Current advances in multiple sclerosis immunopathology

Carlos Fredy Cuevas-García, Nora Hilda Segura-Méndez and Diana Andrea Herrera-Sánchez
Instituto Mexicano del Seguro Social, Centro Médico Nacional Siglo XXI, Specialty Hospital “Dr. Bernardo Sepúlveda Gutiérrez”, Ciudad de México, Mexico

Abstract

Multiple sclerosis is a demyelinating inflammatory disease that affects the central nervous system. Its etiology is the result of a complex interaction between genetic and environmental factors that trigger a deregulated immune response, with the resulting inflammation and neuronal/axonal degeneration. Neuroinflammation is triggered when peripheral leukocytes migrate to the central nervous system and release cytokines such as interleukins 1 and 6 (IL-1 and 6) and tumor necrosis factor (TNF), which act on dwelling cells. The innate immune system plays an important role in the onset and progression of the disease by identifying molecular patterns associated with pathogens and damage, which modulate effector and regulatory functions of the cells where they are expressed, in order to direct the specific immune response. Th17 cells favor the disruption of the blood-brain barrier, which enables the migration of leukocytes to the central nervous system and the triggering of the inflammatory cascade; the Th1 profile (IL-1, IL-6) collaborates to perpetuate it. B-cell function is to produce antibodies and cytokines (IL-6, IL-12 and TNF). Knowledge on multiple sclerosis pathophysiology will enable the development of new therapeutic options that impact on natural history of the disease and its prognosis.


Introduction

Multiple sclerosis (MS) is a chronic autoimmune inflammatory disorder predominantly mediated by T cells, which induce multifocal demyelination and gliosis with loss of oligodendrocytes and axons. It is the result of a complex interaction between genetic susceptibility and environmental factors such as chronic infection with the Epstein-Barr virus (EBV), smoking and vitamin D deficiency.

Its pathophysiology is determined by damage to the myelin sheath that surrounds and protects central nervous system axons induced by autoreactive T cells. Murine models show that experimental autoimmune encephalomyelitis (EAE) can be triggered by the transfer of autoreactive myelin T cells, with consequent neuroinflammation and demyelination.

Over the last two decades, advances in immunology have allowed to know more about humoral and innate immunity, cytokine deregulation and Th17 cells effector profile; strictly speaking, epigenetic changes promote the inflammatory phenotype and demyelination; however, there is still doubt as to how they act on mitochondrial dysfunction, oxidative stress and axonal degeneration.

Multiple sclerosis epigenetics

Epigenetics studies those DNA changes that influence on gene expression without altering the DNA sequence, and that are produced by environmental factors. DNA methylation, histone modification and miRNA silencing are the three most important epigenetic mechanisms.

DNA methylation consists of the binding of a methyl group to a cytosine residue in the nucleotide DNA chain, which produces cytosine-guanine dinucleotides (CpG) that cluster in the genome constituting the so-called CpG islands, a process carried out by DNA methyltransferase enzymes (DNMT). Cytosine residues methylation silences the genes, possibly blocking the binding of transcriptional
factors; consequently, DNA hypomethylation promotes transcription.

Histone modification is another epigenetic mechanism, which consists of acetylation and deacetylation in lysine residues, carried out by histone acetyltransferases (HAT) and histone deacetylases (HDAC), respectively. Histone acetylation facilitates transcriptional factors binding to DNA, i.e., deacetylation, which inhibits transcription, thus influencing on the inflammatory process.

Epigenetic changes can also be generated at the post-transcriptional level by microRNA (miRNA), non-coding, single-chain RNAs that modulate cell differentiation, proliferation and apoptosis; its deregulation has been associated with autoimmunity and inflammation.

Some risk factors identified in the development of MS are viral infections, especially with EBV, belonging to the female gender, vitamin D deficiency, smoking and a family history of MS.

The most important genetic susceptibility is related to major histocompatibility complex genes, specifically the HLA-DRB1*1501 haplotype, which is most often transmitted by mothers and could be mediated by epigenetic mechanisms such as DNA methylation and histone deacetylation.

EBV generates chronic latent viral infection in the lymphocytes and up-regulates DNMT, influencing on cell proliferation and genome stability; however, only its extractable antigen shows association with the onset of MS.

Vitamin D inhibits IL-17 locus by modifying histone deacetylase 2 (HDAC2) at the IL-17A promoter region; consequently, its deficiency favors Th17 profile.

Smoking has been linked to an increase in relapses of MS and the number of active brain lesions in magnetic resonance imaging (MRI). It alters histone modification, DNA methylation pattern and miRNA expression. In addition, it activates neutrophils, macrophages and monocytes.

**Immunopathology**

The presence of the described epigenetic factors is not sufficient to explain MS pathophysiology, where Th1 and Th17 inflammatory phenotypes predominate, with the specific mechanisms that trigger inflammation still remaining to be elucidated.

Most studies on cytokines epigenetic regulation have focused on Th17 profile differentiation, where IL-17 gene promoter regions are shown to undergo histone acetylation and DNA hypermethylation.

Another mechanism by means of which T cell responses are modulated is miRNA-mediated gene regulation. miR-155 and miR-326 levels of expression are closely related to TCD4 cells differentiation into the Th17 phenotype.

Theoretically, the DNA methylation and histone modification processes are susceptible to treatment, targeting enzymes involved in these processes, for example, deacetylase inhibitors; however, they are nonspecific, since epigenetic changes are organ-specific; therefore, MS current treatment continues to be aimed at controlling the inflammatory process.

Next, the function of the most important components of innate and adaptive immunity in MS inflammation will be briefly explained.

**Neuroinflammation and the complex cytokine network**

Neuroinflammatory diseases such as MS occur when peripheral leukocytes migrate to the central nervous system (CNS) and release cytokines such as IL-1, IL-6 and TNF, which act on CNS resident cells (e.g., microglia) to produce more cytokines and perpetuate inflammation (Fig. 1).

Cytokines are proteins that regulate cell behavior. Specifically in MS, they favor the Th1 or Th17 effector profile, which causes inflammation, tissue damage and neurological deterioration.

So far, the most important cytokines in MS pathophysiology are the following:

1) IL-23, produced by antigen-presenting cells (APC) in secondary lymphoid tissues and microglia. Its function is to generate and stabilize a specific Th effector phenotype and stimulate the production of IL-17A, IL-17F, IL-22 and granulocyte-macrophage colony stimulating factor (GM-CSF).

2) IL-17 acts on stromal and endothelial cells, astrocytes and microglia. It destroys blood-brain barrier integrity, which allows leukocyte migration to the CNS and trigger the inflammatory process. Astrocytes respond to IL-17 by producing chemokines that attract neutrophils, which actively collaborate to tissue damage. Mice lacking the IL-17A gene (IL17a -/- mice) are susceptible to EAE; however, they develop a delayed disease, corroborating the importance of this cytokine in pathophysiology.

3) IL-6 is crucial for the development of EAE. It blocks effector Th cells FOXP3 expression, which prevents their regulatory function. It favors IL-1 receptor expression on T cells to stabilize local Th1 profile and counteract the natural tendency towards neuroinflammation resolution. Uninterrupted expression of these cytokines and continuous arrival of leukocytes to the CNS perpetuate inflammation.
4) Interferon gamma (IFN-γ) was central in MS pathophysiology until a couple of decades ago; currently, it is difficult to define its role since it acts on many cell types and has pro- and anti-inflammatory properties that appear to depend on the microenvironment, the dose and the specific moment of exposure (Table 1). In MS, it exerts a predominantly inhibitory effect in the CNS and excitatory in the spinal cord. IFN-γ injection directly into the CNS triggered inflammation in murine models, but administration of blocking antibodies and the lack of IFN-γ inhibitory effect in the CNS and excitatory in the CNS and excitatory in the spinal cord, even in the absence of transcription factor T-bet, which suggests a protecting role on neuroinflammation.

5) Tumor necrosis factor (TNF) is produced by macrophages and TCD4 and TCD8 cells, among others, and has pro- and anti-inflammatory functions (Table 1). Its blockade in patients with MS led to more frequent and serious relapses. The cause was probably the diversity of receptors it can bind to.

6) GM-CSF is a cytokine produced by T cells in response to IL-23 and has been associated with neurodegenerative functions of microglia. It represents the means of communication between T lymphocytes and myeloid cells recruited in response to GM-CSF, with the latter being relevant to the CNS because they phagocytose and induce receptor-mediated apoptosis and are the main source of reactive oxygen species.

7) Transforming growth factor beta (TGF-β) is a cytokine that has three isoforms and the expression of each one is spatially and temporally different. TGF-β1 is present in the immune system and both TGF-β2 and TGF-β3 are expressed in neurons and glia; infiltration of the CNS by Th1 cells in patients with MS is partially regulated by TGF-β functions (Table 2).

The interest in TGF-β within the pathophysiology of MS has increased after the observation that IL-6 + TGF-β drives murine Th17 cell differentiation in vitro, even in the absence of transcription factor T-bet, which regulates Th1 and Th17 profile.

Under normal conditions, the presence of TGF-β in lymph nodes, where naive T lymphocytes typically find the antigen, suppresses T cell activation and differentiation, even in the presence of Th1-promoting cytokines, such as IL-12.

In areas of CNS inflammation of patients with MS, the production of Th1 cytokines can increase, while IL-10 expression is favored, which counteracts Th1 effector cells activity against myelin.

The absence of TGF-β1 signaling results in spontaneous T cell differentiation and autoimmunity. TGF-β induces the Th9 profile, which is associated with Th17 cells negative regulation.

8) IL-9 in the cerebrospinal fluid (CSF) of patients with MS is negatively correlated with inflammation, neurodegeneration and disease progression, which supports its regulatory role. In vitro, the proliferation of oligodendrocyte precursor cells is promoted by the Th9 profile together with IFN-γ, but is inhibited in the presence of IL-17.
Innate immunity

The innate immune system plays an important role in the onset and progression of the disease, through the modulation of T and B cell effector functions; we will focus exclusively on the pattern recognition Toll-like receptors (TLR), autophagy, inflammasomes and natural killer (NK) cells.

**TLR**

TLRs are transmembrane glycoproteins that are expressed in myeloid cells, CPA, T and B lymphocytes. They can bind to a broad range of microbial ligands and endogenous damage-associated molecular patterns (DAMP) or alarms such as HSP70 (Heat Shock Proteins 70) and HMGB1 (high-mobility group protein 1), produced as a result of tissue damage or cell death.

In addition to identification of pathogen-associated molecular (PAM) and DAM patterns, their function is to modulate effector and regulatory functions of cells where they are expressed, i.e. they represent a point of union between innate and adaptive immunity.

In MS, the most widely studied is TLR2, identified in endothelial cells, microglia, astrocytes and oligodendrocytes; it occurs as a homodimer or heterodimer associated with TLR1 or TLR6.27 TLR2 stimulates the production of IL-1, IL-6, IL-12 and IL-23, favoring the Th1 and Th17 profiles; jointly, it influences on T regulatory (Treg) cells function.28

In the CNS, it can contribute to neuroinflammation via a PARP-1-dependent pathway.29

TLR2 is stimulated by infectious agents (pathogens or commensals) that, in case of not being limited, will cause a decrease in Treg cells and escape of preexisting autoreactive T cells. The production of IL-1 and IL-6 as a result of stimulation will inhibit inducible Treg (iTreg) cells differentiation, which are important for peripheral tolerance, contributing to the Treg cell defect that is known in patients with MS.30,31

TLRs play an important role in the regulation of autoimmunity attributable to PRR (pattern recognition receptors) and DAM identification, with the latter being capable of triggering an inflammatory response in the absence of an infectious stimulus.

**Autophagy**

It is a lysosome-dependent degradation pathway that eliminates unnecessary organelles and proteins for energy recycling. There are three subtypes of autophagy: macrophagy, chaperone-mediated autophagy and mitophagy, with the latter being responsible for dysfunctional mitochondria degradation.

Autophagy maintains mitochondrial homeostasis by decreasing reactive oxygen species, which are important in neuroinflammation.32 Autophagy blockage or dysfunction causes inflammasome activation and neurodegeneration.

**Inflammasomes**

These are proteins that form signaling complexes that are sensitive to damage and stress, influence on the production of IL-1β and IL-18 cytokines by means of caspase-1 activation and pyroptosis. They do not possess a signaling sequence for their secretion and are produced from an inactive form, pro-IL-1β and pro-IL-18. The NLRP3

---

**Table 1. IFN-γ and TNF functions in multiple sclerosis**

<table>
<thead>
<tr>
<th>Produced by</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>Inhibits IL-17</td>
</tr>
<tr>
<td>Myeloid cells, lymphocytes</td>
<td>Produces IL-12, IL-23, and IL-21</td>
</tr>
<tr>
<td>BBB integrity</td>
<td>Induces oligodendrocyte apoptosis</td>
</tr>
<tr>
<td>Expression of binding proteins in endothelial cells</td>
<td>Lowers glutamate absorption by astrocytes</td>
</tr>
<tr>
<td>Myelin degradation products phagocytosis</td>
<td>Stimulates adhesion molecule expression in endothelial cells and astrocytes (T cell extravasation through the BBB and brain parenchyma)</td>
</tr>
<tr>
<td>Neurotoxic products accumulation</td>
<td>Synergistically with IFN-γ it promotes the expression of MHC molecules on astrocytes and oligodendrocytes, which magnifies TCD8+ cytotoxicity</td>
</tr>
</tbody>
</table>

**Table 2. TGF-β functions**

<table>
<thead>
<tr>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibits the production of IL-2, IL-12 (Th1)</td>
</tr>
<tr>
<td>Decreases the production of IFN-γ, as a consequence of Th1 blockade</td>
</tr>
<tr>
<td>Promotes Treg differentiation</td>
</tr>
<tr>
<td>Activates TCD8+ cells</td>
</tr>
<tr>
<td>Favors the Th9 profile</td>
</tr>
</tbody>
</table>

TGF-β = transforming growth factor beta.
inflammasome –the most widely studied– promotes leukocyte migration to the CNS in EAE.33 Caspase-1 and IL-1β have been identified in plaques of patients with MS,34 and the levels of IL-1β in the CSF have been proposed to be able to be used as predictors of in situ response to treatment, disease severity and progression.35 Pharmacologically, IFN-β decreases NLRP3 expression36 and IL-1β plasma levels in patients with MS.37

However, even when there are first-line treatments for MS, their use is not successful in all patients. There is a dependence of their effects on NLRP3 and this feature divides the patients in IFN-β responders and non-responders.38

The former show NLRP3 and IL-1β decreased levels before treatment, while non-responders show upregulation 3 months after treatment with IFN-β.39,38 NLRP3-independent EAE is resistant to IFN-β and shows minimal remission due to irreversible neuronal damage.

Natural killer cells

NK cells are part of innate immunity and comprise a subpopulation of lymphocytes that phenotypically express CD16 or CD56 on their surface, in the absence of CD3. NK subpopulations are the following: CD56bright, CD56dim CD16+, CD56dim CD16+ and CD56-.

NK CD56bright cells, which owe their name to the expression of CD56 on their surface (also known as neural cell adhesion molecule), express the inhibitory receptor NKG2A and represent only 10 % of NK cells.

CPAs modulate NK cells by means of receptors for IL-12, IL-15 and IL-18, which lead to the release of molecules such as IFN-γ, IL-10 and IL-13, TNF-β and GM-CSF; their regulatory function is related to the release of these cytokines.40

NK cells cytotoxicity can attenuate inflammation in the acute phase by means of T cell and microglia death, but damaging repair during the late stage through neural stem cell death. Interferon release and natural cytotoxicity by NK cells is crucial to innate defense against viruses. Epidemiological studies suggest that herpesviruses are related to the development of MS, specifically EBV.41

In contrast with the latter, cytomegalovirus (CVM) has been suggested to protect against the development of MS, but study results are contradictory and probably depend on the host-pathogen complex interaction. CMV infection induces an adaptive reconfiguration of NK cells in healthy adults, through the expansion of the NKG2bright subset. In patients with MS and CMV seropositivity, NKG2C expression was associated with lower risk of disease progression, suggesting that it may be beneficial; however, it is uncertain whether NKG2C cells can fully account for the alleged protecting role of CMV in MS.42

Some drugs have benefited from NK cells regulating functions.

Antibody-dependent cytotoxicity by NK CD56dim cells is an essential therapeutic mechanism in T and B cell suppression mediated by alemtuzumab.43 Natalizumab increases the number of total NK and peripheral NK CD56bright cells with a reduced number of NK cells in the CSF.44 On the other hand, daclizumab improves immune tolerance endogenous mechanisms by reducing early T cell activation by blocking the IL-2 receptor that allows phenotype CD56bright expansion. It also favors for NK cells, after binding to T lymphocyte IL-2 receptor, to control direct death, which for CD56bright involves the release of granzyme.45-47

Cell immunity: autoreactivity or regulation?

Th1 versus Th17

For decades, the observation that myelin-specific T cells from patients with MS expressed the Th1 profile validated their predominant role in the pathophysiology of the disease.48

The finding of IL-17 increased expression in peripheral blood, CSF and CNS lesions in patients with MS, associated with the controversial role of IFN-γ and TNF-α have conferred it a higher relevance.49-51

Th1 cells in peripheral blood and CSF are 10-fold higher than Th17 cells; however, the latter have a significant increase during relapses, which has not been observed with Th1.52

IL-17 acts on endothelial, epithelial and myeloid cells and fibroblasts. It potentiates IL-8, CXCL1, CXCL6, IL-1α, IL-6, TNF-β, MIP-2 (macrophage inflammatory protein 2), MCP-1 (monocyte chemotactant protein 1) and GMCS secretion, with the latter being a potent inducer of neutrophil migration53 (Fig. 2).

IL-23 stimulates IL-17 expression in TCD4 memory cells, which are able to co-express IFN-γ (Th1). Both Th1 and Th17 cells cross the BBB; however, IFN-γ IL-17 TCD4 cells in patients with MS appear to cross it more efficiently, i.e., they are more encephalitogenic.54

The above data suggest that it would make sense to neutralize the p40 subunit, shared by IL-12 (Th1-inducer) and IL-23, which would block both profiles. However, in two clinical trials (briakinumab/ustekinumab), the effects...
of this blockade were shown not to be sufficient to justify them as first-line monotherapy.\textsuperscript{55,56}

These results may be due in part to the fact that IL-12 blockade can inhibit the IFN-\(\gamma\)-mediated protective function at early stages of the disease. IFN-\(\beta\) can inhibit Th17 as part of its effects on clinical improvement, reinforcing the importance of the profile.\textsuperscript{57,58}

For reasons unknown, some patients have a dominant profile, Th1 or Th17, which not only will impact on treatment but on the site of lesions: a Th17/Th1 ratio, with predominance of the former will be characterized by TCD4 and macrophage infiltration throughout the brain, while the predominance of the Th1 profile produces localized lesions.\textsuperscript{59}

The role of Th1 and Th17 is not yet fully understood, there are probably not yet defined cytokines and transcription factors that act synergistically in the pathophysiology; for now, the role they play in neuroinflammation remains essential.

\textbf{\(T_{\text{reg}}\) cells}

Keeping autoreactive cells under control to prevent autoimmunity is a task of the so-called "regulatory" cells, out of which the best characterized, although not the only one, is the \(T_{\text{reg}}\) cell, which is mainly induced by TGF-\(\beta\). \(T_{\text{reg}}\) cells express the CD4+CD25+FoxP3+ markers and represent 5% of TCD4+ cells total population; their function is to control T cell expression, by internalizing the IL-2 receptor.

\(T_{\text{reg}}\) cells are dysfunctional in patients with MS due to a restricted TCR repertoire conferred to them by the fact of being inherently prone to autoimmunity due to a lack of diversity in their population.\textsuperscript{60,61}

Additionally, TGF-\(\beta\) signaling is lower in TCD4+ lymphocytes of patients with MS, which causes for these cells to be less sensitive to TGF-\(\beta\); in part, this would explain why therapies directed to this target have failed.\textsuperscript{62}

It is important underscoring that TGF\(\beta\) and the aryl hydrocarbon receptor (AhR) signaling pathway can induce \(T_{\text{reg}}\) or Th17, depending on the microenvironment and inflammatory factors such as TLR2, IL-6 and IFN-\(\gamma\) ligands; this is highly important when considering a \(T_{\text{reg}}\)-inducing therapy, since there is the possibility of Th17 induction.

Finally, glucocorticoids can transiently increase FOXP3 expression, thus favoring immunoregulation.\textsuperscript{63}

\textbf{TCD8+ cells}

MS immunological basis and its experimental model, EEA, have largely focused on TCD4+ cells as mediators...
Humoral immunity

B cells

For decades, B cells role in MS was restricted to the production of antibodies and at this moment there is no doubt that they are important in the pathophysiology of the disease, since they are elevated in CNS lesions and CSF and simultaneously show clonal expansion and hypermutation, as a reflection of their activation. They are responsible for the production of oligoclonal bands, and their blockage by anti-CD20 antibodies such as rituximab and ocrelizumab has shown beneficial effects.59

The autoantigens suggested as targets of these antibodies remain unknown; therefore, the mechanisms that control B cell activation, selection and maturation remain a speculation.

The next question is, how do they migrate to the CNS? The mechanism is influenced by the large number of receptors they express, including CXCL13, a chemokine correlated with the conversion of the clinically isolated syndrome into MS and that facilitates their recruitment to the CNS.70

In comparison with T cells, B cell infiltration in the CNS varies in the course of the disease. The meninges of patients with secondary progressive disease often contain tertiary lymphoid structures with plasma cells, B cells, T cells and follicular dendritic cells, resulting from chronic inflammation.71

Their effector function facilitates demyelination through antibody-dependent cell cytotoxicity, where cells of the innate immune system such as NKs recognize and bind to the antigen-antibody complex through the Fc-gamma receptor, triggering cytotoxicity and lysis; other described mechanisms are opsonization, phagocytosis and complement activation.72

CD20 monoclonal antibodies such as rituximab or ocrelizumab have been shown to be efficacious for reducing relapse rates. These drugs exhaust B cell subsets but not plasma cells, so that their contributions lie in their ability to modulate antigen presentation and IL-6 production.73,74

B cells produce cytokines such as IL-6, which activate microglia and are responsible for dendritic density loss in cortical plates. They also show a regulatory profile through the production of IL-10 and can act as APCs.

The role of B cells in MS pathology is not limited to the production of antibodies, and their function on disease induction, relapse or progression remains to be elucidated.

Conclusions

Despite the great advances in the pathophysiology of MS, many questions remain to be answered. Possibly, future findings in epigenetics and innate immunity will allow to regulate the immune response prior to the release of autoreactive T cells and antibody formation. Therapeutic targets should consider the complex interaction of the immune system, be highly specific and preserve or favor immunoregulation.

References