UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

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TÍTULO: Multiple sclerosis and its relation with immune response

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DEDICATORIA

Dedico esta tesis especialmente a Dios, a la Madre, a mis dos madres que me han apoyado incansablemente a pesar de las dificultades y a cada uno de los que han sido una mano en este camino de preparación.

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"Totus Tuus ego sum et omnia mea Tua sunt"

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RESUMEN

La esclerosis múltiple (EM) es una enfermedad crónica, autoinmune, inflamatoria y desmielinizante del cerebro y la médula espinal, responsable de graves discapacidades físicas en adultos jóvenes, especialmente mujeres. Este trastorno provoca la pérdida de las funciones motoras y sensoriales y su prevalencia se estima entre 15 – 250 per 1000000 personas dependiendo de cada región. Las personas que padecen esclerosis múltiple presentan diversas manifestaciones clínicas: problemas físicos (pérdida de visión, ataxia, espasticidad, fatiga, dolor, incontinencia), cognitivos, psicosociales y de comportamiento.

La patología típica de la esclerosis múltiple (EM) es un aumento de las respuestas inflamatorias con una evidente destrucción de las vainas de mielina junto con una proliferación de astrocitos, una activación de la microglía, gliosis y degeneración axonal. El proceso inflamatorio se asocia a una cascada autoinmune en la que el sistema inmunitario ataca la vaina de mielina de las células nerviosas mediante células T, principalmente mediante células T helper 17 (T_H17).

Las lesiones inflamatorias perivenulares son la característica patológica más distintiva de la EM. Estas lesiones dan lugar a placas de desmielinización en las que el número de células T $CD8^+$ es mayor que el número de células T $CD4^+$ en regiones como la materia gris cortical y la materia blanca.

Los criterios de diagnóstico de la EM han variado en función de la nueva información encontrada, pero los más aceptados son los de McDonald, que recomiendan una punción lumbar, un análisis del líquido cefalorraquídeo y una resonancia magnética (RM). El desarrollo de nuevos biomarcadores y la constante actualización de los criterios diagnósticos son herramientas útiles para un diagnóstico temprano y adecuado.

En este trabajo se realizó una revisión para determinar la estrecha relación entre esta enfermedad neurodegenerativa y la respuesta inmune para reducir la prevalencia de la enfermedad y proporcionar información precisa y actualizada para encontrar un tratamiento adecuado para esta enfermedad.

Palabras Clave: esclerosis múltiple, respuesta inmune, autoinmune, criterios de McDonald, tratamientos, células madre.

ABSTRACT

Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory, demyelinating disease of the brain and spinal cord responsible for serious physical disabilities in young adults, especially women. This disorder causes the loss of motor and sensory functions and its prevalence is estimated from 15 – 250 per 1000000 people depending on each region. Those who have MS show several clinical manifestations: physical (loss of vision, ataxia, spasticity, fatigue, pain, incontinence), cognitive, psychosocial, and behavioral problems.

The pathology typical of multiple sclerosis (MS) is an increase in inflammatory responses with evident destruction of the myelin sheaths together with a proliferation of astrocytes, activation of the microglia, gliosis, and axonal degeneration. The inflammatory process is associated with an autoimmune cascade in which the immune system attacks the myelin sheath of nerve cells by T cells mainly by T helper 17 cells (T_H17).

Perivenular inflammatory lesions are the most distinctive pathologic feature of MS. These lesions give rise to demyelinating plaques in which the number of $CD8^+$ T cells is greater than the number of $CD4^+$ T cells in regions such as the cortical gray matter and white matter.

The diagnostic criteria for MS have varied depending on the new information found, but the most widely accepted are McDonald's, which recommends a lumbar puncture, cerebrospinal fluid analysis, and magnetic resonance imaging (MRI). The development of new biomarkers and the constant updating of diagnostic criteria are useful tools for early and appropriate diagnosis.

This work conducted a review to determine the close relationship between this neurodegenerative disease and the immune response to reduce the prevalence of the disease and provide accurate and updated information to find an appropriate treatment for this disease.

Keywords: multiple sclerosis, immune response, autoimmune, McDonald criteria, treatments, stem cells.

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LIST OF ABBREVIATIONS

AAR	Age-adjusted Risk
ADCC	Antibody-Dependent Cellular Cytotoxicity
AHR	Aryl Hydrocarbon Receptor
AHSCT	Autologous Hematopoietic Stem Cell Transplantation
AKY	Protein Kinase
AP	Autoproliferation
APCs	Antigen Presenting Cells
ApoE	Apolipoprotein E
ASIC1	Acid-sensing ion channel 1
AUC	Analytical Ultracentrifugation
BATF	Basic leucine zipper transcription factor
BBB	Blood-Brain Barrier
BCR	B Cell Receptor
B_{eff}	Effector B cells
BEMS	Best Evidence in Multiple Sclerosis
B_{regs}	Regulatory B cells
CAM	Complementary and alternative therapies
CCAS	Cerebellar cognitive Affective Syndrome
CCL2	CC-chemokine ligand 2
CCR4	CC chemokine receptor 4
CCR8	CC chemokine receptor 8
CD4	The Cluster of Differentiation 4
CD8+ MAIT Cell	CD8 Mucosa- Associated Invariant T Cell
CD16 PE-Cy7-A	Antibody Cyanine7 anti-human

CD45	Transmembrane glycoprotein
CD56^{bright}	NK subset
CD56^{dim}	NK subset
CD56 PE-A	Subset MSC Marker Antibody Anti-human
CD68	Glycosylated glycoprotein
CSF	Cerebrospinal fluid
CFSE	Carboxyfluorescein succinimidyl ester
CIS	Clinically Isolated Syndrome
CNS	Central Nervous System
CSFM	Confocal Scanning Fluorescence Light Microscopy
CXCR3	CXC chemokine receptor
CXCR4	Seven transmembrane domain G protein-coupled surface receptor
Cy	Cyanin
CYP27B1	25- Hidroxivitamina D-1α-Hidroxilasa
DIS	Dissemination in space
DIT	Dissemination in time
DMT	N-dimethyltryptamine
DMTs	Disease-Modifying Therapies
DQA1	HLA-DQ alpha chain
DQB1	HLA-DQ beta chain
DRB1	ATP analogue 1
DR2	Subtype form of HLA
DZ twin	Dizygotic twins
EAE	Experimental Autoimmune Encephalomyelitis
EBNA IgG	Epstein–Barr nuclear antigen Ig
EBV	Epstein-Barr virus
EDSS	Expanded Disability Status Scale

ER	Endoplasmic Reticulum
ERK	Extracellular signal-regulated kinases
eT_{regs}	*ex vivo* autologous T_{regs}
EVI5	Ecotropic Viral Integration Site 5
Fc	Fc receptor
FDA	U.S. Food and Drug Administration
FDC	Follicular Dendritic Cell
FOxP3	Specific marker of natural T regulatory cells
GA	Glatiramer Acetate
GCR	Glucocorticoid Receptor
Gd	Gadolinium
GM	Gray Matter
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GPR	Gene-protein-reaction
GSK239512	Histamine H3 receptor antagonist/inverse agonist
HBV	Hepatitis B virus
HC	Healthy control
HLA	Human Leukocyte Antigen
HLA-DR	Human Leukocyte Antigen – DR isotype
HSCT	Haematopoietic Stem Cell Transplantation
ICD	Immunogenic Cell Death
IFN-β	Interferon beta
IFN-γ	Inferton gamma
IL-4	Interleukin 4
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-13	Interleukin 13
IL-15	Interleukin-15

IL-18	Interleukin-18
IL-27	Interleukin-27
IL-7R	Interleukin 7 Receptor
IL-18R1	Interleukin 18 Receptor 1
IL-21R	Interleukin 21 Receptor
iNOS	Inducible Nitric Oxide Synthase
IPEX	Immune dysregulation, polyendocrinopathy, enteropathy, X-linked
IRF4	Interferon regulatory factor 4
IRF8	Interferon regulatory factor 8
ITIM	Immunoreceptor Tyrosine-based Inhibitory Motif
ITP	Immune thrombocytopenic purpura
LINGO-1	Leucine-rich repeat and Ig-containing Nogo receptor interacting protein-1
Lt-α	Lymphotoxin alpha
LTA	Lipoteichoic Acid
MBP	Myelin Basic Protein
MHC	Major Histocompatibility Complex
MKI67	Marker Of Proliferation Ki-67
MOG	Myelin Oligodendrocyte Glycoprotein
MRI	Magnetic Resonance Imaging
mRNA	messenger Ribonucleic Acid
MS	Multiple sclerosis
MSCs	Mesenchymal Stem Cells
MSFC	Multiple Sclerosis Functional Composite
MZ twin	Monozygotic twins
NDRG1	N-myc Downstream Regulated Gene 1
NK	Natural killers
NKp30	Natural Cytotoxicity Receptor

Nkp46	Natural Cytotoxicity Receptor
NO	Nitric oxide
OCB	Oligoclonal Bands
ODC	Oligodendrocyte
OPCs	Oligodendrocyte progenitor cell
PAI-1	Plasminogen Activator Inhibitor type-1
PCG	Precentral Gyrus
PE	Phycoerythrin
PDC 01	Plasmacytoid Dendritic Cell 01
PLP	Proteolipid protein
PML	Progressive Multifocal Leukoencephalopathy
PPAR-Y	Peroxisome proliferator-activated receptor gamma
PPMS	Primary Progressive MS
PRMS	Progressive Relapsing MS
pT_{regs}	Peripheral T_{regs}
RMS	Remission Multiple Sclerosis
RNA	Ribonucleic acid
RNS	Reactive Nitrogen Species
ROCK	Rho-associated protein kinase
RORC	Retinoic-acid-orphan-receptor-C
ROS	Reactive Oxygen Species
RR	Rate ratio
RRMS	Relapsing-Remitting MS
RXR-α	Retinoid X receptor isoform alpha
RXR-γ	Retinoid X receptor isoform gamma
SNRS	Scripps Neurological Rating Scale
SPMS	Secondary Progressive MS
S1P	Sphingosine-1-Phosphate

SSC	Side scatter
SSC-A	Side-scatter area
STAT1	Signal transducer and activator of transcription 1
T1D	Type 1 Diabetes
T1GAD	Post-contrast T1 gadolinium
TBX21	T-Box transcription factors 21
TCR	T cell Receptor
T_{eff}	Effector T cells
TFA	Trifluoroacetic Acid
TGF-β	Transforming Growth Factor-Beta
T_H	T Helper
T_H1/T_H2	T Helper 1 / T Helper 2
TMA	Thrombotic microangiopathy
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor-alpha
TNF-γ	Tumor Necrosis Factor Gamma
Tr1	Regulatory Type 1
T_{reg}	Regulatory T cell
TRPM4	Transient Receptor Potential Cation Channel Subfamily M member 4
UVB	Ultraviolet B
UVR	Ultraviolet Radiation
VCA IgG	Viral Capsid Antigen
VDR	Vitamin D Receptor
WM	White Matter

1. Introduction

Multiple sclerosis (MS) is a chronic, neuroinflammatory, and degenerative disease of the Central Nervous System (CNS) that affects young adults (Dendrou et al., 2015a; Dobson & Giovannoni, 2019; Eshaghi et al., 2018). In this neurodegenerative disease, peripheral autoreactive immune cells infiltrate the CNS and activate innate immune mechanisms (Haase & Linker, 2021).

Patients with MS show loss of motor and sensory function due to an immune-mediated inflammation process (Karussis, 2014; Morshedi et al., 2019). A patient with this neurological disorder shows temporary loss of vision, fatigue, impairment of bladder, bowel, and sexual functions, and neurocognitive changes (Martin et al., 2016). These affectations are visible in regions called plaques (Tarlinton et al., 2020) in the spinal cord, gray matter, white matter, and thalamus, corroborating inflammatory demyelination and axonal, neuronal, and synaptic loss in MS patients (Grussu et al., 2017).

Despite advances in science, the exact cause of the development of MS remains unknown (Arneth, 2019). However, the development and progression of this disease are due to a combination of genetic predisposition and some environmental factors (P.-P. Axisa & Hafler, 2016; Rijnsburger et al., 2021). The risk of MS increases due to lifestyle, genetic predisposition, epigenetic factors, and environmental factors such as latitude, and viruses (Mi et al., 2021). Indeed, the probability of a person developing MS worldwide is approximately 0.1% (Bishop & Rumrill, 2015).

MS shows different clinical condition depending on the area of CNS affected: Relapsing remitting MS (RRMS), Primary progressive MS (PPMS), Secondary progressive MS (SPMS), and Progressive-relapsing MS (PRMS) (Vidal-Jordana & Montalban, 2017). Each patient is classified according to the presence or absence of relapses, new or enlarging T2 lesions, and gadolinium (Gd)-enhancing lesions (Vidal-Jordana & Montalban, 2017). A patient with MS develops a first demyelinating event known as CIS and subsequently, a relapsing-remitting period follows (Ruprecht, 2021). Approximately 10-15% of patients remain stable in PPMS while other patients show a greater disability and progress to the SPMS phase (Ruprecht, 2021; Weston & Constantinescu, 2015).

Figure 1 shows the 3 main stages of MS starting from a combination of environmental and genetic factors to a progressive state with a prominent loss of brain volume.

The first preclinical stage in MS is linked to the interaction of environmental and genetic factors; then a relapsing-remitting clinical stage involves episodes of neurological dysfunction, sensory

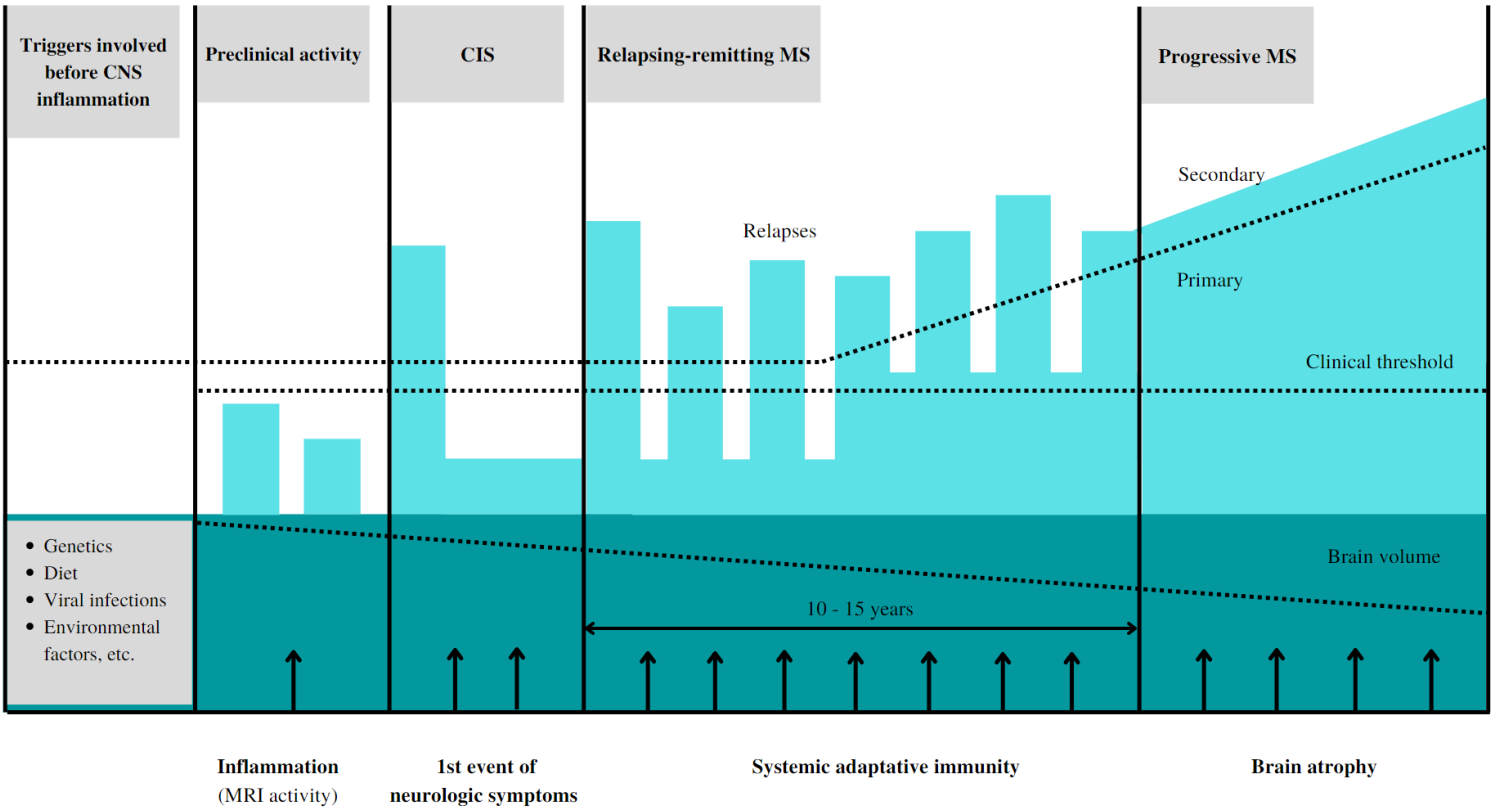

Figure 1. Different stages in Multiple Sclerosis. *MS begins long before it is detected by MRI; this episode is known as clinically isolated syndrome (CIS). It is the first stage characterized by neurological symptoms for at least 24 hrs. Then it progresses to an Relapsing remitting MS (RRMS) stage characterized by relapses that may last for several years (10-15 years). Finally, a patient reaches the progressive stage of MS characterized by loss of brain volume and evident brain atrophy. Adapted from: (Baecher-Allan et al., 2018, Olsson et al., 2016).*

disturbances as well as motor and cerebral disturbances appreciable by MRI, and the last stage in which the neurological dysfunction is advanced and particularly affects gait (Baecher-Allan et al., 2018a) due to the inflammatory process in specific regions of the person mentioned above.

MS is a complex neurological disorder to study and some experimental models have been proposed to obtain essential information and develop new therapies (Martin et al., 2016). One of the most widely used *in vivo* models (Lassmann & Bradl, 2017; van Langelaar et al., 2020) is Experimental autoimmune encephalomyelitis (EAE) (Buzzard et al., 2017; Constantinescu et al., 2011; Lassmann, 2018b). This model has been widely used in autoimmune inflammatory diseases of the CNS whose hallmarks are demyelination and neurological dysfunction (Kubajewska & Ã, 2010). EAE has provided insight into the pathological development of MS in its different stages (Kubajewska & Ã, 2010) especially immunology and brain inflammation (Lassmann & Bradl, 2017), although not all aspects of pathological alteration have been analyzed (Buzzard et al., 2017; Lassmann, 2018). There are different types of EAE capable of providing interesting details on MS and each one offers its particular clinical approach. The EAE model selected depends on the research question of each study (Lassmann & Bradl, 2017).

On the other hand, the immune reaction associated with MS involves different cell types mainly B cells, T cells, NK cells, and myeloid cells (Filippi et al., 2018; Martin et al., 2016; Mi et al., 2021). In the case of T cells, regulatory T cells (T_{regs}) present a deficient regulatory process and there is also the resistance of CNS effector T cells to regulation by T_{regs} (Baecher-Allan et al., 2018b; Filippi et al., 2018). Similarly, in B cells there is an imbalance between proinflammatory B cells and anti-inflammatory B cells (Filippi et al., 2018). These dysregulations between effector cells and regulatory cells lead to a notorious infiltration and damage in the CNS (Dendrou et al., 2015b; A. K. Pröbstel et al., 2015) and an attack on the myelin sheath that forms part of the axons (Aharoni et al., 2021). This immunological attack mainly involves autoreactive effector T cells, T helper 1 (T_H1), and T_H17 (Aharoni et al., 2021).

Modulating the immune response has gained great clinical interest and different Disease-modifying Therapies (DMTs) have been approved for patients with MS (Mi et al., 2021). In addition to the development of new DMTs, treatments targeting vascular comorbidities offer a longer life span for long-lived patients (Vaughn et al., 2019). Likewise, the use of monoclonal antibodies has shown substantial improvements in the patient's life (Sospedra, 2018)and stem cell transplants are currently being used as therapeutic alternatives since they eliminate autoreactive lymphocytes without generating co-stimulatory signals as happens with hematopoietic stem cells (Burt et al., 2019).

The present work is focused on advances in the current situation of multiple sclerosis and its associated immune response to provide relevant information for the treatment of this disease.

2. Methodology

The recommendations of the PRISMA report were followed for this systematic review.
Figure 2 shows the step-by-step process for the preparation of this review.

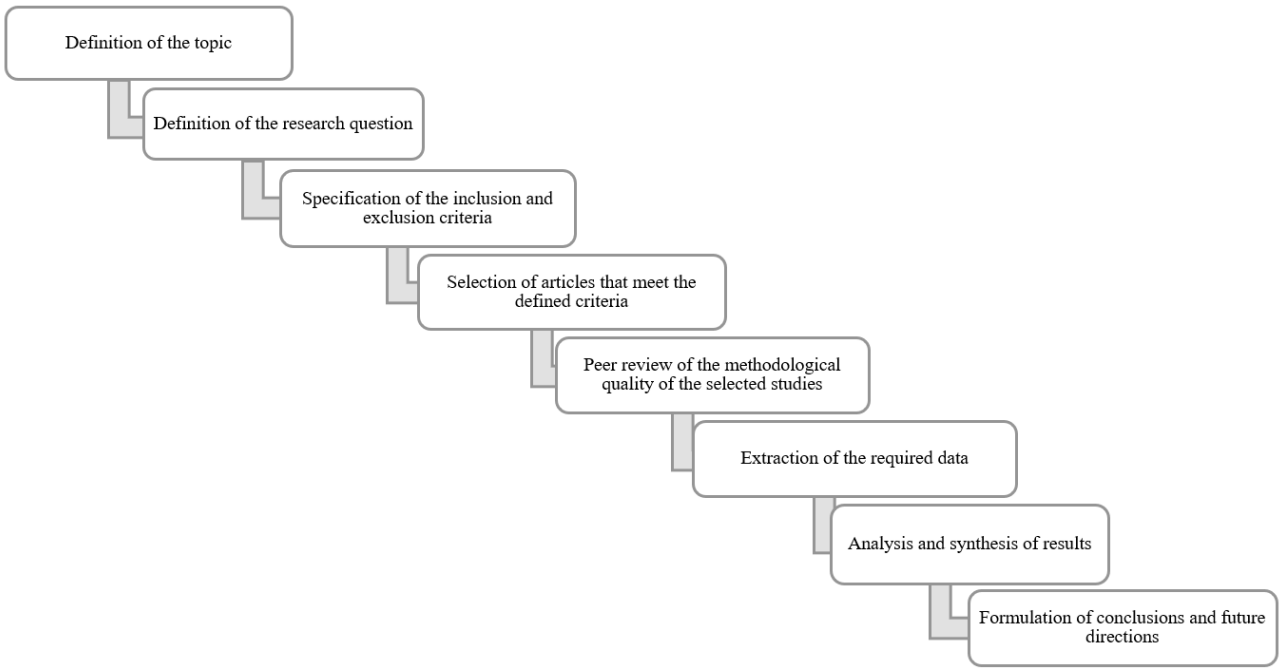

Figure 2. Flowchart of the stages in writing this review.

2.1. Inclusion and exclusion criteria

Articles that met the following characteristics were considered:

- Studies mainly between 2015 and 2022.

- Studies on MS focused on its associated immunological reaction.

- Studies with statistical, pathological and therapeutic data.

- Experimental studies conducted and conducted in humans.

- Studies with a methodological level of 1 on the SIGN scale: meta-analyses, systematic reviews of randomized clinical trials or randomized clinical trials.

- Studies with sufficient methodological quality according to the CASPe instrument for clinical trials and reviews.

All articles in Spanish were excluded.

2.2. Information sources and search strategy

This review analyzed evidence about the current state of MS and the associated immune response. The articles were retrieved from electronic databases including PubMed and Google Scholar. All databases were searched using an identical strategy and search terms such as: "Multiple Sclerosis", "MS immune response", "MS", "B-Cells", "MS genetics", "MS treatments, "MS pathogenesis", "DMTs" and "MS development". The search was limited to original research studies and articles on MS published in English mainly from 2015 to 2022. The abstracts of the identified sources were carefully examined to assess their relevance to the present study. 291 articles were collected and compared each of the findings on the association between MS and its immune response.

2.3. Studies included

Figure 3 shows the PRISMA flow chart for this review. The initial number of articles used was 233, discarding several of them for various reasons until 169 were obtained.

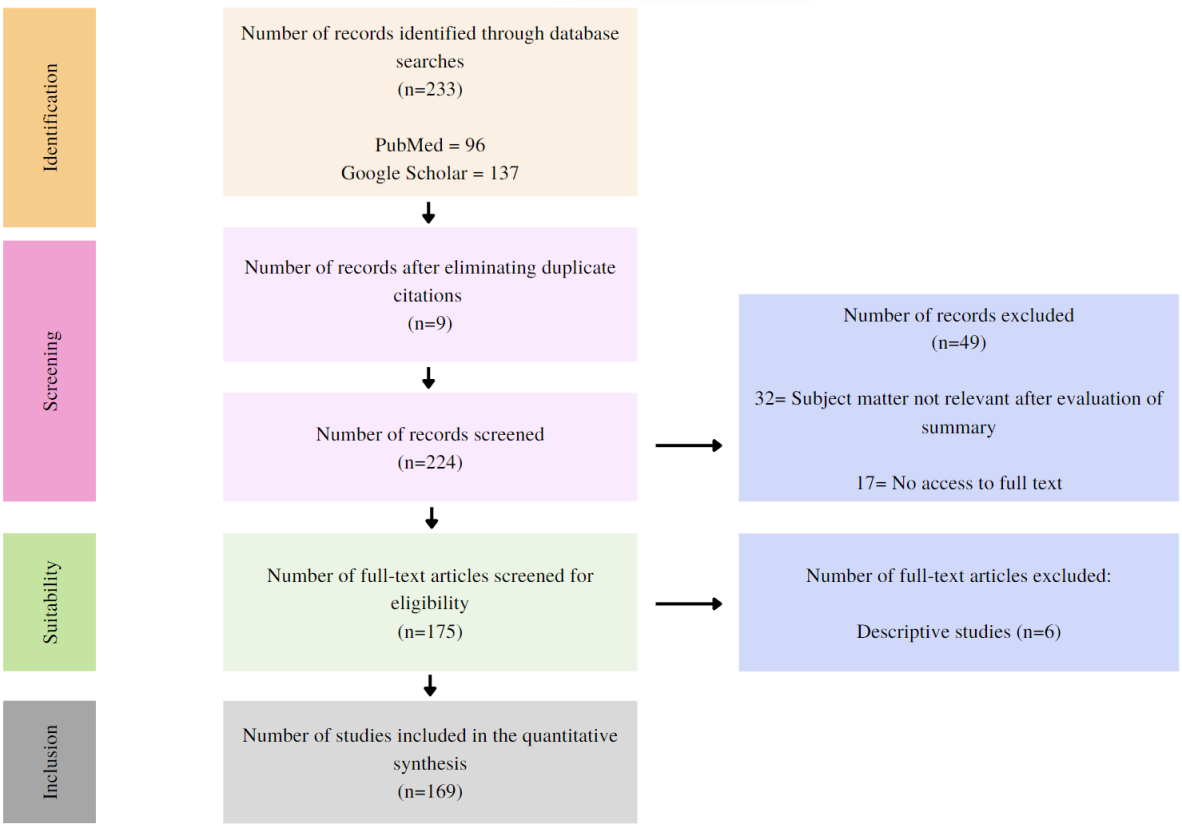

Figure 3. PRISMA Flowchart.

Table 1 shows a summary of the main characteristics of the articles involved according the inclusion criteria.

Table 1. Characteristics of the articles used in this review. *Ia: The evidence comes from meta-analyses of well-designed, randomized, controlled trials. Ib: Evidence comes from at least one randomized controlled trial. IIa: Evidence is from at least one well-designed, non-randomized, controlled trial. IIb: Evidence comes from at least one well-designed, non-fully experimental study, such as cohort studies. This refers to the situation in which the application of an intervention is beyond the control of the investigators, but its effect can be evaluated. III: The evidence comes from well-designed descriptive, non-experimental studies, such as comparative studies, correlation studies or case-control studies.*

Study Level Evidence	Number of participants and country	Design	Follow-up	Objectives	Results
Ahmed et al., 2022 Ia	182 brain donors, Netherlands	ECA	-	To provide arguments on the role of meningeal lymphocytes in subpial cortical lesions and to discover the link between cortical subpial demyelination and pathological mechanisms in subcortical WM.	There is communication between WMLs and meningeal inflammation.
Radaideh et al., 2021 IIa	30 MS patients and 30 HC, Jordan	ECA		To investigate the variety of MRI quantitative metrics.	dGM changes occur in a non-uniform pattern and, almost, do not link directly to MS disease severity
Argento et al., 2021 IIb	20 MS patiens and 18 HC, Italy	ECA		To explore the effects of adding a cognitive task to walking in PMS	Both groups evidenced the presence of cognitive–motor interference (CMI) for both cognitive conditions with a greatereffect of word list generation task in PMS
Astier et al., 2006 Ib	28 MS patients and 18 HC, USA	ECA	10 months	To explore multiple immunologic “hits” required to allow autoimmune diseases to occur	These data demonstrate that human autoimmune diseases can be associated with multiple defects in Treg populations.
Khani et al., 2022 Ia	45 MS patients and 30 HC, Iran	ECA		To investigate the peripheral blood frequency of the CD3−CD56+CD16+NK, CD3+CD56+NKT and CD5+CD19+ B cells and serum IL-10 in IFNtreated and untreated RRMS, NMOSD, and healthy individuals.	The lower proportion of CD3−CD56+CD16+ NK and CD3+CD56+ cells in the peripheral blood of IFN-treated patients with RRMS compared to other groups suggests the importance of immunomodulation in patients with RRMS disorder.
Briggs, 2020 Ia	206 MS cases and 176 unrelated controls, USA	ECA	1 month	To determine if variation in the genes encoding α7 and α9 nAChRs (cholinergic receptor nicotinic alpha 7 (CHRNA7) and alpha 9 (CHRNA9)) will modify MS risk conferred by tobacco smoking.	CHRNA7 and CHRNA9 modifies MS risk conferred by tobacco smoke, where risk among smokers was increased in carriers of the minor CHRNA9 haplotype and in non-carriers the minor CHRNA7 haplotype
Burt et al., 2019 IA	110 MS patients, Canada	RCT	13 years	To compare the effect of nonmyeloablative HSCT vs disease-modifying therapy(DMT) on disease progression.	Patients with relapsing-remitting MS, nonmyeloablative HSCT, compared with DMT, resulted in prolonged time to disease progression.
Piancone et al., 2016 Ia	71 MS patients, Italy	ECA	7 days	To analyze the role of B cells, and in particular of BTLA-expressing B lymphocytes in MS patients affected by different disease phenotypes, and in those whose disease remission is pharmacologically-induced	To confirm and expand these data by showing that, besides Bregs, the peripheral population of BTLA-expressing B lymphocytes is relatively expanded as well by fingolimod

Study Level Evidence	Number of participants and country	Design	Follow-up	Objectives	Results
Cencioni, Ali, et al., 2021 Ib	21 MS patients and 62 HC, UK	ECA		To determine if transitional B-cell-dependent immune regulation could be defective in MS and examined their function in healthy subjects and patients with relapsing-remitting multiple sclerosis (RRMS).	In HC, CD19+CD24hiCD38hi transitional B cells produce more IL-10 than CD19+CD24+CD38+ naive and CD19+CD24hiCD38− memory B cells and are able to suppress CD4+T-cell proliferation and IFNγ and TNFα-production. In subjects with RRMS, CD19+CD24hiCD38hi tran-sitional B cells produce significantly less IL-10 and to fail to suppress effector T-cell function
Eden et al., 2019 IIb	642 MS patients, Canada	ECA		To study the spatial distribution of cervical spine lesions in MS patients characterized by clinical status.	An automatic processing and analysis pipeline which has been made publicly available, minimizing user bias and promoting standardization and reproducibility of scientific results
Jelcic et al., 2018 Ia	32 HC and 50 untreated RRMS patients, Switzerland	ECA		To characterize in detail the cellular interactions that lead to increased AP andprovide evidence for its potential involvement in MS.	There is a link between B cells,T cells with MS pathogenesis and show that the interactions of these two cell types probablyoccur in conjunction with the MS-associated DR15 molecules and that B cells may express antigens, which are also upregulated in the brain and recognized by AP CD4+T cells
Kaufmann et al., 2021 Ia	31 MS patients and 31 HC Germany	ECA	5 months	To investigate whether the therapeutic resistance of progressive MS can be attributed to chronic immune cell accumulation behind the blood-brain barrier (BBB)	A groundwork for a therapeutic strategy to deplete CNS-homing T cells before they can fuel treatment-resistant progression
Koskimäki et al., 2018 IIb	20 patients undergoing treatment with natalizumab for 24–68 months; USA	ECA	36.6 months	To examine whole and segmented gray, white, thalamic, and corpus callosum volume loss in stable patients receiving natalizumab for 2–5years	Brain volume loss in MS is primarily driven by gray matter changes and may be independent of clinically effective treatment
Laroni et al., 2016 Ia	22 MS and 21 HC; UK	ECA		To assess thepossible regulatory function of CD56bright NK cells in healthy people and in patients with MS/CIS.	Possible importance of innate immunity resident in lymph nodes in pre-venting autoimmune responses by controlling autologous T cellactivation, and suggests that the evasion of T cells from CD56brightNK cell control may contribute to the deregulated T cell response observed in MS.
Lifshitz et al., 2016 Ia	52 MS patients and 22 HC, Rusia	ECA	1 month	To analyze ex vivo expanded regulatory T cells CD4+CD25+FoxP3+CD127Low develop strong immunosuppressive activity in patients with remitting-relapsing multiple sclerosis	A demonstrated difference in proportion of regulatory T cells CD4+CD25+FoxP3+CD127low (Tregs) within the same patients' relapse and remission.
Mariottini et al., 2022 III	93 MS patients, UK	ECA		To assess effectiveness of autologous haematopoietic stem cell transplantation (AHSCT) in relapsing–remitting multiple sclerosis (MS)	Superior effectiveness of AHSCT compared to Cy on relapse activity, without differences on disability accrual.
Motl et al., 2021 IIb	62 MS patients, Netherlands, USA	ECA		To establish the presence of cognitive-motor coupling and second exam-ined the possibility that volumes of subcortical gray matter (SGM) structures and aerobic capacity might explain the coupling of cognitive and motor functions in persons with MS.	The strong correlations between cognitive processing speed and walking performance were attenuated in magnitude and not statistically significant when controlling for aerobic capacity alone and aerobic capacity and SGM volumes together. The associations between cognitive processing speed and walking performance remained statistically significant when controlling for SGM volumes alone.

3. Multiple Sclerosis and its current situation in the world

Multiple Sclerosis is a neurodegenerative disease that affects more than 2.3 million people worldwide (Arneth, 2019; Bishop & Rumrill, 2015; Buzzard et al., 2017; Dendrou et al., 2015a; Morshedi et al., 2019; Riemenschneider et al., 2021; Schirmer et al., 2019; Sospedra & Martin, 2016a). During the last years, the rate of patients with MS has increased from 2.1 million to 2.5 million between 2008 and 2013, mainly in Europe, North America, Canada, Latin America, the Middle East, the Mediterranean Basin, and Australia (Correa et al., 2016; Eskandarieh et al., 2016; Vaughn et al., 2019; Wang et al., 2021). For instance, in the United States since 1950 there has been a greater number of patients with MS compared to previous years, attributed in part to new medical technologies for diagnosis such as magnetic resonance imaging (Bishop & Rumrill, 2015a). Similarly, a recent collection of data from more than 100 studies around the world has estimated that the median incidence is 5.2 per 100,000 person-years, the median prevalence is 112.0 per 100,000 person-years and the average illness period is 20.2 per 100,000 person-years (Eskandarieh et al., 2016). Other authors suggest that worldwide there is a median prevalence of 33 per 100,000 people where North America and Europe show the highest prevalence compared to Asia and sub-Saharan Africa (2.2 and 2.1 per 100,000 inhabitants) (Vidal-Jordana & Montalban, 2017). Furthermore, a systematic review demonstrates an increase in the incidence of MS around the world over the years as shown in figure 2 (Lane et al., 2022).

Figure 4 shows the results of a systematic review in which it is evident that the incidence of MS has increased in recent years in several regions of the world.

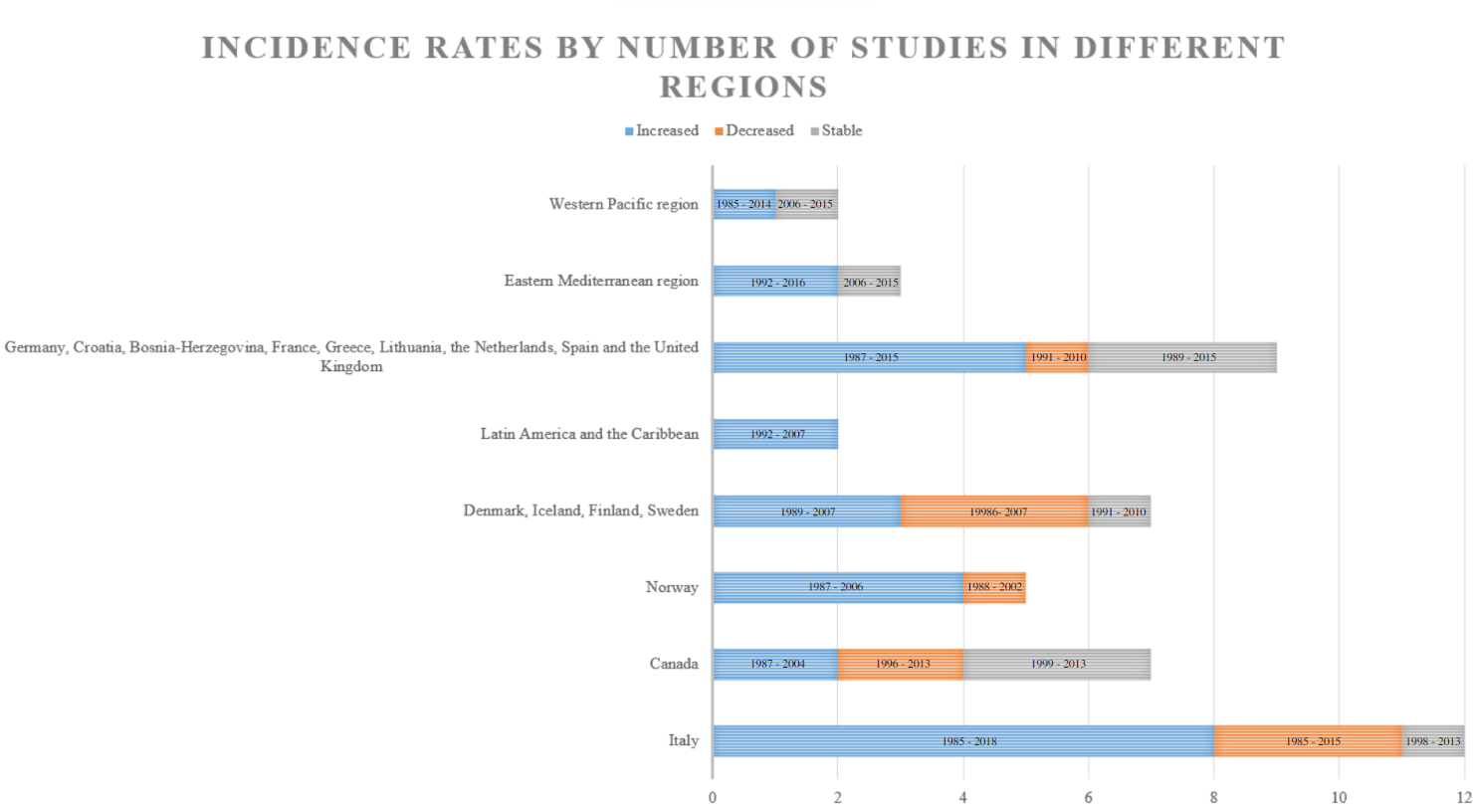

Figure 4. Results of a systematic review of incidence rates of MS. *Sixty-four articles were reviewed to obtain the most up-to-date incidence of patients with MS. Each case was considered if it used consistent case definition: Poser, McDonald, diagnostic codes e.g., ICD, Read or International Classification for Primary Care codes. An increase in incidence in recent years is evident. The x-axis shows the number of articles found for that period of time and the y-axis shows the different regions analyzed in the systematic review. In some regions such as the Eastern Mediterranean region and Western Pacific region and South America, no eligible studies were found. Adapted from: (Lane et al., 2022)*

The data from this systematic review established that the incidence of MS has increased due to the number of studies analyzed. However, when using a consistent case definition throughout the study and a larger population, there was no evidence of an increase in the last three decades. Three of the factors associated with this divergence may be the periods covered, changes in diagnostic criteria for MS, and inequalities in access to health care in some regions.

On the other hand, South America has a low prevalence of MS compared to North America (Correa et al., 2016; Howard et al., 2016). In Latin America, high prevalence ranges are recorded between 25 to 30 patients with MS per 100,000 people in cities such as Nuevo León, and Buenos Aires, while the prevalence is low in cities such Lima (<10 cases per 100,000 people) (Correa-Díaz et al., 2019). Other regions of South America such as Sao Paulo, Buenos Aires, and Argentine Patagonia have a high prevalence of MS (Correa-Díaz et al., 2019; Howard et al., 2016).

Few studies have been carried out in Ecuador on the prevalence of MS in the population. These studies have been carried out in the most important cities and the results show low prevalence in cities such as Quito and Cuenca (5.05 and 3.88 per 100,000 people respectively) (Correa et al.,

2016; Correa-Díaz et al., 2019). Other authors even state that the estimated prevalence is lower (1.2 per 100,000 inhabitants) than that mentioned above (Jácome Sánchez et al., 2018). Although these values of MS prevalence are lower in relation to other places in the world, a slight increase has been evidenced in cities such as Cuenca whose main factors involved are a better knowledge of neurologists and access to medical X-ray equipment not available years ago (Correa-Díaz et al., 2019).

Figure 5 shows the current prevalence of MS per 100,000 people in the world in which North America is the most prominent region for this disease.

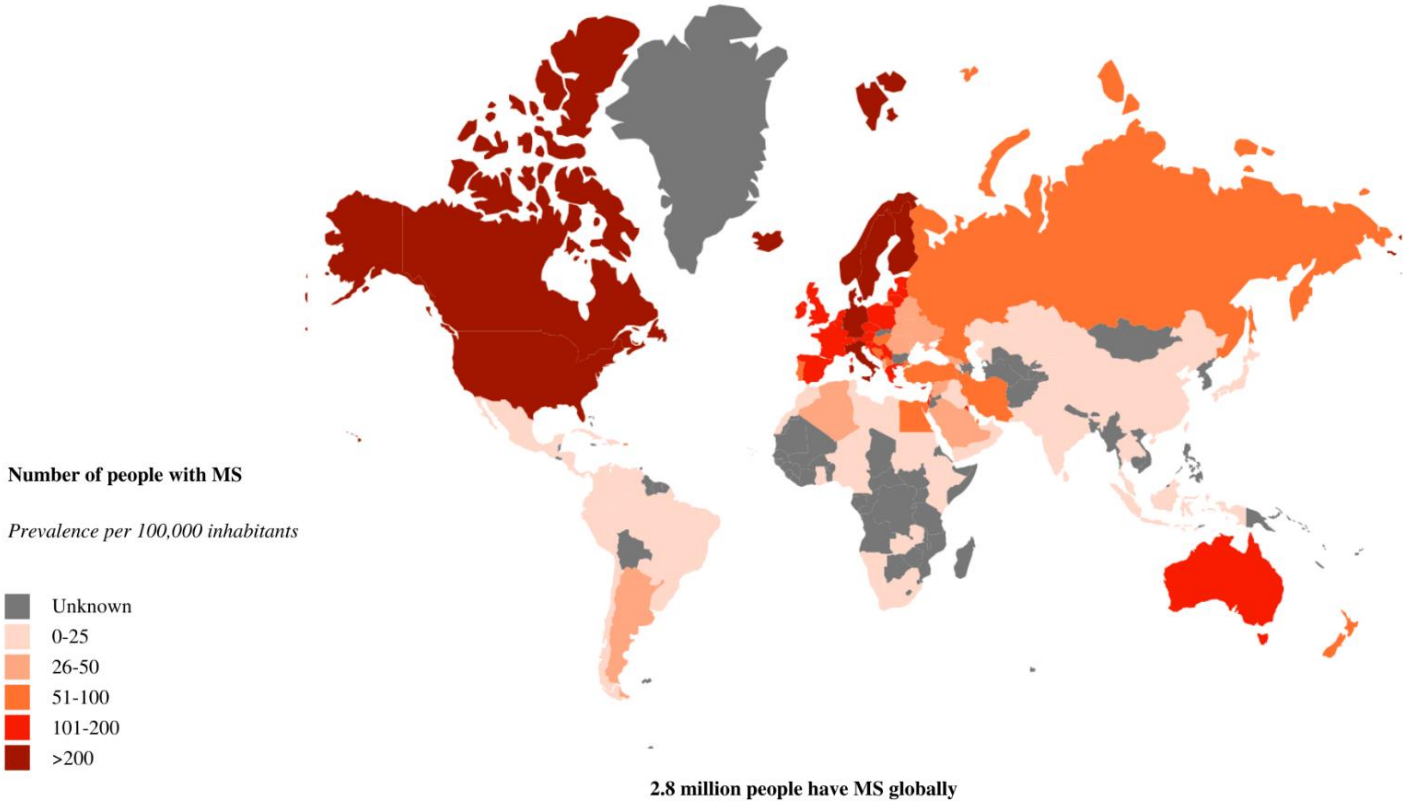

Figure 5. Number of people with MS. *Adapted from: (www.atlasofms.org, Number of people with MS: Atlas of MS 2022)*

This compilation of information is part of the MS Atlas, the largest study of the epidemiology of MS worldwide. Unlike a literature review, this study collected information by asking each country about its current situation concerning MS. Thus, the data suggest that there is an increase in the number of people with MS worldwide and this is mainly evidenced in developed countries because they may have all the diagnostic resources for this neurodegenerative disorder.

Another aspect to consider in patients with MS is the age at which they are diagnosed. The age of patients diagnosed with MS is mainly between 20 and 40 years old (Buzzard et al., 2017; Correa et al., 2016; Klotz et al., 2019; Mathur et al., 2021; Vidal-Jordana & Montalban, 2017), and even patients as old as 50 years have been found (Bishop & Rumrill, 2015b). Recent research suggests that the age range of prevalence has increased over the last few years. Years ago, the age range was documented between 35-39 years but new research shows an increase in reporting a range between 55-59 years (Vaughn et al., 2019). A recent study in the United States found that 14% of patients with MS were 65 years old (Bishop & Rumrill, 2015b), while another study in Italy found that 18% of patients were older than 65 years (Vaughn et al., 2019). Thus, the age range of a patient diagnosed with MS has changed markedly, most likely due to the use of new diagnostic technologies.

In addition, it has been reported that women are the population most affected by MS (Patsopoulos, 2018). The proportion of disease-related sex ratio in the early 1900s remained balanced for both men and women, but lately, it has increased, especially in developed countries (Dobson & Giovannoni, 2019). Different analyzes show that women are 3 times more likely to develop MS than men (Arneth, 2019; Buzzard et al., 2017; Constantinescu et al., 2011; Correa et al., 2016; Martin et al., 2016). However, a recent study of 23 patients found that 14 were women, determining a ratio of 1.5:1 and another similar study found a ratio of 2.8:1, concluding a major prevalence of women (Correa-Díaz et al., 2019; Olek, 2021). Another study from the United States suggests that this sex ratio is rising and that African American women are more likely to have MS than Caucasian-American women or men (Bishop & Rumrill, 2015). The prevalence of women over men is still being investigated, although it is possibly associated with greater complexity of the immune system and the levels of sex hormones such as estrogen.

4. Causes of disease

Although the etiology of MS remains unknown, several factors are associated with the prevalence of MS in the population (Bishop & Rumrill, 2015b; Silva & Ferrari, 2019a). Genetic and environmental factors such as latitude, UVB exposure, and smoking are involved in this disease (Dobson & Giovannoni, 2019). Each of the factors associated with this neurodegenerative disease are detailed below.

4.1. Genetic factors

Several studies have been conducted to determine new relevant aspects associated with MS genetics (Olsson et al., 2016). MHC genes have been recognized for years as the universal genetic locus linked to MS (Constantinescu et al., 2011; Tizaoui, 2018) because one of the factors involved is

autoimmunity and the MHC molecule is self-restricting. Despite this, independent susceptibility variants such as interactions within HLA and its MHC class II genes have also been found (Nikolas et al., n.d.), a distinctive feature in autoimmune diseases. There are two risk factors for MS: MHC genes, which determine the immune repertoire, and non-MHC genes, which determine the regulatory and immune tolerance mechanisms (Baecher-Allan et al., 2018b).

Genes within the HLA complex, located on the small arm of chromosome 6, play an important role in the development of MS (Correa et al., 2016; Olsson et al., 2016; Patsopoulos, 2018). In the early 1970s, HLA alleles were recognized as the genetic factors of MS and partially explained the origin of the disease: HLA class II and I genes (Cotsapas et al., 2018; Olsson et al., 2016). These genes are responsible for encoding cell surface polymorphic glycoproteins involved in immune regulation, and recognizing non-self-intracellular (MHC class I) or extracellular (MHC class II) proteins (Patsopoulos, 2018). Recently it has been found that the HLA-DRB1*1501, DQA1*0102, and DQB1*0602 haplotypes of the major histocompatibility complex (or HLA in humans) are responsible for a high risk of a person having MS and HLA-DR15 is also the most prominent haplotype in MS studies (Brola & Steinborn, 2020; Correa et al., 2016; Dendrou et al., 2015b; Narula, 2016; Nourbakhsh & Mowry, 2019). Surprisingly, the genetic variants associated with MS are found close to genes involved in the regulation of the innate or adaptive system (Nourbakhsh & Mowry, 2019; Olsson et al., 2016; Tizaoui, 2018). A systematic investigation from Argentina, Colombia, Mexico, and Brazil shows that DRB1*1503, DQB1*0602 alleles, DRB1*15, DQB1*06, and DRB1*1501 alleles are involved in MS, highlighting DRB1*15 allele, which was the more common in Caucasian people (33.9%) and white people (24.7%) (Correa et al., 2016). In another study, it was possible to determine that the DRB1*03 allele was significantly present in a group of patients analyzed (Correa et al., 2016). Likewise, alleles such as HLA-A*02, DRB1*14, and DRB1*07 have also been found to have a protective effect against MS (Buzzard et al., 2017; Correa et al., 2016; Narula, 2016; Nourbakhsh & Mowry, 2019). Although the association between HLA and the risk of having MS remains unclear, different theories have been proposed: specificity of peptide-(auto) antigen binding, differential levels of expression, and perturbations in central immune tolerance (Canto & Oksenberg, 2018). Furthermore, the contribution of HLA-encoded products to MS susceptibility has not been identified because the exact associated immunological mechanisms are unknown (Canto & Oksenberg, 2018).

Although HLA genes are important in the immunological aspect of this type of disease, there are also other genes involved in cytokine pathways, co-stimulation, and signal transduction (Tizaoui, 2018).

Table 2 summarizes the most relevant genes associated with MS with the number of polymorphisms present in each case and in which the VDR has a greater number of associated polymorphisms.

Table 2. The most important genes and polymorphisms associated with MS. *Most of the multiple susceptibility loci and their variants in MS are located in genes or near genes important in immune functions. Allele 4 of the ApoE gene would be involved in the neurodegeneration, development, and progression of MS. CD24 is expressed in astrocytes and microglia suggesting a role in the inflammatory process of MS. IL-7R is associated with decisive pleiotropic activities of the immune system. CYP27B1 generates the active form of vitamin D (1,25- dihydroxy vitamin D3). EVI5 is a related oncogene in T-cell lymphomas. VDR is involved in the expression of certain lineage-specific genes. IL-4 is associated with anti-inflammatory activities by regulating T_H1/T_H2 cytokine balance. Adapted from:* (Tizaoui, 2018).

Gene	N° polymorphisms associated
VDR	10
HLA-DR	7
CD24	6
IL4	4
ApoE	3
PAI-1	3
IL7R	2
CYP27B1	1
EVI5	1

Within the results of this meta-analysis, it was shown that HLA-DR, CD58, EVI5, IL2RA, IL4, IL7R, IL6, ApoE, DPP6, PAI-1, CD24, VDR, CYP27B1, IRF5, and mt-DNA are candidate genes for the MS and other SNPs or CNVs need to be considered (Tizaoui, 2018). In addition, many of the associated loci are involved in the different responses of the immune system and these variants are associated with other autoimmune diseases, which could be an aspect to investigate for a future therapeutic alternative.

Recent research shows that the heritability of MS at the familial and global levels is very low. The heritability of MS in the population approaches 0.1%; a child who has a family member with a history of MS has a probability of 2% while for future generations the probability decreases

(approximately 1%) (Buzzard et al., 2017). Furthermore, it has been observed that genetic factors are really important in Europe compared to other places in the world. For example, MS is not commonly found in Chinese, Japanese, black African, and American Indian populations (Correa et al., 2016). The study of heritability has had its emphasis on twins where the results suggest that in monozygotic twins there is a risk of 18% while in siblings there is a risk of 3% (Buzzard et al., 2017). However, a literature review of more than 50 studies found that the pairwise risk for monozygotic twins is 14.3% while the crude risk is 17.25% (O'Gorman et al., 2012).

Table 3 summarizes the most relevant results of the literature review carried out with 18 studies related to family risk and in twins for MS in which the risk is analyzed considering sex and where the prevalence of women over men can be visualized.

Table 3. Results of a literature review of genetic risk for MS in family and twin studies. *500 studies were initially reviewed and 18 were chosen based on the study exclusion criteria. Pairwise risks and AAR for monozygotic and dizygotic twin pairs are presented. The table on the left shows the risks for each sex of monozygotic and dizygotic twins and when they are of the same and different sex. The mean age of the twins studied was 50 years and the DZ/MZ ratio was 1.75 for all couples and 0.95 for same-sex couples. The table on the right shows the crude risk and the AAR analyzing various relationships in which it is seen that the risk for parents and children is lower compared to that of siblings. A 95% confidence interval was used in each case. Taken from:* (O'Gorman et al., 2012).

Relative	Pairwise risk, %	AAR, %
MZ twin	14.3	15.4
Female	16.3	
Male	8.9	
DZ twin	3.7	3.9
Female	4.7	
Male	4.1	
Same sex	4.6	
Opposite sex	3.2	

Relative	Crude risk, %	AAR, %
MZ twin	17.25	18.44
DZ twin	4.37	4.61
Sibling	2.18	2.68
Parent	1.42	1.45
Offspring	0.63	2.07
Niece/nephew	0.32	1.02
Aunt/uncle	0.73	0.75
Cousin	0.6	0.73

The results presented above denote a low probability of having MS in most cases of kinship, except for monozygotic twins because they share the same genome. Thus, the heritability of MS is only 20% or 30% explained, thereby epigenetic factors, gene-gene, or gene-environment interactions must be related (Nourbakhsh & Mowry, 2019).

4.2. Environmental factors

Over the years it has been established that MS susceptibility combines both environmental and lifestyle factors with genetics (Arneth, 2019; Baecher-Allan et al., 2018b; Bishop & Rumrill, 2015b; Brola & Steinborn, 2020; Buzzard et al., 2017). Among the factors that increase the risk of MS such as Epstein-Barr virus and herpes infections, ultraviolet radiation and vitamin D levels, use of tobacco, sex hormones, high sodium intake, obesity, and latitude (Bishop & Rumrill, 2015b; Brola & Steinborn, 2020; Buzzard et al., 2017; Gilmour et al., 2018; Tizaoui, 2018; Vidal-Jordana & Montalban, 2017). Important details on each of the environmental factors associated with MS will be provided below.

4.2.1. Epstein-Barr virus and herpes infections

Epstein-Barr virus (EBV), one of the most common viruses in humans, is strongly associated with a high risk of MS in people of different ethnicity and race (Houen et al., 2021; Nourbakhsh & Mowry, 2019). The immune system fights against EBV infection through Ab-dependent processes, cytotoxic T cells that are evaded by the virus's evasion mechanisms and that are subsequently controlled by the system, describing a cyclical process that can continue at any time (Houen et al., 2020). EBV-infected B cells are responsible for spreading inflammation by transiting not only to the CNS but also to the deep cervical lymph nodes (Bar-or et al., 2020).

MS patients present high amounts of antibodies to EBV compared to control groups. Analyzing the seroprevalence between different studies, 98% was reported for anti-EBNA IgG (OR 4.47, 95% CI 3.26 - 6.11, $p<0.0001$), 92% for VCA IgG (OR 4.51, 95% CI 2.84 – 7.16, $p <0.00001$) (Ruprecht, 2021). Furthermore, evidence has been found that EBV infections in adolescence or adulthood increase the risk of MS (Alfredsson & Olsson, 2019; Nourbakhsh & Mowry, 2019). Although the general population is 95% exposed to EBV, in MS patients the exposure is >99% (Buzzard et al., 2017). Two mechanisms have been proposed to explain the role of EVB in MS: a more specific mechanism involving viral reactivation resulting from inappropriate regulation of latent EBV infection and a more general mechanism involving dysregulation of the immune system (Dendrou et al., 2015b). Despite the data found, further studies are required to know exactly the role of EBV in MS (Alfredsson & Olsson, 2019).

4.2.2. Ultraviolet radiation and vitamin D levels

Investigations suggest a close relationship between a low probability of MS, ultraviolet radiation, and vitamin D (Nourbakhsh & Mowry, 2019). 15% of the world's population lives at high latitudes receiving relatively low amounts of sunlight inhibiting the synthesis of vitamin D so they

are vulnerable to developing MS with high probabilities (Ghareghani et al., 2018). Considering UVR and vitamin D as independent agents, UVR has been shown to reduce the risk for MS, although this protective role is not clear (Alfredsson & Olsson, 2019). On the other hand, vitamin D has important roles within the immune system such as the suppression of B cells, the proliferation of T cells, and the skewing of T cells away from inflammatory responses and toward T_{reg} responses, which is why it also stands out in diseases such as rheumatoid arthritis, type 1 diabetes, and systemic lupus erythematous (Baecher-Allan et al., 2018b). Indeed, it has been found that a person with high vitamin D levels before the age of 20 has a lower chance of developing MS later in life (Alfredsson & Olsson, 2019; Baecher-Allan et al., 2018b; Buzzard et al., 2017; Dobson & Giovannoni, 2019). In addition, it has been shown that a diet rich in vitamin D (fatty fish) contributes significantly to a person having a low probability of MS regardless of sun exposure (Nourbakhsh & Mowry, 2019). Therefore, vitamin D is responsible for maintaining immune system homeostasis although its role in MS is unclear (Buzzard et al., 2017; Ghareghani et al., 2018).

4.2.3. Use of tobacco

Cigarette smoking is another risk factor for MS (Baecher-Allan et al., 2018b). It has been shown that a person who smokes, even if he is a secondhand smoker, has a substantial risk for MS (Nourbakhsh & Mowry, 2019). Even smoking increases the risk by about 60% (Briggs, 2020). A person who smokes has a higher probability of developing MS compared to non-smokers depending on the intensity and duration that can aggravate the situation (Buzzard et al., 2017). Furthermore, Swedish and French control studies showed that smoking increases the risk for MS mainly in women, and that risk increases depending on the duration of exposure (Weston & Constantinescu, 2015).

Table 4 shows the results of the association between parental smoking and the increased risk of the first episode of MS in children (Mikaeloff et al., 2007).

Table 4. Parental smoking at home and increased risk of MS. *The adjusted RR for first-episode MS was 2.12 after classification by family history of MS or other autoimmune diseases and socio-professional status of the head of household. When stratifying by age, the risk increased significantly with greater exposure to tobacco. A 95% confidence interval was used in each case. Taken from:* (Mikaeloff et al., 2007).

Exposure to parental smoking N (%)	Cases	AAR, %	Crude RR	Adjusted RR
Total population	129	1038		
Unexposed	49	570	1	
Exposed	80	468	2.09	2.12
Age at index date ≥ 10 years	90	708		
Unexposed	28	371	1	
Exposed	62	337	2.54	2.49
Age at index date < 10 years	39	330		
Unexposed	21	199	1	
Exposed	18	131	1.37	1.47
Sex male	46	385		
Unexposed	18	214	1	
Exposed	28	171	1.99	2.12
Sex female	83	653		
Unexposed	31	356	1	
Exposed	52	297	2.15	2.17
Low socioprofessional status of head of family	57	638		
Unexposed	22	338	1	
Exposed	35	300	2.40	2.38
Medium or high socioprofessional status of head of family	72	400		
Unexposed	27	232	1	
Exposed	45	168	2.46	2.41

These data reveal that children who live with smoking parents have a higher risk of MS than non-smoking parents. In addition, the duration of exposure also influenced the increased risk. Smoking is associated not only with MS but also with other autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus. In addition, some direct toxic effects derived from some components of cigarette smoke can be considered.

Smoking is a factor that accounts for 40% of the increase in the sex ratio in MS (Dobson & Giovannoni, 2019). Surprisingly, it has been found that the slightest injury to the lungs can cause serious problems, even developing neutralizing antibodies against certain treatments (Alfredsson & Olsson, 2019). Although the relationship between tobacco use and MS has been presented as controversial, a recent study affirms it as a negative agent (Harris et al., 2020). Therefore, further studies are required to analyze the role of cigarette smoke and the constituent elements of cigarette smoke and their association with the risk of MS.

4.2.4. Latitude effect

In addition to the factors mentioned above, geographical latitude plays a decisive role in the development of MS. For those individuals living far from the equator, the probability of MS is higher than in any other region of the world, in other words, the incidence and prevalence increase in high latitude regions (Buzzard et al., 2017; Correa et al., 2016; Nourbakhsh & Mowry, 2019). A higher amount of UVR is linked to a decreasing latitude (Salmen et al., 2020). Latitude is not only a risk factor in itself but also because of its close relationship with vitamin D levels, which partially mediates the effect (Salmen et al., 2020). Regions greater than 40 degrees North and South show a higher prevalence of MS due to low levels of sunlight associated with zero levels of vitamin D synthesis (Ghareghani et al., 2018). Howard and colleagues (2016) collect conclusive data which reported that the prevalence and incidence of MS are low in Africa and high in the northern regions of the British Isles, the Nordic countries, and Australia (Howard et al., 2016). Fifteen percent of the world's population lives at high latitudes and receives low amounts of sunshine, which constitutes a risk factor for vitamin D deficiency affecting the normal development of the immune system against diseases such as MS (Ghareghani et al., 2018). Although latitude as a risk factor was controversial at one time, the work of Risco et al. (2016) showed that just increasing one degree in latitude meant a higher prevalence of MS (Correa et al., 2016). Thus, latitude implies a higher risk of MS as evidenced by the prevalence of this disease in certain geographical areas worldwide.

4.3. Other factors

Different person habits can influence a person's risk of developing MS. Based on different studies, it has been possible to show that a diet high in salt can generate a proinflammatory reaction in experimental models, data that was later corroborated by a study in Argentina (Nourbakhsh & Mowry, 2019). In addition, coffee, alcohol consumption, and shift work are associated with MS, although the data is controversial (Alfredsson & Olsson, 2019; Lu et al., 2020; Nourbakhsh & Mowry, 2019). Studies also suggest that adolescent obesity, especially in women, is an important factor

associated because there is an associated decrease in T_{regs} cells, high levels of leptin which is involved in proinflammatory processes and finally, obesity generates a low vitamin D bioavailability (Alfredsson & Olsson, 2019). Likewise, people with depression can trigger MS pathology (Guerrero-García et al., 2016; Novo & Batista, 2017).

5. Immunology of MS

MS is a highly complex disease to study and experimental models have provided substantial insights into the understanding of immunological mechanisms. Both cells of the innate immune system (macrophages, NK) and the adaptive system such as T cells and B cells develop a wide repertoire in the development of MS (Baecher-Allan et al., 2018b; Ruiz et al., 2019; Thompson et al., 2018).

Figure 6 shows the imbalance recorded between the different populations of T cells, B cells, and NK cells that cause a clinical picture of MS with the secretion of different cytokines.

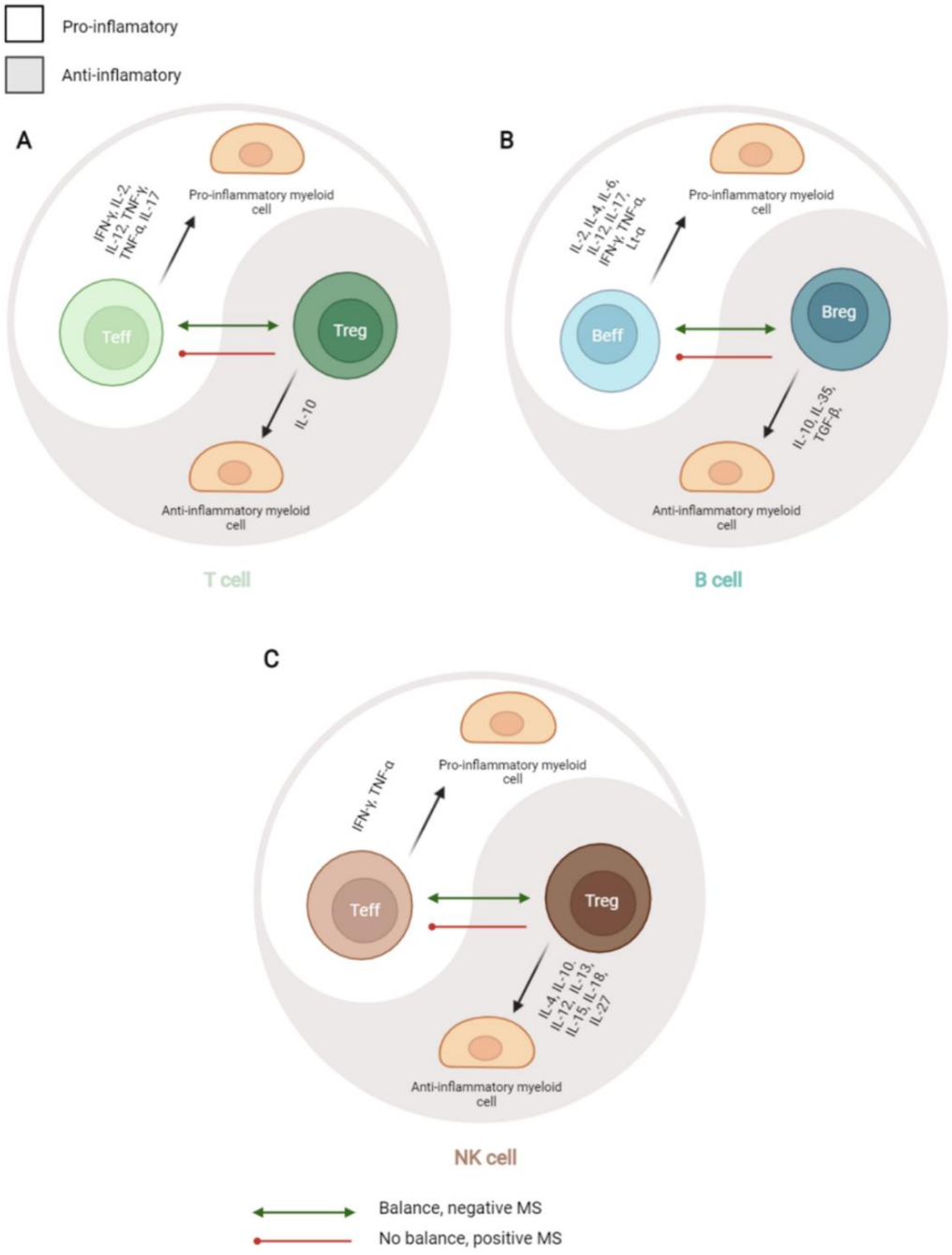

Figure 6. Cellular immunology of MS. *The balance between immune cell subsets maintains the host in a healthy state. Multiple sclerosis begins once the supremacy of autoreactive cells over regulatory cells has been detected. In the graph, it can be seen that abnormalities in the balance between these cells in T cells (A), B cells (B), and NK cells (C) initiate a chain of events that are mediated by different and that lead to MS. Adapted from (Bar-Or & Li, 2021).*

The different types of immunologic agents involved in the development of MS will be discussed below.

5.1. T cells

The first set of immune cells involved in the development of MS and its associated pathology are T cells. In turn, different subsets of T contribute to the immunopathology of this disease. Every detail of each of the T cells will be presented below.

5.1.1. Autoreactive T cells

Autoreactive T cells play a fundamental role in autoimmune diseases due to their regulatory and effector functions. Each of these cells plays a unique role in the progression of this disease as shown below.

5.1.1.1. $CD4^+$ T Cell

People with MS have evidenced high populations of $CD4^+$ T cells both within CNS lesions and in CSF (Baecher-Allan et al., 2018b; Guerrero-García et al., 2016). It is known that the MHC class II locus DR2 (DRB*1501/DQ6) is responsible for regulating the activation of $CD4^+$ T cells (Baecher-Allan et al., 2018b). Moreover, these cells are activated by APCs generating different populations of cells: T_H1, T_H2, and T_H17 and whose activity is mediated by different cytokines (Olcum et al., 2020).

Based on the function of cytokines, $CD4^+$ T cells can be divided into pro-inflammatory (T_H1) and anti-inflammatory (T_H2) (Guo et al., 2008). In this sense, T_H cells associated with MS are T_H1, T_H17, GM-CSF, and follicular Th cells (Bar-Or & Li, 2021; Dendrou et al., 2015a). While T_H1 secretes cytokines such as interferon-gamma (IFN-γ, interleukin-2 (IL-2), interleukin-12 (IL-12), and TNF-γ (cytotoxic activity against oligodendrocyte), T_H17 secretes interleukin-17 (IL-17) (Garg & Smith, 2015; Guo et al., 2008). Recent studies suggest that GM-CSF is responsible for mediating the interactions between subsets of T cells, and it can prolong CNS inflammation and injury once it has activated microglia and inflammasomes in experimental models (Yong et al., 2018). On the other hand, T_H1 and T_H17 cells appear to exhibit pathogenic properties in the development of MS due to low levels of IL-10 expression (T_H17) and high levels of GM-CSF expression (T_H1 and T_H17, mainly those that co-express CXCR4) (Bar-Or & Li, 2021). A recent study found that of all phenotypes involved in increased self-proliferation, a process of autoreactivity of peripheral T_H1 cells, the most prominent T-cell subset is IFN-γ-secreting T_H1 cells (Jelcic et al., 2018).

Figure 7 shows the results obtained from the study conducted by Jelcic et al. (2018) in which the different T_H1 involved in AP, a key process in autoimmune diseases, is evidenced.

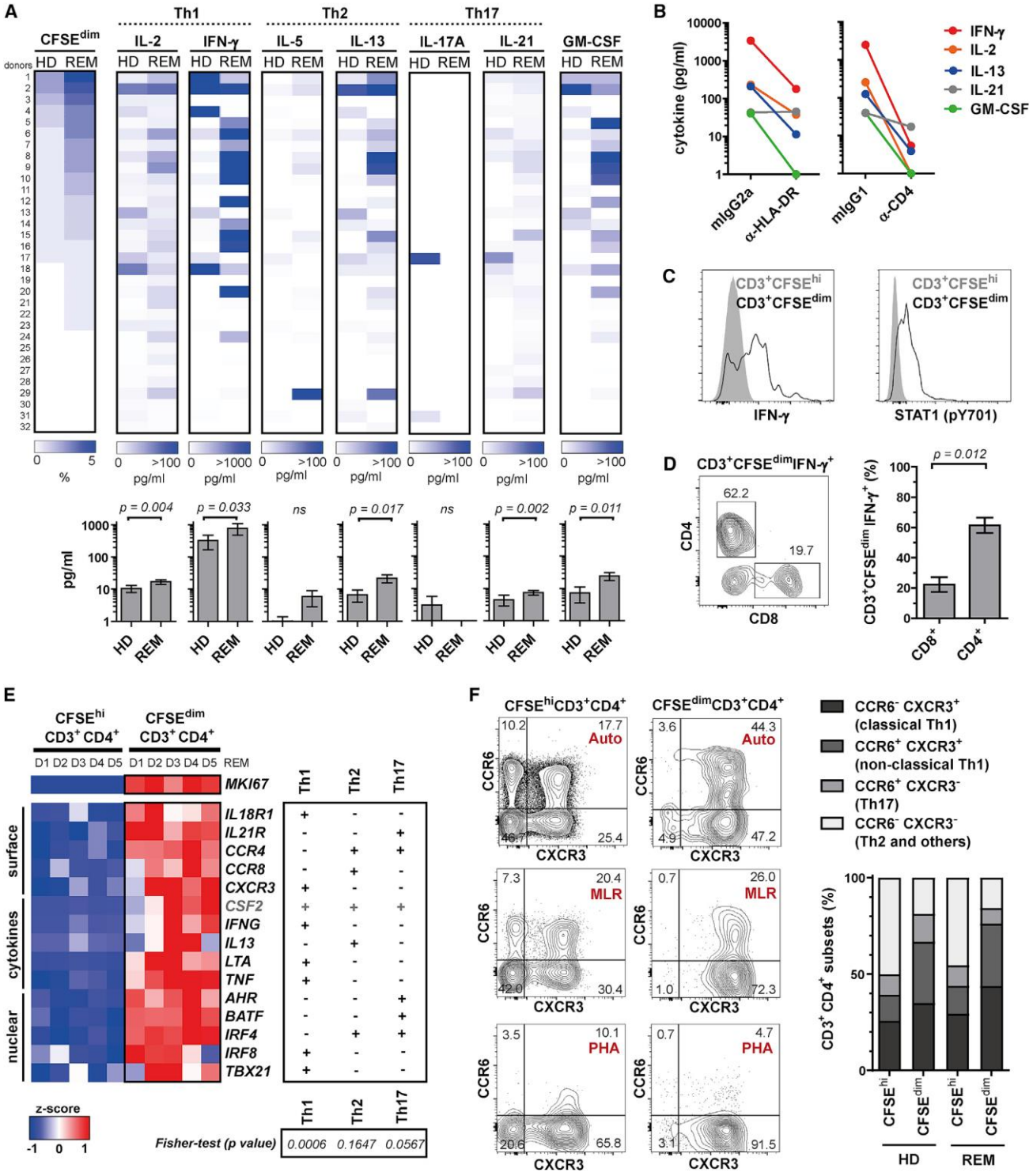

Figure 7. Classical and non-classical T_H1 cells are involved in AP with elevation of proinflammatory response in MS. *In this study the cellular interactions that lead to increased self-proliferation and their association with MS were identified. Peripheral blood mononuclear cells were cultured from RRMS patients in active (REL) and inactive (REM) state and healthy patients free of stimulus and in serum-free conditions. A carboxyfluorescein N-succinimidyl ester diacetate (CFSE) assay was developed to characterize AP cell populations. Following an AP process, culture supernatants exhibited high amounts of IFN-g, IL-2, IL-13, IL-21 and GM-CSF in REM patients compared to healthy patients, whereas low or no amounts of IL-5 and IL-17 were found (Figure 2A). In contrast to AP, IFN-g secretion after activation by conventional T-cell stimulation did not differ between HD and EMR. IFN-g correlated better with AP (Fig. 2A) and was higher in HLA-DR15$^+$ individuals. Cytokines decreased after blocking HLA-DR or CD4, but in vitro*

neutralization of IFN-g and GM-CSF did not inhibit AP. This suggests that cytokine production is the result of AP (Figures 2B and 2D). Intracellular cytokine staining confirmed that AP-CD4$^+$ T cells predominantly expressed IFN-g and also showed STAT1 signaling consistent with a T_H1 phenotype (Figures 2C and 2D). A T_H1-like inflammatory phenotype in REM-AP-CD4$^+$ T cells is supported by RNA-seq revealing a panel of T_H1-specific markers, including TBX21 (T-bet), CXCR3, and IFNG (Figure 2E). T_H2 and T_H17 markers such as GATA3, IL4, RORC, and IL17A were not present or expressed, whereas AHR and BATF such as CSF2 (GM-CSF) were upregulated. Consistent with these results, we observed a high frequency of CD4$^+$ CXCR3 CD4$^+$ T cells expressing T_H17 CCR6-related chemokine receptors in the AP compartment (Figure 2F) called non-classical T_H1 non-classical T_H1 cells (i.e., T_H1/T_H17 or exT_H17-T_H1). These cells are multifunctional and are elevated in MS, and both CXCR3 and CCR6 chemokine receptors are involved in T-cell homing in the brain, consistent with our in vivo results. In addition, an increase in AP was observed in classical T_H1 cells but not in classical cells (Figure 2F). This study used the Mann-Whitney U test. Taken from: (Jelcic et al., 2018)

The data provided by this work show that CD4$^+$ T cells mainly express IFN-γ and that it is directly linked to the T_H1 phenotype as corroborated by RNA sequencing and a high AP was also shown in classical and non-classical T_H1 cells. Thus, these data allow for initializing a possible therapeutic strategy.

Moreover, these autoreactive CD4$^+$ T cells are important in understanding pathogenesis because they recognize myelin-specific antigens such as MBP, PLP, and MOG (Olcum et al., 2020; Sospedra & Martin, 2016a). It is known that the disease begins when CNS antigen-specific CD4$^+$ T cells are activated in the periphery and although the precise site of activation and differentiation is not known, EAE models have established that these may be different lymph nodes (Sospedra & Martin, 2016b). In these models, it has been shown that CD4$^+$ T cells, together with CD11$^+$ dendritic cells, recruit monocytes and activate virgin cells, causing a greater inflammatory condition due to the "epitope spreading" (Dendrou et al., 2015a; Garg & Smith, 2015). Interestingly, T_H17 shows evidence of developing inflammatory processes and T_H1 has a protective role in animal models, which suggests a clear contradiction of the defined pathogenesis of MS (Lazibat et al., 2018). An interesting study showed that the CD4$^+$ T cell population known as T09 plays a decisive role in the pathophysiology of RRMS and SPMS and that this cell type was clustered in both demyelinated areas of the white matter and gray matter (sulcal areas) (Kaufmann et al., 2021).

In Figure 8, it can be seen that the accumulation of T09 cells in the brains of MS patients both in white matter areas linked to relapses and in gray matter areas associated with disease progression.

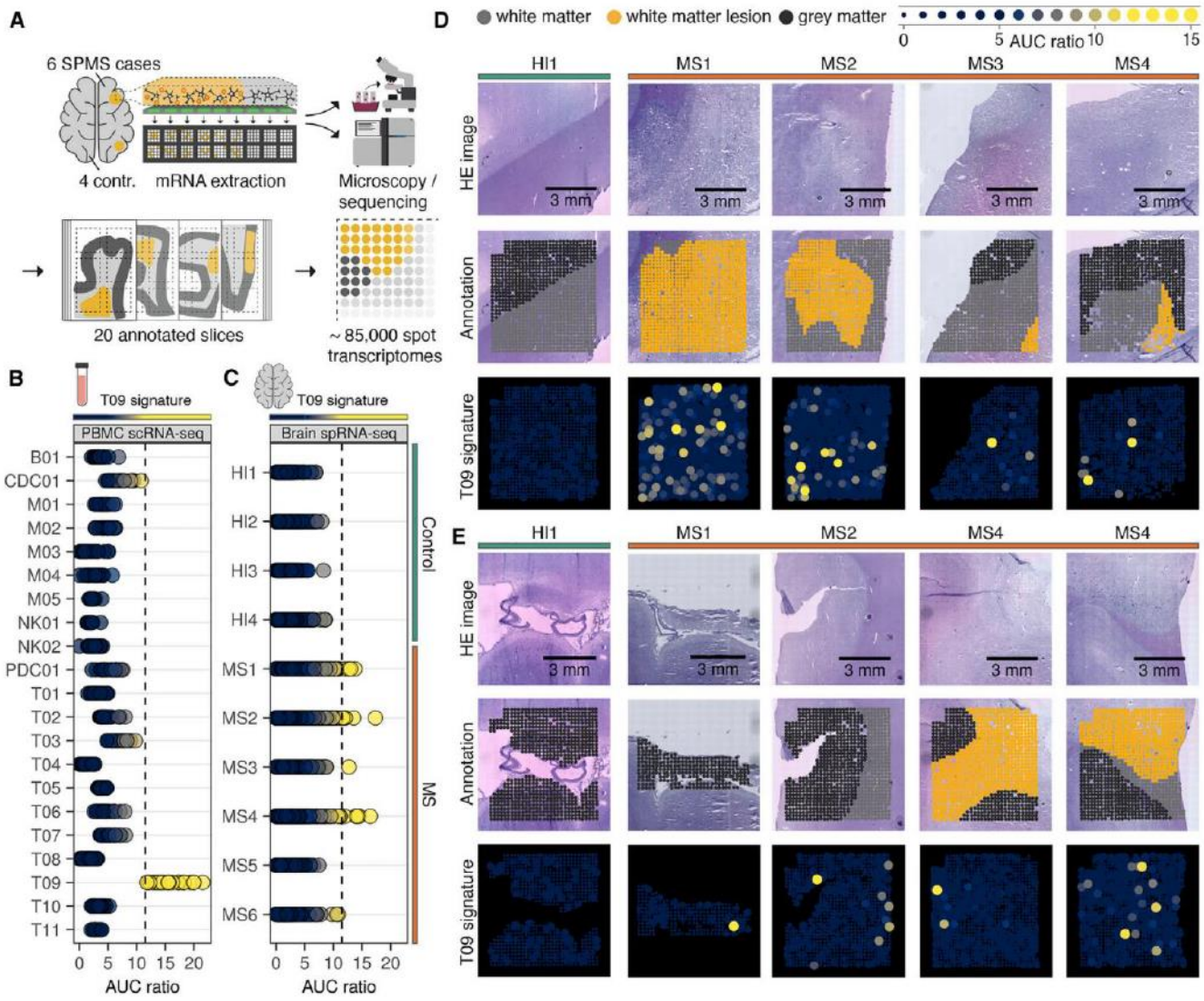

Figure 8. Results of a study that found CNS-homing T09 cells in gray and white matter in SPMS*. In this study, RNA was spatially sequenced to examine the presence and location of T09 cells in postmortem patient brain samples without loss of specificity (Figure 6A). A gene signature was compiled that distinguishes T09 cells from other immune cells in the blood and avoided enrichment of resident cells in the brain (Figure 6B). A speckled enrichment pattern was observed in four of 6 patients and no enrichment was visualized in four controls (Figure 6C-6E). In addition, areas of white matter demyelination coincided with the spatial distribution of T09 enrichment (Figure 6D), and focal enrichment in gray matter in sulcal areas or at the border between gray and white matter was observed (Figure 6E). Taken from:* (Kaufmann et al., 2021)

These data provide direct evidence that the brains of MS patients accumulate T09 cells in both relapse-involved white matter areas and gray matter areas associated with disease progression. Thereby, preventing the establishment of CNS immune cells in the brain at an early stage demands the development of a highly selective therapeutic strategy.

5.1.1.2. $CD8^+$ T Cell

$CD8^+$ T cells have a decisive influence on MS not only because of their functionality but also because of the number of $CD8^+$ T cells present in this type of patient (Alfredsson & Olsson, 2019; Bar-Or & Li, 2021). This population of T cells is responsible for axonal damage and the induction of

neuronal and oligodendrocyte death by the expression of granzyme A (Baecher-Allan et al., 2018b), granzyme B, and perforin to induce the apoptosis process (Bar-Or & Li, 2021; Garg & Smith, 2015; Guo et al., 2008; Olcum et al., 2020). Furthermore, these reactive T cells secrete IFN-γ and IL-17 that enhance the process of endothelial transmigration observed in human and animal models (Baecher-Allan et al., 2018b).

As described for $CD4^+$ T cells, the site of activation of $CD8^+$ T cells is assumed to be the peripheral lymph nodes by APCs, but there is little information on this, and the data obtained to date come from animal models of CNS infection (Sospedra & Martin, 2016a).

Although the antigenic specificity is not clear, myelin and viral antigens are the most strongly accepted (Bar-Or & Li, 2021; Ruprecht, 2021). $CD8^+$ T cells develop high activity secreting INF-γ due to antigen-presenting microglial cells that cross-present exogenous antigens on MHC class I molecules (Baecher-Allan et al., 2018b). This activity of IFN-γ together with that of TNF-α causes BBB malfunction, a key event in patients suffering from MS (Maciak et al., 2021). In EAE, these autoreactive $CD8^+$ T cells are involved in the development of early symptoms of MS such as optic neuritis and mild motor deficits (Yong et al., 2018).

5.1.2. Defective regulatory T cells

Likewise, various types of T_{regs} exist to reverse immune responses of autoreactive T cells and B cells and prevent the onset of autoimmune diseases by maintaining immune homeostasis (Bar-Or & Li, 2021), suppressing and controlling inflammation (Dubois et al., 2019). Impaired function or low levels of T_{regs} promote diseases such as systemic lupus erythematosus, T1D, psoriasis (Knethen et al., 2020), and IPEX syndrome (Lifshitz et al., 2016).

5.1.2.1. CD4 $FoxP3^+$ T_{regs}

$CD4^+$ regulatory T cells play a decisive role in the treatment of inflammation, highlighting $FOxP3^+$ regulatory T cells (nT_{reg} and iT_{regs}) and type 1 regulatory cell (Ruiz et al., 2019). FoxP3 Tregs, called professional suppressor cells, are part of the population of CD4 T cells that circulate in small quantities (<4%) and that have a role in blocking autoimmunity demonstrated in strains of mice (Baecher-Allan et al., 2018b). FOXP3 is an important transcription factor involved in the proper functioning of T_{regs} in mice and humans (Sato et al., 2020). Studies suggest that there is a strong association between a malfunction of $FOXP3^+$ T_{regs} cells (even $CD25_{high}$) and an impending development of MS (Moorman et al., 2019). A recent study demonstrated the proportional difference

of T_{regs} between relapsed, remission, and healthy patients and cell proliferation in the expansion of eT_{regs} (Lifshitz et al., 2016).

In Figure 9, it can be seen that peripheral T_{regs} showed a lower suppression capacity compared to the control group.

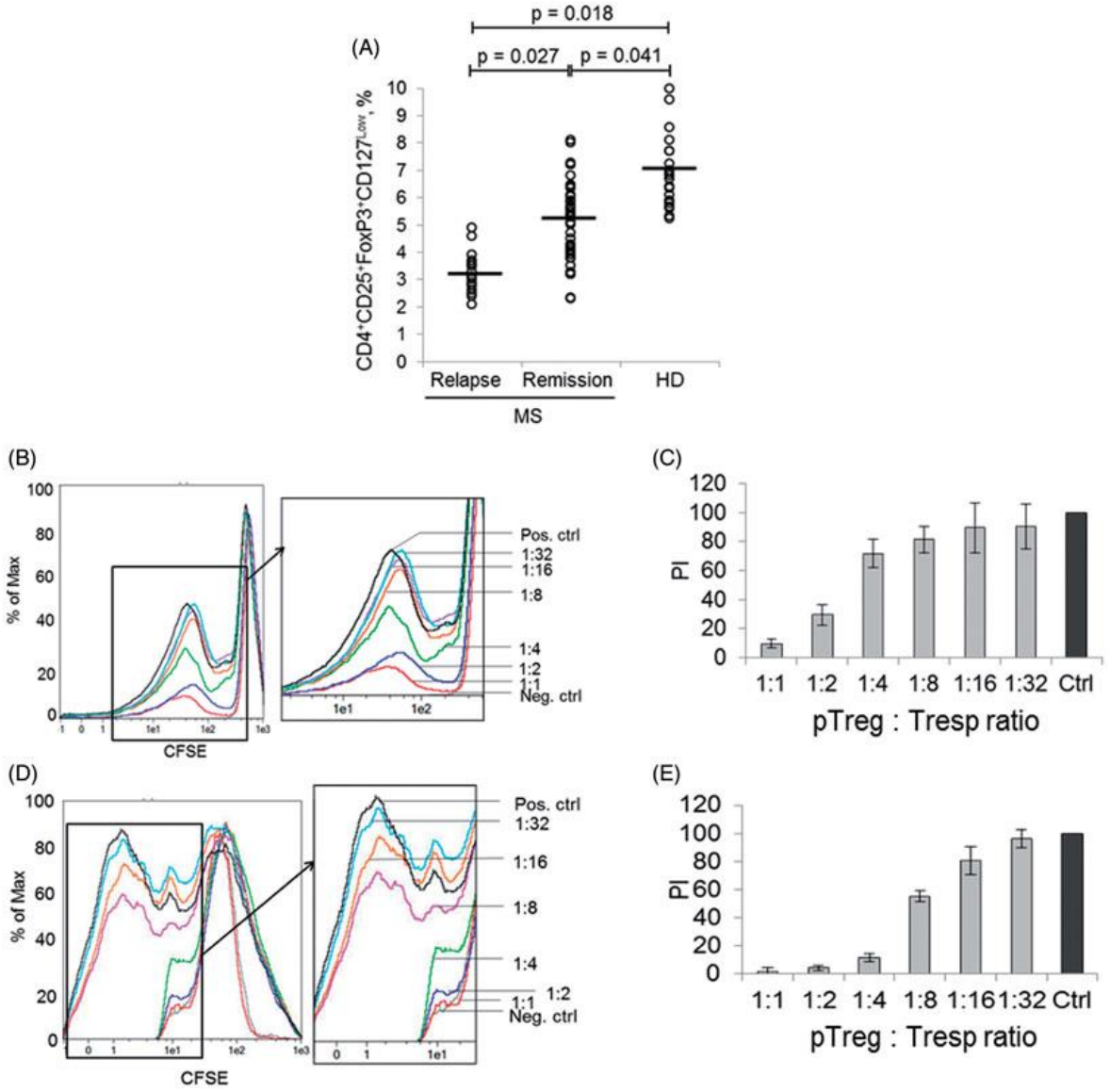

Figure 9. The proportion of peripheral T_{regs} in a population of CD4+ T cells in patients with MS. *This study involved 52 patients with RRMS and 22 healthy individuals. Low numbers of $CD4^+CD25^+FoxP3^+CD127^{low}$ pTregs were found in patients in relapse (3.1%) and remission (5.2%) as opposed to healthy patients (6.7%) (Figure A). The median number of pTregs in 1 ml of blood was 47 for MS patients and 80.5 for healthy patients. In addition, a Tregs suppression assay was performed in which it was evident that MS patients have a lower CD4+ CD25 T-cell suppression capacity (Figures B-E). Taken from:* (Lifshitz et al., 2016).

These results support the protective role of T_{regs} in maintaining tolerance and at the same time provide arguments to affirm that if the number of pT_{regs} decreases, a relapse is coming.

In MS patients, immune regulation by $Foxp3^+$ $CD4^+$ Treg cells is low at the same time that effector T cells are resistant to regulation (Baecher-Allan et al., 2018b; Bar-Or & Li, 2021) in patients

and experimental models (Ferraro et al., 2021). The functional deficits of T_{regs} linked to MS can be explained based on their phenotype: these T_{regs} are prone to developing the T_H1 phenotype, which is less regulatory and whose tendency may be motivated by an environment rich in salt or by a reduction in the T-cell immunoreceptor signaling with immunoglobulin and ITIM domains that is responsible for maintaining the regulatory role of T_{regs} (Bar-Or & Li, 2021). Indeed, studies of patients with autoimmune diseases such as MS have shown that autoreactive T cells resist suppression by T_{regs}, thus further suggesting a T_{reg} defect (Baecher-Allan et al., 2018b; Danikowski et al., 2017).

MS patients show low levels of expression of FoxP3 in T_{regs}, low levels of suppressive function of T_{regs} (Yu-feng et al., 2019) as well as Tr1 as shown by brain biopsies (Danikowski et al., 2017). In this sense, different treatment strategies involving an increase in T_{regs} levels have been tested (Danikowski et al., 2017).

5.1.2.2. $CD4^+$ Tr1 regulatory cells

The most studied population of T_{reg} cells is $FoxP3^+$, but studies have also been carried out on Tr1, which has shown the same problem with $FoxP3^+$ (Baecher-Allan et al., 2018b). Groux et al. distinguished Tr1 cells from $FoxP3^+$ T_{reg} cells by their suppressive activity by IL-10 (Shepard et al., 2021) with pleiotropic attributes on T cells, B cells, and mast cells (Astier et al., 2006). Although the regulatory role of IL-10 has been highlighted in murine models, the role of Tr1 has not yet been investigated *ex vivo* in patients (Astier et al., 2006; Konkle et al., 2020).

5.2. Natural Killer Cells

NK represents an early defense against viruses and tumors by secreting anti-inflammatory cytokines (IL-4, IL-10) and pro-inflammatory cytokines (IFN-γ, TNF-α) (Baecher-Allan et al., 2018b). NK cells represent a great resource of immunoregulatory cytokines whose action may require the participation of other immune cells (Müller et al., 2021). There are two types of NK: $CD56^{dim}$ (90% in peripheral blood, lower frequency in tissue, immediate cytotoxicity) (Gianchecchi et al., 2021), $CD16^{hi}$ or $CD56^{bright}$ (cytotoxicity over time, high frequency in tissues) (Baecher-Allan et al., 2018b; Sospedra & Martin, 2016a).

Figure 10 shows the different subpopulations of NK cells from a flowmetry image sorting process described by the work of Mimpen et al.

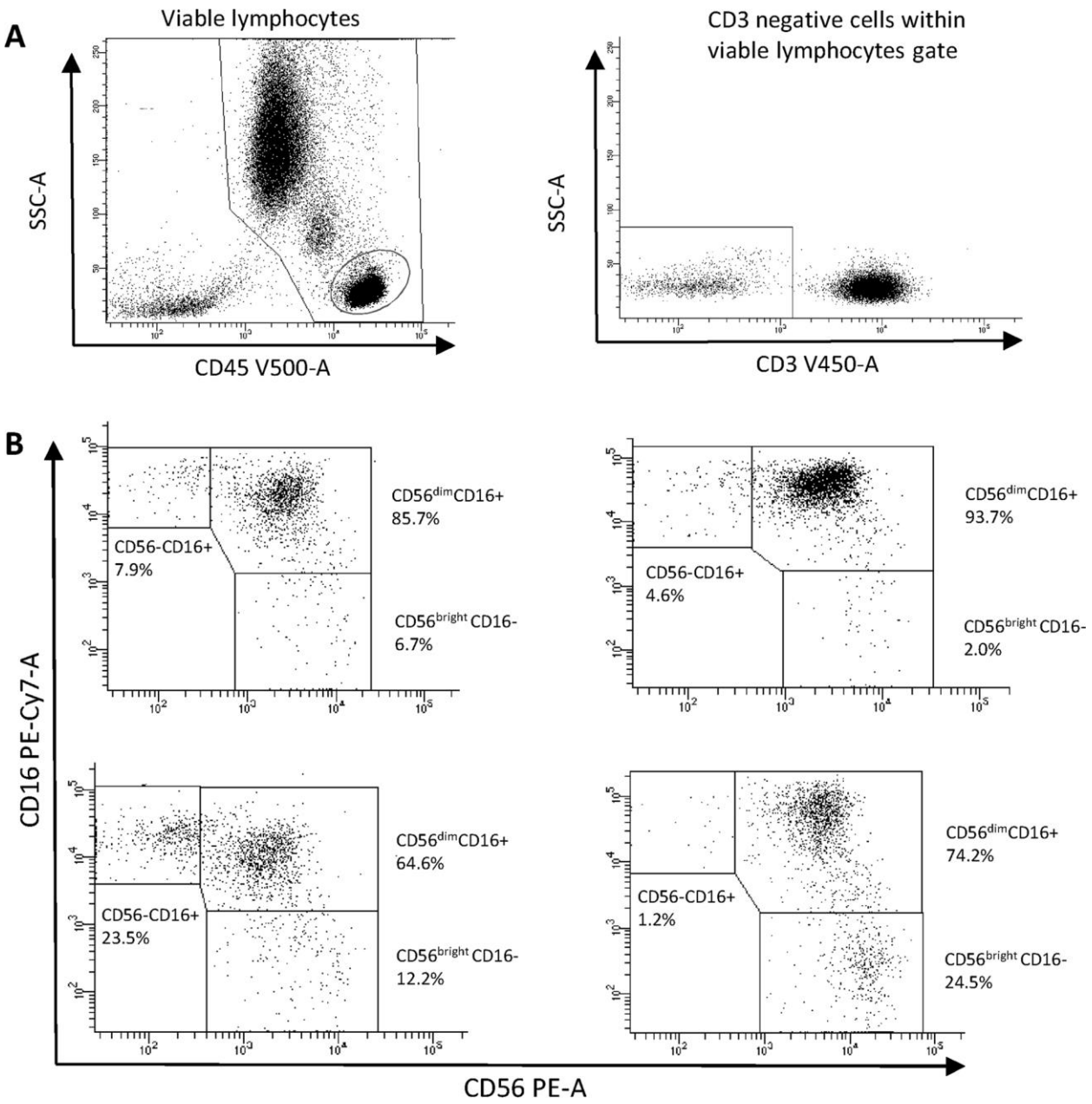

Figure 10. Identification of the different subsets of NK cells. *The identification process was done step-by-step using CD45 to initially identify live cells. Subsequently, T, B and NK cells are differentiated by CD3. CD56-CD16- (B cells), belonging to the CD3- negative population, are discarded and the final result is a strictly NK cell population (Figure A). NK cells are classified into 3 subtypes depending on CD56 and CD16 expression. Thus, the NK cell subpopulations of 4 patients are presented with their corresponding relative proportions. Taken from:* (Mimpen et al., 2020)

$CD56^{bright}$, found in secondary lymphoid organs, develop proliferative properties and secretes more regulatory cytokines compared with $CD56^{dim}$ NK cells (C. C. Gross et al., 2016; Pinar et al., 2020). This population of NK cells has receptors for IL-12, IL-15, and IL-18, which are responsible for the proliferation of $CD56^{bright}$ NK cells and the production of IFN-γ, IL-27, IL-10 and IL-13, TNF-β, and GM-CSF (C. C. Gross et al., 2016). $CD56^{bright}$ NK cells perform different regulatory functions of T cells with different actors: IL-27 inhibits the proliferation of autologous $CD4^+$ T cells as it happens in trials that use daclizumab, a monoclonal antibody (Mimpen et al., 2021) and in some

cases adenosine exerts the same effect (Laroni et al., 2016); with IL-12 and IL-15 it also has the same role as IL-27 but cytotoxic receptors such as NKp30 and Nkp46 are involved (C. C. Gross et al., 2016). Moreover, recent studies have shown that $CD56^{bright}$ NK cells only require a boost from proinflammatory cytokines to exert minor suppression on the proliferative capacity of $CD4^+$ T cells (C. C. Gross et al., 2016).

According to the data provided by a post-mortem study, NK cells destroy myelin through an antibody-dependent mechanism and, on the other hand, $CD56^{dim}$ destroys the CNS (Mimpen et al., 2020). Recent studies confirm a pathogenic role of $CD56^{dim}$ based on data suggesting myelin damage by ADCC in which Fc-γ receptors, present on most $CD56^{dim}$, are involved (Müller et al., 2021).

Evidence has been found that, in addition to having an immunoregulatory role, NK cells were also found in patients with demyelinating lesions (Baecher-Allan et al., 2018b). There is controversial information regarding the role of the different NK cells in MS: it is believed that NK cells are decisive in the polymerization of T_H and thus in the onset and progress of the disease, and on the other hand, different NK subtypes exert varied roles in EAE (B et al., 2022).

Although the role of NK cells in the development of MS is not fully understood (Yang et al., 2021), different NK-based immunotherapies have been promoted (Moreira et al., 2019). Treatments in which a subset of NK cells (mainly $CD56^{bright}$) is enriched or NK activation is induced have shown therapeutic success, as is the case with drugs such as mitoxantrone and monoclonal antibodies such as daclizumab, and alemtuzumab (Moreira et al., 2019; Schwichtenberg et al., 2021).

5.3. B cells

Recently the role of B cells within MS has caused great interest compared to other immune cells (Gregson et al., 2019; A. K. Pröbstel et al., 2015). In MS, B cells appear to exert all possible functions based on data provided from animal and human studies (Staun-Ram & Miller, 2017). B cells are capable of reproducing both pro-inflammatory and anti-inflammatory cytokines (A. Pröbstel & Hauser, 2018; A. K. Pröbstel et al., 2015) and an imbalance of these generates pathological patterns in MS patients (Staun-Ram & Miller, 2017). Failures in the activity of some naïve and memory B-cell cytokines have been observed in MS patients (Cencioni, Mattoscio, et al., 2021). Likewise, B cells appear to be large promoters of antibodies that target specific tissues of the CNS (Wanleenuwat & Iwanowski, 2019).

B cells are a unique set of APCs that can bind, internalize, process, and express antigen fragments via MHC class II (Li, 2016a; A. Pröbstel & Hauser, 2018) and co-stimulatory molecules (Häusser-Kinzel & Weber, 2019). B cells cooperate in the inflammatory process of MS and the

activation process of pathogenic T cells, either by antigen-dependent mechanisms (APC and Ab producers) or by antigen-independent mechanisms (cytokine production) (Staun-Ram & Miller, 2017).

Currently, B cells are implicated in brain lesions and intrathecal synthesis of OCB, a biomarker to diagnose MS, is present in the CSF in the majority of patients (90% or >95%) with MS (Sospedra, 2018; Staun-Ram & Miller, 2017). B cells are more abundant in MS patients than in other inflammatory brain diseases (Li, 2016b). Moreover, B cells have low numbers in the brain and spinal cord and are located mainly in the perivascular cuffs, and can spread to the surrounding parenchyma (Wekerle, 2017).

The development of clinical therapies based on B cells has shown remarkable levels of efficacy (Gregson et al., 2019). Successful studies with agents targeting B cells such as $CD19^{+}CD27^{+}$ memory B cells have been reported (Gregson et al., 2019).

5.3.1. Regulatory B cells (B_{regs})

This type of B cell is known as B_{regs} because it develops a protective role that goes beyond the production of antibodies. Regulatory B cells secrete anti-inflammatory cytokines such as IL-10, IL-35, and TGF-β (Li, 2016a; Staun-Ram & Miller, 2017).

IL-10 is the great protagonist in B_{regs} cells because it reduces inflammatory cells, expresses co-stimulatory molecules, chemokines, and receptors, inhibits the ability to present antigens, and regulates functions autocrine and paracrine of B cells that secrete IL-10 (Staun-Ram & Miller, 2017).

The altered role of B_{regs} in MS is not fully understood from data collected from different studies. Investigations suggest that MS patients have lower levels of IL-10 compared to control patients (Staun-Ram & Miller, 2017). *In vitro* studies have shown that IL-10 production by B_{regs} cells from MS patients was deficient after stimulation by CD40L compared to HC (Cencioni, Mattoscio, et al., 2021). Moreover, a 2020 study reported that in addition to low levels of IL-10, transitional B cells are unable to suppress T_H1 cell effector functions (Cencioni, Mattoscio, et al., 2021). In this study, transitional B cells ($CD19^{+}CD24^{hi}CD38^{hi}$ B cells) show impaired suppressive capacity in MS because these cells were unable to suppress IFN-γ and TNF-α production (Cencioni, Ali, et al., 2021), which is a key clue to understand the duration of CNS inflammation associated with this disease.

The regulatory role of B cells in mice is associated with interactions between B and T cells (Jelcic et al., 2018) because the suppressive capacity is null in B cells lacking MHC class II, CD40, and B7 (Staun-Ram & Miller, 2017). In experimental models (EAE) it has been seen that B cells and

IL-10 B_{regs} are essential to treat inflammation, although these cannot be required to initiate autoimmunity (Staun-Ram & Miller, 2017).

5.3.2. Autoreactive B cells

In addition to their regulatory role, B cells also have effector characteristics. B cells are responsible for stimulating T cells where the antigen is presented by MHC class II and co-stimulatory molecules, generating subsets of pathogenic T cells and myeloid cells to be activated by cytokine secretion (Staun-Ram & Miller, 2017). Autoreactive B cells are responsible for secreting pro-inflammatory cytokines such as IL-2, IL-4, IL-6, IL-12, IL-17, (Matsushita, 2019), IFN-γ, TNF-α, Lt-α (Rahmanzadeh et al., 2018; Staun-Ram & Miller, 2017). Furthermore, once these autoreactive B cells are activated, autoAbs begin to be produced, generating harmful effects for the patient (Matsushita, 2019).

Reports in MS and other autoimmune diseases reveal that B cells show high levels of secretion of proinflammatory cytokines mainly IL-6, which is responsible for the induction of T_H17 cell differentiation (Staun-Ram & Miller, 2017) as well as T_H17 polarization leading to an increase in the severity of the disease (Matsushita, 2019). Furthermore, high levels of IFN-γ and B_{eff} have been found to dull T_{reg} action and enhance T_H cell responses in proteoglycan-induced arthritis while GM-CSF action is high in MS patients (Matsushita, 2019). Likewise, patients with MS have also presented a high frequency of memory B cells, which have expressed GM-CSF, IL-6, and TNF-α, for which a study eliminated these B cells, obtaining a low pro-inflammatory response of IL-6 from macrophages through a GM-CSF-dependent mechanism (Häusser-Kinzel & Weber, 2019).

A recent study in which the levels of cytokines in the different stages of MS (PPMS, SPMS) were analyzed found a greater amount of pro-inflammatory cytokines (TNF-α, IL-12, IFN-γ, IL-6) from B cells and at the same time low levels of anti-inflammatory cytokines (IL-13) (Staun-Ram & Miller, 2017). In this study, high concentrations of CD19+ cells expressing TNF-α were found in PPMS patients compared to RRMS patients ($p=0.017$), SPMS patients ($p=0.01$) and HC ($p=0.005$) (Piancone et al., 2016). In addition, high levels of IFN-γ were found in PPMS patients compared to HC ($p<0.005$), SPMS patients, RRMS, and PPMS patients presented significant amounts of IL-6 compared to HC ($p=0.006$). IL-12 was also found to be present in high amounts in the various stages of MS while low levels of IL-13 were found compared to HC ($p<0.05$) (Piancone et al., 2016).

Figures 11, 12, and 13 show how the different B lymphocytes secrete proinflammatory cytokines in higher amounts and how B_{regs} are found in low amounts in patients with MS.

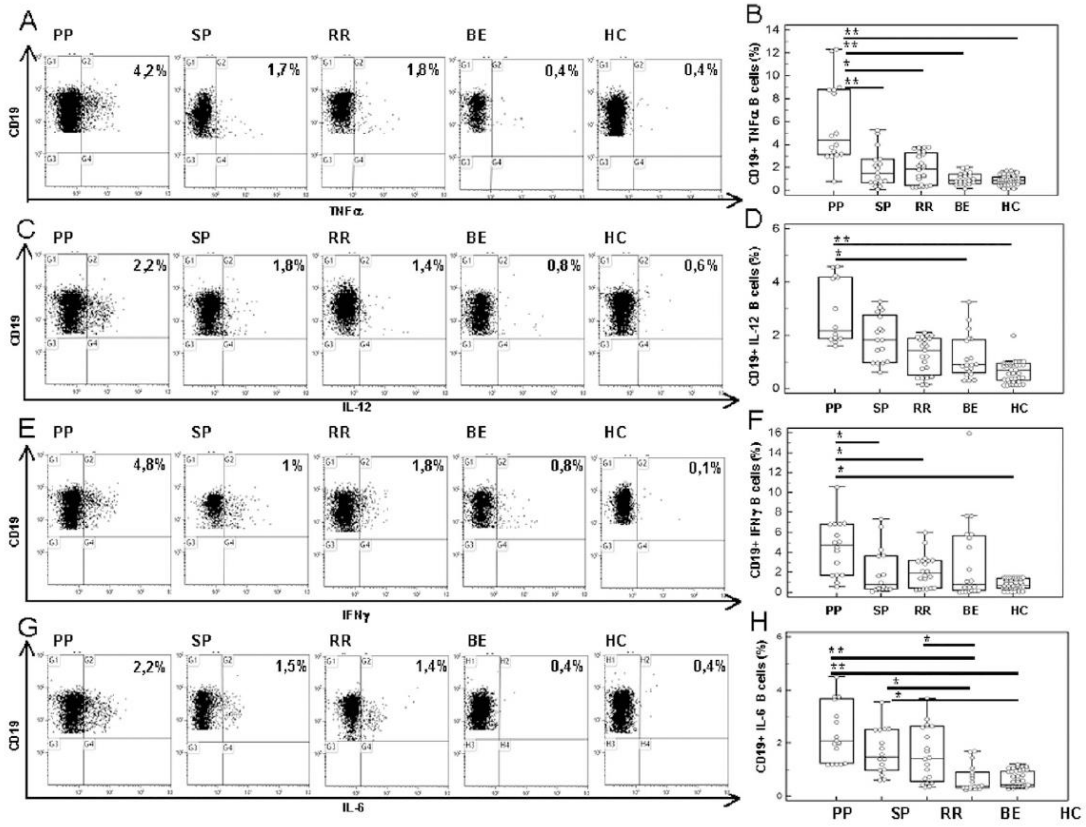

Figure 11. Results of proinflammatory cytokines expressed by CD19+ B lymphocytes. *Lymphocytes were stimulated by MOG from patients with PPMS, SPMS, RRMS, BEMS, and HC matched for age and sex as shown in panels A (TNF-α), C (IL-12), E (IFN-γ), and G (IL-6). In addition, summary results are shown in panels B (TNF-α), D (IL-12), F (IFN-γ), and H (IL-6). Each box ranges from the 25th to the 75th percentile. A line through the box indicates the mean. Lines extending from the boxes indicate extreme values. Statistical significance: * p < 0.05, * * p < 0.01, * * * p < 0.001, Kruskal- Wallis test. Taken from Piancone et al., 2016.*

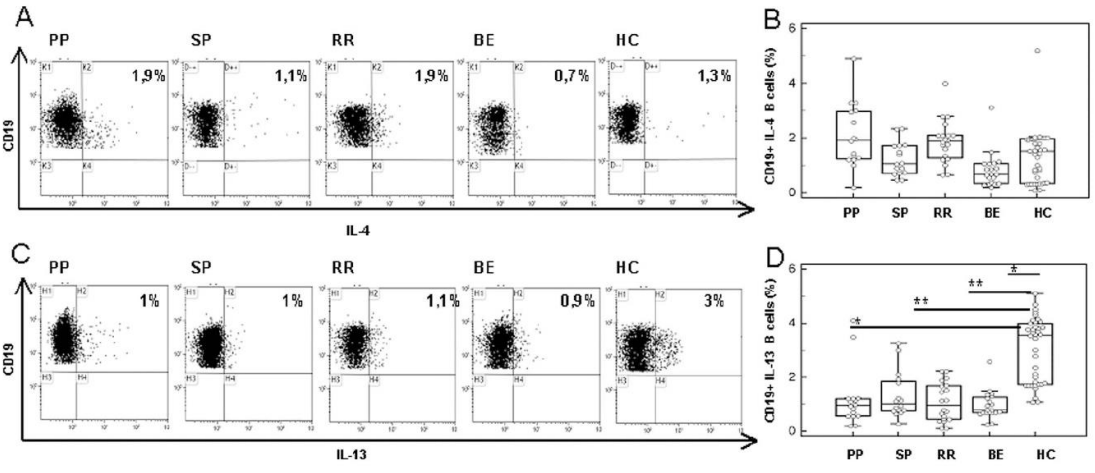

Figure 12. Results of anti-inflammatory cytokines expressed by CD19+ B lymphocytes. *Lymphocytes were stimulated by MOG from patients with PPMS, SPMS, RRMS, BEMS, and HC matched for age and sex as shown in panels A (IL-4), and C (IL-13). In addition, summary results are shown in panels B (IL-4), and D (IL-13). Each box ranges from the 25th to the 75th percentile. A line through the box indicates the mean. Lines extending from the boxes indicate extreme values. Statistical significance: * p < 0.05, * * p < 0.01, * * * p < 0.001, Kruskal- Wallis test. Taken from Piancone et al., 2016.*

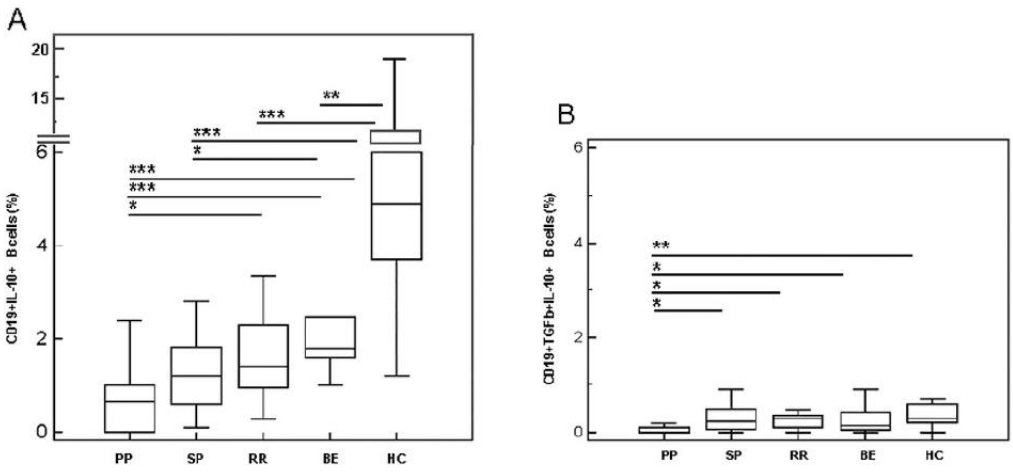

Figure 13. Results of MOG-stimulated CD19+ regulatory B cells*. CD19+ B cells expressing IL-10 are shown in panel A while B cells co-expressing IL-10 and TGFβ are shown in panel B. Lymphocytes were stimulated by MOG from patients with PPMS, SPMS, RRMS, BEMS, and HC matched for age and sex. Each box ranges from the 25th to the 75th percentile. A line through the box indicates the mean. Lines extending from the boxes indicate extreme values. Statistical significance: * p < 0.05, * * p < 0.01, * * * p < 0.001, Kruskal- Wallis test. Taken from Piancone et al., 2016.*

These results reveal that different subpopulations of functional B cells are found in different amounts in healthy patients and MS patients. It was possible to see that the MOG-stimulated B cells of patients with PPMS have high levels of IL-6, unlike the other study groups such as HC. This cytokine is known to enhance the inflammatory process in MS. In addition, low amounts of $CD19^{+}/IL\text{-}10^{+}/TGF\beta^{+}$ B lymphocytes were found in PPMS patients, showing that their absence or low modulation worsens neurological functions due to the lack of control of inflammation. In short, it was possible to see that the role played by B cells is decisive in the immunopathology of MS due to a greater secretion of proinflammatory cytokines.

5.4. Immune response

Once the different immunological agents involved in MS have been defined, it is necessary to define some details about the immunological reaction of this disease both outside and inside the CNS. This immunological disease has associated genetic and environmental factors and each of them contributes significantly to the development and progress of the pathology.

Figure 12 shows the process of development of MS external to the CNS in which the role played mainly by T and B cells can be seen.

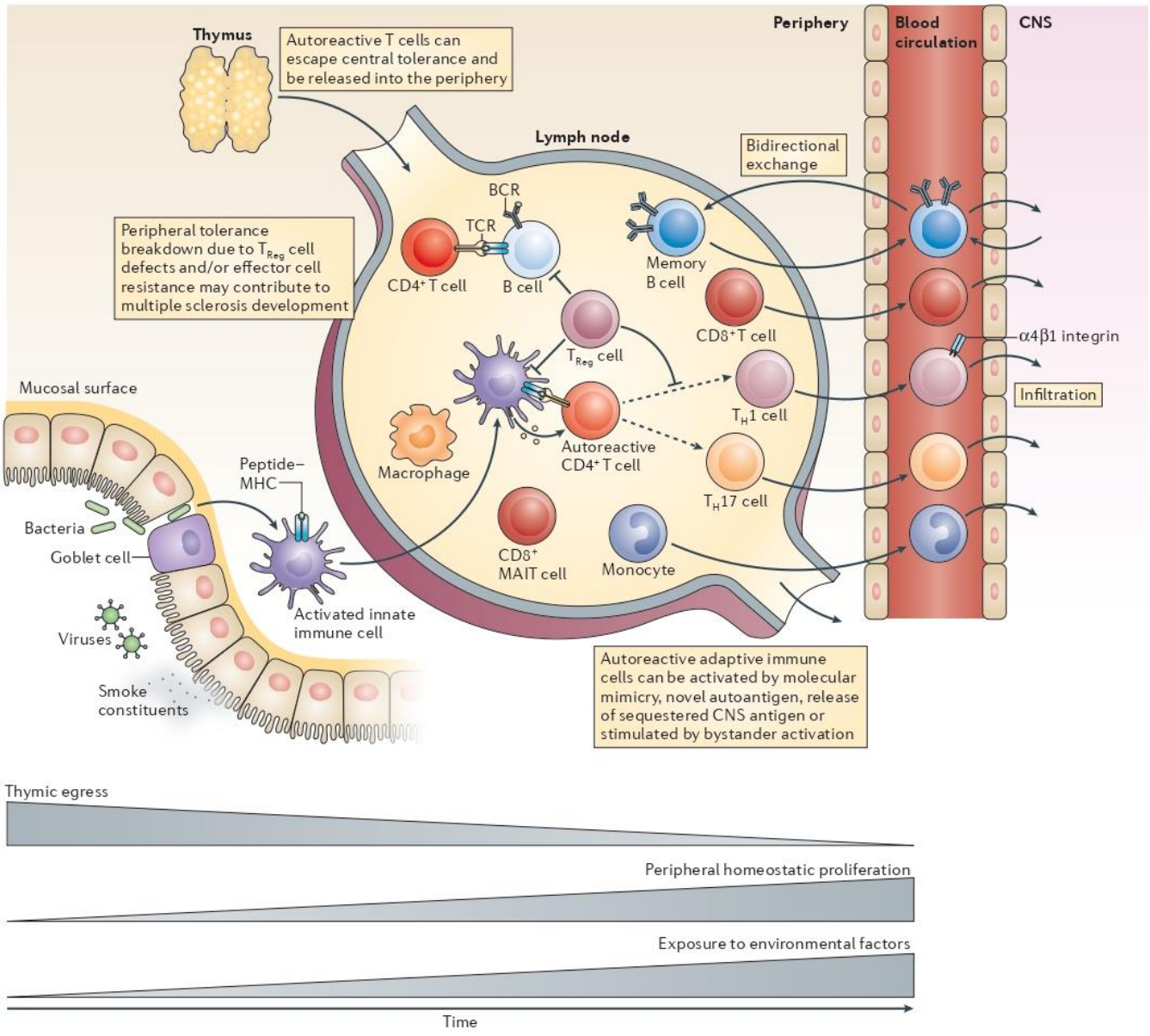

Figure 14. Changes in the immune system outside the CNS. *Reactive T cells are eliminated in the process of establishing central tolerance in the thymus. This process is not precise and some autoreactive T cells are released in the periphery. This tolerance is altered by reduced function of regulatory T cells and/or if the resistance of effector B and T cells to suppressive mechanisms is increased. Thus, autoreactive T and B cells become aggressive effector cells aided by molecular mimicry, presentation of new antigens, and recognition of CNS-sequestered antigens released in the periphery. In addition, environmental and genetic factors converge and contribute to the immune reaction. When these cells are activated, CD8+ T cells, differentiated T helper 1 (TH1) and TH17 cells, B cells, and innate immune cells infiltrate the CNS developing an inflammatory process and tissue damage. Those lymphocytes that leave the CNS undergo an affinity maturation process in the lymph nodes before entering the target organ and generating further damage. Dashed lines indicate differentiation processes. Taken from: (Dendrou et al., 2015).*

One of the environmental factors that generate an increased risk of MS is smoking. Although the role of tobacco in the development of MS is controversial, the most widely accepted idea is that it influences treatment by generating neutralizing antibodies. Although tobacco has this decisive role, it is not the only environmental factor involved in MS and it is combined with genetic factors. On the other hand, failures in central tolerance in the thymus can cause many autoreactive T cells to trigger an aggressive process and exert dominance, also due to the lack of regulation of the T_{regs}.

Those autoreactive T cells interact with the B cells which is a vital process in MS and subsequently, these T cells secrete T_H1 and T_H17 that will stimulate a process of infiltration and attack on the myelin sheath. In addition, monocyte infiltration of the CNS and a bidirectional infiltration of memory B cells from the lymph node are noticeable.

Figure 15 reveals important aspects of inflammation in MS and how this process is associated with the neurodegenerative process.

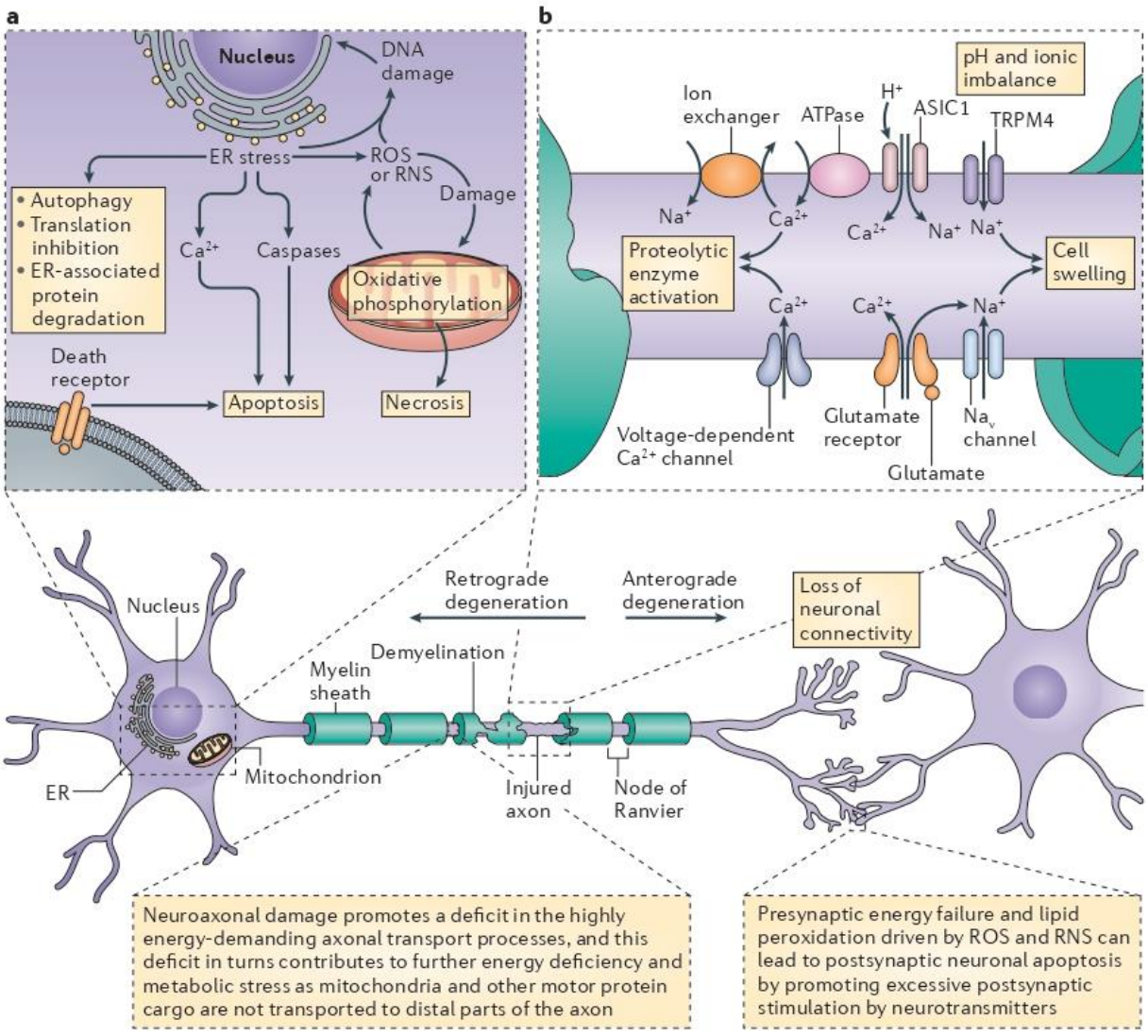

Figure 15. The neurodegenerative process is the result of chronic inflammation. *The production of ROS and RNS likely promotes mitochondrial injury due to the accumulation of damaging mutations in mitochondrial DNA (Figure 13a). This in turn generates metabolic stress, protein misfolding in the ER, loss of neuronal fitness, and energy deficiency. Neuronal ion channels such as ASIC1, TRPMA, and voltage-gated sodium channels such as Nav 1.2 and Nav 1.5 maintain homeostasis by compensatory redistribution. Excess release of glutamate accentuates tissue damage (Figure 13b). Taken from:* (Dendrou et al., 2015b).

A hallmark of MS is inflammation. The release of ROS and RNS mainly generates metabolic stress, and energy deficiency, which critically affects transport to maintain neuroaxonal function because this process requires a large amount of energy. It should be mentioned that also several

mitochondrial and neurometabolic disorders. Excess of the main neurotransmitter, glutamate, promotes an ion imbalance that perpetuates tissue damage. This axonal injury can have degenerative mechanisms that can spread backward known as retrograde degeneration or retrograde neuronal death or can spread to the distal axon terminal known as anterograde degeneration or Wallerian degeneration, even affecting nearby presynaptic and postsynaptic neurons, respectively (Dendrou et al., 2015b). In addition, buffer mechanisms of neuroaxonal injury are activated, such as the expression of genes favorable to survival and the action of the cannabinoid system, but they are quickly annulled and the neuronal damage worsens. This allows possibly elucidating of a therapy based on neuroprotective pathways such as remyelination although anti-inflammatory agents may be required to effectively treat the clinical picture.

Figure 16 shows imbalances of the immune system within the CNS in the early and late stages of MS whose common aspect is the infiltration of different immunological agents.

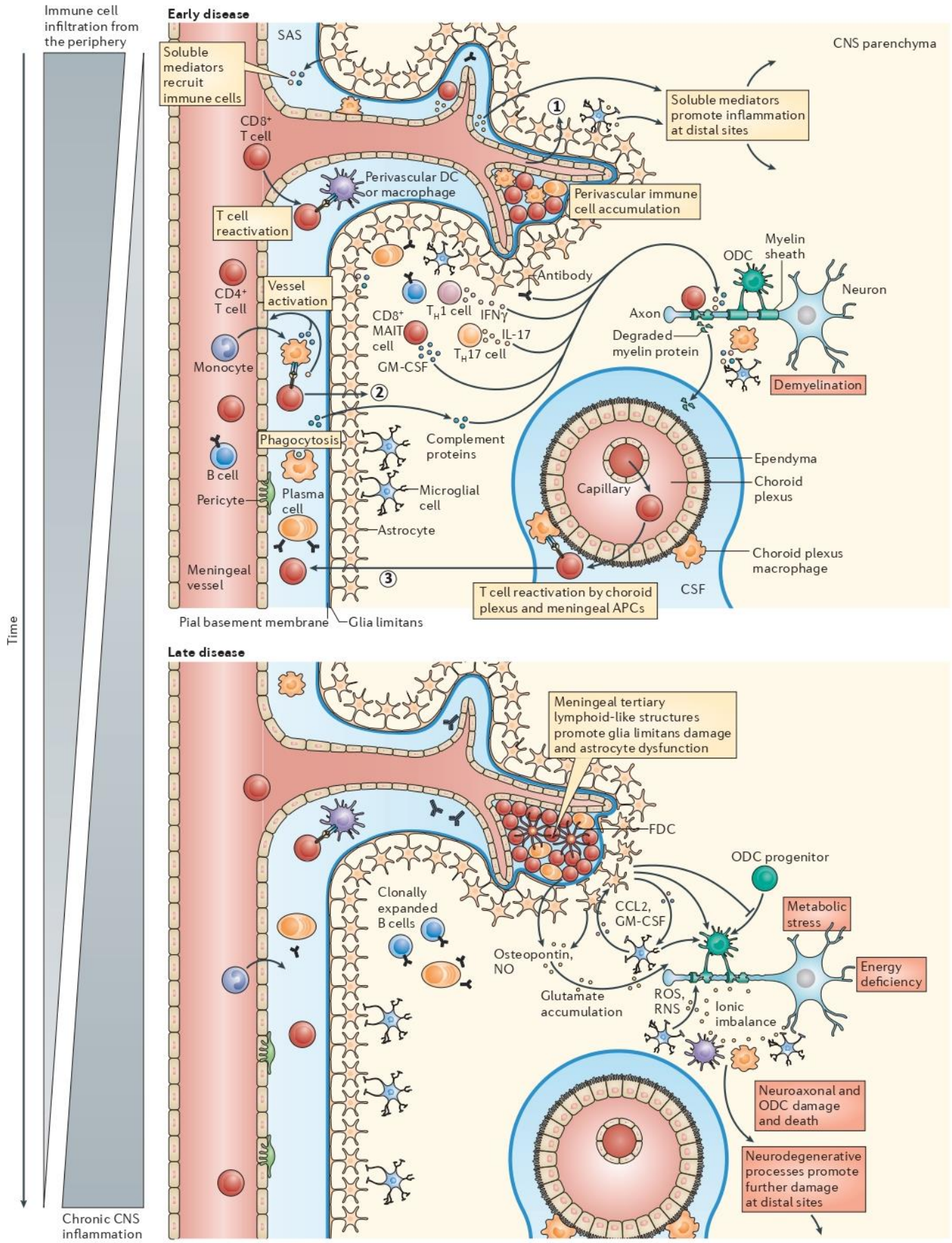
Immune cell infiltration from the periphery
Time
Chronic CNS inflammation
Early disease
SAS
CNS parenchyma
Soluble mediators recruit immune cells
CD8+ T cell
Perivascular DC or macrophage
T cell reactivation
Soluble mediators promote inflammation at distal sites
Perivascular immune cell accumulation
Myelin sheath
ODC
Neuron
Antibody
CD4+ T cell
Vessel activation
CD8+ MAIT cell
TH1 cell
IFNγ
IL-17
TH17 cell
GM-CSF
Axon
Degraded myelin protein
Demyelination
Monocyte
Phagocytosis
Complement proteins
B cell
Pericyte
Plasma cell
Microglial cell
Astrocyte
Ependyma
Choroid plexus
Capillary
Choroid plexus macrophage
Meningeal vessel
T cell reactivation by choroid plexus and meningeal APCs
CSF
Pial basement membrane
Glia limitans
Late disease
Meningeal tertiary lymphoid-like structures promote glia limitans damage and astrocyte dysfunction
FDC
ODC progenitor
Clonally expanded B cells
CCL2, GM-CSF
Metabolic stress
Osteopontin, NO
Energy deficiency
Glutamate accumulation
ROS, RNS
Ionic imbalance
Neuroaxonal and ODC damage and death
Neurodegenerative processes promote further damage at distal sites

Figure 16. The altered immune system within the CNS in the early and late stages of MS. *Infiltration of various immune cells from the periphery occurs from the early stage of MS (upper panel). This infiltration process possibly occurs from the meningeal blood vessels and crosses the BBB (1) or the subarachnoid space (2) or from the choroid plexus through the CSF (3). Peripheral innate and adaptive immune cells possibly accumulate in perivascular spaces and may subsequently enter the CNS parenchyma. In a late stage (lower panel), immune cell infiltration declines although CNS-specific inflammation and neurodegeneration remain. This process is stimulated by microglia, astrocytes, and GM-CSF. Taken from:* (Dendrou et al., 2015b)

The infiltration of immune cells is the beginning of a complex process that will end in neurodegeneration. This infiltration can occur in various ways, although a slightly higher activity has been seen in the early stages compared to the late stages of MS. Peripheral innate and adaptive immune cells, activated CNS-resident microglia, and astrocytes promote demyelination and neuroaxonal and ODC injury by direct mechanisms that depend on cell contact and the intervention of soluble inflammatory and neurotoxic mediators (Dendrou et al., 2015b). In the late stage, infiltration decreases possibly due to the depletion of adaptive immune cells from chronic antigen exposure. In addition, tertiary meningeal lymphoid-like structures that are known to contribute to the secondary progressive state may also contribute to the inflammatory process in late-stage MS.

6. Pathology

The alterations of the immune system in MS are appreciable in lesions that appear in different anatomical regions. The pathological features of MS and hallmarks of this disease are inflammation, demyelination, reactive gliosis, and neuroaxonal damage (Zéphir, 2018). These events are triggered by the infiltration of immune cells in both the spinal cord and the brain (Zéphir, 2018). Proinflammatory leukocytes once infiltrated cause severe damage to the myelin sheath resulting in the neuronal loss (Ruiz et al., 2019). Demyelination affects both white and gray matter regions of the CNS (Lassmann, 2018a). Formerly it was believed that lesions were only present in the white matter but today such lesions are also found in the gray matter (Silva et al., 2021), basal ganglia, brain stem, cortex, and spinal cord (gray matter) (Lassmann, 2018a). The most injured regions in patients with MS are detailed below.

6.1.White matter lesions

The lesions found in the WM show inflammation and demyelination due to the infiltration of monocytes, microglia, and B and T cells (Filippi et al., 2020). Studies suggest that in the early stages of the lesion these immune agents have myelin residues in their cytoplasm confirming that there is a continuous degradation of myelin (Filippi et al., 2020). Indeed, inflammatory demyelination is linked to axonal damage (Filippi et al., 2020).

Although studies suggest a close relationship between WM damage and GM atrophy at certain stages of MS development, other studies reveal partial independence between GM cortical atrophy and WM lesions (Storelli et al., 2021; Zhang et al., 2021). A recent study showed that about 50% of the

data collected showed an independent relationship between cortical atrophy and WM-associated damage (Zhang et al., 2021). In addition, this study showed that WM damage and deep GM atrophy are strongly linked (Zhang et al., 2021).

6.2. Gray matter lesions

WM atrophy does not contribute as much to the development of multiple sclerosis as GM atrophy (Storelli et al., 2021). Aging also denotes brain atrophy but in patients with MS, this process is 2 to 3 times faster (Koskimäki et al., 2018). This atrophy in patients with MS is mostly influenced by lesions in the gray matter causing demyelination in conjunction with a reduction of neurite cross-section and synapse or glial densities (Al-Radaideh et al., 2021; Tsouki & Williams, 2021).

Diffuse abnormalities in the gray matter, specifically in the cortical region, have been evidenced by neuroimaging (Tagge et al., 2021). Evidence shows that atrophy in the brain and the resulting physical disabilities are due to a greater sensitivity of a reduced volume of gray matter compared to low levels of white matter (Cao et al., 2021). From different studies, it has been determined that gray matter volumes in MS patients (specially RRMS) are small compared to control groups (Cao et al., 2021). This volume reduction in regions such as PCG provided strong information about the connection between the patient's disability and the role of gray matter regions in MS (Cao et al., 2021).

Table 5 shows the results of the work by Cao et al. (2021) in which the relationship between volume variations of the gray matter and the disability of patients with MS can be seen.

Table 5. Results of a meta-analysis on gray matter volume changes in a set of RRMS and PPMS patients. *This meta-analysis had statistical significance based on Monte Carlo randomizations and spurious findings were minimized using a stringent threshold of P < 0.0005. 34 datasets of RRMS and PPMS patients and healthy patients were compared and the most relevant ones are included according to the objectives of this review. Taken from:* (Cao et al., 2021)

	Subjects (female, n)		Mean age (years, SD)		Mean NGMV (ml, SD)		Disease duration (years)	EDSS (median)	PASAT z-score (median)
	MS	HC	MS	HC	MS	HC			
1	125 (82)	52 (33)	36.8 (10.7)	37.3 (13.1)	807 (64)	840 (61)	9.6 ± 8.7	2.0 ± 1.5	NA
2	15 (12)	20 (13)	33.3 (7.8)	36.8 (6.8)	679.4 (91.6)	762.4 (89.3)	6.0 ± 2.0	1.5 ± 2.0	NA
3	19 (NA)	19 (NA)	32.9 (8.0)	31.7 (7.5)	711.8	713	1.7 ± 1.4	1.5 ± 1.1a	NA
4	10 (NA)	10 (NA)	36.7 (7.6)	31.7 (7.5)	667.3	713	2.0 ± 1.7	1.7 ± 1.2a	-1.53
5	20 (15)	20 (12)	34.6 (10.4)	33.4 (10.4)	805.3 (56.1)	796.0 (44.1)	6.0 ± 4.3	1.5 ± 1.3	NA
6	20 (14)	20 (12)	33.8 (6.3)	33.4 (10.4)	792.2 (41.37)	796.0 (44.1)	5.3 ± 4.4	1.7 ± 0.6	NA
7	128 (86)	35 (18)	36.1 (9.2)	38.3 (10.9)	607.7 (66.1)	660.1 (49.7)	10.1 ± 7.2	2.8 ± 1.3	NA
8	14 (8)	14 (8)	38.6 (8.5)	38.7 (8.4)	770 (40)	826 (58)	10.6 ± 6.6	1.5 ± 0.4	NA
9	10 (6)	14 (8)	38.0 (7.7)	38.7 (8.4)	795 (66)	826 (58)	8.2 ± 6.2	1.5 ± 0.1	NA
10	23 (16)	61 (35)	36.0 (9.5)	13.6 (3.4)	748 (58)	826 (57)	9.9 ± 8.2	2.0 ± 1.3	NA
11	38 (24)	61 (35)	36.0 (9.5)	13.7 (3.1)	793 (68)	826 (57)	7.1 ± 4.9	1.5 ± 1.0	NA

This meta-analysis concluded that alterations in the GMV in RRMS and PPMS patients in networks such as corticostriatum-thalamus, sensorimotor, and insula may play an important role in the pathophysiology of RRMS and PPMS (Cao et al., 2021). Furthermore, the findings of severe reductions in cingulate and caudate volume in RRMS patients and severe atrophy in the cerebellum in PPMS patients are significant (Cao et al., 2021). The precentral gyrus is possibly the most sensitive region linked to disability. In short, there is solid evidence of the distribution of GMV atrophy in RRMS and PPMS patients.

In addition, a study found that subcortical gray matter presents high microglial activity in patients with SPMS associated with physical disability in contrast to patients with RRMS and those belonging to HC (Singhal et al., 2019). This microglial activity in gray matter is different from the microglial response in the white matter depending on the pathological characteristics of each one (Tsouki & Williams, 2021). Interestingly, inflammation in the white matter is the driver of the development of deep atrophy in the gray matter.

Likewise, gray matter lesions occur in the late stage once symptoms are noticeable which also suggests a low correlation with the duration of MS (Salim et al., 2021). The process of gray matter demyelination along with neuronal loss are observable in the prelude to MS developing irreversible cognitive disabilities (Cao et al., 2021; Koskimäki et al., 2018). It is important to mention that lesions in the gray matter are less inflammatory than those generated in the white matter because microglia in the gray matter heal more efficiently (Tsouki & Williams, 2021).

6.3. Spinal cord lesions

It has been observed that patients with MS present lesions in the spinal cord which is vital to diagnose and prognosticate this disease (Dekker & Wattjes, 2017; Schmierer et al., 2018) as it is one of the main events in the progression of this neurological disorder (Moccia et al., 2020). This spinal cord damage is recorded in about 90% of patients with MS, specifically RRMS (Leguy et al., 2021). This type of lesion allows for the differentiation of MS pathology from other diseases (vascular for example) (Ciccarelli et al., 2019; Dekker & Wattjes, 2017). However, the study of spinal cord damage is limited due to its geometry and anatomical location (Leguy et al., 2021).

The spinal cord, despite its small size, contains all the motor and sensory nerve pathways of the extremities and is therefore associated with the weakening of physical abilities (Leguy et al., 2021; Mariano et al., 2021; Muccilli et al., 2018). Distinctive signs of this debilitation are a lack of coordination or sensory loss and gait impairment (Ciccarelli et al., 2019). On the one hand, emphasis has been placed on spinal cord atrophy, specifically in the cervical spinal cord, obtaining data confirming the link with clinical disability (Dekker & Wattjes, 2017) and on the other hand, post-mortem studies reveal prevalent lesions up to the lumbar region of the spinal cord (Leguy et al., 2021). Even data reveal that 57 to 62% of the spinal cord has been lost in MS patients regardless of axonal size and spinal cord level (Leguy et al., 2021). Data suggest that about 60% of long axons are lost in the spinal cord (Ciccarelli et al., 2019; Rocca et al., 2020) after 30 years of disease diagnosis (Ciccarelli et al., 2019). Moreover, it is known that the rate of atrophy is faster in the spinal cord than in the brain, probably due to different mechanisms (Ciccarelli et al., 2019). Also, while inflammation may play a decisive role in the synaptic loss, it may not influence the lack of connectivity in the spinal cord (Petrova et al., 2020).

However, the location of the lesion in the spinal cord plays an important role in the course of the disease (Leguy et al., 2021). A cross-sectional study evaluating 642 patients with RRMS concluded that those with lesions in the central and lateral regions of the cervical spine had a high EDSS score (Leguy et al., 2021).

Figure 17 shows the frequency of cervical spinal cord injuries using voxelwise injury frequency maps.

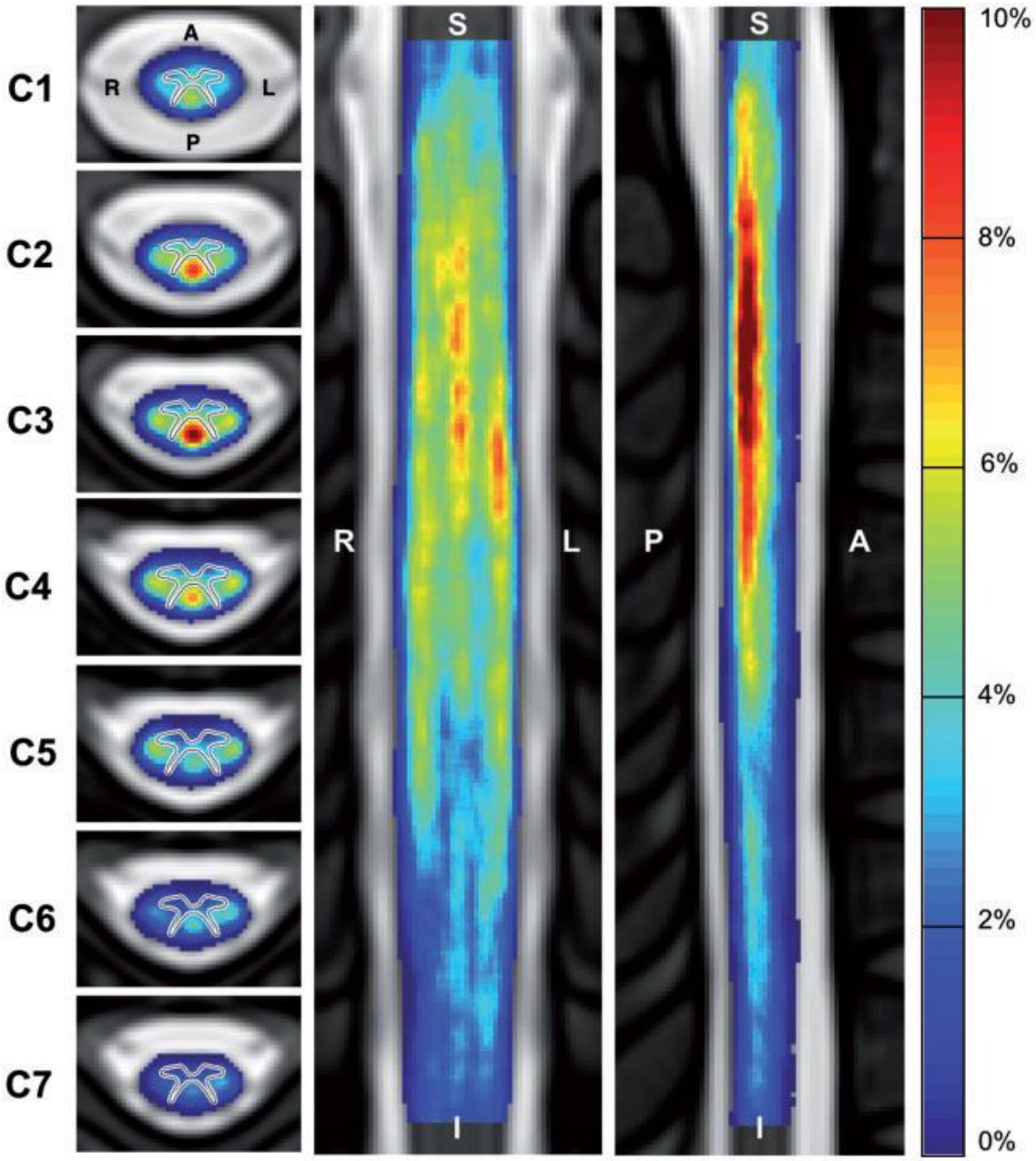

Figure 17. Cervical spinal cord injuries and their frequency in MS patients. *The frequency of lesions is shown in different planes: axial (left), coronal (middle), and sagittal (right). In the case of the axial plane, the frequency of injuries at each vertebral level is averaged. In the axial plane, the contour of the gray matter has been superimposed. Taken from:* (Eden et al., 2019)

From the above image, it could be seen that the most frequent lesions were in the upper cervical spinal cord (C1-C3). In addition, it was observed that the most affected regions were the dorsal column together with the lateral cords. In summary, the lesions affect the dorsal column and the lateral cords more because high frequencies were observed in the center of these regions.

Figure 18 shows the different phenotypes of MS and the frequencies of associated lesions and where it can also be seen that the lesions occurred less in patients with CIS.

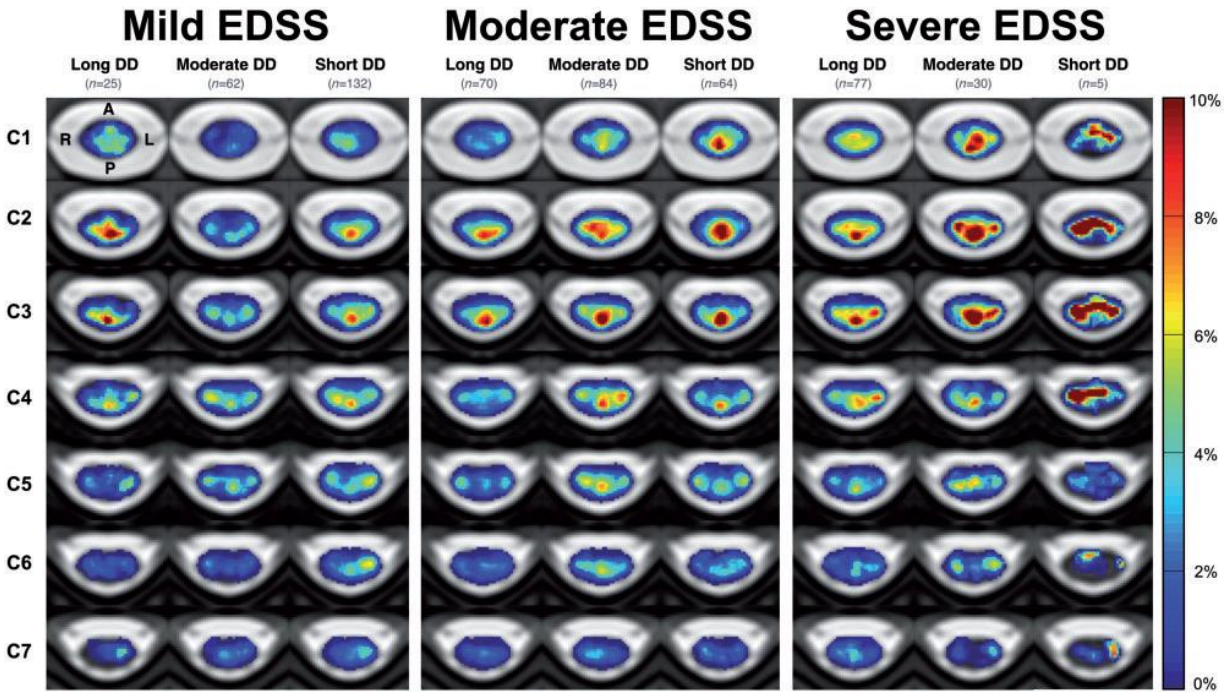

Figure 18. Cervical spinal cord injuries and their frequency in MS patients*. EDSS scores categories: mild (0–2.5), moderate (3–5.5), and severe (>=6), and sub-categorized by disease duration categories: short (0–5 years) moderate (5–15 years), long (>=15 years). A patient whose EDSS score is mild and the duration of the disease, this condition is called benign multiple sclerosis. From:* (Eden et al., 2019)

In this case, the lesions were more prominent in patients with RRMS, SPMS, and PPMS, and vertebral levels C2 and C3 were the most affected. These data show that injury frequency is associated with EDSS score in the lateral cord and central cervical spine regions (Eden et al., 2019). These findings corroborate the state of progression of demyelination and the consequent deterioration and disability.

6.4. Thalamus lesions

The thalamus is the largest region of the diencephalon which is divided into 3 regions both functionally (the relay nuclei, the association nuclei, and the nonspecific nuclei) and anatomically (anterior, lateral, and medial) (Capone et al., 2019). The thalamus presents a large number of cortical connections important in motor, sensory, and executive functions as well as functions related to memory, attention (Amin & Ontaneda, 2021; Weeda et al., 2020) emotion, sleep and wakefulness regulation (Weeda et al., 2020), eye movement regulation and posture maintenance (Trufanov et al., 2021).

Recently the thalamus has gained major interest for its role in the pathophysiology of MS (Capone et al., 2019; Rojas et al., 2018). The role of the thalamus in MS has been elucidated by studies

that, although informative and limited by sample size, retrospective designs, registry biases, and absence of treatment effects, provide interesting details (Minagar et al., 2013). Neuronal damage to the thalamus is caused not only by Wallerian degeneration but also by diffusible cytokines, oxidative stress, CD8 T-cell mediated cytotoxicity, and excitotoxicity (Minagar et al., 2013).

Studies suggest that structural and functional changes in the thalamus are part of the development of MS (Capone et al., 2019; Tona et al., 2014). Studies show that there is volume loss (Amin & Ontaneda, 2021; Capone et al., 2019; Weeda et al., 2020) in both the basal ganglia and frontoparietal cortex associated with cognitive failures (Amin & Ontaneda, 2021) accompanied by demyelinating, inflammatory and neurodegenerative processes in MS patients (Capone et al., 2020). A recent study reveals that volume loss was 0.5% per year and the level of thalamic atrophy was 1% per year in the early stages of the disease (Weeda et al., 2020). Even recent data show that low thalamic volume was found in patients with severe MS (Rojas et al., 2018). In addition, a recent study showed that structural damage in the thalamus and basal ganglia are associated with gait speed failures in patients with MS (Motl et al., 2021) as shown in table 6.

Table 6. Bivariate correlations between cognitive processing speed, walking ability, aerobic capacity, and subcortical gray matter volume in 62 individuals with MS. *In this study, the T25FW was used as a measure of walking speed. In addition, 6MW constitutes a measure of walking endurance and aerobic capacity was measured by peak power output (Wpeak). Significant correlations were found between cognitive performance (SDMT), and gait (T25FW and 6MW). Likewise, SGM volumes were significantly correlated with cognitive processing speed, gait performance, and aerobic capacity. Taken from:* (Motl et al., 2021)

Variable	1	2	3	4	5	6	7	8
1. Symbol Digit Modalities Test (# correct)	–							
2. Timed 25-foot walk (ft/s)	0.59*	–						
3. 6-Minute walk (ft)	0.58*	0.92*	–					
4. Aerobic capacity (W_{peak})	0.64*	0.80*	0.84*	–				
5. Thalamic volume (mm^3)	0.58*	0.47*	0.46*	0.50*	–			
6. Caudate volume (mm^3)	0.42*	0.48*	0.44*	0.45*	0.76*	–		
7. Putamen volume (mm^3)	0.55*	0.41*	0.36*	0.41*	0.71*	0.81*	–	
8. Pallidum volume (mm^3)	0.50*	0.55*	0.52*	0.54*	0.81*	0.84*	0.77*	–

*$p < 0.05$.

The results reveal disability in MS patients as a result of gray matter volume reduction. Significant correlations were found between cognitive performance and gait, revealing the cognitive-motor link. Likewise, cognitive and gait performance was significantly correlated with aerobic capacity (Motl et al., 2021). Also, the volumes of the thalamus, caudate, putamen, and pallidum were correlated with cognitive processing speed, walking performance, and aerobic capacity (Motl et al., 2021). Although these data are revealing, one must be careful with the

interpretation that can be given to these data because the correlation analyzes are not strongly sustainable.

Malfunctioning circuits between the thalamus, cortex, and basal ganglia can cause fatigue, which is one of the prominent symptoms in MS patients affecting up to 80% (Capone et al., 2020). This phenomenon is produced by the high activity of networks involving the thalamus, basal ganglia, and cortex (Capone et al., 2020; Chen et al., 2021).

6.5. Cerebellum lesions

In addition to the previously mentioned areas, the cerebellum is also an area affected by MS. The cerebellum is a structure that is responsible for receiving information from the spinal cord and other brain regions coordinating movements such as balance, posture, speech, and coordination (Argento et al., 2021; Schreck et al., 2018). Because of this, damage to the cerebellum causes loss of memory, attention, and visuospatial, emotional, and language functions (Parmar et al., 2018; Schreck et al., 2018). Unlike brain lesions, this type of cerebellar lesions can be qualitatively visualized instead of a total loss of a certain function such as disorganized movements and unintelligible speech (Parmar et al., 2018).

The cerebellum was a neglected area in the study of MS but new studies have found demyelinated and atrophic regions in the cerebellar cortex (Parmar et al., 2018; Schoonheim et al., 2021). Current studies suggest that the degree of demyelination is greater than in any other area of the brain (the gray matter is five times more demyelinated) (Eshaghi et al., 2018). Today there is talk of a syndrome associated with cerebellar malfunction called CCAS which includes impairments such as executive dysfunction, spatial cognition, failures in verbal fluency, working memory, abstract reasoning (Schreck et al., 2018), planning and visual-spatial skills (Parmar et al., 2018). In addition, overlying inflammation in the cerebellum may play an amplifying role in pathological mechanisms such as secondary retrograde neurodegeneration (Eshaghi et al., 2018).

6.6. Meningeal lesions

Interesting data have been found that reveal that there is an important role for the meninges in the pathogenesis of MS. The meninges play an important role in antigen drainage, immune surveillance, and inflammation (Bevan et al., 2018). Meninges cells secrete proinflammatory cytokines such as TNF, and interleukins such as iNOS and IL-6 in an immune reaction (Silva & Ferrari, 2019b). The role of the meninges is wholly vital to the CNS because it is its first line of defense against threats (Silva & Ferrari, 2019a).

In MS, the inflammatory development of the meninges denotes an accelerating disease process and an indication of the early death of the patient (Silva & Ferrari, 2019a). The inflammatory activity of the meninges is correlated with cortical microglial activity, neuronal degeneration, and demyelination in the developmental stages of the disease (Bevan et al., 2018). Specifically, large numbers of inflammatory leptomeningeal cells such as T and B lymphocytes, plasma cells, and macrophages have been observed in both the cerebral sulci and cerebellar and spinal cord leptomeninges (Bevan et al., 2018). A recent study found high numbers of macrophages (CD68+), T cells (CD3+), and B cells ($CD20^{+}$) (Ahmed et al., 2022; Bevan et al., 2018; Silva et al., 2021) in addition to aggregates of $CD20^{+}$ B cells and $CD3^{+}$ T cells (Bevan et al., 2018) and myeloid cells capable of expressing TNF (Silva & Ferrari, 2019a).

Inflammation of the meninges in MS is directly related to the demyelinating process suffered by the spinal cord, cerebellum, and prosencephalon in patients with acute MS (Bevan et al., 2018) and loss of neurites (Magliozzi et al., 2019) TNF and TNF-y are known to have a synergistic role which causes increased apoptosis in oligodendrocytes and thus release inflammatory mediators involved in demyelination and neurodegeneration (Magliozzi et al., 2019). In line with the above, the severity of spinal cord pathology is linked to meningeal inflammation (Magliozzi et al., 2019). It has been identified that this pathology after 2 years can be decisive (Bevan et al., 2018) even because it is the precedent to the appearance of white matter lesions (Silva & Ferrari, 2019a). On the other hand, a study has recorded that there is a higher density of myeloid cells in the meninges in patients with MS as opposed to control patients (Ahmed et al., 2022).

Figure 19 presents the results of the study conducted by Ahmed et al. which show that the number of $CD3^{+}$ T cells and $CD20^{+}$ B cells per unit length of the meninges increased in patients with MS compared to patients healthy.

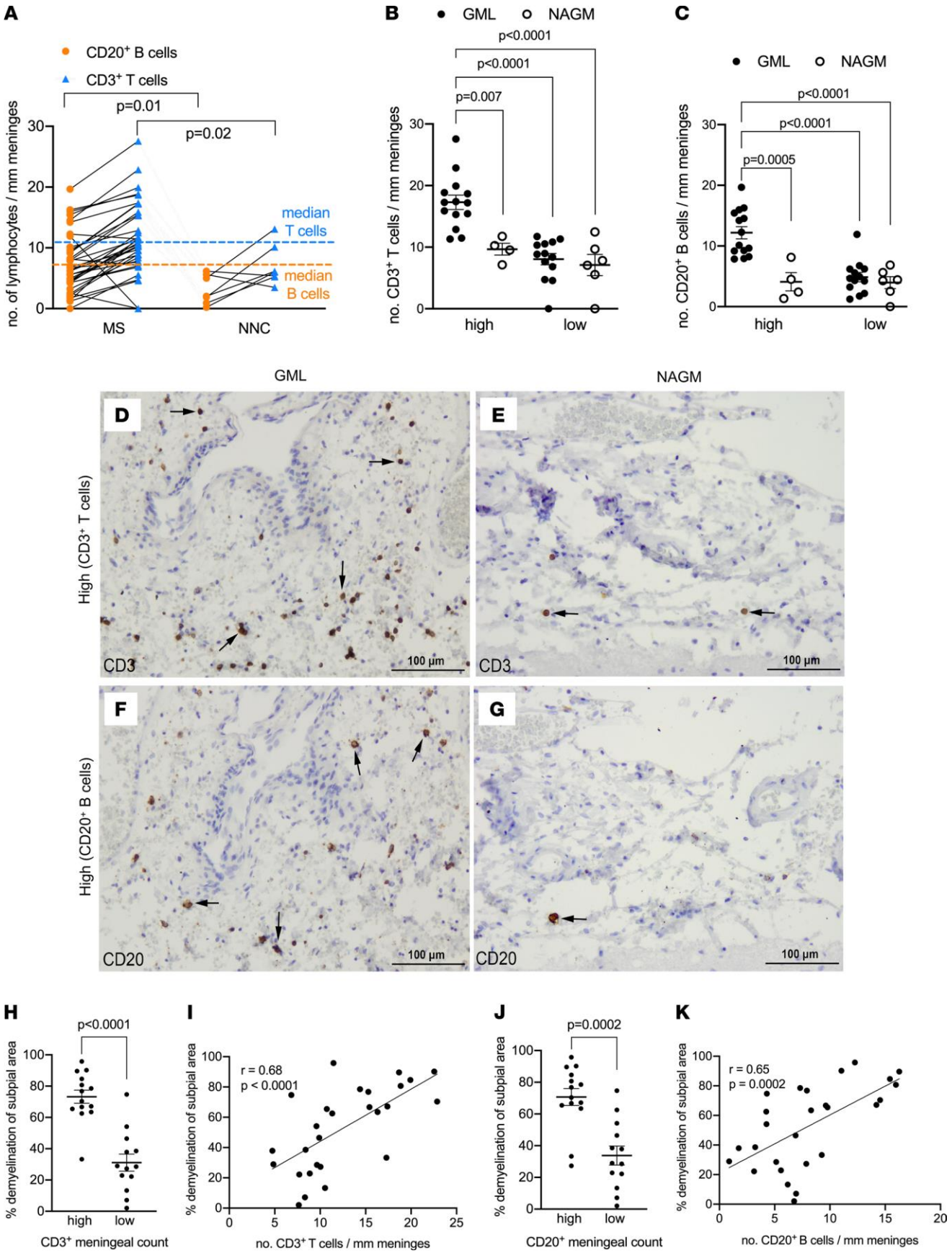

Figure 19. T and B lymphocytes are enriched in MS and are topographically linked to subpial cortical demyelination. *Meningeal CD20+ B cells and CD3+ T cells from 27 MS patients and 9 non-neurological controls were quantified, showing enrichment in MS donors (Figure A). In the count of meningeal CD20+ B cells and meningeal CD3+ T cells, medians were used for each case, represented by orange dotted*

lines and blue dotted lines, respectively. Quantification of $CD3^+$ meningeal T cell numbers showed a significant increase in the meninges adjacent to the LMG compared to NAGM from MS donors with high (n = 14) but not low (n = 13) meningeal T cell numbers. . It shows the accumulation of T cells (Figure B). Quantification of $CD20^+$ meningeal B cell numbers showed higher (n = 14) but not lower (n = 13) meningeal T cell numbers in the meninges adjacent to the LMG compared to NAGM in donors with MS, indicating a significant accumulation (Figure C). Representative immunohistochemistry for CD3 (D and E, arrows) and CD20 (F and G, arrows) in meninges adjacent to LMG or subpial NAGM from MS donors with high numbers of $CD3^+$ or $CD20^+$ meningeal cells. Chemical staining (Figures D–G). Quantification of the percentage of subpial gray matter that did not stain positively for myelin in MS donors with meningeal cell counts CD3+ T high and low (Figure H). Spearman's correlation coefficient between the number of meningeal $CD3^+$ T lymphocytes and the percentage of subpial gray matter that does not stain positively for myelin (Figure I). Quantification of the percentage of subpial gray matter that did not stain positively for myelin in MS donors with high versus low meningeal CD20+ B-cell counts (Figure J). Spearman's correlation coefficient between meningeal $CD20^+$ B-cell counts and the percentage of subpial gray matter that did not stain positive for myelin (Figure K). In A–C, each data point represents the mean cell count (mean ± standard deviation) for all fields analyzed per case. Statistically, significant differences were determined by the Wallis test followed by Dunn's correction for multiple comparisons (B and C). Scale bar: 100 µm (D-G). Taken from: (Ahmed et al., 2022).

These data suggest that there is a high density of meningeal T and B lymphocytes in patients who exhibited greater subpial demyelination compared to patients with low density of T and B lymphocytes, which demonstrates that there is a direct relationship between a high density of lymphocytes T and B and subpial cortical demyelination in patients with PPMS.

7. Clinical features

In the clinical setting, different tools are used to establish the health condition of a patient with MS. Here are some details about the most important ones.

7.1. Biomarkers

A better understanding of MS has been vital through the use of biomarkers (Baecher-Allan et al., 2018b). A biomarker is a measured and analyzed aspect that allows us to know the normal biological, pathological, and pharmacological development within the therapeutic setting (Ziemssen et al., 2019). In this sense, an ideal biomarker does not threaten patient safety, is easy to detect, and is a non-invasive method (Ziemssen et al., 2019). In that sense, we can distinguish both imaging biomarkers and molecular biomarkers (Ziemssen et al., 2019).

The most widely used biomarker in the field of MS study is MRI imaging (Baecher-Allan et al., 2018b). MRI imaging presents relevant information about the age, number, size, and development of lesions in the patient's CNS for diagnostic and therapeutic monitoring (Ziemssen et al., 2019). In addition, MRI imaging provides insight into the degree of inflammation before and after the use of various drugs to regulate the treatment and reduce the number of lesions (Baecher-Allan et al., 2018b).

Figure 20 show the most relevant lesions in MS marked with arrows can be observed by MRI presented in different views.

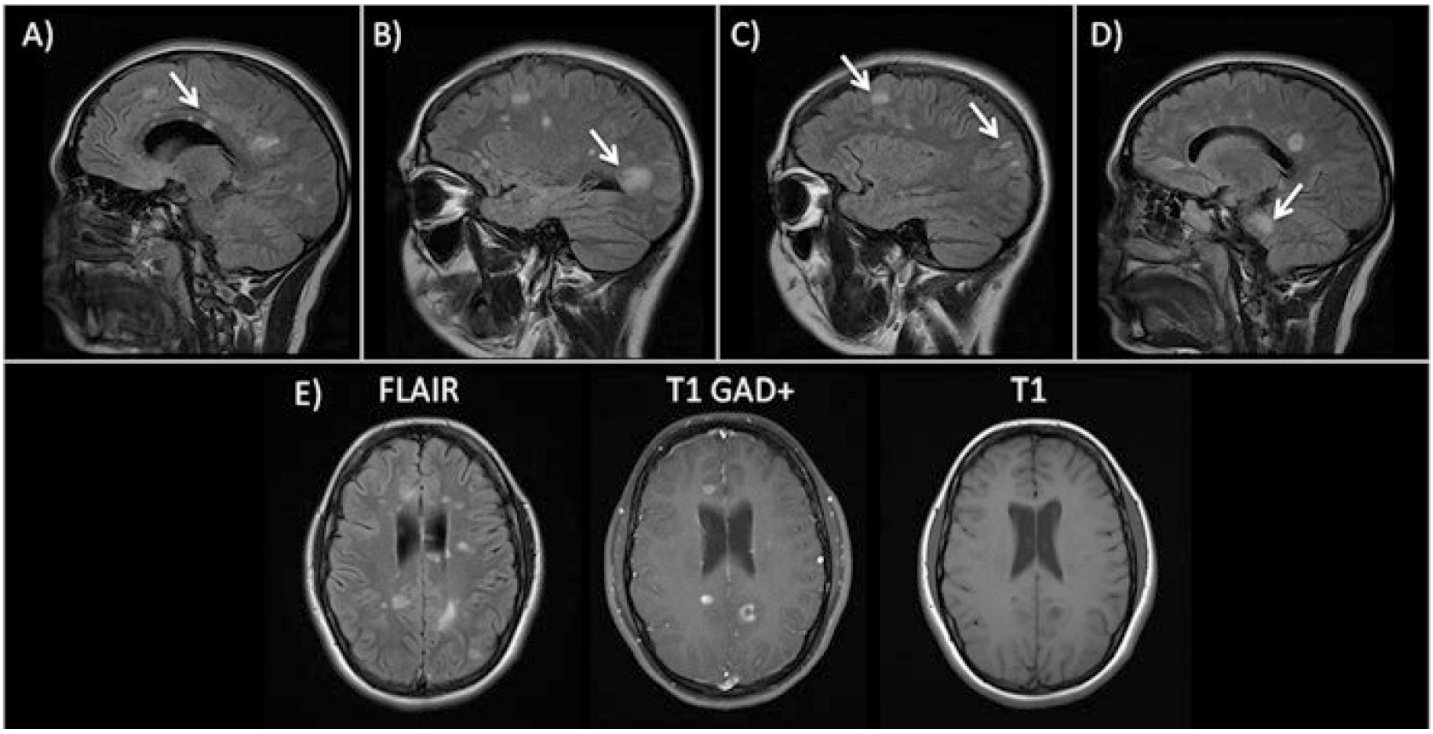

Figure 20. Common lesions in patients with MS by MRI. *Sagittal FLAIR (Figures A-D) and axial (Figure E) MRI brain of a patient with lesions disseminated in space and time according to McDonald criteria are shown. Demyelinating lesions are present in regions such as the corpus callosum (Figure A), periventricular region (Figure B), juxtacortical region (Figure C), and infratentorial region (Figure D). Simultaneous contrast-enhanced and non-contrast-enhanced lesions are also present according to the single scan criteria for DIT. Taken from:* (Buzzard et al., 2017)

Lesions in patients are visualized by MRI after a single clinical attack. The figure above shows lesions scattered over time together with asymptomatic lesions enhanced with Gd and without enhancement. Within the diagnostic criteria, an assessment can be made after two clinical attacks when the MRI shows scattered lesions in space and a demyelinating lesion in locations associated with MS such as periventricular, juxtacortical, infratentorial or spinal cord (Buzzard et al., 2017). In addition, although MRI images are indeed important, paraclinical tests such as the search for an intrathecal synthesis of oligoclonal bands can support a positive diagnosis for MS.

7.2. Diagnostic criteria

The criteria for MS diagnosis have been refined over time according to the amount of data obtained for analysis. Years ago, criteria such as Schumacher's and Poser's were used. Currently, the McDonald criteria are used, which include clinical, laboratory, and radiographic data (Karussis, 2014). Similar to the Poser criteria, the McDonald criteria required 2 clinical attacks varying in both time (DIT) and space (DIS) within 30 days (Howard et al., 2016) to determine a definitive diagnosis (Petrou et al., 2020). The following table summarizes the most important aspects of McDonald's criteria until their latest revision.

Table 7 compares the different aspects to be considered within the McDonald's criteria for MS according to the last two reviews.

Table 7. McDonald's criteria for MS. *Over the years these criteria have changed to provide more effective diagnostic. Undoubtedly, the revision in 2010 laid the groundwork for MS since in 2017 the criteria did not undergo major modifications Adapted from: (Dekker & Wattjes, 2017; Thompson et al., 2018).*

Diagnostic criteria for multiple sclerosis		
McDonald Criteria	**Dissemination in time (DIT)**	**Dissemination in space (DIS)**
2010	• A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MR imaging with reference to baseline scan, irrespective of the timing of the baseline MR imaging • Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time	DIS demostrated by ≥ 1 T2 lesion in at least 2 of the following central nervous system areas: • Periventricular • Juxtacortical • Infratentorial • Spinal cord
2017	• Simultaneous presence of gadolinium-enhanced and non-enhanced lesions at any time or by a new T2-hyperintense or gadolinium-enhanced lesion on follow-up MRI, with reference to a baseline scan regardless of the time of the baseline MRI.	• Same as above • There is no need to distinguish between symptomatic and asymptomatic MRI lesions as in the 2010 McDonald criteria.

These criteria have been revised in 2010 to diagnose MS early (Dekker & Wattjes, 2017). These new criteria require a single clinical attack with the condition that the initial MRI reveals disseminated lesions over time along with the presence of asymptomatic lesions with or without gadolinium enhancement (Buzzard et al., 2017). In that sense, it is worth mentioning that diagnostic criteria can be applied if the patient presents with typical CIS symptoms (Vidal-Jordana & Montalban, 2017).

The 2001 McDonald criteria have high specificity and sensitivity over 1-3 years compared to the Poser criteria (Karussis, 2014). These criteria revised in 2005 gave a sensitivity of 60% and a specificity of 88% (Karussis, 2014) and after the recent revision in 2011 an earlier diagnosis is expected (Garg & Smith, 2015). In line with the above, the 2017 revision of the McDonald criteria incorporated the spinal cord as an anatomical location to be considered (Ciccarelli et al., 2019). This revision further considers necessary in the diagnosis of 2 relapses along with 2 objective signs over time or failing that, it requires 1 relapse as a CIS along with 2 specific clinical signs and MRI images (Olek, 2021b).

The gradual retrospective study of the history of worsening disability is a decisive clinical component to knowing the patient's status (Filippi et al., 2020). The clinical observation of the MS patient can be done for months (>=6) as in the case of RRMS patients or years (>=12) as in the case of PPMS patients (Filippi et al., 2020).

Within the clinical study, tools such as the EDSS (Vidal-Jordana & Montalban, 2017) are used to know the state of disease progression (Filippi et al., 2020; Sumowski et al., 2018). EDSS is a scale that measures the patient's physical disability with values between 0 (no physical disability) and 10 (major physical disability or death) whose score progresses by a value of 0.5 (Sumowski et al., 2018). For example, a score of 6 implies unilateral assistance (100m walk) (Buzzard et al., 2017; Vidal-Jordana & Montalban, 2017). In addition, this scale provides insight into the neurological disability of 8 functional systems: pyramidal, sensory, cerebral, cerebral, visual, brainstem, cerebral/mental, and bladder/bowel (Buzzard et al., 2017). It is important to note that there are limitations within this scale such as low reliability, limited sensitivity, and confidence in locomotor functions higher than 4.0 (Filippi et al., 2020).

However, at the moment it has been difficult to establish a scale to determine the state of cognition, making it impossible to study cognitive tests and, therefore, to develop possible treatments (Sumowski et al., 2018). Today we know that cognitive monitoring will be an important piece to monitoring (Sumowski et al., 2018) this type of neurodegenerative diseases. Cognitive impairment stands out as the behavioral factor that exclusively indicates an active disease state (Sumowski et al., 2018).

On the other hand, there is also a test capable of providing information on limb movement. MSFC provides scores regarding leg, arm, and hand function through different mechanisms (Filippi et al., 2020). This CSFM is sensitive and effective for clinical severity and progression in patients with MS (Filippi et al., 2020).

8. Therapies and treatments

Over the years, different medications and therapeutic alternatives have been developed for MS and many of them have been approved by the FDA. Some therapeutic options and their latest advances are presented below.

8.1. Drugs used in Multiple Sclerosis

Within the category of injectable treatments, some drugs that can be administered under this procedure have been tested. Initially, 3 IFN-β products were administered intramuscularly or subcutaneously (Gholamzad et al., 2019): IFN-β -1a, IFN-β-1a SC, IFN-β-1b (Krupp et al., 2019). The

latter was the first treatment approved in 1993 for patients with relapsing MS (P. Axisa & Hafler, 2021). In general, IFN-β is responsible for increasing the production of anti-inflammatory cytokines (IL-10) (Baldassari & Fox, 2018; Tsur et al., 2021) has a limiting role in leukocyte migration across the BBB, and results in increased production of nerve growth factor (Tsur et al., 2021). A synthetic copolymer composed of 4 amino acids called Copaxone has shown immuno-dissolving effects in patients with MS reducing the fall rate by up to 30% (Gholamzad et al., 2019).

A study showed that therapies based on Copaxone and IFN-β obtained a reduction in the progression of disability in patients after 6 years of medication (Gholamzad et al., 2019). Although this treatment is presented as safe and reliable, there are limitations such as long administration times and periodic self-injections (Gholamzad et al., 2019). On the other hand, adverse effects of using Copaxone-based products (unwanted injection site reactions) and IFN-β (increased liver enzyme levels, unwanted injection site reactions, flu-like symptoms, myalgia, fever) have been reported (Gholamzad et al., 2019; R. H. Gross & Corboy, 2019).

Humanized monoclonal antibodies have also been tested with very promising results. Examples are daclizumab, natalizumab, alemtuzumab, and mitoxantrone (Gholamzad et al., 2019). Studies suggest that administration of natalizumab reduces more than 44% (Baldassari & Fox, 2018) and up to 65% of relapses over 2 years of treatment and greater than 90% suppression of new inflammatory lesions (P. Axisa & Hafler, 2021). Even the application of natalizumab offers greater effectiveness compared to IFN-β-1a or placebo (Baldassari & Fox, 2018). Likewise, a double-blind study with 194 patients evidenced an improvement in all the analyses performed in those patients who received doses of mitoxantrone for 2 years (Baldassari & Fox, 2018). Table 3 shows the best-known DMTs according to their efficacy according to clinical trials and other outstanding treatments for this disease.

However, this type of drug has also presented patient safety problems. Complications caused by alemtuzumab are autoimmune (Gholamzad et al., 2019; R. H. Gross & Corboy, 2019) neoplasms, strokes, arterial dissection (R. H. Gross & Corboy, 2019) and those derived from the administration of natalizumab are progressive multifocal leukoencephalopathy headaches, fatigue, allergic reactions (R. H. Gross & Corboy, 2019). In addition, the use of daclizumab has in some cases caused liver damage, skin reactions, and colitis (Gholamzad et al., 2019) and after administration of mitoxantrone, cardiotoxicity, acute leukemia (Gholamzad et al., 2019) and cardiomyopathy (R. H. Gross & Corboy, 2019) were observed in the patients administered.

8.2. Immunomodulators

Treatments based on oral immunomodulators have offered a promising alternative for people with MS (Krupp et al., 2019). These immunomodulators such as fingolimod, dimethyl fumarate, teriflunomide, and cladribine offer new mechanisms of action (Krupp et al., 2019). Oral administration of dimethyl fumarate causes a reduction in the number of proinflammatory subsets and memory B cells (Tsur et al., 2021). Treatment with fingolimod generates low levels of relapses up to 60% and a reduction of recorded MRI activity (P. Axisa & Hafler, 2021) most likely because it prevents the entry of lymphocytes from secondary lymphoid organs and blocks the MS activity after administration of fingolimod or dimethyl fumarate over 18 months in contrast to the low effect of teriflunomide (Derfuss et al., 2020; Krupp et al., 2019). Teriflunomide, which inhibits pyrimidine synthesis (R. H. Gross & Corboy, 2019), reduces the proliferation of T and B lymphocytes (Tsur et al., 2021).

The adverse effects of this type of immunotherapy are reported in some publications. Teriflunomide can cause hepatotoxicity, hypersensitivity, alopecia, peripheral neuropathy, and high blood pressure in patients administered with this inhibitor (R. H. Gross & Corboy, 2019). On the other hand, dimethyl fumarate may develop lymphopenia (R. H. Gross & Corboy, 2019). In the case of fingolimod use, patients have reported bradycardia, low lung diffusing capacity of carbon monoxide, fungal infections (R. H. Gross & Corboy, 2019), lymphopenia (P. Axisa & Hafler, 2021), macular edema, disseminated varicella-zoster virus, cryptococcal infections (Baldassari & Fox, 2018).

GA is a synthetic polypeptide compound (Hauser & Cree, 2020) capable of generating an increase in anti-inflammatory agents through the differentiation of $CD4^+$ T cells into T_H cells (Tsur et al., 2021). Studies reveal that this compound has slightly decreased the progression of disability (Hauser & Cree, 2020) in male patients with MS in a 3-year study of 943 people (Baldassari & Fox, 2018). The administration of GA is considered an effective alternative to IFN-β. Adverse effects are also notorious in this treatment: allergic reactions at the injection site, palpitations, chest tightness, flushing, anxiety after injection, dyspnea, and rarely, lipoatrophy (Hauser & Cree, 2020).

Table 8 shows the most accepted DMTs due to their efficacy together with other treatments that have recently been explored as therapeutic alternatives such as CAM.

Table 8. Current treatments and therapies available for MS. *The most important disease-modifying therapies (DMTs) used to treat MS according to their efficacy are presented at the top of the table. In addition, other treatments such as HSCT and suggested alternative therapies such as reiki, and yoga is presented at the bottom. Adapted from: (www.atlasofms.org, https://www.mssociety.org.uk/about-ms/treatments-and-therapies)*

DMTs used by efficacy category		
High efficacy	**Good efficacy**	**Moderate efficacy**
Alemtuzumab	Cladribine (oral)	Glatiramer acetate
Natalizumab	Dymethyl fumarate	Interferon-beta 1a
Ocrelizumab	Fingolimod	Interferon-beta 1b
	Siponimod	Peginterferon-beta 1a
		Teriflunomide
Other treatments		
Haematopoietic stem cell transplantation (HSCT)	Physiotherapy and cannabis	Complementary and alternative therapies (CAM)

Currently, the use of DMTs has shown significant results for MS patients. However, new studies are being carried out to determine the effectiveness of each of them. The most effective DMTs are monoclonal antibodies. In addition, today it is being analyzed to include alternative therapies such as yoga, and mindfulness, within the recovery of the patient's life.

Recent studies suggest the great benefit obtained in patients with MS after the administration of monoclonal antibodies such as ocrelizumab, rituximab, and ofatumumab. Ocrelizumab, a monoclonal antibody has brought great benefit in patients with MS by reducing relapses by up to 47% (Gholamzad et al., 2019) and silent progression in RMS and preventing the emergence of new white matter lesions (Hauser & Cree, 2020). Rituximab, a chimeric mouse-human monoclonal antibody has been used in different clinical trials and approved for diseases such as rheumatoid arthritis (Greenfield & Hauser, 2018). Despite the divergence of data on the efficacy of rituximab, it can be highlighted that it has contributed to the de-inflammatory process (Baldassari & Fox, 2018) and the reduction of lesions obtained via MRI up to 91% (Gholamzad et al., 2019). On the other hand, ofatumumab administration obtained a 99% reduction in MRI activity and no significant adverse effects were found (Gholamzad et al., 2019). Furthermore, ofatumumab has shown similar efficacy to ocrelizumab while rituximab has been widely used in both clinical trials and real-world experience

despite not having official approval. Overall, rituximab, ocrelizumab, and ofatumumab all exert a significant role in reducing relapses, MRI-recorded brain lesions, and evident efficacy following drug cessation (Greenfield & Hauser, 2018).

However, adverse effects of administering ocrelizumab are evident such as breast cancer, and complications associated with herpes virus infections (Hauser & Cree, 2020). In the case of ocrelizumab, there are risks associated with lymphopenia, and reactivation of the hepatitis B virus (Tsur et al., 2021).

Patients with MS who have received doses of cladribine suffered significant improvement in their condition. Cladribine, which is a purine analog, demonstrated efficacy of cladribine after 12 months of administration in improving the severity and prevalence of relapse (Gholamzad et al., 2019) according to EDSS and SNRS (Baldassari & Fox, 2018). A combinatorial study between IFN-β and cladribine showed a 23% reduction in relapses (Gholamzad et al., 2019). On the other hand, adverse effects of this treatment include bone marrow suppression, profound prolonged lymphopenia, neutropenia, anemia with persistent macrocytosis, plastic anemia (Baldassari & Fox, 2018), and HBV reactivation (Tsur et al., 2021).

This modulator has been approved to treat relapsing forms of MS especially SPMS, patients with relapse episodes or with significant MRI lesions (Hauser & Cree, 2020). Patients with MS after the administration of siponimod have evidenced improvements in health status (Derfuss et al., 2020). Specifically, patients with SPMS after receiving 2mg caused a reduction in the risk of disability progression over 3 and 6 months, and reduced volume loss and inflammatory MRI activity (Derfuss et al., 2020). In addition, other data suggest that RRMS patients had fewer relapses and the number of MRI brain lesions was reduced (Gholamzad et al., 2019).

Another drug used for treatment is a neuromodulator called ozanimod. This selective S1P receptor modulator is safe and tolerable in patients with RMS (Hauser & Cree, 2020). Like siponimod, studies suggest that the application of this drug in patients with SPMS reduces the relapse rate per year, improves the number of lesions and the rate of brain volume loss (Derfuss et al., 2020) as demonstrated by MRI data (Gholamzad et al., 2019). Compared to other treatments such as IFN-1a, ozanimod is superior (Derfuss et al., 2020).

Other immunomodulators such as laquinimod and ponesimod offer important considerations in MS. Laquinimod has been used in neurodegenerative disease trials. This carboxamide derivative has demonstrated neuroprotective properties (Baldassari & Fox, 2018) in experimental models that it can reduce inflammation, demyelination, and axonal damage (Gholamzad et al., 2019). However,

there are conflicting results regarding the clinical efficacy of laquinimod (Derfuss et al., 2020). In the case of ponesimod, a very effective modulator, after application, it has generated a reduction in the number of lesions in patients with SMPS (Derfuss et al., 2020). However, in some trials, adverse effects such as dyspnea or respiratory problems have been seen and this treatment has been discontinued (Derfuss et al., 2020).

Favorable effects similar to ponesimod are recorded in patients with SPMS, and it is noteworthy that there were no adverse cardiac effects (Derfuss et al., 2020). Similarly, the use of ceralifimod for the treatment of SPMS showed a reduction of lesions of up to 92% (Derfuss et al., 2020). Additionally, CS-0777 which is an S1P agonist has shown great promise because of the weekly administration intervals it offers (Derfuss et al., 2020).

Table 9 below shows the different medications used to treat MS with the most outstanding details of each one.

Table 9. Disease-modifying therapies for MS. *The use of DMTs has been shown to reduce the frequency and severity of relapses and the development of new areas of CNS damage leading to disability. Pharmaceutical names, chemical compounds and dosages associated with the various adverse effects can be seen. Adapted from: nationalMSsociety.org/DMT*

Treatment	Chemical name	Dose/Route of administration	Side effects
Avonex®	Interferon beta-1a	30 mcg intramuscular (into a large muscle) injection once weekly	Injection site reactions, heart problems, blood problems, seizures, TMA, autoimmune diseases
Betaseron®	Interferon beta-1b	0.25 mg subcutaneous (under the skin) injection every other day	Injection site reactions, heart problems, seizures, flu-like symptoms
Copaxone®	Glatiramer acetate	20 mg subcutaneous (under the skin) injection every day, or 40 mg subcutaneous injection three times per week	Immediate post-injection reaction, chest pain, lipoatrophy, skin necrosis
Extavia®	Interferon beta-1b	0.25 mg subcutaneous (under the skin) injection every other day	Injection site reactions, heart problems, seizures, flu-like symptoms
Glatopa®	Glatiramer acetate, generic equivalent of Copaxone	20 mg subcutaneous (under the skin) injection every day, or 40 mg subcutaneous injection three times per week	Immediate post-injection reaction, chest pain, lipoatrophy, skin necrosis, hepatic injury
Kesimpta®	Ofatumumab	20 mg subcutaneous (under the skin) injection at weeks 0, 1 and 2, followed by 20 mg once monthly starting at week 4	Infections (HBV, PML, weakened immune system)

Table 4. Disease-modifying therapies for MS continued.

Treatment	Chemical name	Dose/Route of administration	Side effects
Plegridy®	Pegylated interferonbeta-1a	63 mcg subcutaneous (under the skin) or intramuscular (into a large muscle) injection on day 1, 94 mcg on day 15, and 125 mcg on day 29 and every 14 days thereafter	Serious allergic reactions, injection site reactions, heart problems, blood problems, TMA, seizures
Rebif®	Interferon beta-1a	22 mcg or 44 mcg subcutaneous (under the skin) injection three times per week	Behavioral health problems, liver problems, skin reactions
Aubagio®	Teriflunomide	7 mg or 14 mg pill by mouth once daily	Allergic reactions, serious skin reactions, fever, chills, nausea
Bafiertam™	Monomethyl fumarate	95 mg capsule by mouth twice daily for 7 days and 190 mg twice a day thereafter	Allergic reaction, PML, liver problems, nausea, flushing
Gilenya®	Fingolimod	0.5 mg capsule by mouth once daily for adults and children weighing greater than 40 kg or 0.25 mg once daily by mouth for children weighing less than or equal to 40 kg	Slow heart rate, PML, macular edema, infections
Mavenclad®	Cladribine	Tablet given by mouth in two treatment courses, once per year for two years. Each treatment course has two cycles, which are 4-5 days long and about one month apart. The exact dose will depend on your weight	Risk of cancer, birth defects

Table 4. Disease-modifying therapies for MS continued.

Treatment	Chemical name	Dose/Route of administration	Side effects
Mayzent®	Siponimod	Increases each day over 4-5 days to the ongoing (maintenance) dose of a 1mg or 2 mg pill by mouth once daily. Your healthcare provider will do a blood test to determine whether you will take the 1 mg or 2mg maintenance dose and give you specific instructions for increasing the dose each day to reach the maintenance dose.	Slow heart rate, infections, macular edema, fever, body aches, chills, nausea, tiredness
Ponvory™	Ponesimod	2mg pill by mouth on day one, increased incrementally to a maintenance dose of 20mg on day 15 taken once daily thereafter. Your healthcare provider will give you specific instructions for increasing the dose each day to reach the maintenance dose.	Upper respiratory tract infection, hepatic transaminase elevation, and hypertension
Tecfidera®	Dimethyl fumarate	120 mg capsule by mouth twice daily for one week, followed by 240 mg capsule twice daily thereafter	Allergic reaction, PML, liver problems, herpes zoster infections, loss of appetite, severe tiredness
Vumerity®	Diroximel fumarate	231 mg capsule by mouth twice daily for one week, followed by two 231 mg capsules taken twice daily thereafter	
Zeposia®	Ozanimod	0.23 mg capsule by mouth once daily for days 1-4, followed by 0.46 mg once daily for days 5-7, then increased to 0.92 mg once daily on day 8 and thereafter.	Infections, PML, slow heart rate

Table 4. Disease-modifying therapies for MS continued.

Treatment	Chemical name	Dose/Route of administration	Side effects
Lemtrada®	Alemtuzumab	12 mg per day intravenous infusion (a needle placed in your vein) for five consecutive days, followed by 12 mg per day on three consecutive days one year later	Serious autoimmune problems, ITP, kidney problems, serious infusion reactions, stroke, tears, certain cancers
Novantrone®	Mitoxantrone	12 mg/m² intravenous infusion (a needle placed in your vein) every 3 months. Lifetime cumulative dose limit of approximately 8–12 doses over 2–3 years (140 mg/m2)	Menstrual disorder, nausea, alopecia, amenorrhea, upper respiratory tract infection, urinary tract infection, stomatitis, arrhythmia, back pain, sinusitis, headache
Ocrevus®	Ocrelizumab	600 mg intravenous infusion (a needle placed in your vein) every 6 months (first dose: 300 mg on day one and 300 mg 2 weeks later)	Infusion reactions, PML. decreased immunoglobulins
Tysabri®	Natalizumab	300 mg intravenous infusion (a needle placed in your vein) once every 28 days. Must take place in an approved infusion facility	Herpes infections, liver damage, allergic reactions, low platelet counts, lung infection, depressionm urinary tract infection, headache

Inyectable treatments	Oral treatments	Intravenous infusion treatments

8.3. Stem cells therapies

In addition to the drugs used for MS, bone marrow transplantation has been shown to offer a viable alternative. Studies in animal models suggest that transplanting the marrow syngeneically offers antigen-specific tolerance (Gholamzad et al., 2019). Although phase I clinical trials show improvements in disease activity after autologous hematopoietic stem cell transplantation (AHSCT), there has also been a high rate of adverse events with bone marrow transplantation in patients with MS and this therapy has been reserved for cases with poor prognosis or when other treatments have failed to respond (Gholamzad et al., 2019).

Hematopoietic stem cell transplantation has attracted attention among therapies for beneficial results in patients with MS. A compilation of various studies and clinical trials of AHCT with various designs, study populations, transplantation protocol, and control groups showed that the majority of MS patients experienced a reduction in long-term inflammatory activity (Cohen et al., 2019).

Likewise, there are promising data in phase II trials after HSCT. One such trial showed that 70% of patients after aggressive ablative treatment with graft autoreactive lymphocyte depletion did not demonstrate disease progression due to a lack of relapses, new MRI lesions, and EDSS progression (Gholamzad et al., 2019). Despite the promising results, further in-depth studies are required. In a study with 103 patients divided into two groups, the effect of nonmyeloablative HSCT and DMT was compared; in the HSCT group, only 3 patients suffered disease progression while 34 patients in the DMT group (Burt et al., 2019). In addition, the subsequent follow-up of each group showed that patients in the HSCT group improved the mean EDSS score while patients in the DMT group suffered an increase denoting a worsening (Burt et al., 2019).

Figure 21 shows a schematic analyzing the progression of the disease between DMT and HSCT of the above-mentioned study.

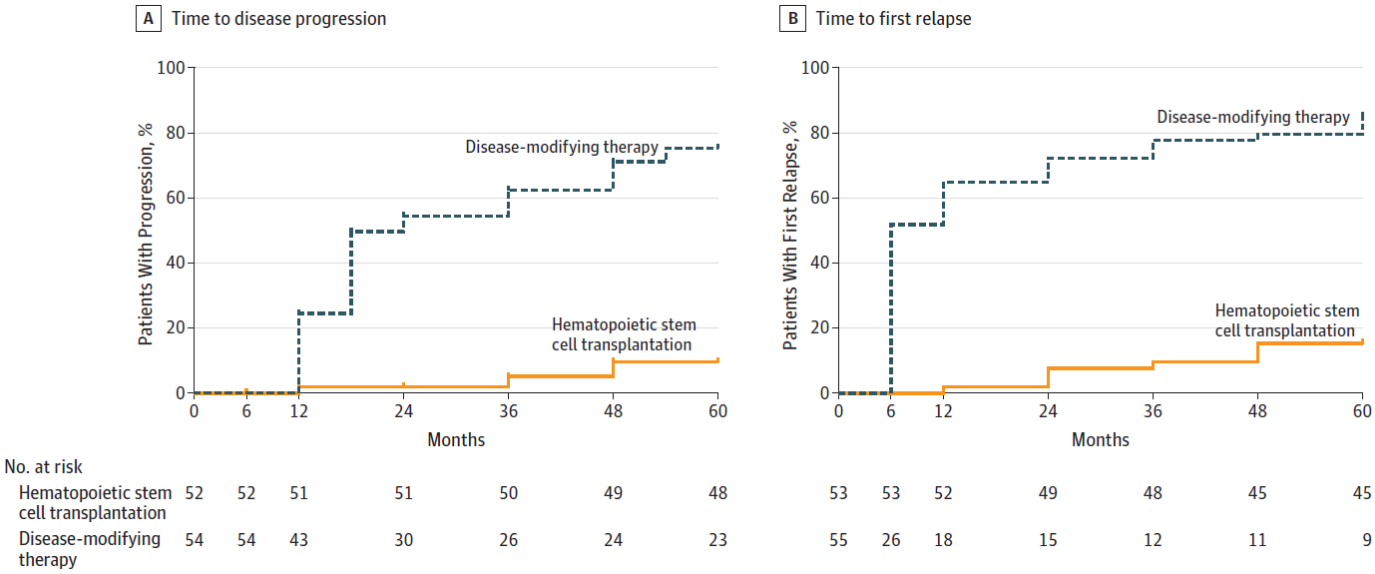

Figure 21. Time to disease progression and first relapse in patients undergoing hematopoietic stem cell transplantation compared with disease-modifying therapy. *In this study, 110 patients were randomized into two groups: HSCT and DMT. Patients in the DMT group received the drug as prescribed by their neurologist as follows: natalizumab, dimethyl fumarate, fingolimod, glatiramer acetate, interferon beta-1a, mitoxantrone, and teriflunomide. In addition, 38 patients received methylprednisolane, rituximab, plasmapheresis, intravenous immunoglobulins, or intravenous cyclophosphamide. Disease progression according to the EDSS score was visualized in 3 patients in the HSCT group and 34 patients in the DMT group (figure 2A). Likewise, the first relapse in the DMT group was observed at 6 months while in the HSCT group a value could not be obtained due to the paucity of events (figure 2B). Taken from Burt et al., 2019.*

The results of using HSCT for MS are promising. In the previous figure, it is visualized that its use in patients prolonged the time until the progression of the disease. Likewise, positive results were evidenced in EDSS, NRS, and MSFC, and a decrease in the volume of the lesion weighted in T2 by MRI (Burt et al., 2019). This suggests that the application of this therapeutic alternative should be further explored to definitively guarantee its use.

Likewise, AHSCT has been used in recent years as a therapeutic alternative for MS because it has shown a significant reduction in activity-improving disability (Patti et al., 2022), especially in patients with RRMS (Sharrack et al., 2020), relapses in patients with SPMS (Mariottini et al., 2022) and promising survival curves (Rush et al., 2019). On the other hand, small studies in progressive MS have been developed indicating that there is good safety and tolerability although adverse effects such as low fevers, headaches, infusion reactions, and aseptic meningitis have also been reported (Baldassari & Fox, 2018).

On the other hand, one of the stem cells studied for MS is MSCs (Bezukladova et al., 2022). MSCs belong to the group of pluripotent cell precursors that stand out mainly in the study of therapies for progressive MS because of their neuroprotective properties and repair-promoting functions (Baldassari & Fox, 2018). The MESEMS trial, the best-known

trial to verify the efficacy of MSCs as therapy in MS, failed to provide conclusive affirmative data for its application in patients with this neurodegenerative disease (Uccelli et al., 2019). Therefore, further studies should be carried out to obtain better protocols and to obtain the greatest benefit from the use of MSCs.

8.4. Strategies to restore myelination

A distinctive aspect of the pathology of the developmental process of MS is demyelination. New treatments and therapies have sought to address this situation with new drugs and above all relying on the potential of stem cells (Villoslada & Steinman, 2020).

Considering the biology of oligodendrocytes, some therapies have been tested to generate remyelination. Experimental data in mice suggest that administration of the tocopherol derivative TFA-12, a member of the vitamin E family, causes OPCs to differentiate causing myelin to repair (Gholamzad et al., 2019). Remyelination from mature oligodendrocytes constitutes the first line of natural defense against demyelination (Villoslada & Steinman, 2020). In line with the above, indomethacin which is a non-steroidal anti-inflammatory drug crosses the BBB promoting the differentiation of OPCs into mature cells and can thus generate remyelination via Wnt/B-caterin (Gholamzad et al., 2019). Preventing demyelination is possible within the first 10 years of disease due to the survival capacity of oligodendrocytes and the regenerative capacity of oligodendrocyte precursor cells (Villoslada & Steinman, 2020).

One of the most analyzed therapeutic targets that have shown efficacy in stimulating remyelination is LINGO-1 (Simkins et al., 2021). This protein is expressed by oligodendrocytes and axons where it exerts a regenerative role. Thus, antibodies targeting LINGO-1 may generate relief of axonal lesions in MS patients (Gholamzad et al., 2019; Villoslada & Steinman, 2020). An example of this treatment is the administration of opicinumab which is responsible for promoting remyelination and does not register considerable adverse effects (Gholamzad et al., 2019; Simkins et al., 2021) except for weight gain and dose hypersensitivity reactions (Baldassari & Fox, 2018). Phase 2 trials reveal that administration of this drug reduced disability in RRMS and SPMS patients (Baldassari & Fox, 2018; Villoslada & Steinman, 2020).

In this sense, other drugs have been analyzed to promote remyelination whose mechanism modulates neurotransmitter channels, and ion channels, or considers

cholesterol synthesis (Pablo Villoslada & Lawrence Steinman). Drugs such as clobetasol, guanabenz, benztropine, nimodipine, liothyronine, clemastine, simvastatin (Villoslada & Steinman, 2020), bexarotene, GSK239512, biotin stand out (Cunniffe & Coles, 2021). Simvastatin has shown great efficacy in clinical trials (Villoslada & Steinman, 2020). New approaches are being developed considering intracellular receptors (GCR, RXR-Y, VDR, ROCK, PPAR-Y), key kinases in OPC maturation to improve myelination (AKY, ERK, NDRG1, Notch/Jagged, WNT, GPR or Gli) and to develop new drugs (Villoslada & Steinman, 2020).

The efficacy of MSC transplantation in promoting endogenous neurogenesis and remyelination has also been analyzed (Bezukladova et al., 2022). MSCs have also been tested for myelin repair although these were not able to promote OPC differentiation and remyelination in the manner that was expected (Koutsoudaki et al., 2020).

8.5. Physical therapy

Despite the different pharmacological treatments that MS patients may receive, in certain cases the possibility of recovery also involves rehabilitation. In this sense, physiotherapy comprises kinesitherapy, hydrotherapy, physical therapy, and massage (Bethoux, 2007).

Physiotherapy seeks to achieve the mobility of the person by activating effector and behavioral capacities allowing in turn to recover functionality but not movement (Bethoux, 2007). In this way, physical activity was achieved by reducing the negative effects of akinesia, and increasing functional capacities without taking into account the development of the disease (Bethoux, 2007). A physical rehabilitation plan for a patient with MS should consider the stage of the disease, neurological deficits, degree of disability, etc. (Řasová et al., 2020).

Hydrotherapy is another therapeutic alternative with substantial benefits. Water has physical properties that allow performing gymnastic exercises discharged from the motor system without pain and with a greater range of motion (Bethoux, 2007). Furthermore, an aquatic environment is favorable for balance exercises and symmetry of the body in imbalance (Amedoro et al., 2020).

9. Conclusions

The study of MS has progressed greatly in recent years. These advances have made it possible to identify abnormal immune responses and improve diagnostic therapies. MS is characterized as an autoimmune disease whose pathology combines genetic and environmental factors such as EBV, latitude, vitamin D, smoking, and lifestyle. It affects more than 2.3 million people diagnosed, mainly between 20-40 years old and female. Relevant aspects of the pathology are currently known, including the role played by the different T cells, B cells, NK cells, demyelination mechanisms, and tissue damage, although information is limited. In this sense, $CD4^+$ regulatory T cells are involved in the neuroinflammatory process, a malfunction of $FoxP3^+$ Tregs and IL-10-producing Tr1 cells and $CD4^+$ $CD25^+$ $FoxP3^+$ deficiency have been observed in patients with MS. Th cells, in particular, T_H1-T_H17 and GM-CSF-producing $CD4^+$ T cells are also associated with the initiation and progress of inflammatory responses and give rise to the neurodegenerative process. In addition, these patients demonstrate a clear infiltration of B cells in the CNS, abnormal production of cytokines, and a link between T cells and B cells in the development of pathogenesis has been shown. Neurodegeneration, a distinctive aspect of MS, has been observed in gray matter and white matter, generating a progression of physical and cognitive disability. The different experimental models such as EAE constitute the primary source of information on the pathology, testing, and validation of drugs in MS. On the other hand, the MRI image is a very useful tool to monitor the progress of the disease in the various affected areas such as the spinal cord, thalamus, gray matter, white matter, etc., and provide early diagnosis according to the latest revision of the McDonald criteria. Until now approved treatments such as DMTs have shown effectiveness in reducing clinical and radiological activity such as alemtuzumab, cladribine, interferon-beta 1a, etc. However, the implementation of HSCT and stem cells have shown greater efficacy over time than standard treatments, although challenges remain. Improvements in biomedical equipment, in equipment in laboratories, have made it possible to approach a more accurate diagnosis, although today new forms of earlier diagnosis are being investigated. Therefore, it is necessary to delve into the clinical study of MS due to its increasing prevalence and incidence in recent years to guarantee a better life condition for patients affected by this disorder.

10. Future directions

Over the last century, MS has gone from being an unknown and untreatable disease to a disease with diverse therapeutic options and there are still many avenues to explore. Future studies including large cohorts of MS patients and healthy controls are required to clarify various pathological details and prevent disease progression by developing new biomarkers. Because the symptoms are so varied and unpredictable, the discovery of new details of the disease should lead to the development of more effective treatments and provide comprehensive treatment. It is even necessary to link immunological therapies with strategies that attack remyelination disability. In addition, future research should focus on identifying markers of the different populations of B cells to understand and modulate the inhibitory and immunostimulatory effects and develop new therapeutic alternatives. In addition, improvements in MRI technologies that allow the quantification of the different lesions continues to be a challenge to be solved. It is also important to focus on a deeper study of the various stem cell-based therapies to substantiate their therapeutic potential in MS.

11. References

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