ became interesting as a target for neuroprotective therapy (Planey et al. 2014).

Several studies showed that prophylactic administration of the ERβ ligand 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN) decreases clinical features of EAE, is neuroprotective, stimulates endogenous myelination, and improves axon conduction without altering peripheral cytokine production or reducing CNS inflammation (Crawford et al. 2010). Further studies assessed the effects of therapeutic DPN treatment during peak EAE disease and investigated the mechanism of action of DPN treatment-induced recovery during EAE (Kumar et al. 2013). Given that prophylactic and therapeutic treatments with DPN during EAE improved remyelination-induced axon conduction, and that ER (α and β) and membrane (m)ERs are present on oligodendrocyte lineage cells, a direct effect of treatment on oligodendrocytes is likely. DPN treatment of EAE animals resulted in phosphorylated ERβ and activated the phosphatidylinositol 3-kinase (PI3K)/serine–threonine-specific protein kinase (Akt)/mammalian target of rapamycin (mTOR) signaling pathway, a pathway required for oligodendrocyte survival and axon myelination (Kumar et al. 2013). These results make DPN and similar ERβ ligands potential therapeutic candidates for demyelinating disease.

Thyroid hormone, progesteron and testosteron have been investigated in preclinical studies as potential modulators of myelination (Schumacher et al. 2012; Zhang et al. 2015a, b), however treatment with natural hormones can lead to a variety of systemic side effects. Thus, hormone analogs with tissue selective actions are exciting drug candidates for myelin repair. Thyroid hormone levels are essential for brain development and myelination and exerts their effect through intracellular thyroid hormone receptors (TR). During development, thyroid hormones signals OPCs to stop proliferating and start differentiating into OLs. Several studies demonstrated that thyroid hormones promote myelin repair on cuprizone model and EAE model in rodents and primates (Calza et al. 2010; Harsan et al. 2008; D'Intino et al. 2011).

Progesterone and its reduced metabolite allopregnanolone are both involved in OPCs proliferation and differentiation during development.

Late neural stem cells (NSCs) and OPCs produce higher levels of progesterone than differentiated oligodendrocytes. In early NSCs, allopregnanolone is produced by these cells and acts in an autocrine manner as a positive allosteric modulator of GABAA receptors to enhance NSC and OPC proliferation. In contrast, in OPCs and pre-oligodendrocytes, progesterone exerts its proliferative effects through intracellular progesterone receptors (PRs) (Schumacher et al. 2012; Hartley et al. 2014). Studies performed on animal models of EAE showed that progesteron decrease demyelination, disease severity and neurological deficits (Schumacher et al. 2012). Also PR agonist elcometrine has shown the ability to mimic progesteron activity in vitro (Hussain et al. 2011).

Testosterone has also been implicated as potential therapy for MS. Studies on EAE models showed that testosterone can have beneficial effects on disease course that was originally thought to occur due to immunomodulatory and anti-inflammatory mechanisms (Hussain et al. 2013). However, recent studies on cuprizone models of demyelination showed that testosterone enhances remyelination through the neural androgen receptor and a synthetic testosterone analog, 7α- methyl-19-nortestosterone fully mimicked testosterone action in the models (Hussain et al. 2013). Clinical trials determined that, in MS patients, testosterone treatment was correlated to reduced inflammation and improved cognition (Sicotte et al. 2007; Gold et al. 2008).

7.4.11 Other Factors That Influence OPCs Migration and Differentiation

A recent study have found that the myelin proteolipid protein is critical to regulating OPC migratory responses to the neurotransmitter glutamate through modulation of cell-surface expression of the calcium-impermeable GluR2 subunit of the AMPA glutamate receptor and increased intercellular Ca(2+) signaling. Altered glutamate homeostasis has been reported in demyelinated lesions. Therefore, understanding how OPCs respond to glutamate has important implications for treatment after white matter injury and disease (Harlow et al. 2015). Another factor that decreases migration, proliferation, and survival of OPCs, and reduces their differentiation into OLs in the MS lesions is acidic extracellular pH (Jagielska et al. 2013). Further, OPCs exhibit directional migration along pH gradients toward acidic pH. These in vitro findings support a possible in vivo scenario whereby pH gradients attract OPCs toward acidic lesions, but resulting reduction in OPCs survival and motility in acid decreases progress toward demyelinated axons and is further compounded by decreased differentiation into myelin-producing OLs. These results suggest that lesion acidity could contribute to decreased remyelination.

7.5 Oligodendrocytes as Immunomodulatory Cells in MS

There is increasing evidence that oligodendrocytes are capable of expressing a wide range of immunomodulatory molecules. They express various cytokines and chemokines (e.g.Il-1β, Il17A, CCL2,CXCL10), antigen presenting molecules (MHC class I and II) and co-stimulatory molecules (e.g.CD9,CD81), complement and complement receptor molecules (e.g.C1s,C2 and C3,C1R), complement regulatory molecules (e.g.CD46,CD55,CD59), tetraspanins (e.g. TSPAN2), neuroimmune regulatory proteins (e.g.CD200,CD47) as well as extracellular matrix proteins (e.g.VCAN) and many others. Their potential immunomodulatory properties can, at specific times and locations, influence ongoing immune processes (Zeis et al. 2015). Therefore, oligodendrocytes are well capable of immunomodulation, especially during the initiation or resolution of immune processes in which subtle signaling might tip the scale. Moyon et al. have recently demonstrated that OPCs isolated from the brain of mice undergoing cuprizone-induced demyelination express high levels of CCL-2 and IL-1b (Moyon et al. 2015). CCL-2 has a critical role in recruiting monocytes (Deshmane et al. 2009), while IL-1b is a powerful inflammatory

cytokine involved in many aspects of the immune response (Sims and Smith 2010). While authors did not explore the role of these mediators on immune cell recruitment/ activation, they discovered that CCL-2 promotes OPC migration in vivo and is also expressed by OPCs present in active multiple sclerosis (MS) lesions (Moyon et al. 2015).

7.6 Promoting Remyelination – Perspectives for Regenerative Therapies in MS

All current treatments for MS used in clinical practice act primarily on modulating or suppressing the immune system, to prevent relapses, but there is still sparse evidence about the role of these drugs or of another potential agents on remyelination and neuroprotection in MS. Here we present the evidence about the remyelination potential of immunomodulatory drugs approved or still in clinical trials for the treatment of MS.

7.6.1 S1P Modulation Agents

S1P is a bioactive sphingolipid that mediates a wide range of biological effects in different cells and tissues. Research from different laboratories revealed direct actions of S1P at different maturational stages along the oligodendroglial lineage (Coelho et al. 2010). Mature OLs preferentially express S1P5 and may express S1P1, S1P2, and S1P3 at lower levels, while OPCs show high levels of S1P1 gene expression and lower levels of S1P5 and S1P3 expression (Novgorodov et al. 2007; Jung et al. 2007; Miron et al. 2008a, b). There is also evidence for the existence in oligodendrocytes of interactions between S1P and signaling by factors which, like neurotrophin-3 (NT-3) and platelet-derived growth factor (PDGF), have profound effects on oligodendrocyte development and myelination (Coelho et al. 2010).

The effects of S1P on oligodendrocyte lineages include differentiation, migration and survival, depending on the developmental stage (Jaillard et al. 2005; Jung et al. 2007).

Fingolimod (FTY720; GILENYA™, Novartis Pharma AG, Basel, Switzerland) is a modulator of S1P receptors and is the first oral disease-modifying therapy approved for relapsing forms of MS (Miron et al. 2008a, b). Fingolimod is phosphorylated in vivo by sphingosine kinase, particularly SphK2, to produce the active metabolite fingolimod phosphate (fingolimod-P). Fingolimod and fingolimod-P are structural analogs of sphingosine and S1P, respectively. Being a structural analog of S1P enables fingolimod-P to bind to and activate four of the five S1P receptor subtypes: S1P1, S1P4, S1P5 and S1P3, but has shown essentially no activity at S1P2 (Groves et al. 2013). Due to modulation of S1P1 on lymphocytes, fingolimod is thought to retain circulating pathogenic lymphocytes in the lymph nodes, thereby preventing their infiltration into the CNS (Cohen and Chun 2011). Fingolimod-P initially acts as an S1P1 agonist; however, chronic exposure to fingolimod-P leads to irreversible receptor internalization resulting in "functional antagonism" of S1P1-mediated S1P signaling (Groves et al. 2013). Fingolimod does not significantly affect activation and proliferation of redistributed naive and central memory T cells and does not block the egress from lymph nodes of effector memory T cells that are CCR7-negative, a distinct subpopulation of T cells that are important for immunosurveillance (Matloubian et al. 2004).

In vitro studies showed that fingolimod-P exerts diverse effects on cultured OL lineage cells, depending on developmental stage, treatment dose and duration of exposure (Novgorodov et al. 2007; Miron et al. 2008a, b; Jaillard et al. 2005; Coelho et al. 2007). Studies on animal models of MS showed that fingolimod crosses the BBB due to its lipophilic nature (Kipp and Amor 2012; Miron et al. 2008a, b) and exerts direct effects on OL lineage cells, which have S1P receptors (Miron et al. 2008a, b; Jung et al. 2007), leading to remyelination in the CNS after demyelination (Rossi et al. 2012). However, there is controversy in the literature regarding the contribution of Fingolimod to remyelination (Hu et al. 2011; Groves et al. 2013).

A recent study performed on EAE mouse model showed that a dose of 0.3 mg/kg body weight, corresponding to a dose of 0.024 mg/kg body weight in the adult, produced a significant increase in CNS myelination derived probably from both myelin protection and remyelination (Zhang et al. 2015a, b). Zhang et al. (2015a, b) demonstrated that fingolimod treatment, initiated after EAE symptoms appearance, significantly improved neurological functional recovery and that fingolimod promoted proliferation and differentiation of OPCs. The authors suggest that Shh signaling pathway may mediate the effects of fingolimod on OPCs.

Clinical evidence about Fingolimod effects on neuroprotection came from the two randomized, double-blind, phase 3 clinical trials: FREEDOMS (FTY720 Research Evaluating Effects of Daily Oral Therapy in Multiple Sclerosis; a placebo-controlled trial of 1272 patients) and TRANSFORMS (Trial Assessing Injectable Interferon versus FTY720 Oral in Relapsing–Remitting Multiple Sclerosis; comparing fingolimod with an interferon in a total of 1292 patients) (Cohen et al. 2010; Kappos et al. 2010). Imaging outcomes currently provide the best in vivo measures of neuroprotection and possibly repair in MS (Barkhof et al. 2009). Percentage change in brain volume in 1 year, a sensitive measure of neuroprotection, has been reported to be a strong predictor of future disability (Barkhof et al. 2009) and the reduction of brain volume in MS reflects axonal loss and myelin damage (Simon 2006). In phase 3 studies fingolimod 0.5 mg significantly reduced brain volume loss by 31 % over 1 year compared with intramuscular interferon-beta 1a (pb0.001; TRANSFORMS) (Cohen et al. 2010), and by 35 % over 2 years compared with placebo (pb0.001; FREEDOMS) (Kappos et al. 2010). Subgroup analyses from FREEDOMS confirmed that these effects over 2 years were independent of the presence or absence of gadolinium(Gd)-enhancing lesions, T2 lesion load, previous treatment status, or level of disability (Simon 2006). These findings suggest that fingolimod may have other effects than peripheral immunomodulatory actions.

7.6.2 Alemtuzumab

Alemtuzumab is a monoclonal antibody that destroy lymphocytes via CD-52 recognition. Two randomised controlled phase III clinical trials investigated the efficacy of alemtuzumab compared with interferon-beta 1a in patients who had not received other primary treatment (CARE-MS I [Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis]) and who had failed on other disease-modifying medication (CARE-MS II) (Cohen et al. 2012; Coles et al. 2012). Both trials showed that patients on alemtuzumab treatment were less likely to experience a relapse over the course of the 2 years. In addition, patients treated with alemtuzumab in CARE-MS II (only) showed a 42 % reduction in sustained accumulation of disability. This reduction in disability may be directly related to fewer episodes of demyelination or, indirectly, to longer periods of remission allowing spontaneous remyelination to occur, or to a potential effect on remyelination/neuroprotection. To support the last hypothesis, a recent study showed that cultures of peripheral blood mononuclear cells, in particular T cells, produce increased concentrations of neuronal growth factors when treated with alemtuzumab and stimulated with myelin basic protein. In addition, media from these cultures promote survival of neurons and OPCs and enhance OL differenciation and myelination (Jones et al. 2010; Munzel and Williams 2013). Other two ongoing clinical trials (NCT01395316, NCT01307332) were designed to identify possible mechanisms by which alemtuzumab acts to protect the brain from inflammation and how it may enhance repair through remyelination.

7.6.3 Dimethyl Fumarate

Dimethyl Fumarate (DMF) is a methyl ester of fumaric acid that is both immunomodulator and upregulates the transcription factor Nrf2 that induces a cascade of cytoprotective (Scannevin et al. 2012) and antioxidant pathways (Huang et al. 2015). Additionally, DMF can suppress the

transcription factor NF-kb that mediates pro-inflammatory signaling. However the mechanism of action of DMF is not yet well understood (Wang et al. 2015; Linker et al. 2011). Phase 3 clinical trials (DEFINE, CONFIRM) have indicated the efficiency of DMF in a dosage of 240 mg twice and three times daily in significantly reducing relapse rate and development of brain lesions in RRMS, reducing the risk of disability progression at 2 years (Fox et al. 2012; Gold et al. 2012a, b). The trials provided evidence for the safety and tolerability of DMF, but there is no direct evident for the effect of DMF on remyelination and neuroprotection. The related anti-psychotic compound of DMF, quetiapine, a fumarate salt, does possess pro-remyelination and neuroprotective properties in rodents (Zhornitsky et al. 2013). Quetiapine produces an increase in differentiation of rodent progenitor cells into oligodendrocytes and a greater extent of myelination in cortical aggregate cultures after treatment with quetiapine (Xiao et al. 2008). Moreover, in vivo experimental models demonstrate reduced demyelination and loss of oligodendrocytes (Bi et al. 2012; Mei et al. 2012) with quetiapine treatment, as well as faster return of myelin proteins (Zhang et al. 2012).

7.6.4 Human Monoclonal IgM Antibody 22

The human monoclonal IgM antibody 22 (rHIgM22) is a recombinant antibody, usually present in the serum of MS patients, that was able to induce spinal cord remyelination in a virus-mediated mouse model of demyelinating disease (Mitsunaga et al. 2002). Similar increases in remyelination have been reported in MRI studies using the same experimental animal model (Pirko et al. 2004). The pro-myelination effect of rHIgM22 is independent of immunomodulation (Ciric et al. 2004) and is associated to anti-apoptotic signaling in pre-myelinating OL (Howe et al. 2004). A phase I multi-center, double-blind, randomized, placebo-controlled study (NCT01803867) designed to evaluate safety, tolerability, pharmacokinetics, and immunogenicity of a single dose of rHIgM22 in participants with any type of MS who were clinically stable for at least 3 months, has been recently completed. All participants remained on their existing MS treatment regimens, including disease-modifying therapies. Across all of the study groups, 55 participants received one of the five doses of rHIgM22 and 17 received placebo. There were no dose-limiting toxicities and no serious adverse events (SAE) in any of the five rHIgM22 dose levels in the study. There was one SAE of squamous cell carcinoma in a placebo-treated participant.

7.6.5 Glatiramer Acetate (GA)

GA is developed from Copolymer 1 (Cop 1), a synthetic polypeptide with amino acids analogous to those of the autoantigen MBP. Studies performed on cuprizone models of multiple sclerosis revealed that GA, in addition to its immunoregulatory properties, has also an effect on resident immuno-response and produce modulation of microglia activation by promoting increased secretion of IL-10 and decrease in TNF-α. This promotes OPCs proliferation, recruitment and maturation and leads mature OLs towards demyelination repair (Rosato Siri et al. 2013; Pul et al. 2011). GA has been described to be degraded in the periphery after subcutaneous injection; degradation products are taken up by dendritic cells and released into the CNS while the integrity of the BBB is not compromised (Liu et al. 2007a, b). Therefore, modulation of MG activation could be achieved either through a peripheral mechanism or a direct entry of GA into the CNS. Aharoni et al. (2008) have observed that GA treatment reduce myelin breakdown and tissue damage and stimulate repair processes in an EAE model in mice. It has been also shown that GA has a beneficial effect on oligodendrogenesis and myelination in the developing nervous system under non-pathological conditions probably due to an increase in insuli-like growth factor (IGF-1) and brain-derived neurotrophic factor (BDNF) (From et al. 2014). A recent study which included 40 RRMS patients

aimed to determine the evolution of T1 unenhanced hypointense lesions (acute or chornic "black holes")by measuring their magnetization transfer ration (MTR) changes over 12 months. GA treatment significantly recovered MTR in acute and chronic black holes, possibly indicating a greater potential for remyelination (Zivadinov et al. 2012).

7.6.6 Laquinimod (LQ)

LQ is an oral immunomodulatory agent with potential neuroprotective properties. On animal models of EAE, prophilactic and therapeutic treatment with LQ increased OLs number and myelin density and improved axon conduction indicating significant neuroprotective and neurorestorative effects (Moore et al. 2013). In Phase II clinical trials, LQ demonstrates a favorable safety profile and significant reduced disease activity by decreasing the number of active lesions (Polman et al. 2005; Comi et al. 2008). in Phase III ALLEGRO and BRAVO clinical studies, LQ reduced annual relapse rate, significantly reduced disability progression and brain atrophy by 35 % and reduced the risk of sustained disability (Comi et al. 2012). Also, LQ increased levels of BDNF in the serum of MS patients (Thone et al. 2012).

7.7 Conclusion

OLs are the essential cells for the myelination of the CNS and they originate from OPCs in several zones of the CNS. The process of myelination starts during development and continues throughout life being controlled by an exquisite genetic mechanism. OPCs are present in the adult CNS and, under certain conditions such as inflammation or brain injury, they proliferate, migrate and differentiate into mature OLs. The interaction between OLs and axons is important for myelination. In MS, the immune process induces a chain of pathological pathways that conduce to injury of both OLs and OPCs generating demyelination and reduced re-myelination. Oxidative stress, mitochondrial dysfunction, activation of astrocytes and microglia, excitotoxicity, disturbed balance of ion channel function concur to OLs apoptosis, axonal damage and neurodegeneration. Re-myelination in MS is triggered by inflammation and there are some evidence that follows similar steps as myelination during development, however, due to local molecular factors, it is incomplete and the myelin sheaths are thinner in the re-myelinated zones. Re-myelination in MS is mainly supported by OPCs. Disease modifying drugs in MS have been proven to be efficient immunomodulators but the evidence about certain re-myelinating properties of some of these molecules is only emerging. There is a lot of interest in developing drugs that may target certain pathways involved in re-myelination, with the aim to obtain efficient regenerative therapies for this disease, that may act not only in RRMS but also in SPMS or PPMS. The identification of key molecules and pathways controlling the migration and differentiation of OPCs and myelination has provided clues for potential targets of drug candidates that may trigger re-myelination and neuroprotection. Some of these molecules have been already tested on animal models of MS. However, translation across species is a major concern and the efficacy of this strategy remains to be evaluated in clinical trials.

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Clinical Neurophysiology of Multiple Sclerosis

8

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Abstract

Different neurophysiological methods such as evoked potentials (EP), testing of the autonomic nervous system (ANS) or polysomnography have the potential to detect clinically silent lesions or to confirm the existence of an association between a clinical symptom and multiple sclerosis (MS); previously undetected by MRI. Therefore, in the most recent MRI criteria for the diagnosis of MS (MAGNIMS consensus guidelines), neurophysiological confirmation of optic nerve dysfunction (slowed conduction on visual EP), support dissemination in space and, in patients without concurrent visual symptoms, dissemination in time. In this chapter we will review the existing evidence regarding the role of different neurophysiological tests (specifically the role of EPs, autonomic nervous system testing and sleep testing in MS) in the diagnosis and management of MS.

Keywords

Autonomic nervous system • Evoked potentials • Multiple sclerosis • Sleep disorders

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Abbreviations

ANS	autonomic nervous system
BAEP	brainstem auditory EP
CIS	clinically isolated syndrome
CMCT	central motor conduction time
DIS	dissemination in space
DIT	dissemination in time
EP	evoked potentials
HRV	heart rate variability

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mEPs	Multimodal evoked potentials
MEPs	motor evoked potentials
MS	multiple sclerosis
OH	orthostatic hypotension
ON	optic neuritis
OSA	obstructive sleep apnea
PoTS	orthostatic tachycardia syndrome
RBD	REM sleep behavior disorder
RLS	restless legs syndrome
SSEP	short latency somatosensory EP
VEMP	vestibular evoked myogenic potentials
VEPs	pattern reversal visual Eps

8.1 Introduction

Multiple sclerosis (MS) is a chronic idiopathic demyelinating illness of the central nervous system and it is the leading cause of disability in young adults. In the diagnosis of MS, three main principles are applied: demonstration of dissemination in space (DIS), demonstration of dissemination in time (DIT), and reasonable exclusion of alternative explanations for the clinical presentation. The demonstration of DIS and DIT is heavily influenced with MRI, and since its introduction, this method has become the cornerstone in the diagnosis of MS with various MRI criteria applied over time (Poser et al. 1983; Polman et al. 2011). The last version of the McDonald criteria allows making a diagnosis of MS in patients with typical clinically isolated syndrome (CIS).

Despite these advancements, there is still a poor correlation between clinical symptoms and MRI findings in a substantial proportion of MS patients (Habek 2013). Different neurophysiological methods such as evoked potentials (EP), testing of the autonomic nervous system (ANS) or polysomnography have the potential to detect clinically silent lesions or to confirm the existence of an association between a clinical symptom and MS; previously undetected by MRI. A nice example of the latter are EP, which have been widely used in MS, although their clinical use has been reduced after the introduction of MRI. This is not always justifiable since the information provided by evoked potentials is more related to function, unlike the information provided by MRI which is more related to anatomy. (Comi et al. 1998) Therefore in the most recent MRI criteria for the diagnosis of MS (MAGNIMS consensus guidelines), neurophysiological confirmation of optic nerve dysfunction (slowed conduction on visual EP), support dissemination in space and, in patients without concurrent visual symptoms, dissemination in time. (Filippi et al. 2016).

In this chapter we will review the existing evidence regarding the role of different neurophysiological tests (specifically the role of EPs, autonomic nervous system testing and sleep testing in MS) in the diagnosis and management of MS.

8.2 Evoked Potentials in Multiple Sclerosis

The role of EPs in the evaluation of MS has changed over time primarily due to advances in neuroimaging technology, dominantly the MRI. In contrast with MRI, EPs provide information about functionality and pathophysiological involvement of a certain neuroanatomic pathway (Chiappa 1997) and their clinical utility is based on their ability to reveal subclinical involvement of a sensory system in the presence of signs/symptoms suggestive for demyelinating disease (Walsh et al. 2005). In routine clinical practice, most frequently used EPs are: pattern reversal visual EPs (VEPs), brainstem auditory EP (BAEP), short latency somatosensory EP (SSEP), and motor evoked potentials (MEPs) (Chiappa 1997).

VEPs are widely used in assessment of patients with clinical signs of optic neuritis (ON) as well as in evaluation of asymptomatic involvement of visual pathways in patients with MS. The most common finding in acute ON is delayed latency of wave P100 together with amplitude reduction (Chirapapaisan et al. 2015). With recovery from ON, the amplitude improves but latency usually remains increased. The sensitivity of VEPs in patients with MS and a history of

optic neuritis is about 77–100 % while the frequency of abnormal VEPs in overall patients with MS varies between studies; from 42 to 100 % (Movassat et al. 2009; Palace 2001). According to new MAGNIMS criteria, VEP is reintroduced as part of diagnostic MS criteria (Filippi et al. 2016).

BAEPs are used to detect and approximately localize symptomatic, as well as asymptomatic, dysfunctions of the auditory pathways within the auditory nerve and brainstem. The most common BAEP pathological findings in patients with MS reflect dysfunction of the upper or lower brainstem, including increased wave I-III (lower brainstem) or III-V (upper brainstem) interlatencies (La Mantia et al. 1982). According to published literature, overall sensitivity in evaluation of brainstem involvement is low (Walsh et al. 2005; Ivanković et al. 2013; Comi et al. 1993) and it is inferior to MRI and vestibular evoked myogenic potentials (VEMP) (Ivanković et al. 2013).

SSEP, elicited from the upper and lower limbs, evaluate dorsal columns and the thalamo-cortical sensory system. The diagnostic value of SSEP is most pronounced in diagnostic evaluation of patients with no evidence of demyelinating lesions on the spinal MRI. Tibial SSEP is considered to be among the most valuable EPs (Djuric et al. 2010), giving pathological findings in up to 80 % of patients with MS who do not have sensory symptoms and signs (Kraft et al. 1998). Pathological findings commonly found in tibial SSEP are increased latencies of upper thoracic and cortical response. SSEPs of the median nerve add additional value because through the P14 wave they provide information about the degree to which the lower brainstem is affected. Abnormalities of P14 were found to be a significant contributor to the functional brainstem assessment battery.

MEPs are evaluating the corticospinal tract and together with SSEPs represent a valuable neurophysiological method for evaluation of the spinal cord. Beside its diagnostic value, MEP studies in MS serve as an indication of corticospinal pathway dysfunction (Magnano et al. 2014). The pathological finding in MS is an increased central motor conduction time (CMCT), which is found to be related with EDSS values (Fuhr et al. 2001) and can predict long term disability (Schlaeger et al. 2014a).

Vestibular evoked myogenic potentials (VEMPs) have been proven to be useful in the assessment of brainstem involvement in MS (Habek 2013). VEMP presents a myogenic response to a loud acoustic stimulus and is divided into two parts, depending where the myogenic response is measured: cervical VEMP (ipsilateral sternocleidomastoid muscle, waves P13 and N23), which provides information about vestibulospinal pathways; and ocular VEMP (contralateral ocular muscle, waves N10 and P13), which provides information about the functionality of vestibuloocular reflex. The sensitivity for MS patients varies from 30 to 100% and results are characterized with an absent response, prolonged latencies and reduced amplitudes of major waves (Murofushi et al. 2001; Versino et al. 2002). According to some studies, VEMP is superior to clinical examination, MRI and BAEP in detection of brainstem lesions (Skorić et al. 2014).

8.2.1 The EP Score

Different modalities of evoked potentials show correlation with disability and disease progression in MS patients, so it could be assumed that a combination of different evoked potential could provide even more useful information. VEP, BAEP, SSEP and MEP could be combined into a multimodal EP score, a specific scale calculated according to normative values for each of the EPs. A different degree of the significance is assigned to each of the types of abnormalities (prolonged latencies, reduced amplitude, absent response), and the level of significance is specific for every study. If the EP score consists of VEP, SSEP of upper and lower extremities where a normal response is scored with 0, prolonged latency with 1, reduced amplitude with 2 and an absent response with 3; then a patient with prolonged VEP latencies on the left side, a reduced amplitude of P40-N50 complex on the left side

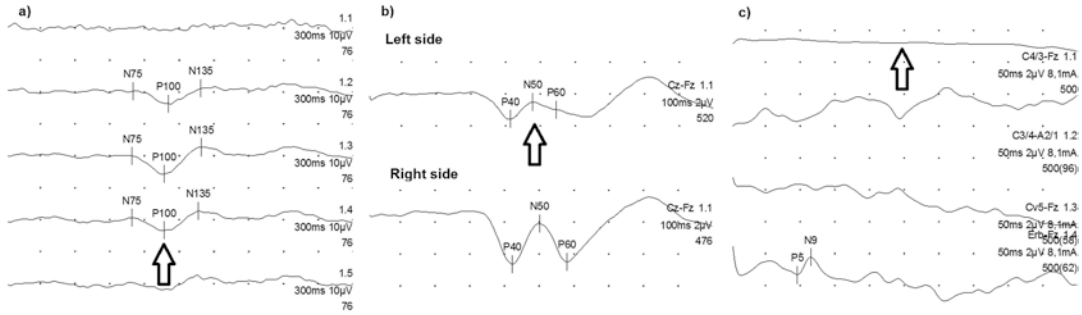

Fig. 8.1 Example of EP score (VEP, SSEP of *upper* and *lower* extremities) where normal response is scored with 0, prolonged latency with 1, reduced amplitude with 2, and absent response with 3: presented EP score has value of 6 (1+2+3), (**a**) VEP: Prolonged latency of P100 wave = 1; (**b**)Tibial nerve SSEP: Reduced amplitude of P40-N50 complex on the left side = 2 and (**c**) Medial nerve SSEP: Absent response on the right side = 3

for tibial nerve SSEP and an absent response on the right side for the medial nerve SSEP has an EP score of 6 (1+2+3), as presented on Fig. 8.1. The EP score could be defined with ordinal values calculated according to normative values or with different transformations of raw EP data (z transformation).

Multimodal evoked potentials (mEPs measure and moderately predict clinically relevant disease activity in patients with early relapsing remitting MS (Jung et al. 2008). The mEPS at baseline has shown correlation with EDSS after 24 months and changes in mEPS correlated with changes in EDSS, where patients with EDSS progression showed stronger mEPD deterioration than clinically stable patients (Jung et al. 2008). A combination of VEP, BAEP, SSEP and MEP results gathered in an EP score has demonstrated a significant correlation between the EP score and EDSS score at the time of neurophysiological study and at 1, 3 and 5 years of follow-up, particularly for MEP and SEP; thus, giving rise to the evidence that EPs, particularly MEP and SEP, have significant value in predicting neurological disability (Invernizzi et al. 2011). Patients with an EP score at a baseline higher than the median value had an 72.5 % increased risk of disability progression at follow-up; meanwhile, patients with a lower EP score had a risk of only 36.3 % (Leocani et al. 2006), suggesting a predictive role of the multimodal EP score. This was confirmed by the fact that patients with worsening at follow-up had a significantly worse global EP score at baseline in comparison with patients without worsening (Leocani et al. 2006).

Different EPs (VEP, BAEP, SSEP and MEP) associated with the EP score have shown moderate and useful correlation with clinical status in patients with primary progressive MS – PPMS (Canham et al. 2015). The numerical score based on VEP, SSEP and MEP results correlates well with disability in PPMS and allows some prediction of the disease course over 3 years (Schlaeger et al. 2014b). Combination of VEP, BAEP and SSEP could be used as an outcome variable for determining the efficiency of a particular treatment (Margaritella et al. 2015). Treatment effects did not show any significance for EDSS, but there was improvement in EP score (mainly because of the significant decrease in VEP score) between different treatment groups (Margaritella et al. 2015). Finally, brainstem involvement in MS patients is very important in the prediction of disease progression. In an EP score that includes BAEP (Leocani et al. 2006), it has not been shown to have any statistically significant correlation between BAEP and EDSS, neither on baseline or follow-up suggesting that BAEP is insufficient in the neurophysiological evaluation of the brainstem in MS and it is necessary to include another measure of brainstem dysfunction.

It is known that VEMP is superior to BAEP in detection of brainstem involvement and because of that, the VEMP score was designed. The VEMP score presents interpretation of VEMP

results quantified according to cut-off values (0 = normal response, 1 = prolonged latency, 2 = reduced amplitude, 3 = absent response) calculated separately for every recording position and combined in a unique score, with a minimal value of 0 and maximal of 12. The VEMP score is higher for MS patients with clinical signs of brainstem involvement, correlates with EDSS and disease duration and, according to multiple regression analyses, the VEMP score is a statistically significant predictor for EDSS (Gabelić et al. 2015). These results indicate that the VEMP score is sensitive to brainstem involvement and it could replace BAEP in the EP score and improve its sensitivity to brainstem involvement.

8.3 Autonomic Dysfunction in Multiple Sclerosis

The importance of the autonomic nervous system (ANS) is well appreciated, as it is paramount for regulating function of each and every organ in the body. However, our capacity to test its activity somewhat lags behind its significance. The reason is that the ANS is unavailable for direct assessment. As we are unable to test it directly, we must rely on testing its reflexes. This kind of testing is mainly related to cardiovascular and sudomotor autonomic reflexes. The cardiovascular autonomic system is tested by the following methods: blood pressure and heart rate response to Valsalva maneuver, heart rate variability during deep breathing and blood pressure and heart rate changes during tilt table testing (Freeman 2006). Sudomotor function is most precisely assessed by the Quantitative Sudomotor Axon Reflex testing (Low et al. 1983). Combining all of these tests we can quantify the severity of ANS dysfunction using the Composite Autonomic Scoring Scale and render the impairment more precisely using the adrenergic, cardiovagal and sudomotor indexes (Low 1993). Another method of ANS assessment, nowadays gaining popularity, is the analysis of heart rate variability (HRV). In this method differences in sympathetic and parasympathetic effects on cardiac activity, reflected in the variability of beat-to-beat R-R intervals on ECG, are exploited to estimate the level of activity of each ANS branch (Shaffer et al. 2014).

Cardiovascular ANS dysfunction is commonly present in multiple sclerosis (MS) (Adamec and Habek 2013). Furthermore, it is recognized in the early stages of the disease as the clinically isolated syndrome (Crnošija et al. 2016). Altogether, it affects up to two thirds of patients during the course of the disease (Acevedo et al. 2000). It is mainly caused by demyelinating lesions located in the periventricular region of the fourth ventricle that affect the autonomic nuclei, as well as due to the descending and ascending autonomic pathways in the medulla also being affected (Vita et al. 1993; De Seze et al. 2001). Half of MS patients experience orthostatic intolerance with presenting symptoms that can be insidious and nonspecific such as dizziness, lightheadedness and general malaise (Adamec et al. 2013a). The failure of blood pressure to remain stable in an upright position in MS patients is due to impaired sympathetic vasocostrictory reflex that is responsible for maintaining adequate blood pressure during postural change (Flachenecker et al. 1999). This, in turn, results in orthostatic hypotension (OH), a significant and sustained decrease of blood pressure upon standing (Freeman et al. 2011) (Fig. 8.2). The symptoms are caused by cerebral hypoperfusion and are typically induced by standing and quickly resolve when lying flat. If the fall of blood pressure is sufficiently pronounced it can lead to falls and even loss of consciousness with the hazard of traumatic injuries. Patients with OH are commonly fatigued and, using the HRV analysis, it has been found that reduced sympathetic activity during standing correlates with the Modified Fatigue Impact Scale in MS patients (Flachenecker et al. 2003). Another variety of orthostatic intolerance is the postural orthostatic tachycardia syndrome (PoTS). It is characterized by sustained heart rate increase on orthostatic challenge without concomitant OH (Freeman et al. 2011). PoTS is recognized to be present in MS more frequently than in healthy controls and its presence is explained by demyelinating brainstem and hemispheral lesions disrupting the

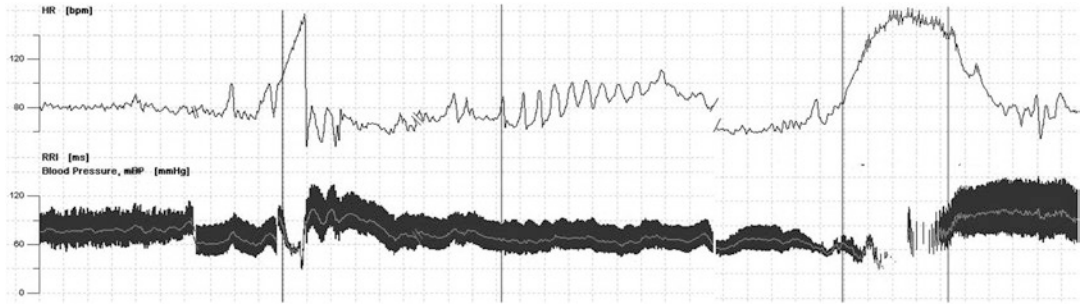

Fig. 8.2 Autonomic nervous system testing in an MS patient showing abnormal blood pressure response to Valslava maneuver and significant drop in blood pressure upon tilt-up

physiological heart rate variability modulation (Adamec et al. 2013b; Kanjwal et al. 2010). Although the true significance of PoTS in MS is not completely elucidated, it is known that PoTS patients have a restricted ability to exercise and an increased sensation of fatigue, which may aggravate preexisting symptoms in MS. Another factor adding to the problem of fatigue in MS is reduced vagal activity that is seen to occur at a younger age in MS (Keselbrener et al. 2000).

MS patients can also present with more severe cardiovascular symptoms, which can actually be secondary to disease activity. Acute central nervous system lesions, including demyelinating lesions, can induce an increased release of catecholamines causing necrotic changes in cardiac myocytes and disrupt the endocardial conduction system leading to arrhythmias such as sinus bradycardia or paroxysmal atrial fibrillation (Sörös and Hachinski 2012; Juric et al. 2012; Chagnac et al. 1986). There have even been reports of cardiogenic shock and pulmonary edema as a presenting symptom of MS due to active lesions in the brainstem affecting the solitary tract nucleus (Midaglia et al. 2016). Furthermore, studies have shown that HRV is reduced in MS patients compared to healthy subjects (Mahovic and Lakusic 2007a; Brezinova et al. 2004) and this reduction seems to be related to disease duration (Mahovic and Lakusic 2007b). This is important since it has been found that reduced HRV is associated with an increased risk of cardiac events (Tsuji et al. 1996). Therefore, the occurrence of cardiac symptoms in an MS patient with no known cardiac disease should prompt consideration of MS relapse as a possible etiology. An interesting finding is that HRV analysis may also be useful in predicting the known cardiac side effects of fingolimod, an immunomodulatory treatment, in an individual MS patient. (Rossi et al. 2015) Reduced sweating ability has also been documented in MS patients. Quantitative assessment has shown a lower sweating response compared to healthy controls without a disease specific pattern (Saari et al. 2009). The MRI lesion load as well as neurologic disability is associated with development of thermoregulatory hypohydrosis. However, sweating impairment can already be seen in the early stage of multiple sclerosis, the clinically isolated syndrome (Crnošija et al. 2016). These abnormalities of sweating to heat exposure seem to result from the disruption of central sudomotor pathways connecting the anterior hypothalamus with the intermediolateral columns of the spine (Davis et al. 2010). It is important to stress that heat intolerance in MS patients can lead to pseudorelapses, the so called Uhthoff phenomenon, which underlines the importance of adequate thermoregulation in MS.

Although autonomic dysfunction is usually considered as a consequence of MS activity, the interaction is more complex and not completely one-sided. Namely, the ANS participates in the regulation of the immunological system via adrenergic and cholinergic receptors on the immune cells (Kohm and Sanders 2001). Its anti-inflammatory effect is mainly based on sympathetic activity that inhibits production of Th1-derived proinflammatory cytokines while stimulating production of Th2-dervied antiin-

flammatory cytokines (Sternberg 2016). Thus, sympathetic dysfunction, which is more pronounced in the relapsing remitting phase, increases inflammation and further potentiates MS activity. Therefore, research of ANS dysfunction in MS is not only important for the assessment of disease manifestation but also contributes to unfolding the complex mechanisms of interaction between MS and the immune system.

8.4 Sleep Disorders in Multiple Sclerosis

Sleep disorders in multiple sclerosis (MS) are more common than in the general population and, depending on the study, they account from 25 to 54 % of cases (Barun 2013). The immunological background of disease development in both multiple sclerosis and sleep disorders has been proposed as a possible common pathophysiological mechanism and recent findings of disrupted melatonin pathways in MS patients suggest a multi-level causative mechanism of the development of sleep disorders in MS. Importantly, sleep disorders are considered to be one of the crucial etiological factors in development of fatigue, a common and debilitating symptom of MS. More precisely, decreased sleep efficiency detected by overnight polysomnography significantly correlated with fatigue and lack of energy in MS patients compared to controls (Braley et al. 2012a). Furthermore, a recent study showed that obstructive sleep apnea and sleep disturbance in MS patients were significantly associated with multiple-domain cognitive impairment such as visual memory, verbal memory, executive function, attention, processing speed, and working memory (Braley et al. 2016). However, sleep disorders are commonly undiagnosed and untreated in the MS population (Brass et al. 2014).

Although almost all of the major subgroups of sleep disorders such as insomnia, sleep disordered breathing, REM sleep behavior disorder, narcolepsy and restless legs syndrome have been described in MS patients, a higher prevalence in the MS population than in healthy controls was well established for insomnia, obstructive sleep apnea, and restless legs syndrome (RLS) (Merlino et al. 2009; Braley et al. 2014; Italian REMS Study Group et al. 2008a). Insomnia is more frequent in patients with multiple sclerosis (40 %) than in the general population (10–15 %) and it has been proposed that insomnia in MS occurs due to a multifactorial etiology associated with MS *per se* like nocturia, spasticity, pain and depression (Ferini-Strambi et al. 1994).

Sleep-disordered breathing are disorders characterized by respiratory abnormalities during sleep. The most common among them is obstructive sleep apnea (OSA) which is characterized by repeated collapse of the upper airway during sleep with consecutive sleep fragmentation and intermittent hypoxia resulting in increased daytime sleepiness and higher risk for development of atherosclerosis. Multiple sclerosis brainstem lesions could be additional risk factors for development of OSA (Braley et al. 2012b). One study included 62 MS patients and 32 healthy controls who where evaluated by overnight polisomnography, showed the prevalence of obstructive sleep apnea was 58 and 47 %, respectively (Kaminska and Kimoff 2012).

A high prevalence of restless leg syndrome (RLS) in MS patients has been confirmed in several studies (Auger et al. 2005; Deriu et al. 2009; Italian REMS Study Group et al. 2008b; Manconi et al. 2008) and they have been correlated with disease duration, older age and cervical cord lesions. Distinguishing RLS from other motor and sensory symptoms in MS can be difficult. Unlike leg discomfort encountered in RLS which is worse in evening, leg spasms, often seen in MS patients, are worse on awakening and can occur at any time of the day.

The prevalence of REM sleep behavior disorder (RBD) in the general population ranges from 0.38 to 0.5 % (Frenette 2010). RBD is a parasomnia characterized by loss of muscle atonia during REM sleep and consecutive abnormal motor or verbal behaviors associated with unpleasant dreams (American Academy of Sleep Medicine et al. 2005). A study that investigated prevalence of RBD in 135 MS patients and 118 healthy individuals using RBD questionnaires found four

(2.9 %) MS patients and none of the healthy controls having RBD (Gómez-Choco et al. 2007). There are also case reports of RBD in MS patients which suggest that a MS lesion in the proximity of the penduculopontine nucleus causes this disorder of REM sleep (Tippman-Peikert et al. 2006; Plazzi and Montagna 2002).

In addition to the case series describing narcolepsy features in MS patients (Poirier et al. 1987), a study on the secondary causes of narcolepsy has revealed that MS is the fourth most common cause after inherited disorders, CNS tumors and brain injury; in this study, 12 % of the cases of secondary narcolepsy were due to MS (Nishino and Kanbayashi 2005). The fact that both of these diseases are related to human leukocyte antigen DQB1*0602 might suggest that similar autoimmune process may be important in development of narcolepsy and MS. Finally, hypothalamic MS lesions resulting in low CSF hypocretin levels have been described to cause hypersomnia in affected patients (Oka et al. 2004).

Several humoral immunologic factors, such as IL-1 and TNF alpha, have been implicated in development of sleep disorders and sleepiness. Since MS is proven to be characterized by immune abnormalities, the notion that MS and sleep disorders share a similar background seems plausible. However, sleep disorder should be viewed separately due its differing etiopatological grounds. Considering the fact that sleep disorders largely contribute to development of fatigue, the most common and debilitating symptom of MS, assessment of sleep disorders in multiple sclerosis is important.

8.5 Conclusion

In conclusion, EPs are reliable procedures to predict disability in MS patients. The index of global EP alteration (EP score), which combines alterations in VEP, BAEP, motor and somatosensory EP, shows significant correlation with the EDSS score at the time of neurophysiological study and at 1, 3 and 5 years of follow-up. Furthermore, autonomic nervous system dysfunction can lead to an array of clinical symptoms often observed in MS patients. There is a connection between dysfunction of autonomic cardiovascular reflexes and development of cardiac side effects of several drugs that are used in MS treatment; and cardiovascular and thermoregulatory autonomic dysfunctions in MS have considerable potential to adversely affect exercise. Finally, sleep disorders largely contribute to fatigue in MS, making formal assessment of sleep important.

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Multiple Sclerosis Epidemiology in Europe

9

Daiana Bezzini and Mario A. Battaglia

Abstract

Multiple sclerosis is characterized by a non-homogeneous distribution around the world. Some authors in past described a latitude gradient, with increasing risk from the equator to North and South Poles, but this theory is still controversial. Regarding Europe, there are many articles in the literature concerning the epidemiology of this disease but, unfortunately, they are not always comparable due to different methodologies, they do not cover all countries in the continent, and most of them reported data of small areas and rarely at a national level. In 2012 there were 20 national registries that could help to describe the epidemiology of the disease and, in addition, there is an European Register for Multiple Sclerosis that collect data from already existing national or regional MS registries and databases. Another valid alternative to obtain epidemiological data, also at national level, in a routinely and cost-saving way is through administrative data that are of increasing interest in the last years.

Keywords

Administrative data • Epidemiology • Europe • Multiple sclerosis • Registries

Abbreviations

HLA	human leukocyte antigen
MS	multiple sclerosis
MSIF	Multiple Sclerosis International Federation
UK	United Kingdom
WHO	World Health Organization

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9.1 Introduction

Epidemiology is "the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the prevention and control of health problems" (Last 2001). The measure of the disease frequency requires, as numerator, the number of disease cases and, as denominator, the population at risk. Commonly used measures are incidence, i.e. new cases of disease in a population during a specified time interval; prevalence, i.e. current cases in a population at a specified point in time or over a specified period of time; and mortality, i.e. deaths in a population during a given time interval (www.cdc.gov).

Regarding MS, WHO/MSIF MS Atlas estimated a global median prevalence of 35 cases and a median incidence of 4 cases per 100,000, with a total of 2.3 million people with MS worldwide (Atlas of MS 2013), but with important geographical differences. In a recent literature review, the authors reported a median estimated prevalence of 112.0, and a median estimated incidence of 5.2 per 100,000, but the dispersion was very wide, indicating an uneven worldwide distribution, with a range of 5.2–335 for prevalence and 0.5–20.6 for incidence (Melcon et al. 2014). Kurtzke, in Kurtzke 1964, described three zones of high, medium and low frequency of MS, and later assumed that the distribution directly correlates with latitude (Kurtzke 1964, 1975). Nevertheless Rosati, in two review in Rosati 1994 and Rosati 2001, deduced that the increasing gradient from equator to poles was an oversimplification and assumed that the uneven worldwide distribution was due not only to environmental factors but also to different genetic susceptibilities (Rosati 1994, 2001). Also Zivadinov and colleagues, in their meta-analysis study of Zivadinov et al. 2003, demonstrated that the latitude gradient became less remarkable when they age- and sex- adjusted prevalence rates, published between 1980 and 1998 (Zivadinov et al. 2003).

In 2010, Koch-Henriksen and Sørensen reported a weak association between prevalence and latitude in Europe and North America, while in Australia and New Zealand there was no association (Koch-Henriksen and Sørensen 2010). On the contrary, in the same year, Simpson et al., demonstrated with a comprehensive meta-analysis of studies from 1923 to 2009 a strong and significant latitudinal gradient for prevalence, also with age standardization, but only among nations of largely European descent and with some exceptions (Simpson et al. 2011). In fact, it was found a statistically significant inverse gradient in Scandinavia due to a high dietary intake of vitamin D particularly at the northern latitudes in Scandinavia (Kampman and Brustad 2008; Freisling et al. 2010; Brustad et al. 2004; Burgaz et al. 2007) and in Italy to a unique distribution of HLA-DRB1 alleles (Simpson et al. 2011). Also incidence published data are in contradiction. Zivadinov reported no correlation between age-adjusted incidence rates and latitude, and also Koch-Henriksen and Sørensen in their meta-analysis in Western Europe and North America (Zivadinov et al. 2003; Koch-Henriksen and Sørensen 2010). On the contrary, Alonso and Hérnan found a significant association between them, although moderated after 1980 (Alonso and Hérnan 2008).

To study MS epidemiology, it is preferable to use population-based studies. These studies are useful also to describe comorbidities, care pathways, burden of this disease and to plan the management strategies and resource allocation necessary to cope with it (Lix et al. 2008; Di Domenicantonio et al. 2014). In Europe, several MS national and local registries and databases have been developed for this purpose (Flachenecker and Stuke 2008). Registries are useful platforms not only for studying temporal and geographical distribution of the disease, but they enable long-term follow-up of patients in a relatively inexpensive manner, compared to randomized controlled clinical trials, also monitoring large patient populations, and studying disease characteristics and long-term outcomes in everyday clinical practice. In addition to the systematic collection of patients data, they gather information on different healthcare systems, as well as on their impact on patient health and quality of life, allowing to compare the benefits

of different types of healthcare. In other words, data collected by registries can be used to improve patients care (Flachenecker 2014).

In the last review on European MS registries, Flachenecker and colleagues found 20 national registries (Austria, Bosnia-Herzegovina, Croatia, Czech Republic, Denmark, France, Germany, Greece, Iceland, Italy, Malta, Netherlands, Norway, Russia, Serbia, Slovenia, Spain (Catalonia), Sweden, Switzerland and United Kingdom). Ten registries were hospital-based, five were population-based, with three being hospital- and population-based together. Nine registries were created to collect all patients in the country, whereas four registries collected patients from representative regions. The main aims of the registries were researches on epidemiology (n = 10), healthcare (n = 10), long-term therapy (n = 8) and support for clinical trials (n = 8) (Flachenecker et al. 2014). Four to 13 registries started their recording activity in the 5 years before that review, indicating the increasingly interest on them due to their ability to provide data that cannot be captured in any other way (Hurwitz 2011).

In 2010, an international consortium created the European Register for Multiple Sclerosis (EUReMS) with the aim of collecting, analyzing and comparing MS data longitudinally collected from already existing national or regional MS registries and databases. This register has four areas of action: epidemiology, long-term therapy outcome, healthcare and quality of life of people with MS (Flachenecker et al. 2014). In Italy, there is the Multiple Sclerosis Database Network based on iMed, an electronic clinical database, used by 48 MS clinical centres, that included 28,479 patients at the end of 2015. There are also two regional population MS registries, in Tuscany and in Liguria, both promoted by the Italian Multiple Sclerosis Foundation (FISM). At the beginning of 2016, from the collaboration of the University of Bari and FISM with the partnership by the Mario Negri Institute for Pharmacological Research, it was founded the Italian Multiple Sclerosis Registry that will collect patients from MS clinical centres of all country.

Unfortunately the present European registries differ in terms of objectives, resources and coverage: in fact, not all are representative of the entire MS population. A valid alternative to obtain data on entire MS population is from administrative sources (Lix et al. 2008; Krysko et al. 2015; Marrie et al. 2010, 2012, 2013; Bezzini et al. 2016). Administrative data are routinely collected in a standardized way for the health system management and for reimbursement by the National Health Service (NHS) and covers all the resident population, enrolled in publicly funded health systems, such as the Italian system (Marrie et al. 2013), and all the services covered by the NHS. These data are less expensive than registries or epidemiological studies but have some limitations: the quality of data is not always perfect, it may exclude some population subgroups and it does not collect clinical data. To have a comprehensive view of the disease and to monitor care pathways, it will be useful to link clinical and administrative registries (Krysko et al. 2015).

9.2 Epidemiology in Europe

In Europe there are more than 600,000 patients estimated, with a median prevalence of 100 cases and a median incidence of 5.5 cases per 100,000 (Atlas of MS 2013). Many are the epidemiological studies about European epidemiology. The most recent review is the one of Kingwell et al., that included studies published from January 1st 1985 to January 31st 2011 (Kingwell et al. 2013). The aim of this chapter was to collect new epidemiological data regarding MS prevalence and incidence across Europe. This search included all original studies not included in any review: we considered studies published from February 1st 2011 until March 1st 2016. The search terms "multiple sclerosis", "incidence", "prevalence" and "epidemiology" were entered in MEDLINE database. Prevalence data are shown in Table 9.1. and incidence data in Table 9.2. Tables collect several information: region and year of study, diagnostic criteria, number of cases/population, crude and standardized rates in both sexes, in male and in female, F:M ratio, and references.

Table 9.1. Prevalence data from included studies, stratified by region

Study region, Year of prevalence estimate	Diagnostic criteria	Cases (Male, Female)/ *population*	Crude prevalence[a] [Standardized prevalence][a] Total	Crude prevalence[a] [Standardized prevalence][a] Male	Crude prevalence[a] [Standardized prevalence][a] Female	F:M ratio	References
Nordic region							
Iceland 2000	Poser et al. (1983)	345 (104 M, 241F)/*279,049* Including PO and PP	123.63 (±13.04) [131.72±13.89]	74.46 (±14.31) [81.47±15.65]	172.90 (±21.81) [181.60±22.90]	2.2	Sveinbjornsdottir et al. (2014)
Norway 2012	Poser et al. (1983) and Polman et al. (2011)	10,121 (3147 M, 6,974F)/ *4,985,870*	203 (199–207) [195 (191–199)]	126 (122–130) [119 (115–124)]	280 (274–287) [272 (266–279)]	2.2	Berg-Hansen et al. (2014)
Norway 2012	Poser et al. (1983) and Polman et al. (2011)	European country background: 9885 (3066 M, 6819F)/*4,653,984*	212 (208–217)				Berg-Hansen et al. (2015)
		non-European immigrant: 236 (81 M, 155F)/*331,886*	71 (63–81)				
Hordaland County (Western Norway) 2013	Poser et al. (1983) and Polman et al. (2011)	1035/ *490,570* Including PP	211.4 (198.3–224.2)	151.8 (136.8–167.9)	270.9 (250.6–292.3)	1.8[b]	Grytten et al. (2016)
Oppland County (Southern Norway) 2002	Poser et al. (1983) and Polman et al. (2005)	474/ *183,235*	174.1 definite ^185.6 including PP^	98.0 ^104.7 definite+ PP^	247.7 ^265.0 definite+ PP^	2.5	Risberg et al. (2011)
Vest-Agder county (Southern Norway) 2007	Poser et al. (1983)	295/ *163,702* Including PP	180 (161–202) [186 (166–209)]	119 (98–146) [120 (99–147)]	240 (209–276) [255 (221–293)]	2.0[b]	Vatne et al. (2011)
Sweden 2008	Poser et al. (1983), McDonald et al. (2001) and Polman et al. (2005)	17,485 (5220 M, 12,265F)/ *9,256,347* Including PP	188.9 (186.1–191.7)	113.4 (110.3–116.5)	263.6 (258.9–268.3)	2.35	Ahlgren et al. (2011)

Sweden 2008	Poser et al. (1983), McDonald et al. (2001) and Polman et al. (2005)	Immigrant pop: 1327 (407 M, 920F)/*940,357*	128.36				Ahlgren et al. (2012)
		Including PP					
		Native-born Swedish pop: 15,840/*7,974,766*	198.6	118.5	278.6	2.36	
		Including PP					
Västerbotten County (Northern Sweden) 2010	Poser et al. (1983), McDonald et al. (2001), Polman et al. (2005) and Polman et al. (2011)	430/ *259,286*	215 (198–233)	131 (113–152)	299 (271–331)	2.3	Svenningsson et al. (2015)
Northern Ostrobothnia (Northern Finland) 2007	Poser et al. (1983) and McDonald et al. (2001)	397 (121 M, 276F)/ *386,972*	103 (93–113)	62 (52–74)	144 (128–162)	2.17	Krökki et al. (2011)
British Isles							
Donegal (Northwest Ireland) 2007	Polman et al. (2005)	329/ *113,347*	290.3 (238.7–255.5) [257.8 (253.2–262.4)]	157.6 (152.7–162.6) Age-standardized	358.3 (350.3–366.5) Age-standardized	2.1	Lonergan et al. (2011)
Wexford (Southeast Ireland) 2007		173/ *119,442*	144.8 (138.3–151.9) [152.0 (148.4–155.6)]	119.0 (114.8–123.4) Age-standardized	176.0 (170.4–181.7) Age-standardized	1.5	
South Dublin (Ireland) 2007		130/ *101,721*	127.8 (131.6–146.7) [123.2 (120.0–126.4)]	84.2 (80.6–87.9)	160.6 (155.2–166.1)	2.6	
Aberdeen city (Scotland) 2009	Poser et al. (1983), McDonald et al. (2001) and Polman et al. (2005)	442 (130 M, 312F)/ *205,446* PP	215 (196–236) [229 (208–250)]	126 (105–149) [134 (119–151)]	306 (273–341) [318 (306–356)]	2.4	Visser et al. (2012)
Orkney (Scotland) 2009		82 (23 M, 59F)/ *20,000* PP	410 (326–509) [402 (319–500)]	232 (147–349) [226 (165–302)]	584 (44–753) [569 (481–698)]		
Shetland (Scotland) 2009		66 (17 M, 49F)/ *22,656* PP	291 (225–371) [295 (229–375)]	148 (86–236) 148 (102–207)]	440 (326–582) [429 (367–547)]		
United Kingdom 2010	Phys-diag	126,669	203.4	113.1 (108.6–117.7)	285.8 (278.7–293.1)	2.5[b]	Mackenzie et al. (2014)

(continued)

Table 9.1. (continued)

Study region, Year of prevalence estimate	Diagnostic criteria	Cases (Male, Female)/ *population*	Crude prevalence[a] [Standardized prevalence][a] Total	Crude prevalence[a] [Standardized prevalence][a] Male	Crude prevalence[a] [Standardized prevalence][a] Female	F:M ratio	References
Isle of man 2011	Polman et al. (2005)	152	179.89 (153.45-210.89) [167.76 (143.10–196.67)]	100.07 (73.95–135.41) [90.96 (67.22–123.08)]	258.67 (214.57–311.82) [241.88 (200.65–291.58)]	2.59	Simpson et al. (2015)
Central Europe							
Lorraine (Northeastern France) 2008	Poser et al. (1983)	4001 PP	170.9 (165.7–176.3), [137.4]	99.2 (93.5–105.2).	239.2 (230.5–248.1)	2.4	El Adssi et al. (2012)
Bavaria (Germany) 2009	Phys-diag	18,176 (4857 M, 13,319F)	175 (172–177)	100 (97–103)	240 (236–244)	2.4[b]	Höer et al. (2014)
Csongràd (Southeastern Hungary) 2013	Poser et al. (1983) and McDonald et al. (2001)	379 (93 M, 286F)/ *421,827*	89.8 [83.7]	46.6 [42.3]	128.6 [122.6]	3.08	Zsiros et al. (2014)
Iberian peninsula							
La Rioja (Northern Spain) 2011	Poser et al. (1983) and Polman et al. (2005)	210 (69 M, 141F)/ *322,955*	65 (56–74)	42.70	87.37	2	Bártulos Iglesias et al. (2015)
Osona shire, Catalonia (Northeastern Spain) 2008	McDonald et al. (2001)	120 (49 M, 71F)/ *150,139*	79.9 [74.2]	64.9	95.1	1.5[b]	Otero-Romero et al. (2013)
Malaga (Southern Spain) 2008	Poser et al. (1983)	1921/ *1,528,851*	125 (102–169)			1.97	Fernández et al. (2012)
Northern Seville District of Andalucía (Spain) 2011	Poser et al. (1983)	*163,324* pop. at 2001	90.2 (75.6–104.8)	54.2 (38.0–70.4)	127.6 (103.5–151.8)	2.4[b]	Izquierdo et al. (2015)
Benfica, Pontinha and Odivelas (Portugal) 2009	McDonald et al. (2001)	Capture method	41.4 [56.20 (46.88–65.52)]				de Sá et al. (2012)
Braga (Northwestern Portugal) 2009	Poser et al. (1983)	345/ *866,012*	39.84 (27.47–52.21)			1.79	Figueiredo et al. (2015)

Italy							
Padua (Veneto-Northeastern Italy) 2009	McDonald et al. (2001)	1173 (829 M, 344F)/ *841,597*	139.5 (131.3–147.5)	83.9 (77.7–90.2)	192.0 (182.5–201.5)	2.3	Puthenparampil et al. (2013)
Verona (Veneto-Northeastern Italy) 2001	McDonald et al. (2001)	270 (82 M, 188F)/ *253,208*	106.6 (94–120) [96.0]	68.5	140.8	2.3	Gajofatto et al. (2013)
Genoa (Liguria-Northwestern Italy) 2007	Poser et al. (1983)	1312 (431 M, 881F)	148.5	103.1	189.1	1.8[b]	Solaro et al. (2015)
Tuscany (Central Italy) 2011	Phys-diag	6890 (2152 M, 4,738F)/ *3,667,780*	187.9	122.3 [121.1]	248.3 [251.3]	2.0[b]	Bezzini et al. (2016)
Lazio (Central Italy) 2011	Phys-diag	7377 (2427 M, 4950F)/ *5,653,008*	130.5 (127.5–133.5) [119.6 (116.8–122.4)]	89.7 (86.1–93.3) [81.4 (78.1–84.7)]	167.9 (163.3–172.6) [155.5 (151.1–160.0]	1.9	Bargagli et al. (2016)
Campobasso (Molise-Southern Italy) 2009	Poser et al. (1983) and McDonald et al. (2001)	47 (17 M, 30F)/ *51,663*	91.02 (66.35121.78) [90.91]	68.62 (39.93–109.86)	111.68 (75.38–159.47).	1.76	Bellantonio et al. (2013)
Catania (Sicily-Southern Italy) 2004	Poser et al. (1983)	398/ *313,110*	127.1 (115.1–140.4) [136.8]	104.7 (89.9–122.9)	147.2 (129.6–167.2)	1.41[b]	Nicoletti et al. (2011)
Mount Etna (Sicily-Southern Italy) 2009	Poser et al. (1983)	Western flank 53 (13 M, 40F)	94.3 (68.7–119.3) [100.7]	48.0 (26.7–84.4)	137.3 (99.4–188.9)	2.86[b]	Nicoletti et al. (2013)
		Including PP					
		Eastern flank 65 (20 M, 45F)	137.6 (104.5–171.5) [137.8]	72.2 (45.3–113.7)	230.3 (170.5–305.3)	3.19[b]	
		Including PP					
Provinces of Cagliari and Carbonia-Iglesias (Sardinia-Southern Italy) 2009	Phys-diag	*25,885*	224 (170–290)	137 (78–222)	296 (214–401)	2.16[b]	Sardu et al. (2012)
Provinces of Carbonia-Iglesias (Sardinia-Southwestern Italy) 2007	McDonald et al. (2001) and Polman et al. (2005)	292/ *138,765*	210.4 (186.3–234.5)	138 (110.1–165.8)	280.3 (241.4–319.3)	2.03[b]	Cocco et al. (2011)

(continued)

Table 9.1. (continued)

Study region, Year of prevalence estimate	Diagnostic criteria	Cases (Male, Female)/ *population*	Crude prevalence[a] [Standardized prevalence][a] Total	Crude prevalence[a] [Standardized prevalence][a] Male	Crude prevalence[a] [Standardized prevalence][a] Female	F:M ratio	References
South East Europe							
Čabar (Croatia) 2001	Poser et al. (1983)	29/*4387*	205.7			1.11	Perković et al. (2010)
		Including PP					
Sumadija (Central Serbia) 2006	McDonald et al. (2001)	194 (72 M, 122F)/ *298,778*	64.9 (56.1–74.4) [60.3 (52.3–69.4)]	49.3 (44.1–54.6) [45.0 (35.3–57.2)]	79.9 (72.8–88.3) [75.1 (62.6–91.0)]	1.7	Toncev et al. (2011)
Kosovo 2003–2012	McDonald et al. (2001)	412 (128 M, 284F)	19.6			2.3	Zeqiraj et al. (2014)
Tirana and Saranda (Albania) 2006–8	McDonald et al. (2001)	3	0.3 (0.0–0.6) [0.3 (0.0–0.6)]				Kruja et al. (2012)
Kandira (Turkey)	Poser et al. (1983)	5 (1 M, 4F)/ *8171*	61.2			2.85	Türk Börü et al. (2011)
		Including PO					
Geyve (Turkey)		7 (2 M, 5F)/ *17,016*	41.1				
		Including PO					
Erbaa (Turkey)		15 (4 M, 11F)/ *28,177*	53.2				
		Including PO					
Crete 2008	McDonald et al. (2001) and Polman et al. (2005)		108	84	137	Urban: 1.8	Kotzamani et al. (2012)
			Urban: 137			Rural: 1.3	
			Rural: 61.5				
Russia							
North-Western Administrative District (SZAO) of Moscow 2008–2012			53.38			2.61	Boĭko et al. (2013, 2014)
Nalchik (Kabardino Balkaria Rep) 2010			13.7				Zikhova et al. (2013)
Prokhladnensky (Kabardino Balkaria Rep) 2010			19.8				
Primosky Krai 2010	Phys-diag		11.45				Gavrilenko et al. (2012)
Vladivostok 2010			16.2				

PO Poser Possible, *PP* Poser Probable
Phys-diag: diagnosis by physicians
[a]Prevalence/100,000 (95 % CI)
[b]F:M ratio calculated by available data

Table 9.2 Incidence data from included studies, stratified by region

Study region, Interval period	Diagnostic criteria	Cases (Males, Females)/ *person-years or mean population*	Crude incidence[a] [Standardized incidence] Total	Crude incidence[a] [Standardized incidence] Male	Crude incidence[a] [Standardized incidence] Female	F:M ratio	References
Nordic region							
Iceland 1990–1999	Poser et al. (1983)	136 (42 M, 94F)/ *2,669,780* Including PO and PP	5.06 (4.12–6.11)	3.10 (2.26–4.24)	7.03 (5.71–8.64)	2.3[b]	Sveinbjornsdottir et al. (2014)
Iceland 2002–2007	Poser et al. (1983)	136 (34 M, 102 F)/ *1,781,102*	7.6 (6.4–9.0) [8.2]	3.8	11.5	3.0[b]	Eliasdottir et al. (2011)
Hordaland County (Western Norway) 2003–2007	Poser et al. (1983) and Polman et al. (2011)		8.5 (7.3–9.7)			1.8	Grytten et al. (2016)
Oppland County (Southern Norway) 1994–1998	Poser et al. (1983) and Polman et al. (2005)	68 (26 M, 42F)/*182,826* average population	7.4 (5.8–9.4) [7.6]	5.7 (3.7–8.4)	9.1 (6.6–12.3)	1.6[b]	Risberg et al. (2011)
Vest-Agder county (Southern Norway) 2001–2006	Poser et al. (1983)	Including PP	7.5 [8.0 (4.6–14.2)]	5.1 [5.6 (2.1–14.9)]	9.6 [10.3 (5.1–20.6)]	1.9[b]	Vatne et al. (2011)
Sweden 2001–2008	Poser et al. (1983), McDonald et al. (2001) and Polman et al. (2005)	*9,054,658* average population Including PO	10.2	6.2	14.0	2.26	Ahlgren et al. (2014)
Västerbotten County (Northern Sweden) 1998–2010	Poser et al. (1983), McDonald et al. (2001), Polman et al. (2005), Polman et al. (2011)	201	6.0 (5.2–6.9)	3.9 (3.0–5.0)	8.1 (6.8–9.6)	2.1[b]	Svenningsson et al. (2015)

(continued)

Table 9.2 (continued)

Study region, Interval period	Diagnostic criteria	Cases (Males, Females)/ *person-years or mean population*	Crude incidence[a] [Standardized incidence] Total	Crude incidence[a] [Standardized incidence] Male	Crude incidence[a] [Standardized incidence] Female	F:M ratio	References
Northern Ostrobothnia (Northern Finland) 1992–2007	Poser et al. (1983) and McDonald et al. (2001)	374	6.3 (5.2–7.2)	3.9 (3.1–4.7)	8.6 (7.0–10.2)	2.2[b]	Krökki et al. (2011)
British Isles							
United Kingdom 2010	Phys-diag	6003	9.64	4.84 (4.54–5.16)	11.52 (10.96–12.11)	2.4	Mackenzie et al. (2014)
Isle of Man 2006–2011	Polman et al. (2005)		6.86 (4.77–9.88) [6.62 (4.60–9.52)]	4.29 (2.23–8.24) [4.07 (2.12–7.82)]	9.41 (6.07–14.58) [9.07 (5.85–14.07)]	2.19	Simpson et al. (2015)
Central Europe							
Netherlands 1996–2004a 2007–2008b	Phys-diag	84/ *2,344,724*	4.8 (3.9–6.0) a: 4 (3–5) b: 9 (6–16)	2.1 (1.3–3.3)	7.5 (5.9–9.6)	3.6[b]	Kramer et al. (2012)
Lorraine Region (Northeastern France) 2008	Poser et al. (1983)	104 Including PP	4.4 (3.6–5.4)				El Adssi et al. (2012)
France 2001–2007	Phys-diag	28,682/ *52,449,871*	7.6 (7.5–7.6) [6.8]	4.3 (4.2–4.4) [3.7]	10.5 (10.3–10.6) [9.8]	2.4[b]	Fromont et al. (2012)
Brittany (Northwestern France) 2000–2001	Poser et al. (1983) and Polman et al. (2011)	249 Including PP	4.28 [4.41 (3.32–5.51)]	2.23 [2.21 (1.12–3.31)],	6.22 [6.68 (4.75–8.60)],	2.8[b]	Yaouanq et al. (2015)
Germany 2009–2011	Polman et al. (2005)	227 subjects <15 years	0.64 (0.56–0.73)				Reinhardt et al. (2014)
Iberian peninsula							
La Rioja (Northern Spain) 2001–2011	Poser et al. (1983) and Polman et al. (2005)	107/ *306,357* mean population	3.5 (2.8–4.2)				Bártulos Iglesias et al. (2015)

Northern Seville District of Andalucia (Spain) 1991–2010	Poser et al. (1983)	*163,324* pop at 2001	4.6 (4.1–5.1)	2.5 (2.0–2.9) in 1991–2000	4.3 (3.6–4.9) in 1991–2000	1:1 in 1991–2000	Izquierdo et al. (2015)
				2.8 (2.3–3.4) in 2001–2010	8.8 (7.84–9.69) in 2001–2010	4:1 in 2001–2010	
Girona, Catalonia (Spain) 2009–2013	Polman et al. (2005) and Tintoré et al. (2003)	182 Including CIS + probable MS	3.6 (2.4–5.3) [3.29 (3.2–3.3).] CIS: 6.8 (5.08–8.97)	2.9 (1.4–5.2) CIS: 6.3	4.3 (2.5–7.1) CIS: 7.3	1.5	Otero-Romero et al. (2015)
			Probable MS 5.1 (3.60–6.98)				
Benfica, Pontinha and Odivelas (Portugal) 1998–2007	McDonald et al. (2001)	62/ *1,963,120*	3.16 [3.09 (2.32–3.87]	1.38 [1.30 (0.59–2.01)]	4.79 [4.79 (3.44–6.13)]	3.5[b]	de Sá et al. (2014)
			CRM-adjusted, 4.53 (3.13–5.94); and age- and CRM-adjusted, 4.48 (3.54–5.41)				
Braga (Northwestern Portugal) 1998–2009	Poser et al. (1983)		2.74				Figueiredo et al. (2015)
Italy							
Padua (Veneto-Northeastern Italy) 2000–2009	McDonald et al. (2001)		5.5 (5.0–6.0)	3.5 (2.9–4.1)	7.4 (6.6–8.2)	2.1	Puthenparampil et al. (2013)
Genoa (Liguria-Northwestern Italy) 1998–2007	Poser et al. (1983)	575/*879,005*	6.6 (5.0–8.5) [7.2 (5.4–9.1)]	4.4 (2.6–6.9) [4.6 (2.6–6.8)]	8.6 (6.2–11.7) [8.6 (6.7–12.8)]	2.22	Solaro et al. (2015)
Campobasso (Molise-Southern Italy) 1996–2000 2001–2005	Poser et al. (1983) and McDonald et al. (2001)	1996–2000: 28 (8 M, 20F)/*51,633*	10.84 (1.01–15.99)	6.45 (2.77–12.07)	14.89 (9.09–23)	2.31[b]	Bellantonio et al. (2013)
		2001–2005: 11 (5 M, 6F)/*51,633*	4.26 (2.12–7.62)	4.03 (1.3–9.4)	4.46 (1.63–9.7)	1.11[b]	
Catania (Sicily-Southern Italy) 2000–2004	Poser et al. (1983)	108/ *309,916*	7.0 (4.3–10.2) [6.8]	5.3 (2.3–10.4)	8.4 (4.6–14.1)	1.6	Nicoletti et al. (2011)

(continued)

Table 9.2 (continued)

Study region, Interval period	Diagnostic criteria	Cases (Males, Females)/ *person-years or mean population*	Crude incidence[a] [Standardized incidence] Total	Crude incidence[a] [Standardized incidence] Male	Crude incidence[a] [Standardized incidence] Female	F:M ratio	References
Mount Etna (Sicily-Southern Italy) 1980–2009	Poser et al. (1983)	Western flank 51 (13 M, 38F)/ *52,561* Including PP	3.2 (2.4–4.2) [2.8]	1.7 (0.9–2.9)	4.7 (3.3–6.4)	2.8[b]	Nicoletti et al. (2013)
		Eastern flank 60 (19 M, 41F)/ *43,797* Including PP	4.6 (3.1–5.9) [3.9]	2.7 (1.6–4.2)	6.7 (4.8–9.1)	2.5[b]	
Provinces of Carbonia-Iglesias (Sardinia-Southwestern Italy) 2003–2007	McDonald et al. (2001) and Polman et al. (2005)		9.7 (3.4–13.2)	4.7 (2.4–17.0)	14.6 (11.8–34.8)	3.1[b]	Cocco et al. (2011)
South East Europe							
Čabar (Croatia) 1948–2004	Poser et al. (1983)	29/*4387* Including PP	5.52 (3.27–8.72)				Perković et al. (2010)
Kosovo 2003–2012	McDonald et al. (2001)		0.95				Zeqiraj et al. (2014)
Crete 1980–2008	McDonald et al. (2001) and Polman et al. (2005)			1980–1984: 1.5	1980–1984: 1.5	1980–1984: 0.88	Kotzmani et al. (2012)
				2005–2008: 3.4	2005–2008: 7.3	2005–2008: 2.15	
Russia							
North-Western Administrative District (SZAO) of Moscow 2008–2012			2.16				Boĭko et al. (2013)
Primosky Krai 2010			2.49				Gavrilenko et al. (2012)
Ukraine 2005–2010			3.0–3.3				Kolosynska (2013)

PO Poser Possible, *PP* Poser Probable
Phys-diag: diagnosis by physicians
[a]Incidence/100,000 over time period specified (95 % CI)
[b]F:M ratio calculated by available data

9.2.1 The Nordic Region

Twelve studies from the Nordic region were included in our review, five from Norway (Berg-Hansen et al. 2014, 2015; Grytten et al. 2016; Vatne et al. 2011; Risberg et al. 2011) four from Sweden (Svenningsson et al. 2015; Ahlgren et al. 2011, 2012, 2014), one from Finland (Krökki et al. 2011) and two from Iceland (Sveinbjornsdottir et al. 2014; Eliasdottir et al. 2011). All these studies used the Poser diagnostic criteria alone or in combination with others. Half of them utilized data from National/Local MS Registry and National Patient Registry (Berg-Hansen et al. 2014, 2015; Ahlgren et al. 2011, 2012, 2014; Svenningsson et al. 2015). Prevalence rates ranged from 103/100,000 in Northern Ostrobothnia (Finland) (Krökki et al. 2011) to 215 in Västerbotten County (Sweden) (Svenningsson et al. 2015). The highest annual incidence rate was found in Sweden for the 2001–2009 time period (Ahlgren et al. 2014) whereas the lowest one was in Iceland for 1990–1999 time period (Sveinbjornsdottir et al. 2014). In all studies, women had a prevalence/incidence rates approximately double then men. Many studies in this region reported an increasing prevalence in Norway (Berg-Hansen et al. 2014; Grytten et al. 2016 and Risberg et al. 2011), in Sweden (Svenningsson et al. 2015) and in Iceland (Sveinbjornsdottir et al. 2014); and an increasing incidence in Finland (Krökki et al. 2011) and in Iceland (Sveinbjornsdottir et al. 2014). Two studies, one in Sweden and one in Norway highlighted lower prevalence among immigrants *vs* native people and among non-European immigrants *vs.* European people (Ahlgren et al. 2012; Berg-Hansen et al. 2015).

9.2.2 The British Isles

Four studies, one from Ireland (Lonergan et al. 2011), one from Scotland (Visser et al. 2012), one from the Isle of Man (Simpson et al. 2015), and one from the United Kingdom (Mackenzie et al. 2014) were included in this review. The first two articles compared rates of different geographical areas (Lonergan et al. 2011; Visser et al. 2012). Two studies capture cases from General Practitioner Research Database alone (Mackenzie et al. 2014) or with other sources (Visser et al. 2012). Crude prevalence rates ranged from 128/100,000 in South Dublin in 2007 (Lonergan et al. 2011) to 410 in the islands of Orkney in 2009 (Visser et al. 2012). Regarding incidence, only two studies calculated this rate with 9.7 in the UK and 6.9 in the Isle of Man (Simpson et al. 2015; Mackenzie et al. 2014). Most of studies found a female:male ratio of around 2.5 (Simpson et al. 2015; Mackenzie et al. 2014; Lonergan et al. 2011; Visser et al. 2012). The study of Visser and colleagues reported increasing prevalence and F:M ratio in Scotland, especially in the islands of Orkney (Mackenzie et al. 2014).

9.2.3 Central European Countries

Seven of the studies included in this review were from Central Europe, covering France (El Adssi et al. 2012; Fromont et al. 2012; Yaouanq et al. 2015), Netherlands (Kramer et al. 2012), Germany (Höer et al. 2014; Reinhardt et al. 2014) and Hungary (Zsiros et al. 2014). Three of them were based on National/Local Health Insurance System, alone (Fromont et al. 2012; Höer et al. 2014) or in combination with other data sources (i.e. hospitalization records and a local MS registry) (El Adssi et al. 2012), while the study conducted in Hungary utilized data from a local MS Register (Zsiros et al. 2014). The prevalence study conducted in France utilized the capture-recapture method to reduce case underestimation (El Adssi et al. 2012). Crude prevalence estimates from this region ranged from 90 to 175/100,000. The lowest rate originated from Hungary in 2013 (Zsiros et al. 2014), while the highest one was found in Bavaria (Germany) in 2009 (Höer et al. 2014). Incidence was studied in Netherlands and in France with rates ranged from 4.3 in Brittany (2000–2001) to nine in the Netherlands (2007–2008) (Yaouanq et al. 2015; Kramer et al. 2012). In addition, an incidence national study conducted in Germany among

pediatric MS cases (children and adolescent <15 years old) registered an annual rate of 0.6/100,000 (Reinhardt et al. 2014). An increasing trend was observed for prevalence in Germany (Höer et al. 2014) and for incidence in France (El Adssi et al. 2012) and in the Netherlands (especially in women) (Kramer et al. 2012).

9.2.4 Iberian Peninsula

Three studies from Portugal (de Sá et al. 2012, 2014; Figueiredo et al. 2015) and five from Spain (Bártulos Iglesias et al. 2015; Fernández et al. 2012; Otero-Romero et al. 2013, 2015; Izquierdo et al. 2015) were included. No studies included the entire country of either Spain or Portugal. Considering data sources, one incidence study in Catalonia (Spain) utilized a local MS Registry (Otero-Romero et al. 2015). Four studies used the capture-recapture method to adjust for incomplete ascertainment (de Sá et al. 2012, 2014; Fernández et al. 2012; Otero-Romero et al. 2015). Prevalence was around 40/100,000 in Portugal (de Sá et al. 2012; Figueiredo et al. 2015), while it ranged from 65 to 125 cases/100,000 in Spain (Bártulos Iglesias et al. 2015; Fernández et al. 2012). Regarding incidence, the lowest rate was in Braga (Portugal) with 2.7/100,000 (Figueiredo et al. 2015), and the highest one was in Andalucia (Spain) with 4.6 (Izquierdo et al. 2015). In Spain, many authors reported increasing prevalence (Otero-Romero et al. 2013; Izquierdo et al. 2015), female:male ratio (Fernández et al. 2012), and incidence, especially in female (Izquierdo et al. 2015).

9.2.5 Italy

Italy is classified as a high-risk area for MS, with highest rates in the island of Sardinia, and no evidence of the latitude gradient (Rosati 2001). The Italian MS patient society (AISM) in 2014 estimated that in Italy there were more than 75,000 prevalent cases with an incidence of more than 2000 cases per year (Bilancio sociale AISM 2014). In this review were included ten articles from Italy, but no one incorporated the entire country, but only small area (Mount Etna) (Nicoletti et al. 2013), provinces (Solaro et al. 2015; Gajofatto et al. 2013; Bellantonio et al. 2013; Puthenparampil et al. 2013; Sardu et al. 2012; Cocco et al. 2011; Nicoletti et al. 2011) or regions (Bezzini et al. 2016; Bargagli et al. 2016). Nicoletti and colleagues carried out an ecological survey to determine if crater gas could affect MS epidemiology among populations living in Mount Etna. In particular they analyzed incidence and prevalence on the two (eastern and western) flanks of the volcano which are differently exposed to crater gas emissions (the eastern flank is more exposed to trace elements than the western one) (Nicoletti et al. 2013).

The most recent two studies, regarding two entire regions (Tuscany and Lazio), were based on administrative data and the Tuscan one validated the case-finding algorithm (Bezzini et al. 2016; Bargagli et al. 2016). The reported prevalence ranged from 94 in the western flank of Mount Etna in 2009 (Nicoletti et al. 2013) to 188 in Tuscany in 2011 (Bezzini et al. 2016), and to 224 in Sardinia where the risk of MS is higher than continental Italy and Sicily (Sardu et al. 2012). Sardinia population was characterized by an elevated risk to autoimmune diseases, such as MS, due to an homogeneous genetic background coming from past isolation from other population (Lampis et al. 2000; Marrosu et al. 2002).

Considering incidence, the rates were lower in the Mount Etna (3.2) for the period 1980–2004 (Nicoletti et al. 2013) but higher in Catania (7.0) for the period 2000–2004 (Nicoletti et al. 2011) and in Sardinia (9.7) in 2003–2007 (Cocco et al. 2011). Some authors reported an increasing prevalence in the provinces of Genoa, Padua (North) and in Catania (South) and also an increasing incidence in the last two provinces (Solaro et al. 2015; Puthenparampil et al. 2013; Nicoletti et al. 2011).

9.2.6 South East Europe

Six of the studies included in this review were from South East Europe, covering Kosovo (Zeqiraj et al. 2014), Serbia (Toncev et al. 2011), Croatia (Perković et al. 2010), Albania (Kruja et al. 2012), Turkey (Türk Börü et al. 2011) and Crete (Kotzamani et al. 2012). Two studies used a door-to-door sampling (Kruja et al. 2012; Türk Börü et al. 2011), while the one on the island of Crete identified patients using the MS epidemiology program project of Crete. Prevalence rates were very low in the two examined Albanian communities (0.3 cases/100,000) (Kruja et al. 2012) and in Kosovo (19.6) (Zeqiraj et al. 2014), slightly higher in Turkey (with rates ranged from 41 to 61) (Türk Börü et al. 2011), in Serbia (65 cases/100,000) (Toncev et al. 2011) and higher in the island of Crete (108) (Kotzamani et al. 2012). The highest prevalence was found in Croatia, with 206 cases per 100,000 but more than half of patients (53 %) were familiar case indicating a familiar pseudocluster in the city of Čabar (Perković et al. 2010). Toncev and colleagues observed an increasing prevalence in Serbia (Toncev et al. 2011), whereas Kotzamani and his group found an increasing incidence in the island of Crete and a very higher risk in women residing in urban centers than people living in the countryside (Kotzamani et al. 2012).

9.2.7 Eastern Europe

Four studies from Eastern Europe were included, three local studies from Russia (Boĭko et al. 2013, 2014; Zikhova et al. 2013; Gavrilenko et al. 2012) and one national incidence study from Ukraine (Kolosynska 2013). Prevalence rates were low (from 11.5 to 53.4) nonetheless Gavrilenko and colleagues found an increasing trend. Incidence rates ranged from 2.2 to 3.3 with higher values in some areas of Ukraine (Gavrilenko et al. 2012; Boĭko et al. 2013; Kolosynska 2013). The aim of the study of Kolosynska was to estimate MS incidence in population living in contaminated areas after the Chernobyl accident compared to other population groups, and they found an incidence of 3.0–3.3 cases per 100,000 with significantly higher values (up to 7.5) in contaminated areas.

9.3 Conclusion

This search identified and catalogued MS incidence and prevalence studies across Europe between February 1st 2011 and March 1st 2016. Some European countries have undergone several epidemiological studies, such as Norway and Spain with five studies and Italy with ten, whereas, in many European regions, MS epidemiology is not well documented. In general, we can observe an increasing prevalence probably due to increasing survival and maybe an increasing incidence, as reported from some authors.

Much of the literature focused on specific cities, provinces or regions within a given country, whereas a few studies reported countrywide data (Sveinbjornsdottir et al. 2014; Eliasdottir et al. 2011; Berg-Hansen et al. 2014, 2015; Ahlgren et al. 2011, 2012, 2014; Mackenzie et al. 2014; Kramer et al. 2012; Fromont et al. 2012; Reinhardt et al. 2014; Zeqiraj et al. 2014; Kotzamani et al. 2012; Kolosynska 2013) due to difficult to collect data from extensive population of many European countries. Administrative data can be used to obtain countrywide data, and if obtained through a validated case-finding algorithm, data could be compared at national or international level (Culpepper et al. 2006; Marrie et al. 2010; Bezzini et al. 2016). Ethnic differences were presented only in two studies (Ahlgren et al. 2012; Berg-Hansen et al. 2015). Prevalence rates tended to be lower in Portugal, Serbia, Kosovo, Albania, Turkey and Russia, and incidence was lower in Kosovo and Russia (de Sá et al. 2012; Figueiredo et al. 2015; Toncev et al. 2011; Zeqiraj et al. 2014; Kruja et al. 2012; Türk Börü et al. 2011; Boĭko et al. 2013, 2014; Zikhova et al. 2013; Gavrilenko et al. 2012; Kolosynska 2013). The comparison of estimates between regions is difficult due to differences in the size of the studied population, the quality of the studies, the source of data and the diagnostic criteria that were used. The Poser criteria were the most

widely used (either alone or in combination with other criteria), but several studies included only definite cases, whereas some others included probable and/or possible cases. Despite the breadth of the literature on the European MS epidemiology, the reported data cannot be easily compared due to different methodologies of inclusion and diagnostic criteria utilized by single studies, and to their different quality. In this search, we did not evaluate the quality of included studies, but Kingwell and colleagues in a recent review reported different methodologies, diagnostic criteria and also a lack of appropriate standardization of included articles, limiting the inter-study comparison (Kingwell et al. 2013). In conclusion, more epidemiological studies, especially at national level and with a similar standardization, are needed for the evaluation and the comparison of MS epidemiological figures in European continent.

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10 Timing of Future Remyelination Therapies and Their Potential to Stop Multiple Sclerosis Progression

Burcu Zeydan, Moses Rodriguez, and Orhun H. Kantarci

Abstract

Prior to the onset of demyelination in multiple sclerosis (MS), early oligodendrocyte injury, axonal degeneration and astroglial scarring occur. The irreversible progressive phase of MS begins when the axonal loss threshold is reached. Progressive disease onset has the highest impact on a poor prognosis in MS. Conversion to progressive disease is essentially an age-dependent process independent of disease duration and initial disease course. Although prevention of relapses has been the primary approach in the disease management, incomplete recovery from even the first relapse correlates with the long-term neurodegenerative phenotype of progressive MS onset. Therefore, the provider should review each patient's potential for relapse-related disability and start DMDs with the goal of preventing relapses. Existing immunomodulatory medications used to prevent MS relapses do not prevent long-term disability, which requires agents focused on remyelination and axonal repair. If applied immediately after a relapse rather than during the progressive phase of MS, remyelination-stimulating strategies may result in full recovery and prevention of long-term neurodegeneration and progressive disease course.

Keywords

Axonal degeneration • Progressive MS • Remyelination

Abbreviations

ACTH	adrenocorticotropic hormone
Ca2+	calcium
CIS	clinically isolated syndrome
DMD	disease-modifying drug
EAE	experimental autoimmune encephalomyelitis
IgM	immunoglobulin M

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LINGO-1	leucine-rich repeat neuronal protein 1
MRI	magnetic resonance imaging
MS	multiple sclerosis
NAbs	naturally occurring antibodies
OPCs	oligodendrocyte progenitor cells
PPMS	primary progressive multiple sclerosis
RIS	radiologically isolated syndrome
RRMS	relapsing remitting multiple sclerosis
SAMS	single attack multiple sclerosis
SAPMS	single attack progressive multiple sclerosis
SPMS	secondary progressive multiple sclerosis

10.1 Introduction

Multiple sclerosis (MS) is an idiopathic, immune-mediated demyelinating disease of the central nervous system (CNS). MS is the most common chronic demyelinating disease of the CNS and predominantly affects young adults in their most productive years. Besides displaying a broad spectrum of radiological features, treatment responses, and neuropathology, MS has a wide range of clinical manifestations. Prior to the clinical manifestations, especially in early disease phase, MS is mainly a subclinical process associated with alterations in the tissue structure of the brain and/or spinal cord (Novotna et al. 2015b). The main clinical features in MS are relapses and progression. Progressive disease onset has the highest impact on the poor prognosis in MS. Poor recovery from a relapse accelerates the accumulation of disability (Weinshenker 1998; Rotstein et al. 2015). Existing immunomodulatory medications used to prevent relapses in MS do not prevent long-term disability, which requires agents focused on remyelination and axonal repair. This review focuses on indication and timing of such treatment modalities in the future.

10.2 Neuropathology of MS

As a result of disease phase, clinical phenotype and lesion activity stage, MS displays an evident heterogeneity in terms of neuropathology. The disease is characterized by the interplay between inflammation-demyelination, remyelination, and axonal loss (Kantarci et al. 2014). Four pathological patterns of new lesion development have been identified that involve different degrees of remyelination in addition to demyelination (Lassmann et al. 2001; Lucchinetti et al. 2000). The patterns include T cell-mediated mechanisms, antibody- and complement-mediated demyelination, distal oligodendrogliopathy and apoptosis, and primary oligodendrocyte degeneration (Lassmann et al. 2001). Two of the patterns, which primarily affect myelin, also manifest a 30 % loss of oligodendrocytes; in the other two patterns, the oligodendrocytes seem to be the main target (Lassmann et al. 2001; Lucchinetti et al. 2000). Although the initial cause of oligodendrocyte injury remains unknown, early oligodendrocyte injury, which may exist years prior to the clinical presentation of MS, must occur before the onset of the chronic demyelination (Barnett and Prineas 2004; Cannella et al. 2007; Lucchinetti et al. 2000; Rodriguez and Scheithauer 1994; Rodriguez et al. 1993). In addition to early oligodendrocyte injury, axonal degeneration and astroglial scarring also occur prior to the onset of demyelination (Kuhlmann et al. 2002; Kutzelnigg et al. 2005; Seehusen and Baumgartner 2010; Trapp et al. 1998). However, due to T-cell cytotoxicity and neurotrophic failure, demyelination can also cause axonal damage (Rodriguez 2003). As the threshold is reached for the axonal loss, MS patients enter the irreversible progressive phase (Novotna et al. 2015b).

10.3 Potential Treatment Strategies of MS

Because the immune activation mechanisms of MS relapses have been extensively studied through experimental autoimmune encephalomyelitis (EAE), this model has influenced the development of many treatment options. However, there is limited information on the remission phase after each relapse, which is mainly provided by oligodendrocytes and the astrocytes. Although

prevention of relapses has been the primary approach in MS care based on original natural history studies, incomplete recovery from even the first relapse correlates with the long-term neurodegenerative phenotype of progressive MS onset (Novotna et al. 2015a, b). Lack of repair and remyelination after a clinical or subclinical relapse is the key indicator of long-term disability accumulation in MS.

10.3.1 Remyelination

Mechanisms for functional recovery include axonal plasticity, synaptic plasticity, and remyelination (Irvine and Blakemore 2008; Jeffery and Blakemore 1997; Murray et al. 2001). Remyelination is a cellular process involving oligodendrocytes, demyelinated axons, and immune cells that infiltrate the CNS to form new myelin sheaths and internodes for demyelinated axons (Rodriguez et al. 2013). Remyelination in CNS occurs spontaneously; however, it is typically incomplete. The resulting myelin is very thin for the corresponding axon, and the internodes are shorter (Blakemore 1974). But despite a shorter internodal distance, the remyelinated lesions provide saltatory conduction (which is lost after demyelination) and almost-normal conduction velocity in both experimental animals and computer simulations (Weiner et al. 1980). Remyelination has been shown in autoimmune, viral and toxic models of demyelination (Rodriguez et al. 2013). In MS, CNS remyelination of oligodendrocytes and peripheral nervoussystem remyelination by Schwann cells can occur. It is most commonly observed in acute lesions of pattern I and II and is absent in patterns III and IV (Lucchinetti et al. 2000).

Remyelination can be promoted in three basic ways: triggering endogenous repair mechanisms; using exogenous cells, which form myelin; or simply decreasing myelinated cell damage (Novotna et al. 2015b). However, the failure in progenitor-oligodendrocyte recruitment and differentiation, delayed growth-factor expression, and damaged axon reception can lead to decreased remyelination capacity with age and disease progression as shown in animal studies of CNS demyelination (Hinks and Franklin 2000; Ozawa et al. 1994; Sim et al. 2002; Zhao et al. 2006). This supports the concept that MS remyelination is more noticeable early in the disease course, especially in acute lesions. As the disease progresses, remyelination becomes less prominent (Ghatak et al. 1989; Lucchinetti et al. 1999).

Remyelination may be one of the most effective forms of neuroprotection (Murray et al. 2001). Observation of animal models suggests that demyelinated axons restore neurophysiological function by remyelination (Smith et al. 1979; Smith et al. 1981). Complete myelin repair is possible in non-inflammatory animal models of demyelination. This is similar to what was observed in humans with early-phase MS (Patrikios et al. 2006). While many treatment options, such as corticosteroids, plasma exchange, and immunosuppressants, have proven successful in acute treatment of MS relapses, treatment should also include remyelination (Kantarci et al. 2014). If immediately applied after a relapse, remyelination-stimulating strategies may result in full recovery and prevention of long-term neurodegeneration and progressive disease course (Kantarci et al. 2014).

10.3.2 Naturally Occurring Human Monoclonal Antibodies

Recent studies have shown that natural immunoglobulins from humans or mice promote CNS remyelination. When used in experimental models of demyelination, naturally occurring antibodies (NAbs) against oligodendrocytes demonstrate remarkable remyelination (Warrington et al. 2000; Xu et al. 2013). In contrast to conventional MS therapies, NAbs uniquely target the cells responsible for myelin-sheath production. The application of NAbs before the onset of permanent axonal damage may reverse neurological deficits. Given that early remyelination is a determinant of recovery, it is important to recognize and treat the early onset of the disease.

10.4 MS Phases (Sub-Phenotypes)

Different phases of MS range from asymptomatic to progressive disease. The 2010 McDonald criteria diagnose each phase of MS. There are six clinical MS sub-phenotypes, which are defined by the relationship between presence of relapses and the progressive phase. Occasionally, asymptomatic patients seeking magnetic resonance imaging (MRI) for unrelated reasons will present with white matter lesions; after exclusion of other possible etiologies, these patients are designated as having either preclinical MS or radiologically isolated syndrome (RIS). The high MS risk phase evolves into RIS (Lebrun et al. 2008; Okuda et al. 2009; Siva et al. 2009), and some individuals with RIS evolve into clinically isolated syndrome (CIS). However, most patients present the first time with CIS. Once the first symptom as a clinical attack suggestive of inflammatory-demyelination is observed, the patient is diagnosed with CIS. CIS may evolve into basically two phases in an age-dependent nature, either as multiple symptomatic attacks (relapsing-remitting MS or RRMS, which is characterized by multiple relapses with or without ongoing MRI activity) or new asymptomatic MRI activity (single attack MS or SAMS). If the patient shows an insidiously worsening of neurological dysfunction as opposed to discrete clinical attacks, we can conclude that the patient has a progressive disease course. Progressive disease may begin as an initial presentation of a SAMS-type onset (single attack progressive MS or SAPMS) (Tutuncu et al. 2013), or it may follow RRMS, in which case it is labeled as secondary progressive MS (SPMS). Patients who develop progression after a typical age and who are free of symptomatic relapses have primary progressive MS (PPMS) (Lublin et al. 2014; Tutuncu et al. 2013). Careful definition and classification of MS phases are critical because different treatment approaches are linked to each MS phase.

10.5 Management of MS

The main factors associated with poor long-term disability are high frequency of early relapse, older age at MS onset, male sex, and spinal cord syndrome at MS onset; however, onset of progressive disease is the key determinant of long-term disability as confirmed by natural history studies (Confavreux et al. 2000; Kantarci et al. 1998; Runmarker and Andersen 1993; Weinshenker et al. 1989). We also know that conversion to progressive disease is essentially an age-dependent process independent of disease duration and initial disease course (Confavreux and Vukusic 2006; Koch et al. 2007; Tutuncu et al. 2013). Recent data prove that better relapse recovery during the first 5 years of the disease course will delay progressive MS onset; the patients with severe brainstem, cerebellar and spinal cord syndrome have the worst prognosis for recovery (Novotna et al. 2015a). Patients with multiple relapses prior to the onset of progressive phase are more prone to accumulate disability than patients with SAPMS or asymptomatic attacks before the onset of progressive MS (Paz Soldan et al. 2015). Significantly, the extent of relapse recovery decreases with age and length of time from disease onset.

10.5.1 Treatment of Acute MS Relapses

Of the three main clinical phenomena (relapses, pseudo-relapses and progressive disease course) in MS, treatment of acute relapses should receive the highest priority in terms of management. A pseudo-relapse typically lasts less than 24 h and can be described generally as the recurrence of an existing symptom without development of any objective lesion or new finding in the neurological examination. The usual causes are infections, stress, fatigue or increased body temperature. A true relapse, which usually evolves over hours or days, has a plateau stage that persists for several hours to weeks and ends in partial or complete recovery. The established approach for treating relapses starts with high-dose corticosteroids (and adrenocorticotropic hormone or ACTH), which address cell-mediated immune responses and bloodbrain barrier repair. Administration typically follows an MS relapse with 1000 mg per day intravenous methylprednisolone for a period of 3–7 days; the peak effect often is

observed between days 7 and 10. High-dose of intravenous corticosteroids speed recovery faster but have a limited effect on long-term recovery from individual relapses (Ciccone et al. 2008). Providers should assess the efficacy of treatment at least 2 weeks after the initiation of intravenous corticosteroids. Approximately 15 % of patients do not respond to therapy. Plasma exchange, in which the mechanism of action is antibody deletion, is the treatment of choice in cases that do not respond to corticosteroids. Completion of a seven-session course is not recommended unless significant improvement occurs by the fourth exchange. A noticeable improvement is unlikely to occur spontaneously. Common predictors for improvement following plasma exchange are early initiation of treatment, preserved reflexes, male sex, and certain radiological findings of MS lesions, i.e., ring enhancement and mass effect of the lesion (Keegan et al. 2002; Magana et al. 2011). Despite the lack of a well-performed clinical trial, strong immunosuppressive therapies, such as cyclophosphamide in addition to corticosteroids, frequently comprise the third step when patients fail both intravenous corticosteroids and plasma exchange. Prior to starting this, providers should re-evaluate the possibility of alternative diagnoses and add a rehabilitation program (Elkhalifa and Weiner 2010; Weiner et al. 1984). Other alternatives, such as alemtuzumab and natalizumab, have serious adverse effects. Intravenous immunoglobulin is another option to treat acute MS relapses in some centers, but there is no evidence-based material on the subject (Noseworthy et al. 2000).

10.5.2 Prevention of Acute MS Relapses

To prevent MS relapses in MS, providers should review the natural history studies and predictors of an individual's relapse-related disability potential before deciding when to start disease-modifying drugs (DMDs). DMDs have a stronger impact on subclinical lesion development than on relapses, so background MRI activity can also guide treatment decisions. Relapse prevention starts with tier-1 drugs including interferon-beta, glatiramer acetate, dimethyl fumarate and teriflunomide (medications with weak-to-moderate efficacy and long-term safety). Tier-2 medications (natalizumab, fingolimod, cyclophosphamide, mitoxantrone) have a higher efficacy (moderate-to-significant) but also carry more safety concerns. Future strategies to prevent MS relapses include monoclonal antibodies such as rituximab, daclizumab and alemtuzumab. Since most patients do not experience relapse 5 years after the onset of progressive MS or by 59 years of age, DMDs should be discontinued when one of these time points is reached (Paz Soldan et al. 2015).

10.5.3 Management of Progressive Phase of MS

Since progressive MS is responsible for the vast majority of the disability burden in MS (Confavreux et al. 1980; Confavreux and Vukusic 2006; Kantarci et al. 1998; Kantarci and Weinshenker 2005; Runmarker and Andersen 1993; Weinshenker et al. 1989; Wolinsky 2003), avoiding the progressive phase is a very important management goal. Each relapse increases irreversible myelin injury and axonal loss, which correlate to the severity and the duration of the relapse as well as the individual's ability to recover (De Stefano et al. 2001; Traboulsee 2007). Since poor recovery from the first relapse especially can predispose an individual to an earlier onset of progressive MS, achieving good recovery from relapses should be the ultimate goal (Novotna et al. 2015a). The key elements of treatment strategy should be preventing relapse in early phases of MS (even in RIS) and in the early, recurrent relapse phases (Novotna et al. 2015b). Another, maybe the most important, future strategy is proactive promotion of recovery from any type of relapse. As mentioned before, although a symptomatic relapse can result in sustained long-term disability (but only in low-to-moderate levels) (Paz Soldan et al. 2015), recovery from individual relapses and number of relapses have a greater impact on the long-term

severity resulting from relapses. To decrease the disability impact of MS, the treatment strategies should also involve (a) prevention of MS during the high-risk phase before it starts; (b) prevention of clinical conversion during RIS; (c) prevention of relapses by redefining the timing of already existing DMDs; (d) prevention of progressive MS; (e) slowing or stopping progressive MS after it starts; and (f) designing repair strategies for both the active and inactive periods of MS (Novotna et al. 2015b). Despite the current therapies to eliminate future demyelination, direct targeting of demyelinating axons has shown no benefit on long-term disability. Unfortunately there are no current proven strategies for axonal preservation, myelin repair, oligodendrocyte stimulation or neuronal sprouting (Novotna et al. 2015b). To complement the protocols of corticosteroids, plasma exchange and immunosuppressives, future MS treatment strategies should focus particularly on remyelination and axonal repair with the aim of achieving potential full recovery from a relapse and avoiding long-term neurodegeneration and development of progressive disease (Kantarci et al. 2014).

10.5.4 Future Treatment Directions of Remyelination

The two agents that have shown promising effects on myelin repair are anti-LINGO-1 and human immunoglobulin M (IgM) 22. Anti-Lingo-1, a mammalian anti-peptide antibody, is directed against a CNS protein called leucine-rich repeat neuronal protein (LINGO)-1. This protein inhibits oligodendrocyte differentiation, myelination and axonal regeneration (Mi et al. 2005). In animal models, the novel product against LINGO-1 promotes CNS remyelination and neuroaxonal protection (Mi et al. 2007). The second agent is a naturally occurring monoclonal antibody, human IgM22 (sHIgM22), the first IgM antibody with the characteristics of classic NAbs, promoting significant remyelination in vivo. It was isolated from sera of patients with monoclonal gammopathies lacking neurologic or antibody-associated pathologies (Wootla et al. 2013). In the Theiler's virus model, animals with active demyelinating disease surprisingly showed remyelination in demyelinated spinal cord lesions after the injection of antisera and antibodies generated against myelin components in healthy animals (Lang et al. 1984; Rodriguez et al. 1987). Then human serum samples with high immunoglobulin concentrations such as multiple myeloma were screened for NAbs. Two IgM antibodies (sHIgM22 and sHIgM46) promoted remyelination in vivo (Wootla et al. 2015). rHIgM22 was produced by cloning the sHIgM22 antibody variable DNA sequence into an IgM expression vector frame (Mitsunaga et al. 2002; Warrington et al. 2007). This remyelination-promoting IgM antibody induces calcium ($Ca2^{+}$) signaling, which results in a $Ca2^{+}$ influx in astrocytes (glial, fibrillary, acidic protein-positive cells), oligodendrocyte progenitor cells (OPCs) and immature oligodendrocytes (Paz Soldan et al. 2003). As the proposed mechanism suggests, the antibody binds specifically to the lipid rafts on the surface of live oligodendrocytes and brings all related molecules together to construct a signaling complex (Watzlawik et al. 2010; Wootla et al. 2013). As a result, rHIgM22 promotes proliferation of progenitor oligodendrocytes. Finally, the blood-brain barrier breakdown allows the high-molecular-weight antibody to enter and accumulate in the demyelinated lesion (Filippi et al. 1998; Kermode et al. 1990) and induce maximal remyelination within 5 weeks after a single dose in an animal model of MS (Pirko et al. 2004; Warrington et al. 2007). rHIgM22 has also undergone phase-1 randomized, double-blind, placebo-controlled clinical trial, evaluating pharmacokinetics and immunogenicity of the drug as well as the safety and tolerability in MS patients (Wootla et al. 2013). Overall, the combination of two novel agents, anti-LINGO-1 and rHIgM22 with different mechanisms of action sounds promising. They can complement each other to promote remyelination. Another candidate natural monoclonal IgM antibody (sHIgM12) may be useful in the treatment of neurodegenerative diseases associated with axonal injury as well as MS (Warrington et al. 2004). It differs from sHIgM22 by having no effect on either

remyelination in vivo or $Ca2^{+}$ influx; it does not induce calcium in glial cells, consistent with the fact that glial cells are not the targets for the antibody. Whether sHIgM12 induces a calcium response in neurons or axons remains to be tested (Warrington et al. 2004). Other neurologic diseases in which the recombinant, autoreactive, naturally occurring human IgM antibodies represent a potential therapeutic regimen are Parkinson's disease, amyotrophic lateral sclerosis, Lewy body dementia, and Alzheimer's disease (Wootla et al. 2015).

10.6 Conclusion

Prior to the onset of demyelination in MS, early oligodendrocyte injury, axonal degeneration and astroglial scarring occur. Once the axonal loss threshold is reached, the irreversible progressive phase of MS begins. Progressive disease onset has the highest impact on a poor prognosis in MS. Conversion to progressive disease is essentially an age-dependent process independent of disease duration and initial disease course. Although prevention of relapses has been the primary approach in the disease management, incomplete recovery from even the first relapse correlates with the long-term neuro-degenerative phenotype of progressive MS onset. Therefore, the provider should review each patient's potential for relapse-related disability and start DMDs with the goal of preventing relapses. Treatment of acute relapses is the highest priority in MS management because poor recovery significantly accelerates the accumulation of disability. Irreversible myelin injury and axonal loss increase with each relapse and directly correlate to the severity and the duration of the relapse as well as the individual's ability to recover. In addition, the extent of relapse recovery decreases with age and length of time from disease onset. The main treatment options, corticosteroids, plasma exchange, and immunosuppressants, have already been proven effective in acute treatment of MS relapses. Despite the existing therapies focusing on the elimination of future demyelination, direct targeting of demyelinating axons does not prevent long-term disability. The ideal treatment should also enhance remyelination, since lack of remyelination after a clinical or subclinical relapse is the key indicator of long-term disability accumulation in MS. Remyelination is one of the most effective forms of neuroprotection. Future MS treatment strategies should focus particularly on remyelination and axonal repair to achieve full recovery from a relapse and to prevent progressive disease.

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11 Neuroplasticity-Based Technologies and Interventions for Restoring Motor Functions in Multiple Sclerosis

Sofia Straudi and Nino Basaglia

Abstract

Motor impairments are very common in multiple sclerosis (MS), leading to a reduced Quality of Life and active participation. In the past decades, new insights into the functional reorganization processes that occur after a brain injury have been introduced. Specifically, the motor practice seems to be determinant to induce neuroplastic changes and motor recovery. More recently, these findings have been extended to multiple sclerosis, in particular, it has been hypothesized that disease progression, functional reorganization and disability are mutually related. For this reason, neuroplasticity-based technologies and interventions have been rapidly introduced in MS rehabilitation. Constraint-induced movement therapy (CIMT), robotics and virtual reality training are new rehabilitative interventions that deliver an intensive e task-specific practice, which are two critical factors associated with functional improvements and cortical reorganization. Another promising strategy for enhancing neuroplastic changes is non-invasive brain stimulation that can be used with a priming effect on motor training. The aims of this chapter are to review the evidence of neuroplastic changes in multiple sclerosis and to present technologies and interventions that have been tested in clinical trials.

Keywords

Constraint-induced movement therapy • Multiple sclerosis • Non-invasive brain stimulation • Robotics • Use-dependent neuroplasticity • Virtual reality

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Abbreviations

BWSTT	body weight support training on a treadmill
CIMT	constraint-induced movement therapy
CNS	central nervous system
CPGs	central pattern generators
FES	functional electrical stimulation
ICT	intensive comparison therapy
MS	multiple sclerosis
NIBS	non-invasive brain stimulation
PAS	paired associative stimulation
RAGT	robot-assisted gait training
RT	robotic training
rTMS	repetitive transcranial magnetic stimulation
tDCS	transcranial direct current stimulation
UC	usual care
VR	virtual reality

11.1 Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease that might induce several symptoms such as motor impairments, cognitive deficits, spasticity, fatigue, pain and bladder disorders (Kister et al. 2013). Among those, motor impairments are very common and highly disabling: 80 % of them experience gait and mobility impairments, 75 % suffer from balance disorders (Feinstein et al. 2015) and 76 % report some disability regarding manual dexterity (Johansson et al. 2007). Disease-modifying drugs limit central nervous system (CNS) inflammation and reduce relapses rate. However, they are unable to prevent disease progression and disability (Feinstein et al. 2015). Recently, it has been demonstrated how the cerebral cortex might adopt functional reorganization mechanisms that might prevent functional loss and maintain the ability to learn a motor task (Tomassini et al. 2011). We might hypothesize that clinical progression partially occurs when the mechanisms above mentioned fail. This new perspective leads to the application of rehabilitative interventions that might promote functional reorganization and recovery. Functional recovery in MS is achieved by the resolution of inflammation and the development of functional reorganization processes. Despite the widespread pathology, limited but definite evidence support an adaptive role of functional reorganization mechanisms that might limit the adverse effects of MS on motor behaviors (Tomassini et al. 2012; Rocca et al. 2005).

In the past two decades, new insights and findings in neuroscience fields lead to a paradigm shift in neurorehabilitation, which included new therapeutic opportunities for people who suffered from a CNS damage (Warraich and Kleim 2010). Animal and human models provided new evidence that the human brain can change and modulate itself according to external experiences and behaviors, leading to physiological and anatomical changes (Kleim et al. 2004; Nudo et al. 1996; Remple et al. 2001). Recently, *bottom-up* and *top-down* approaches have been described to enhance cortical reorganization and motor recovery. The former included multimodal, external inputs that act at a peripheral level (bottom) with the aim of influencing CNS and neuroplastic changes. They are mainly represented by sensory-motor training. The letter use brain functions and post-lesional reorganizations mechanisms to drive rehabilitative interventions (i.e. brain-computer interface machines) (Belda-Lois et al. 2011). The bottom-up approach is based on the belief that post-lesional CNS might regain functions and motor skills and that behavioral experiences and exercises might shape it. However, the underlined patterns and paradigms are still unclear, and the dose, type, and modality of exercises are far to be outlined. Thus understanding the fundamental principle of spontaneous recovery are essential to design effective rehabilitative interventions. Constrained-induced movement therapy (CIMT), robotics and virtual reality are new approaches that offer high potential for neurorehabilitation. Another promising strategy for enhancing motor recovery is non-invasive brain stimulation. Two non-invasive techniques of inducing electrical currents into the brain have proved to produce long-lasting plastic changes in motor systems (repetitive transcranial magnetic stimulation and transcranial direct current stimulation). Furthermore, brain stimulation combined with motor practice could lead to a more remarkable

and outlasting clinical gains in rehabilitation (Liew et al. 2014). This new class of interventions and techniques which are based on principles of use-dependent neuroplasticity and mechanisms of motor recovery after CNS lesions (Warraich and Kleim 2010) are emerging in clinical settings as potential tools for increasing functional recovery. However, well-established evidence from large-scale clinical trials and meta-analysis on the efficacy of these interventions are still lacking, and further studies are essential to drive definitive conclusions. The aims of this chapter are threefold, to discuss: i) the evidence of motor cortical reorganization processes in multiple sclerosis; ii) the principles of use-dependent neuroplasticity; iii) the state-of-the-art of neuroplasticity-based technologies and interventions in MS.

11.2 Functional Recovery in Multiple Sclerosis

Functional recovery in MS is achieved by repair of damage through remyelination, with a resolution of inflammation and functional reorganization. Evidence from brain systems supports an adaptive role for neuroplastic changes in MS despite the widespread pathology. Specifically, it may limit the negative effects of MS on behavior (Reddy et al. 2002; Tomassini et al. 2012). The extent and type of neuroplastic changes vary across phases and stages of the disease (Rocca et al. 2005). Rocca et al., in an fMRI study, showed that patients with a clinically isolated syndrome presented a more widespread recruitment of the contralateral hemisphere (local cortical reorganization) during a simple motor task (fingers flexion-extension). Conversely, in a relapsing-remitting form and some disability, an activation of the ipsilateral sensorimotor networks occurs (lateralization shift). As the disease advances toward secondary progression, patterns of functional reorganization show an increasingly bilateral distribution and, even for simple motor tasks, involve higher-control sensorimotor areas that are recruited for a novel or complex tasks in healthy individuals (association areas). Zeller et al. tested LTP-like rapid-onset central motor plasticity in MS using paired associative stimulation (PAS) and motor learning paradigms, their findings suggested that the enhancement of cortical excitability due to PAS and training-induced improvement were preserved even in disabled MS (Zeller et al. 2010). Similarly, Tomassini et al. found improvements in both short- and long-term motor learning in MS population, despite the disability level (Tomassini et al. 2011). However, functional reorganization processes could be limited by MS-specific characteristics and the accumulation of structural CNS damage. As postulated by Schoonheim et al. (Schoonheim et al. 2010), brain damage, functional reorganization processes, and disability are mutually related throughout the disease progression (Fig. 11.1).

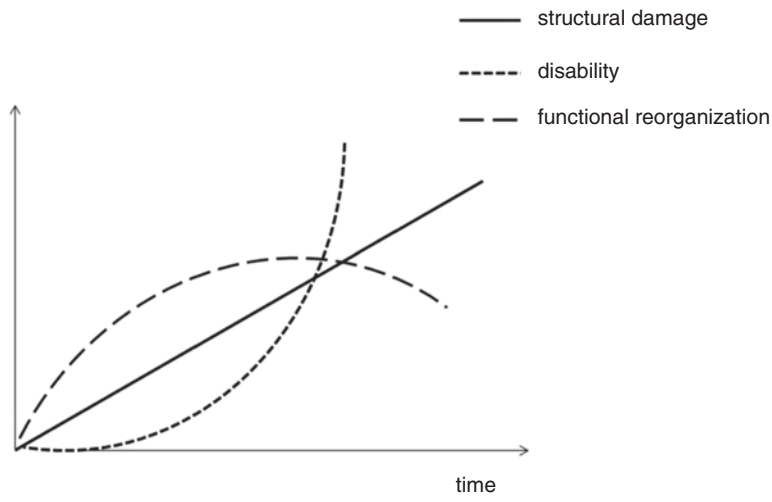

Fig. 11.1 Multiple sclerosis disease progression hypothesis (Adopted by Schoonheim et al. 2010)

The effects of neuroplasticity-based technologies and interventions, virtually beneficial for functional recovery, have been poorly tested so far. Recently, upper extremity task-oriented rehabilitation, but not arm passive motion, has been showed to influence white matter integrity in the corpus callosum and corticospinal fiber bundles (Bonzano et al. 2014). In conclusion, limited but clear evidence of functional recovery in MS exists and the developing of therapeutic interventions that induce adaptive plasticity are encouraged (Tomassini et al. 2012).

11.3 Principles of Use-Dependent Neuroplasticity

Plasticity refers to "an intrinsic property of the human brain and represents evolution's invention to enable the nervous system to escape the restrictions of its own genome and thus adapt to environmental pressures, physiologic changes, and experiences" (Pascual-Leone et al. 1995). Neural plasticity is believed to be the basis for both learning in the intact brain and relearning in the damaged brain that occurs through physical rehabilitation. It is now well established how experiences and practices play a fundamental role in neural reorganization processes in the healthy and damaged brain. Plasticity can be considered multi-levels phenomena that involve: brain (neurons and glia cells), cortical networks (changes in neuronal activation and cortical maps), intra (i.e. mitochondrial functions) and inter-cellular mechanisms (changes in synaptic strength, including sprouting), genome.

Motor behavior is remarkably adaptive and may change during motor experiences; the components of motor training (skills, strength, and endurance) could have specific effects on plasticity-related events. Skill training, which refers to the acquisition of new and complex movements' combination, can induce a substantial cortical network reorganization that leads to a synaptogenesis process with increased synaptic number, an increased synaptic strength, and changes in the cortical topography closely related to the trained movement. These findings have been highlighted both in animal (Adkins et al. 2006) and human's models (Pascual-Leone et al. 1995). We should bear in mind that cortical reorganization occurs only if the tasks are challenging and quite new. In rat models, motor skill level increases rapidly over the first few days of skill training (Kleim et al. 1996, 2004). The early phase of skill training is characterized by an increase in the synthesis of various proteins, including the immediate early gene c-fos (Kleim et al. 1996) and the cAMP response element binding protein. Later phases of skill training are accompanied by significant increases in synapse number (Kleim et al. 1996, 2004) and motor map reorganization (Kleim et al. 2004). In humans, Pascual – Leone et al. demonstrated as an intensive five-fingers "like piano" motor training was able to modify significantly finger cortical motor maps (Pascual-Leone et al. 1995). Although an influence of CNS might be expected even in strength training that preferentially leads to an increased muscle power, it does not result in any form of cortical reorganization (Remple et al. 2001). Finally, endurance training, in which motor outputs are prolonged, can induce new angiogenesis and increase cerebral flow without any effect on motor maps (Swain et al. 2003).

Neuroscience research has made significant advances in understanding experience-dependent neural plasticity, and these findings are beginning to be integrated with research on the degenerative and regenerative effects of brain damage. A relevant example of the integration of basic neuroscience, rehabilitation practice and research are the ten experience-dependent plasticity principles postulated by Kleim and Jones (Kleim and Jones 2008) as reported in Table 11.1. These principles should be incorporated in clinical rehabilitation, with the aims of improving functional recovery, activities and quality of life.

11.4 Robotics

Robotic devices allow a repeated, intensive practice of skilled motor tasks; among different phases of rehabilitation (acute, sub-acute and chronic) they might assist human movements

Table 11.1 Principles of experience-dependent neuroplasticity

Principle	Description
1. Use it or lose it	Failure to drive specific brain functions can lead to functional degradation.
2. Use it and improve it	Training that drives specific brain function can lead to an enhancement of that function.
3. Specificity	The nature of the training experience dictates the nature of the plasticity.
4. Repetition matters	Induction of plasticity requires sufficient repetition.
5. Intensity matters	Induction of plasticity requires sufficient intensity.
6. Time matters	Different form of plasticity occur at different times during training.
7. Salience matters	The training experience must be sufficiently salient to induce plasticity.
8. Age matters	Training-induced plasticity occurs more readily in younger brains.
9. Transference	Plasticity in response to one training experience can enhance the acquisition of similar behaviors.
10. Interference	Plasticity in response to one training experience can interfere with the acquisition of other behaviors.

Adopted by Kleim and Jones (2008)

providing various types of guidance (passive, active, supported) according to cognitive and motor impairments. Moreover, during robotic sessions, kinematic and spatio-temporal parameters (i.e. velocity, smoothness) can be recorded with the aim of monitoring online performances and acquiring clinical information on subjects' characteristics. Finally, it is supposed that subjects' motivation and engagement are sustained during a robotic training. The rationale of robotics in neurorehabilitation is based on several principles and since 1994 robotic devices have been introduced in clinical settings. Firstly, robot-assisted devices might deliver a repetitive motor practice; the dose of exercise (i.e. more than 1000 reps for upper extremity) is closer, compared to conventional therapy (Lang et al. 2009), to task repetition numbers required to induce neuroplastic changes and post-lesional recovery. Secondly, robotic devices are usually designed to train subjects in task-specific motor tasks, such as gait, or reaching tasks. Thirdly, these new technologies might reduce rehabilitation costs or increase cost-effectiveness by reducing physiotherapists' workload.

11.4.1 Gait Training with Robotics

Body weight support training on a treadmill (BWSTT) is an example of an high-intensity, task-oriented intervention to restore locomotor functions in people who suffered from CNS lesions, such as stroke (Veerbeek et al. 2014; Duncan et al. 2011), spinal cord injury (Dobkin et al. 2006) or multiple sclerosis (Giesser et al. 2007). The conceptual basis of BWSTT is based on central pattern generators (CPGs) activation at lumbar spine level in addition to use-dependent principles of motor learning (Kleim and Jones 2008), such as intensity, progression, and specificity. Subjects are trained on a treadmill supported by a harness connected with a weight support system placed on the treadmill. However, this training can be high physically demanding for physical therapists and subjects, especially when subjects with severe gait impairments and markedly reduced mobility are trained. Even though a well-sound rationale of BWSTT is still recognized and several preliminary small unpowered randomized-controlled studies (< 50 subjects) reported potential benefits, two recent trials (Duncan et al. 2011; Dobkin et al. 2006) failed to prove a clear superiority of BWSTT regarding conventional overground gait training or home-exercise programs. In the past years, robot-assisted gait training devices have been introduced in the clinical setting to overcome BSWTT limits and try to induce more motor-learning related changes. Clinical robotic gait machines can be divided into two groups: (i) end-effectors and (ii) exoskeleton devices. Gait Trainer (Reha-Stim, Berlin, Germany) represents the most popular end-effector device and is an alternative approach to treadmill-centered technology with electromechanically driven footplates that guide the feet

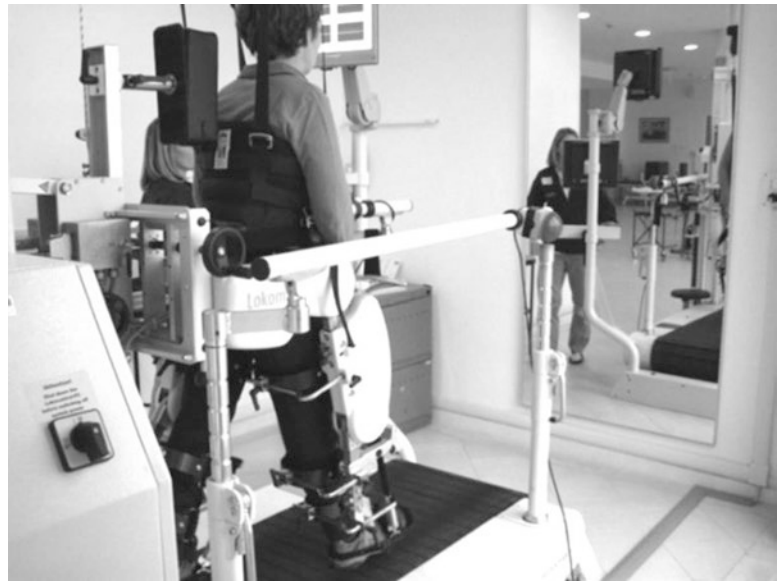

Fig. 11.2 Robot-assisted training with exoskeleton device (Lokomat, Hocoma, Switzerland)

and reproduce gait trajectories with a varying degree of support provided (Hesse and Uhlenbrock 2000). Pilot studies demonstrated the effects of Gait Trainer on locomotor function in stroke survivors (Pohl et al. 2007). The Lokomat (Hocoma, Switzerland) is the most popular exoskeleton device (See Fig. 11.2). Subjects wear a harness attached to a system to provide body weight support and walk on a treadmill with the help of a robotic-driven gait orthosis. The torque of the knee and hip drives (guidance) can be adjusted from 100 % to 0 % for both legs. The speed of the treadmill is set from 0 to approximately 3 km/h and body weight support ranged from 0 to 100 %. As training progresses, adjustments in the guidance, the level of body weight support and treadmill speed are adjusted according to subject performance.

A recent review on the effects of electromechanically-driven gait orthosis in stroke survivors concluded that people who receive robotic gait training in combination with physiotherapy are more likely to achieve independent walking than people who receive gait training without this type of devices (Mehrholz et al. 2013). Specifically, some positive but inconclusive results are reported in the subacute phase and in subjects who are not able to walk (Mehrholz et al. 2013). Moreover, a Phase III multi-center study is ongoing; probably it will shed light on the effects of this technology and it will help drawing definitive conclusions about robot-assisted gait training (Wirz et al. 2011). So far, several studies tested the effects of robot-assisted gait training (RAGT) (Beer et al. 2008; Lo and Triche 2008; Vaney et al. 2012; Schwartz et al. 2012; Straudi et al. 2013; Gandolfi et al. 2014; Straudi et al. 2016) or a combination of RAGT and BWSTT within a session (Ruiz et al. 2013) in MS population. Beer et al. (2008) in severe MS patients (EDSS 6–7.5), found an improvement in walking endurance; Lo and Triche (2008) reported overall improvements in walking speed and endurance after RAGT or BWSTT. Vaney et al. (Vaney et al. 2012) and Schwartz et al. (Schwartz et al. 2012) postulated that RAGT is not superior to overground walking training. In a pilot study (Straudi et al. 2013), we observed that patients who underwent RAGT had an improvement in gait speed and walking endurance compared to a control group. More recently, in a multi-center RCT on progressive MS, we found an RAGT larger effect size on walking endurance compared to an usual conventional physiotherapy (Straudi et al. 2016). Gandolfi et al. (Gandolfi et al. 2014) tested an end-effector device with favorable effects on balance. Finally, a novel training paradigm has been recently proposed by

Ruiz et al. (2013) which combines both RAGT and BWSTT within each session, with significant effects on walking endurance and balance.

In conclusion, small but positive effects on functional status or quality of life in a heterogeneous sample of MS subjects have been highlighted; nevertheless, RAGT superiority on another specific gait training with the same amount of practice has not been proven. Moreover, different devices were used, heterogeneous MS subgroups (RR, PP, SP) with a broad range of gait disabilities (EDSS 3–7.5) were tested and different training protocols (12–15 sessions over 3–6 weeks) were delivered.

11.4.2 Upper Limb Motor Training with Robotics

In the past decades, many robotic devices have been developed for upper extremity stroke rehabilitation. Robotic assistance may increase sensory inputs, reduce muscle tone with an overall increased subjects' confidence in performing movements and tasks that, without assistance, might be frustrating or impossible to achieve. Existing robotic upper-limb devices (i.e. MIT-MANUS) (Krebs et al. 1999) primarily train the proximal portions of the upper limb (arm), while few devices provide therapy to the hand and fingers. Takahashi et al. have shown that a robot-based therapy may offer improvements in hand motor function in chronic stroke subjects combined with a cortical reorganization of motor maps (Takahashi et al. 2008). To date, several studies (Lum et al. 2002; Volpe et al. 1999; Fasoli et al. 2003; Lo et al. 2010) and meta-analysis (Veerbeek et al. 2014) highlighted how robot-assisted therapy would improve arm motor function after stroke. Also, robots can be used to understand the stroke recovery process, such as the anticipatory control of arm movement (Takahashi and Reinkensmeyer 2003) or motor synergies (Dipietro et al. 2007). Hogan et al. provided meaningful clinical recommendations on the delivery of upper limb robotic therapy. They suggested that the form of robotic therapy (active participation, progressive training based on motor coordination) may be more important than its intensity (Hogan et al. 2006).

The first and most popular upper extremity robotic system, named MIT-MANUS, has been developed at MIT (Cambridge, MA) in 1989 by Hogan and Krebs (Krebs et al. 1998), and commercialized in 1994 as InMotion (Interactive Motion Technologies, Inc. Boston, MA). The fist device was a 2-degree of freedom endpoint manipulator, in which intensive reaching practice could be performed. In 1990s–2000s, clinical trials have been conducted in subacute and chronic stroke survivors and the preliminary hypothesis that robotic therapy was superior to usual care was stated (Volpe et al. 1999; Fasoli et al. 2003). More recently, additional distal modules have been introduced to train wrist and hand movements as well. In 2010, the VA-ROBOTICS study (Lo et al. 2010) included 127 chronic stroke survivors and hypothesized that robotic training (RT), which involved both proximal and distal arm components, might reach further gains compared with usual care (UC) and intensive comparison therapy (ICT). The robotic group received three sessions per week over 12 weeks of shoulder-elbow-wrist-hand movements (about 1024 movements per sessions); the results of these large-scale well-designed three arms RCT reported a superiority of RT and ICT over UC and RT higher long-term effects. The Mirror Image Movement Enhancer, named MIME, is a six-degree of freedom endpoint manipulator (Puma 560) that helps patients during multiplanar reaching tasks. Compared to conventional therapy, this device seems to be more efficient regarding muscle strength, smoothness and impairments (Lum et al. 2002). The Bi-Manu-Track (Reha-Stim, Berlin, Germany) is a robotic device focused on the bilateral forearm and wrist movements with positive effects on motor function (Hesse et al. 2005). The Reo Therapy System (Motorika Medical Ltd., Israel) is an adjustable arm-trainer highly diffused in a clinical setting; it is based on a robot manipulator which assists the arm during goal-directed movements. The efficacy of this device has been explored in a non-controlled trial with chronic stroke survivors (Bovolenta et al. 2009) (Fig. 11.3).

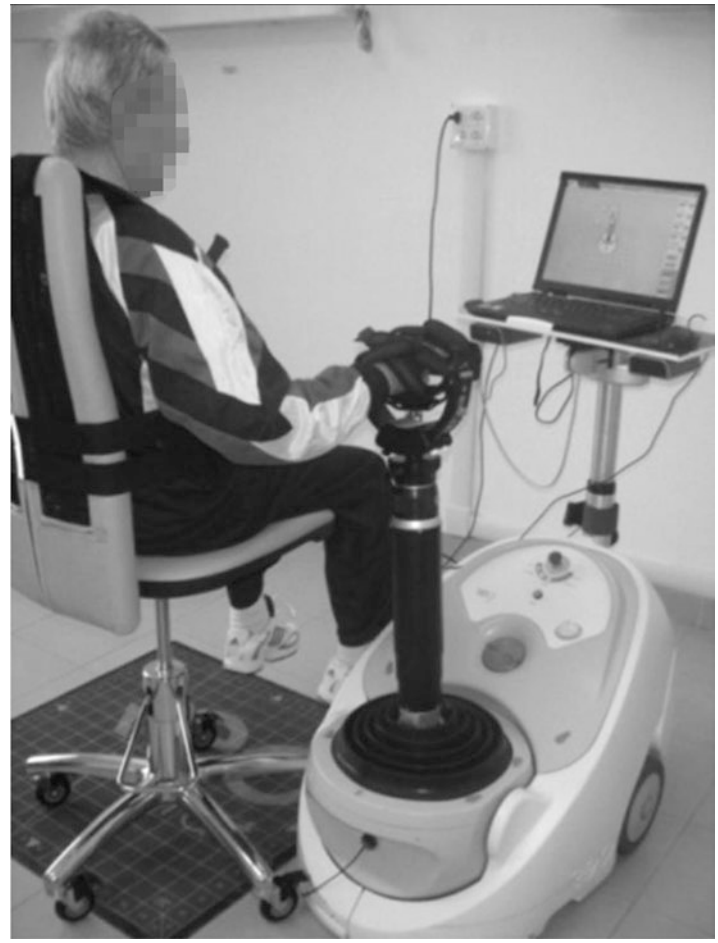

Fig. 11.3 Reaching training with endpoint manipulator (Reo Go Therapy, Motorika, Israel)

In addition to endpoint manipulator, cable suspensions and exoskeletons are relatively "young technologies", such as ARMEO spring and ARMEO power (Hocoma, Switzerland). The Therapy Wilmington Robotic Exoskeleton (T-WREX), commercialized as ARMEO spring (Hocoma, Switzerland), is five degrees of freedom passive device that delivers a repetitive, task-oriented arm movements in virtual reality environments (Rahman et al. 2006). Providing external support to the paretic arm has been shown to improve motor performance of arm reaching in stroke survivors (Housman et al. 2009), encouraging a progressive control of voluntary movements (Beer et al. 2007). The seven degrees of freedom exoskeleton robot ARMin, commercialized as Armeo Power (Hocoma, Switzerland), supports the physiological movements of the arm and the opening and closing of the hand, providing an intensive and task-specific motor training in a virtual environment (Nef et al. 2009). See Fig. 11.4. Klamroth-Marganska et at. found positive effects of ARMin on motor function in chronic stroke survivors (Klamroth-Marganska et al. 2014).

Even if solid evidence on their effects has not been established jet, these new technologies are extremely promising despite their higher degrees of complexity. With the VA-ROBOTICS trial (Lo et al. 2010), upper limb robotic technologies in 2010 have reached a "tipping point" moving the field into the mainstream. In this case, Krebs et al. hypothesized that robotics can be considered a "disruptive technology" where disruptive

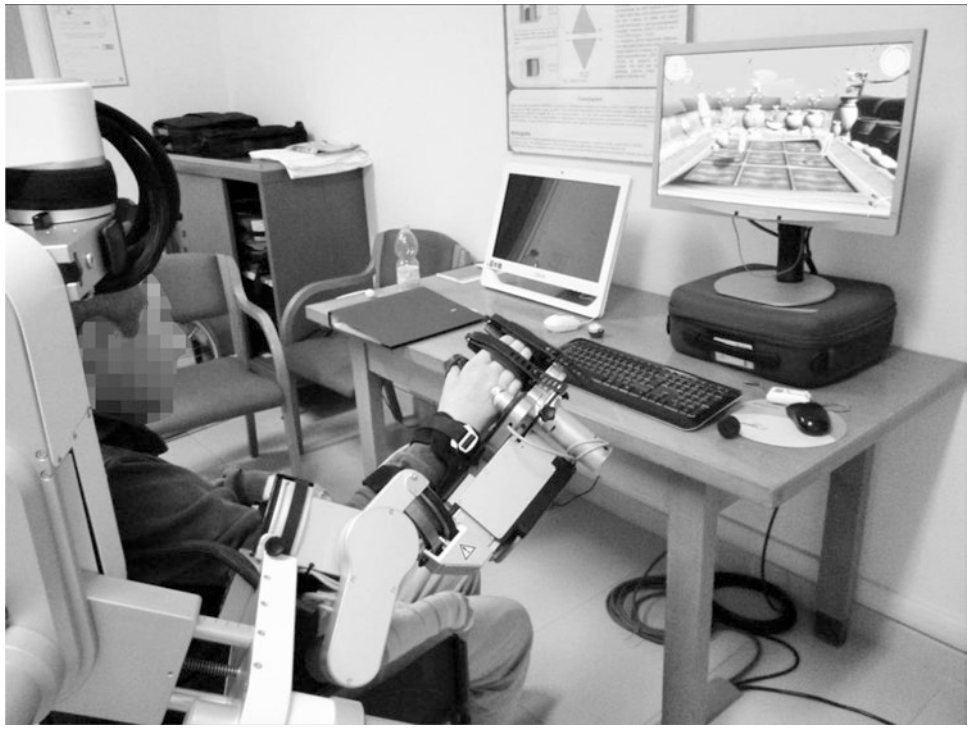

Fig. 11.4 Task-oriented arm training with Armeo Power exsoskeleton (Hocoma, Switzerland)

technology is a term to characterize an innovation that disrupts an existing market or way of doing things and creates a new value network (Krebs and Hogan 2012). However, even if the effectiveness of arm robotics (specifically the first generations, the end-manipulators) has been proved, their clinical use is still very infrequent. So far, few studies tested the effects of arm robotics in MS population (Carpinella et al. 2009; Feys et al. 2015; Gijbels et al. 2011; Sampson et al. 2016). Carpinella et al. run an open trial (Carpinella et al. 2009) and reported positive effects on reaching spatio-temporal parameters and smoothness using an end-effector, 2 degrees of freedom device, Braccio di Ferro (Casadio et al. 2006). More recently, the same group tested two robot-assisted therapy protocols, with and without manipulation, in a pilot RCT (Carpinella et al. 2012). Feys et al. in a pilot RCT tested the effects of an additional robot-assisted training (HapticMaster robot within an individualised virtual learning environment) compared to conventional treatment alone. They reported a more efficient transporting and reaching execution, without any significant gain in clinical tests (Feys et al. 2015). Gijbels et al., in an uncontrolled pilot study, delivered arm therapy with a gravity-supporting exoskeleton (Armeo Spring) reporting positive results on arm function (Gijbels et al. 2011). Furthermore, the additional use of functional electrical stimulation (FES) could improve arm movement accuracy (Sampson et al. 2016).

11.5 Constraint-Induced Movement Therapy

The constraint-induced movement therapy (CIMT) is a family of neurobehavioral techniques that aim to restore functional abilities in hemiplegic subjects. It is based on basic neuroscience and behavioral research with animal models that demonstrated how CIMT produces unique functional reorganization processes

of the CNS (Uswatte and Taub 2013). It has been applied to upper extremity and, more recently, to lower limb rehabilitation. The aims are twofold: to overcome the "learned non-use" phenomena in hemiparesis, that is the inhibition of purposive movement in everyday life, through the compensation by the healthy arm and to induce functional reorganization processes in CNS with repetitive, task-oriented practice (Mark et al. 2006). The CIMT essential components are: repetitive task practice with the affected arm; shaping of training tasks; restraint of the healthy hand with a padded mitt for 90 % of the waking hours and the "transfer package", a set of behavioural techniques designed to facilitate transfer of the functional gains from the therapeutic setting to everyday life. Even though CIMT has been mostly tested on stroke survivors (Corbetta et al. 2015), some preliminary results have been published in MS population in open small trials. CIMT protocols have been administered both for upper (Mark et al. 2008) and lower extremities (Mark et al. 2013) leading to an improvement in real-world motor performances.

11.5.1 Virtual Reality (VR)

In recent years, VR technologies have begun to be used as a treatment tool in rehabilitation for their low-cost, high portability, off-the-shelf software and devices available and for the chance to deliver an engaged, high-repetitive, standardized, active learning. Moreover, this technology could objectively measure motor behavior in ecologically sound environments while maintaining control over the stimulus delivered (Rizzo 2002). VR has been defined as the "use of interactive simulations created with computer hardware and software to present users with opportunities to engage in environments that appear and feel similar to real-world objects and events (Weiss et al. 2006)". Two fundamental concepts in VR are *presence* and *immersion*: presence is considered the subjective feeling of being present in a simulated environment, whereas immersion is a measure of the VR platform related to the ability to induce a sensation of the real world in the users (Weiss et al. 2006). In virtual rehabilitation, simple devices (i.e. joystick) or complex systems using capture motion systems, sensors or haptic feedback are used to interact with the environments. VR scenario usually reproduces real life activities where practice can be adjusted on user's characteristics. More recently, gaming console (i.e. Nintendo Wii, Kinect X-box) have been introduced in clinical and research settings as a low-cost way to deliver virtual reality. See Fig. 11.5.

A Cochrane review has been published so far exploring the effectiveness of VR-based interventions in stroke survivors, reporting how the use of virtual reality and video gaming may be beneficial in improving upper limb function and ADL function (Laver et al. 2015). In MS population, VR technologies have been tested so far for improving balance or gait with inconclusive results (Peruzzi et al. 2016; Nilsagard et al. 2013; Brichetto et al. 2013; Kramer et al. 2014; Prosperini et al. 2013). In an open trial, Peruzzi et al. evaluated the effects of a VR scenario combined with a treadmill training on gait, reporting positive results on gait speed and ability in negotiating obstacles (Peruzzi et al. 2016). Interactive visual-feedback exercises with Nintendo Wii balance were tested for improving balance and mobility in MS patients with mixed conclusions. Nilsagard et al. reported no significant differences compared to no intervention, even if moderate effect size has been highlighted (Nilsagard et al. 2013). Conversely, Brichetto et al. postulated that Wii training could be more effective than the current standard protocol in improving balance disorders in MS (Brichetto et al. 2013). Finally, Prosperini et al. proposed the Wii balance training as a potentially useful home-based treatment (Prosperini et al. 2013). Kramer et al. combined exergames with an unstable platform to improve balance; they found how it was superior to other treatments especially in dual-task conditions (Kramer et al. 2014).

Fig. 11.5 Balance training with Nintendo Wii Balance board

11.6 Non-invasive Brain Stimulation (NIBS)

Non-invasive brain stimulation techniques, such as repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS), are novel therapeutic approaches to induce motor recovery in neurorehabilitation (Lafaucheur et al. 2014; Kang et al. 2015). rTMS can modulate cortical plasticity and brain network activities via the production of electromagnetic currents delivered by a coil placed over the scalp (Pscual Leone et al. 1998). tDCS applies weak direct currents to the scalp via sponge electrodes that modify cortical excitability for up to 90 min from the end of stimulation (Nitsche and Paulus 2000). It has advantages over rTMS, such as the greater portability and lower cost, the ability to stimulate both hemispheres simultaneously (Wagner et al. 2007; Williams et al. 2010), the long-lasting effects on cortical excitability with no significant adverse effects, and the lower level of discomfort experienced by patients. Moreover, tDCS can be combined with a behavioral training with a priming effect on motor learning (Fritsch et al. 2010). Recently, few studies tested the effects of tDCS (Meesen et al. 2014; Cuypers et al. 2013) or rTMS (Elzamarany et al. 2016; Burhan et al. 2015; Koch et al. 2008) on motor recovery in MS population. High-frequency rTMS on M1 increases hand dexterity in MS subjects with cerebellar dysfunction (Koch et al. 2008; Elzamarany et al. 2016), whereas in prefrontal cortex improves gait performance in a single case (Burhan et al. 2015). tDCS induces changes in motor cortex excitability (Cuypers et al. 2013); however these effects seem not to be transferred into hand dexterity gains (Meesen et al. 2014). The application of NIBS in MS population is still very preliminary; no randomized sham-controlled clinical trials have been conducted so far. Specific MS-related characteristics could have a role in determining clinical effects of NIBS. Moreover, the target area, stimulation parameters (i.e. intensity, duration) and the combination with behavioral interventions (i.e. physiotherapy, robotics, virtual reality) have to be tested to optimize motor performance.

11.7 Conclusion

Up to date, it is reasonably demonstrated that functional reorganization processes occur even in MS patients and that they could be positively modulated by motor practice. In this chapter, we summarized the preliminary evidence of the application of neuroplasticity-based technologies and interventions, such as robotics, virtual reality, CIMT and non-invasive brain stimulation, in multiple sclerosis for restoring motor function. So far, positive effects of these interventions were documented in arm function, gait, mobility and balance and subsequently on Quality of Life and participation. However, these findings are preliminary and corroborated by small open trial or pilot RCT.

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