Chapter

Peripheral Biomarkers in Multiple Sclerosis Patients Treated with Interferon-Beta

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Abstract

Multiple sclerosis is a relapsing and eventually progressive disorder of the central nervous system that continues to challenge researchers who try to understand the pathogenesis of the disease and prevent its progression. Interferon-beta is the most widely prescribed treatment for MS. Peripheral blood seems to mirror the immunological disturbances that underlie MS, which could represent the migration patterns between periphery and other tissues according to the clinical phase of the disease. Based on this assumption, several studies point to significant alterations in peripheral blood homeostasis of different subpopulations of T cells, like γδ T cells or Th1, Th2 and Th17 functional subsets; of B cells subpopulations; and of innate cells like monocytes and dendritic cells. The main goal of this chapter is to make an in-depth review of the major findings described in the literature that correlate specific alterations on different leukocytes subpopulations with disease status, and which therefore have the potential to constitute a peripheral biomarker of disease progression.

Keywords: biomarkers, T cells, B cells, dendritic cells, monocytes

1. Introduction

Around 2.8 million people are diagnosed with multiple sclerosis (MS) worldwide. MS is an autoimmune demyelinating disease of the central nervous system (CNS) of unknown etiology. Hallmarks of MS include focal inflammatory infiltrates, demyelinating plaques, reactive gliosis, and axonal damage [1, 2].

The mechanism of MS pathology involves complex interactions between systems and cell types including neurons, glia, and immune cells, accompanied by permeability of the blood–brain barrier (BBB). Autoreactive T cells activated outside the CNS cross the BBB and are reactivated by local antigen-presenting cells. Secretion of proinflammatory cytokines stimulates microglial cells and astrocytes, recruits additional inflammatory cells, and induces antibody production by plasma cells [3].

Recombinant interferon-β (IFN-β) remains the most widely prescribed treatment for relapsing–remitting MS (RRMS) and a valid approach because of its good benefit/risk profile. Despite widespread use of IFN-β, its therapeutic mechanism is still partially understood. The efficacy of IFN-β treatment has been shown by a decreased annual relapse rate, disability progression and inflammatory brain lesions resulting in the approval of different IFN-β preparations [4].

IFN-β is a highly pleiotropic cytokine which antagonizes the proinflammatory milieu by inhibiting expression of proinflammatory molecules, while increasing production of anti-inflammatory factors. It inhibits leukocyte trafficking, regulates the adhesion molecule expression and inhibits matrix metalloproteinase activity. The mechanism of action of IFN-β is complex and multifactorial but has been shown to reduce the biological activity of RRMS in several clinical class I trials [5].

The identification of peripheral markers that could reflect the clinical course of MS and the efficacy of treatment is a stimulating field of research and debate. An ideal biomarker is characterized by high sensitivity and specificity as well as a simple, cost effective, reproducible, and non-invasive detection method [6]. For instance, there are reports focusing molecules and autoantibodies as potential biomarkers in the MS disease course. Our focus in this chapter is on circulating leucocytes that can be considered during the follow of RRMS patients in remission *versus* relapse phase.

2. Multiple sclerosis

MS is an autoimmune disease of the brain and the spinal cord characterized by chronic inflammation, demyelination, gliosis and neuronal loss. The demyelination consists of the damage of the myelin sheath surrounding nerves, consequently affects the function of the nerves. The pathological hallmark of chronic MS is the demyelinated plaque or lesions, which consists of a well-demarcated hypocellular area characterized by the loss of myelin sheaths or oligodendrocytes, relative preservation of axons, and the formation of astrocytic scars [1].

The etiology of MS remains elusive, with a complex multifactorial system implicated, in which environmental factors are hypothesized as interacting with genetically susceptible individuals. MS causes a heterogeneous array of symptoms and signs because of the differential involvement of motor, sensory, visual and autonomic systems with serious physical disability in young adults, especially women [2, 4, 7].

The CNS is frequently described as an immune-privileged site, evidence supports the notion that the CNS receives limited immune surveillance by peripheral lymphocytes under physiological conditions. New findings provide a mechanism by which large particles and immune cells can drain from the brain and interface directly with the peripheral immune system [8, 9].

MS is triggered in the periphery or in the CNS. The CNS-extrinsic (peripheral) model is the most widely accepted and is consistent with the method used to induce experimental autoimmune encephalomyelitis (EAE), the animal model for neuroinflammation. The autoreactive T cells from MS patients may become activated in the periphery as a result of a molecular mimicry, gain access to the CNS, and T cells generated against non-self-epitopes (viral or microbial antigens) cross-react with self-myelin epitopes of similar sequence [10–12].

85% of patients present a RR form of MS, characterized by discrete episodes of neurological dysfunction (relapses) separated by clinical stable periods with lack of disease progression (remissions). More than 30% remain in the RRMS form of the disease into old age [7, 11–13].

Relapse is the clinical result of an acute inflammatory focal lesion and is typically discernible using magnetic resonance imaging. Relapse is defined as newly appearing neurological symptoms in the absence of fever or infections that last for more than 24 hours and are separated from the previous event by at least one month. The frequency of relapses can vary widely among patients as well as during different periods during an individual patient's disease. The relapse tends to be present for a limited time – days or weeks – and can lead to full recovery or can leave sequelae.

At present time, no clinical features or biomarkers that are predictive of relapse rates have been identified. The signs and symptoms that occur during relapses are also diverse and unpredictable [3, 8, 11].

Immunological characteristics of MS lesions have been reflected in circulating immune cells of MS patients. Peripheral blood provides a 'window' into the immunopathogenesis of MS. The immunological disturbances that underlie MS can be observed not only in the CNS, but also through examination of peripheral immune cells [14].

3. Therapeutic management

IFN-β and glatiramer acetate have been used as first-line disease-modifying therapy for RRMS. More than two decades have passed since IFN-β was found to be effective in the management of MS. IFN-β treatment efficacy has been shown by a decrease in the annual relapse rate, in disability progression and in inflammatory brain lesions, resulting in the approval of different IFN-β preparations [15–17].

IFNs are naturally occurring cytokines, secreted by various cells such as fibroblasts, NK cells, leukocytes, and epithelial cells in response to pathogens such as bacteria, viruses, parasites, and tumor cells, as well as other foreign substances. They have a wide range in anti-inflammatory processes, regulation of cell growth and modulation of immune responses [18, 19].

IFN-β binds to the interferon receptor, activates the Janus kinase/signal transducer and the activator of transcription (STAT) pathway to phosphorylate STAT1 and STAT2. The activation of interferon-stimulated genes leads to the production of antiviral, antiproliferative, and antitumour products. The effectiveness of IFN-β in the treatment of MS may rely on both anti-viral and immunomodulatory aspects [20, 21].

IFN-β was the first immunomodulatory therapy approved by the U.S. Food and Drug Administration and is the most widely prescribed treatment for MS; it is generally well tolerated and overall reduces the relapse rate by 30% in patients with RRMS [4].

Several IFN-β preparations have been approved with differing structures (glycosylated IFN-β-1a vs. non-glycosylated IFN-β-1b), formulation (lyophilized vs. liquid), used excipients (e.g., containing serum albumin or not), modification (pegylation), dosage (protein load and bioactivity), route of administration (subcutaneous vs. intramuscular), or frequency of injection (ranging from bi-weekly to every other day). IFN-β shows high tissue distribution; however, it is not supposed to cross the BBB and exerts its immunomodulatory mechanism in the peripheral compartment. IFN-β is cleared via renal and hepatic pathways, in which catabolism seems to be important rather than simple excretion [15].

The therapeutic benefit of IFN-β in MS has been proven in several large clinical trials, with the effect of IFN-β therapy being more studied on T and B cells [22]. In spite of this, it is known that the biological functions of IFN-β act in both innate and adaptive immune responses and may influence phenotype and functions of all MS-relevant immune cells [23].

4. Peripheral blood leukocytes as potential biomarkers of disease activity

A biomarker is defined as a characteristic that can be objectively measured and evaluated and serves as an indicator of normal biological processes, pathological processes or pharmacological reactions to therapy. An ideal biomarker is

characterized by high sensitivity and specificity as well as a simple, cost effective, reproducible, and non-invasive detection method [6].

In this section we synthesize and integrate the most relevant data regarding the characteristics of the selected immune cells that could be considered as IFN-β treatment-related biomarkers. The main goal of this work is an attempt to help researchers to perform a good assessment of immune cells in future studies. The presented data is a result of a compilation of several studies and findings.

4.1 Antigen-presenting cells

Antigen presenting cells (APCs) are considered key players in the immune surveillance of CNS and, at the same time, they are critically involved in the pathogenesis of CNS autoimmune diseases. They are a morphologically and functionally diverse group of cells that links the innate and adaptive immune responses. These cells are specialized in the presentation of antigens to lymphocytes, particularly T cells. Included among such cells are dendritic cells (DCs), monocytes and macrophages (derived from monocytes that migrated from the blood stream to tissues). B lymphocytes that specifically capture antigens via their clonally expressed membrane immunoglobulin can also function efficiently as APCs to T cells [24].

4.1.1 Dendritic cells

In humans, DCs comprise two major subsets: plasmacytoid DCs (pDCs) and myeloid (mDCs). Through nucleic acid-sensing, pDCs activate toll-like receptors (TLR), such as TLR7 and TLR9, rapidly producing type I IFN. mDCs are dedicated APCs that have a characteristic dendritic morphology, express high levels of MHC class II molecules and recognize pathogen-derived lipids, proteins and nucleic-acids by TLR2, TLR4 and TLR3 respectively [25].

The DCs subsets may be helpful as biomarker between remission and relapse of RRMS patients treated with IFN-β. The circulating mDCs subset reduces in remission and increase in relapse RRMS patients. On the other hand, the pDCs frequency are maintain across the different phases of disease. Usually, these subsets present a low frequency in systemic circulation, so the mDCs/pDCs ratio is a good representative of the alteration observed in the DCs subsets. The mDCs/pDCs decreases in remission RRMS patients and is re-established in relapse RRMS patients, constituting a potential peripheral biomarker [26, 27].

The involvement of DCs in MS arises from studies that demonstrate the abundant presence of these cells in the inflamed CNS lesions and in the CSF of MS patients [23].

One of the immunomodulatory effects of IFN-β in the EAE model is the reduction in antigen presentation, particularly myelin-specific antigens, leading to reduced T-cell responses [25, 28]. In contrast with these effects, in remission phase it was observed that the DCs subsets increase the expression of HLA-DR and decrease in the relapse phase. The variation in HLA-DR expression is more evident in the mDCs subset. The same subset reduce the mRNA gene expression of CX3CR1; fractalkine is known to be upregulated and released in response to pro-inflammatory stimuli and induces adhesion, chemoattraction, and activation of leukocytes [27].

The activation status of the mDCs subset could discriminate between RRMS phases. This subset shown a highest activated status in remission than in relapse phase, through the increased HLA-DR expression and a reduced migratory capability, since reduce the mRNA gene expression of CX3CR1.

4.1.2 Monocytes

Monocytes represent a heterogeneous population of primary immune effector cells with distinct phenotypical and functional characteristics; their differential roles in steady-state immune surveillance and the pathogenesis of human CNS disease are poorly understood [20].

The differential expression of CD14 (part of the receptor for lipopolysaccharide) and CD16 (also known as FcγRIII) allows monocytes to be segregated into three subsets. The major subset designated "classical" monocytes ($CD14^{++}CD16^{-}$, cMo), corresponds to 80–90% of circulating monocytes. CD16 expressing monocytes are divided into a named "intermediate" monocyte ($CD14^{++}CD16^{+}$, iMo) and a subset classified as "non-classical" monocytes ($CD14^{+}CD16^{++}$, ncMo); each of these subsets corresponds to 5–10% of circulating monocytes [21, 29].

Patients with MS display high levels of monocyte-secreted inflammatory molecules in serum compared to healthy individuals, demonstrating a role for peripheral monocytes in the progression of the disease. Increased levels of serum tumor necrosis factor (TNF) α and β have been reported in MS relapse. Monocytes and microglia are known to act as major effectors in the demyelinating process through direct interaction and the production of proinflammatory cytokines and mediators (e.g., IL-1b, nitric oxide). $CD16^{+}$ monocytes may contribute to the breakdown of the BBB by facilitating T cell trafficking into the CNS [20, 24, 30].

Research performed on monocyte pool in RRMS patients is scarce and ambiguous. A recent work achieved a significant decrease of the ncMo subset in both phases of RRMS patients, although in a higher extension in remission patients [27].

The frequency of monocytes subsets does not allow us to identify different phases of RRMS, but the HLA-DR expression could constitute a potential important biomarker between remission and relapse phases. A significant increase in HLA-DR expression in all monocyte subsets in the remission group when compared with healthy and relapse groups, has been described [27]. IFN-β enhances HLA-DR expression in circulating monocytes, but inside the CNS, one prominent model is based on the observation that IFN-β inhibits the IFNγ upregulation of MHC class II molecules on cell surface of macrophages and glial cells and therefore diminishes antigen presentation [28]. In the periphery, Kantor et al. report that the increase of MHC Class II expression in monocytes induced by IFN-β may contribute to the positive immunomodulatory effect in MS [31]. These findings were reinforced by the observation that when IFN-β-stimulated monocytes were used to stimulate autologous T cells, there was an increased secretion of anti-inflammatory cytokine IL-13 [32].

4.2 T cells

4.2.1 $CD4^{+}$ and $CD8^{+}$ T cells

T cells are central regulators of the adaptive immune response, they help B lymphocytes to produce antibodies and secrete cytokines that provide efficient protection against pathogens. Distinct T helper (Th) cell subsets, producing one or more lineage-defining cytokines and expressing master transcription factors and homing receptors. Th subsets are differentiated from naive $CD4^{+}$ T cells in response to a specific class of pathogenic microorganisms and to the cytokine milieu. This occurs in peripheral lymph nodes by mature DCs that present pathogen-derived peptides associated to MHC class II. With the involvement of their costimulatory molecules, DCs promote T cell proliferation and produce polarizing cytokines. In turn T cell was differentiated in distinct Th cell subsets, such as Th1, Th2, Th17, regulatory T (T reg) and T follicular helper (Tfh) [33].

The $CD4^+$ T cells have been the most studied in the pathogenesis of MS, although $CD8^+$ T cells are the dominant lymphocyte population in all stages of disease and lesions of MS patients. Naive $CD8^+$ T cells follow a similar differentiation programme of $CD4^+$ T cells [34, 35].

Th1 cells are described as being the pathogenic subset of T cells, whereas Th2 cells are reported to exert inhibitory effects [5]. Previous studies have pointed to a reduction in pro-inflammatory capability promoted by IFN-β therapy, consisting of a reduction of the expression of Th1-induced cytokines while enhancing Th2 responses [18]. Concerning the T cytotoxic (Tc) subsets, it has been reported the same behavior, in remission a downregulation of pro-inflammatory Tc1 responses and up-regulation of anti-inflammatory Tc2 with a beneficial effect on disease activity [36]. This dichotomy Th1, Th2 subsets and Tc1, Tc2 subsets could contribute to discriminate between remission and relapse phases.

The identification of Th17 cells helped to resolve some in adequacies of the original Th1/Th2 concept that had dominated T cell immunology research filed for almost 20 years. For a long time, it was thought that the IL-12/IFNγ pathway and Th1 cells were central to the development of autoimmune disease [37].

Both Th1 and Th17 cells have been implicated in the initiation and progression of disease in RRMS and its experimental model EAE [19]. The link between Th17 cells, IL-17 and MS relapses comes from the observation that in humans, Th17 cells are able to cross the BBB in MS lesions, enhancing neuroinflammation. In vitro studies have revealed that IL-17 blocks the differentiation and reduces the survival of oligodendrocyte lineage cells. In EAE model, it has been suggested that Th17 cells interact directly with neurons, forming antigen-independent, immune, synapse-like contacts [7, 38].

It is assumed that the inhibition of Th17 cells in RRMS patients attenuates the disease, however conflicting data have been published. Axtell et al. reported that IFN-β treatment effectively blocked disease symptoms in mice with EAE induced with Th1 cells. Otherwise, in EAE induced with Th17 cells the IFN-β treatment worsened disease [19].

In RRMS patients, it is not clear whether a more specific blockade of the Th17 pathway has beneficial effects in MS patients. Treatment with an antibody directed against IL-12p40 and therefore neutralizing both IL-12 and IL-23 did not result in a significant reduction of disease activity [39].

A meta-analysis pointed out several limitations across studies that assess the levels of peripheral Th17 cells and serum Th17-related cytokines. Like the severities of the disease and clinical subtypes in MS patients; the disease duration from relapse; and that the MS treatments were not consistent; and it was postulated that most studies selected MS patients with high disease activity. There were differences in experimental methods between studies and a lack of detailed standardized methods to identify the Th17 cells and Th17-related cytokines [40].

A recent in vivo study observed an increased frequency of circulating Th17 and Tc17 cells, accompanied by increased serum levels of IL-17 in remission RRMS patients treated with IFN-β [41]. This contradiction underlines the need to clarify the role of the IL-17-producing T cells in RRMS patients.

It has been demonstrated that a significant proportion of Th17 cells convert into IFN-γ-producing T cells and have chemokine receptors from both Th17 and Th1 subtypes, referred as Th17.1 cells. The enhanced potential of Th17.1 cells to infiltrate the CNS was supported by their predominance in CSF of early MS patients and their preferential transmigration across human brain endothelial layers [42, 43]. In remission RRMS patients, it was observed that Th17 and Tc17 cells exhibited a higher degree of Th1 plasticity since there were higher frequencies of those cells simultaneously producing intracellular IL-17 and IL-2 or IFNγ or TNFα [41].

Another subset of T cells, the Tregs, are characterized by high expression of CD25 and the transcription factor *Foxp3*, which is critical for their development, lineage commitment, and regulatory functions. Tregs are a very heterogeneous population with suppressive functions that maintain tolerance to harmless food/self-antigens and prevent autoimmune disease. Numerous studies have identified Tregs as important immunoregulators in many inflammatory and autoimmune disease conditions including asthma, MS, and type-I diabetes [37, 44].

In MS patients, both reduced or normal frequency of Tregs was observed. Libera et al. described a significant decrease in Treg cells in remission RRMS patients [45]. Haas et al. state that the frequency of Treg cells was normal in MS patients but with a lower suppressive function on autoreactive T cells [46]. Venken et al. described that RRMS patients treated with IFN-β showed restored naive Treg numbers as compared with age- and disease-duration-matched untreated patients [47].

Recently identified, the Tfh subset expresses the chemokine receptor CXCR5 as well as CD279 [48], is specialized in helping B cells to produce antibodies in the face of antigenic challenge and plays a crucial role in orchestrating the humoral arm of adaptive immune responses. Tfh cells have the unique ability to migrate into follicles in secondary lymphoid organs where they colocalize with B cells to deliver contact-dependent and soluble signals that support survival and differentiation of the latter cells. There is no complete and thorough understanding of how naïve Th cells differentiate into mature Tfh [49, 50].

Tfh cell levels are elevated in the blood of MS patients and this population is positively correlated with the progression of disability. One potential mechanism through which Tfh cells can contribute to disease is promoting the inflammatory B-cell activities, suggesting that Tfh cells cooperate with Th17 cells to induce inflammatory B cell responses in the CNS and increase disease severity [49].

The increased frequencies of Th1 cells, activated Tfh- and B-cells parallel findings from pathology studies, along with the correlation between activated Tfh- and B-cells, suggest a pathogenic role of systemic inflammation in progressive MS [51].

A similar frequency of Tfh cells between RRMS patients and healthy subjects was reported. However, this subset tend to exhibit a more proinflammatory activity, since higher frequencies of TNF-α^+ Tfh cells have been observed [41]. It is well known that Tfh cells play an important role in T/B interactions in germinal centres (GC) and one potential mechanism through which Tfh cells can contribute to MS is in promoting inflammatory B-cell activities [49]. The Tfh subset and others follicular like T cells subsets, like Treg/follicular cells, are promising targets in the study of T cells in pathophysiology of MS.

4.2.2 γδ T cells

γδ T cells develop in the thymus together with αβ T cells but rearrange a different TCR, consisting of a TCR-γ and TCR-δ chain. One of the most striking characteristics of γδ T cells is their inherent ability to secrete pro-inflammatory cytokines very rapidly, which influences adaptive immunity, they carry out immediate effector functions as well as mounting a memory response upon microbial reinfection. This fast response can be explained by γδ T cells exiting the thymus already with the functional competence to produce cytokines with no need of APCs cells [52, 53].

In MS, their potential importance is increased by the finding that γδ T cells accumulate in demyelinating CNS MS plaques; these cells show evidence of oligoclonal expansion indicating a local response to currently unknown antigens. γδ T cells have been shown to be present in both MS lesions and in CSF, and sequencing studies have shown that the major γδ T subsets present in the lesion differ from those in the CSF, suggesting specific functions for these cells in lesion development.

In more chronic lesions, γδ T cells may become the most prevalent type of T cell in the lesion. γδ T cells isolated from the CNS can be expanded but only in patients with relapse disease, not chronic MS patients, suggesting that these cells may have differential roles during various phases of the disease [54, 55].

The frequency, the migratory pattern, the activation status of γδ T cells in RRMS patients are unclear. Between remission and relapse RRMS patients, the γδ terminally differentiated effector memory T cells (T_{EMRA}) and the $CCR5^{+}$ γδ T_{EMRA} decrease in relapse when compared with remission RRMS patients [56], constituting a good biomarker between phases of the disease. Probably as a result of the migratory pattern describe for this phase of MS, preferentially toward RANTES and MIP-1α, whose expression is increased during relapses [57, 58].

The decrease of Eomesodermin and granzyme B mRNA expression in $CD27^{-}$ γδ T cells suggests a reduction in the cytotoxic potential of the circulating pool of γδ T cells, particularly in relapsing RRMS patients [56].

4.3 B lymphocytes

The most consistent immunodiagnostic feature and hallmark immunologic finding in MS patients is the presence of oligoclonal bands (OCB) in the CSF and their absence in peripheral circulation. Consequently, the pathogenic function of B cells in MS has been traditionally associated with antibody production. However, B cells have three putative biological roles: production of proinflammatory or regulatory cytokines, function as APCs and antibody production [59].

In MS, the memory B cells, plasmablasts and plasma cells preferentially cross the BBB and migrate into the CNS, where they dominate the B cell pool and exert different effector functions. B cells seem to be abnormally polarized toward a more proinflammatory phenotype [60].

More recent, somatic hypermutation studies have demonstrated that identical B cell clones can be shared between the CNS and the periphery in individual patients. These studies provide evidence of bidirectional trafficking of distinct B cell clones (both into and out of the CNS). The patterns suggest that B cells can travel back and forth across the BBB and commonly re-enter GC (in the meninges or cervical lymph nodes) to undergo further somatic hypermutations. These findings change our view of lymphocytic surveillance of CNS tissue and underline that B-cell trafficking is an important topic for future research and therapy strategies [60–62]. This news about recirculation of B cells through the BBB alters the perception of the role of B cells in MS.

B cells are released in the peripheral blood, recirculate between the secondary lymphoid tissues, and dying after a few days. According to phenotypic profile of B cell subsets, which also reflects their functional abilities and behavior, four major maturation-associated subsets can be identified in the human peripheral blood: immature/transitional, naive, memory and plasmablast [63].

In remission RRMS patients submitted to IFN-β, the percentage of immature/transitional B cells increases. This increase can be seen as an attempt to increase anti-inflammatory cytokines. Meanwhile, a decrease in the proportion of circulating class-switched memory B cells was reported [64, 65].

The relapsing RRMS patients exhibited distinct changes in B cell subsets homeostasis, resulting in a decrease in the total population of B cells, including a decrease of the immature/transitional and naïve B cell subsets when compared with remission RRMS patients. On the other hand, the plasmablast B cell subset presented an increase in relapse RRMS patients. The ratio between immature/transitional B cells and plasmablasts can thus be considered as a potential biomarker between phases of RRMS patients. The remission RRMS patients and the healthy subjects presented a similar ratio, and the relapse RRMS patients present a decreased ratio [66].

According to the new and recent data about the recirculation of B cells in RRMS, it seems that the increase of plasmablasts in circulation of relapsing episodes may be due to a migration of these cells from cervical lymph nodes and/or from B cell aggregates described in the meninges of MS patients to the blood marrow in an attempt to promote the immune response [67].

5. Effects of IFN-β in circulating cells

An ever-expanding body of literature, sometimes difficult to integrate, defines the intricate pathways by which IFN-β mediates its broad effects. To resume the effects of IFN-β in circulating immune cells a table listing the relevant studies and findings was performed (**Table 1**).

	Effects of IFN-β
Antigen presenting cells	• reduces mDCs frequency, pDCs frequency remains unchanged, mDCs/pDCs ratio decreases [25, 27, 65]; • activated pDCs decreased TLR9 consequently decreases Th1 cell differentiation, reduced pro-inflammatory IL-6, TNF-α, IFNγ secretion, expression of CCR7 and increased IL-10 secretion [20, 25–27, 66] • activated status mDCs trough the expression of HLA-DR and mRNA gene expression of CX3CR1 reducing their migration pattern [27] • pDCs showed reduced expression of the maturation markers CD83 and CD86 molecules and lower secretion of proinflammatory cytokines, including IFN-α, and a decreased ability to stimulate allogeneic T cells in response to maturation stimuli [24, 65]; • enhances HLA-DR expression in circulating monocytes [27, 28]
T cells	• reduces T-cell activation, downregulating MHC class II and costimulatory molecules, prevents the interaction of B7/CD28 and CD40/CD40L decreases the activation of myelin-reactive T cells [4, 5, 25]; • inhibits proinflammatory IFNγ, TNFα and IL-17, increasing the production of IL-10 [5, 68]; • increases levels of Th1 cytokines during RRMS relapse, whereas Th2 cytokines increases during remission in RRMS patients [5, 25, 40]; • prevents T-cell adhesion and extravasation across the BBB [4, 5]; • induces Treg cells [4, 5, 22]; • Mediates the chemokine receptor CCR7, channel autoreactive T cells into secondary lymphoid tissue rather than to CNS [5]; • the $CCR5^{+}\gamma\delta$ T_{EMRA} cells decreases with a reduction in the cytotoxic potential in relapse when compared to remission [56]
B cells	• induces expression of the B-cell survival factor B-cell-activating factor, with a shift toward less mature circulating B cells [65]; • reduces of memory B cell frequency exerted by the induction of a FAS-R-mediated caspase 3-dependent apoptosis [16]; • downregulates costimulatory molecules, CD40 and CD80 becoming less efficient APCs and less able to induce T-cell proliferation [23]; • inhibits proinflammatory cytokines, IL-1β and IL-23, anti-inflammatory IL-10 is upregulated in B cells [24, 64]; • the ratio between immature/transitional B cells and plasmablasts decreases in relapse when compared to remission RRMS [66]

Table 1.
Main effects of IFN-β in circulating immune cells in MS.

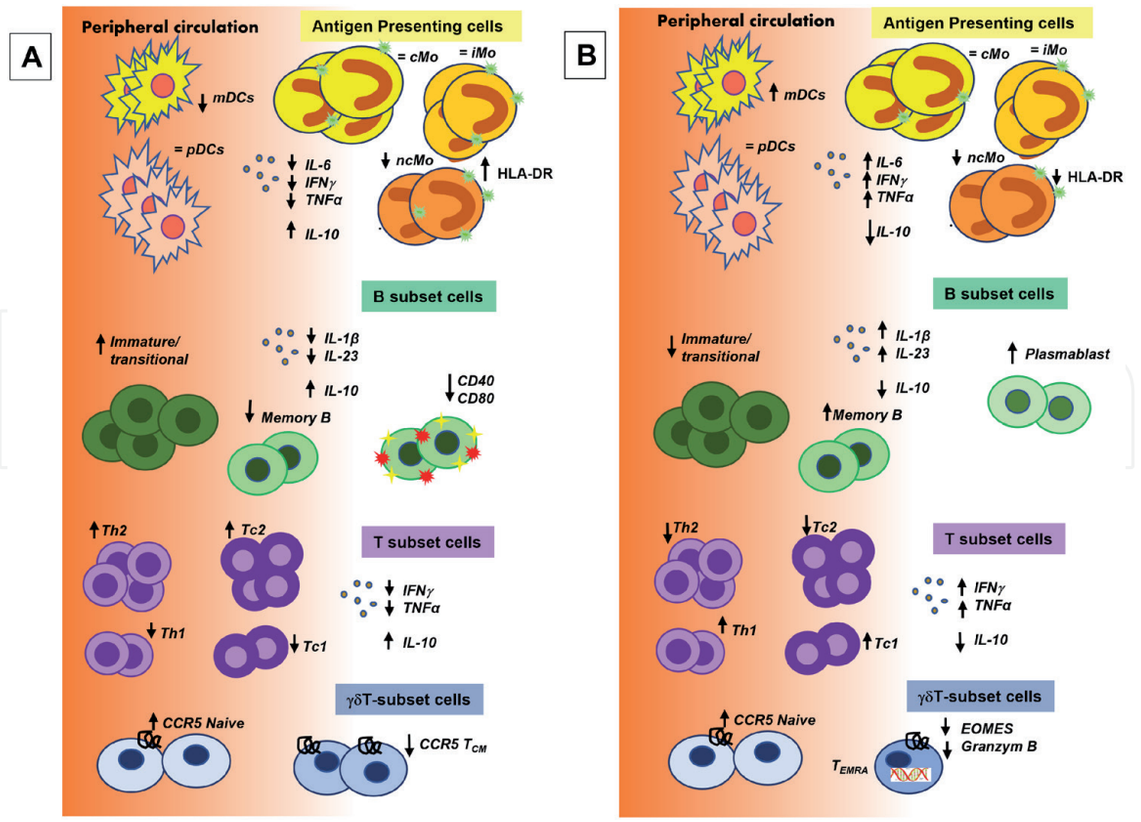

Figure 1.
Main effects of IFN-β in RRMS patients (a) remission phase and (B) relapse phase. mDC – Myeloid dendtitic, pDC – Plasmacytoid dendtitic cell, cMO – Classical monocytes, iMo – Intermediate monocytes, ncMo – Non-classical monocytes.

A major role for IFN-β is the induction of a priming state through which production and regulation of mediators, including cytokines, are affected by synergistic or antagonistic interactions. In the treatment of MS, the most important IFN-β mechanisms of action appear to be mediated mainly by the increased expression and concentration of anti-inflammatory agents, in turn, down-regulating the inflammatory state observed in the patients both in the periphery and in the brain tissue (**Figure 1**) [23].

6. Methodology

The work from our group started with the selection of the RRMS patients and collected blood from each one after assigned an informed consent. By flow cytometry performed direct immunofluorescence membrane and intracytoplasmic staining protocols to identify and characterize the circulating subsets. To functional assessment of the cells was measured intracellular cytokines at single cell level, after in vitro stimulation. To evaluation of gene expression, RNA isolation and quantitative real-time reverse transcriptase-polymerase chain reaction was performed.

In our group publications, one can be find the flow strategy with the description of the antibodies used and the mRNA gene expression studies performed in APCs [27], in T cell subsets [41], in γδ T cells [56] and in B cell subsets [66].

The literature search was performed using the PubMed electronic bibliographic database. The search was restricted to English and publications between 2010 and 2021. The keywords used were: multiple sclerosis, IFN-β, antigen presenting cells, T cells and B cells alone or in conjugation. The bibliographies of retrieved articles and previous review articles were hand searched to obtain additional articles.

7. Conclusion

In demyelinating diseases, mainly in relapse phase of RRMS, the BBB suffer a profound disturbance, so as the exchanges and ultimately the CNS itself. Despite CNS suffered an immune response, immune abnormalities could be found in the peripheral immune compartment.

The periphery assumes an extremely important role in the study of MS. In remission phase is establish an equilibrium between CNS and systemic circulation. In this chapter we have attempted to contribute to highlight the more relevant data regarding circulating cell subsets that could potentially be considered as peripheral biomarkers in RRMS patients treated with IFN-β.

Some circulating immune cells assume differences between the remission and relapse phases of RRMS. These differences may be used as disease activity biomarkers to measure inflammatory and/or neurodegenerative components of disease and helpful to discriminate between phases of RRMS.

Technological advances of flow cytometry have greatly increased the strength of analysis achievable at the single-cell level. These developments can be applied to understand more clearly the immunopathology of MS and the identification of consistent, safe and reproducible biomarkers in the periphery.

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References

[1] Lassmann H, Brück W, Lucchinetti CF. The immunopathology of multiple sclerosis: An overview. In: Brain Pathology. 2007. p. 210-218. DOI: 10.1111/j.1750-3639.2007.00064.x

[2] Noseworthy JH, Lucchinetti C, Rodriguez M W, BG. Multiple Sclerosis. N Engl J Med. 2000;343:938-952. DOI: 10.1056/NEJM200009283431307

[3] Goverman J. Autoimmune T cell responses in the central nervous system. Nat Rev. Immunol. 2009;9(6):393. Doi:10.1038/nri2550

[4] Haji Abdolvahab M, Mofrad MRK, Schellekens H. Interferon Beta: From molecular level to therapeutic effects. Vol. 326, international review of cell and molecular biology. Elsevier Inc.; 2016. 343-372 p. DOI: 10.1016/bs.ircmb.2016.06.001

[5] Dhib-Jalbut S, Marks S. Interferon-β mechanisms of action in multiple sclerosis. Neurology. 2010;74(SUPPL.). DOI: 10.1212/WNL.0b013e3181c97d99

[6] Ziemssen T, Akgün K, Brück W. Molecular biomarkers in multiple sclerosis. J Neuroinflammation. 2019;16(1):1-11. DOI: 10.1186/s12974-019-1674-2

[7] Hauser SL, Oksenberg JR. The Neurobiology of Multiple Sclerosis: Genes, Inflammation, and Neurodegeneration. 2006;61-76. DOI: 10.1016/j.neuron.2006.09.011

[8] Kamm CP, Uitdehaag M, Polman CH. Neuro-Update: Multiple Sclerosis Multiple Sclerosis: Current Knowledge. 2014;132-141. DOI: 10.1159/000360528

[9] Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Vol. 15, nature reviews immunology. Nature Publishing Group; 2015. p. 545-558. DOI: 10.1038/nri3871

[10] Comabella M, Khoury SJ. Immunopathogenesis of multiple sclerosis. Clin Immunol. 2012;142(1):2-8. DOI: 10.1016/j.clim.2011.03.004

[11] Vaughn CB, Jakimovski D, Kavak KS, Ramanathan M, Benedict RHB, Zivadinov R, et al. Epidemiology and treatment of multiple sclerosis in elderly populations. Vol. 15, Nature Reviews Neurology. Springer US; 2019. p. 329-42. DOI: 10.1038/s41582-019-0183-3

[12] Simmons SB, Pierson ER, Lee SY, Goverman JM. Modeling the heterogeneity of multiple sclerosis in animals. Vol. 34, Trends in Immunology. 2013. p. 410-422. DOI: 10.1016/j.it.2013.04.006

[13] Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. Lancet. 2018;391(10130):1622-1636. DOI: 10.1016/S0140-6736(18)30481-1

[14] Jones AP, Kermode AG, Lucas RM, Carroll WM, Nolan D, Hart PH. Circulating immune cells in multiple sclerosis. Vol. 187, clinical and experimental immunology. Blackwell Publishing Ltd.; 2017. p. 193-203. DOI: 10.1111/cei.12878

[15] Hegen H, Auer M, Deisenhammer F. Pharmacokinetic considerations in the treatment of multiple sclerosis with interferon-β. Vol. 11, Expert Opinion on Drug Metabolism and Toxicology. 2015. p. 1803-1819. DOI: 10.1517/17425255.2015.1094055

[16] Rizzo F, Giacomini E, Mechelli R, Buscarinu MC, Salvetti M, Severa M, et al. Interferon-β therapy specifically reduces pathogenic memory B cells in multiple sclerosis patients by inducing a FAS-mediated apoptosis. Immunol Cell Biol. 2016 Oct 1;94(9):886-894. DOI: 10.1038/icb.2016.55

[17] Dendrou CA, Fugger L. ScienceDirect Immunomodulation in multiple sclerosis: promises and pitfalls. Curr Opin Immunol. 2017;49:37-43. DOI: 10.1016/j.coi.2017.08.013

[18] Mendes A, Sá MJ. Classical immunomodulatory therapy in multiple sclerosis: How it acts, how it works. Arq Neuropsiquiatr. 2011;69(3):536-543. DOI: 10.1590/S0004-282X20 11000400024

[19] Axtell RC, Raman C, Steinman L. Type i interferons: Beneficial in Th1 and detrimental in Th17 autoimmunity. Vol. 44, Clinical Reviews in Allergy and Immunology. 2013. p. 114-120. DOI: 10.1007/s12016-011-8296-5

[20] Baufeld C, O'Loughlin E, Calcagno N, Madore C, Butovsky O. differential contribution of microglia and monocytes in neurodegenerative diseases. Vol. 125, Journal of Neural Transmission. Springer Vienna; 2018. p. 809-826. DOI: 10.1007/ s00702-017-1795-7

[21] Wong KL, Yeap WH, Tai JJY, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: Implications for health and disease. Immunol Res. 2012;53(1-3):41-57. DOI: 10.1007/ s12026-012-8297-3

[22] Kasper LH, Reder AT. Immunomodulatory activity of interferon-beta. Vol. 1, Annals of Clinical and Translational Neurology. 2014. p. 622-631. DOI: 10.1002/acn3.84

[23] Severa M, Rizzo F, Giacomini E, Salvetti M, Coccia EM. IFN-β and multiple sclerosis: Cross-talking of immune cells and integration of immunoregulatory networks. Cytokine Growth Factor Rev. 2015;26(2):229-239. DOI: 10.1016/j. cytogfr.2014.11.005

[24] Waschbisch A, Schröder S, Schraudner D, Sammet L, Weksler B, Melms A, et al. Pivotal role for CD16 + monocytes in immune surveillance of the central nervous system. J Immunol. 2016;196(4):1558-1567. DOI: 10.4049/jimmunol.1501960

[25] Boltjes A, van Wijk F. Human dendritic cell functional specialization in steady-state and inflammation. Front Immunol. 2014;5(APR):1-13. DOI: 10.3389/fimmu.2014.00131

[26] Pennel L, Fisher E. IFN-β effects on dendritic cells in EAE. Immunology. 2016;38(1):42-49. DOI: 10.1111/ imm.12781

[27] Monteiro A, Rosado P, Rosado L, Fonseca AM, Coucelo M, Paiva A. Alterations in peripheral blood monocyte and dendritic cell subset homeostasis in relapsing–remitting multiple sclerosis patients. J Neuroimmunol. 2021;350(September 2020):577433. DOI: 10.1016/j. jneuroim.2020.577433

[28] Bergh FT, Dayyani F, Ziegler-heitbrock L. Impact of type-I-interferon on monocyte subsets and their differentiation to dendritic cells An in vivo and ex vivo study in multiple sclerosis patients treated with interferon-beta. 2004;146:176-188. DOI: 10.1016/j.neuroim.2003.10.037

[29] Ziegler-Heitbrock L. Blood monocytes and their subsets: Established features and open questions. Front Immunol. 2015;6(AUG):1-5. DOI: 10.3389/fimmu.2015.00423

[30] Zang YC, Skinner SM, Robinson RR, Li S, Rivera VM, Hutton GJ, et al. Regulation of differentiation and functional properties of monocytes and monocyte-derived dendritic cells by interferon beta in multiple sclerosis. Mult Scler. 2004;10(5):499-506. DOI: 10.1191/135245804ms1081oa

[31] Kantor AB, Deng J, Waubant E, Lin H, Becker CH, Lacy JR, et al.

Identification of short-term pharmacodynamic effects of interferon-beta-1a in multiple sclerosis subjects with broad- based phenotypic profiling. J Neuroimmunol. 2007;188(1-2):103-116. DOI: 10.1016/j.jneuroim.2007.05.009

[32] Marckmann S, Wiesemann E, Hilse R, Trebst C, Stangel M. Interferon-b up-regulates the expression of co-stimulatory molecules CD80, CD86 and CD40 on monocytes: significance for treatment of multiple sclerosis. 2004;499-506. DOI: 10.1111/j.1365-2249.2004.02624.x

[33] Kunkl M, Frascolla S, Amormino C, Volpe E, Tuosto L. T Helper cells: The modulators of inflammation in Multiple Sclerosis. Cells. 2020;9(2):1-20. DOI: 10.1191/1352458504ms1081oa

[34] Machado-Santos J, Saji E, Tröscher AR, Paunovic M, Liblau R, Gabriely G, et al. The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue-resident CD8+ T lymphocytes and B cells. Brain. 2018 Jul 1;141(7):2066-2082. DOI: 10.1093/brain/awy151

[35] Kaskow BJ, Baecher-allan C. Effector T cells in Multiple Sclerosis. Cold Spring Harbor Perspectives in Medicine 2018;1-14. DOI: 10.1101/cshperspect.a029025

[36] Peelen E, Thewissen M, Knippenberg S, Smolders J, Muris AH, Menheere P, et al. Fraction of IL-10+ and IL-17+ CD8 T cells is increased in MS patients in remission and during a relapse, but is not influenced by immune modulators. J Neuroimmunol. 2013;258 DOI: 10.1016/j.jneuroim.2013.02.014

[37] Stadhouders R, Lubberts E, Hendriks RW. A cellular and molecular view of T helper 17 cell plasticity in autoimmunity. J Autoimmun. 2018;87:1-15. DOI: 10.1016/j.jaut.2017.12.007

[38] Cipollini V, Anrather J, Orzi F, Iadecola C. Th17 and Cognitive Impairment: Possible Mechanisms of Action. 2019;13(November):1-12. DOI: 10.3389/fnana.2019.00095

[39] Sie C, Korn T, Mitsdoerffer M. Th17 cells in central nervous system autoimmunity. Exp Neurol. 2014;262(Part A):18-27. DOI: 10.1016/j.expneurol.2014.03.009

[40] Li YF, Zhang SX, Ma XW, Xue YL, Gao C, Li XY. Levels of peripheral Th17 cells and serum Th17-related cytokines in patients with multiple sclerosis: A meta-analysis. Mult Scler Relat Disord. 2017;18:20-25. DOI: 10.1016/j.msard.2017.09.003

[41] Monteiro A, Rosado P, Rosado L, Fonseca AM, Paiva A. Alterations in circulating T cell functional subpopulations in interferon-beta treated multiple sclerosis patients: A pilot study. J Neuroimmunol. 2020;339. DOI: 10.1016/j.jneuroim.2019.577113

[42] Rodrigues G, Passos D, Sato DK, Becker J, Fujihara K. Th17 cells pathways in Multiple Sclerosis and Neuromyelitis Optica Spectrum disorders: Pathophysiological and therapeutic implications. Hindawi. 2016;2016. DOI: 10.1155/2016/5314541

[43] Van Langelaar J, Van Der Vuurst De Vries RM, Janssen M, Wierenga-wolf AF, spilt IM, Siepman TA, et al. T helper 17.1 cells associate with multiple sclerosis disease activity: Perspectives for early intervention. Brain. 2018;141(5):1334-1349. DOI: 10.1093/brain/awy069

[44] Raphael I, Nalawade S, Eagar TN, Forsthuber TG. Cytokine T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine. 2014. DOI: 10.1016/j.cyto.2014.09.011

[45] Libera DD, Di Mitri D, Bergami A, Centonze D, Gasperini C, Grasso MG,

et al. T regulatory cells are markers of disease activity in multiple sclerosis patients. PLoS One. 2011;6(6). DOI: 10.1371/journal.pone.0021386

[46] Haas J, Fritzsching B, Trübswetter P, Korporal M, Milkova L, Fritz B, et al. Prevalence of Newly Generated Naive Regulatory T Cells (T reg) Is Critical for T reg Suppressive Function and Determines T reg Dysfunction in Multiple Sclerosis. J Immunol. 2007;179(2):1322-1330. DOI: 10.4049/jimmunol.179.2.1322

[47] Venken K, Hellings N, Broekmans T, Hensen K, Rummens J, Stinissen P. Natural naive CD4 + CD25 + CD127 low regulatory T cell (Treg) development and function are disturbed in Multiple Sclerosis patients: Recovery of memory Treg homeostasis during disease progression. J Immunol. 2008;180(9):6411-6420. DOI: 10.4049/jimmunol.180.9.6411

[48] Scherm MG, Ott VB, Daniel C. Follicular helper T cells in autoimmunity. Curr Diab Rep. 2016;16(8). DOI: 10.1007/s11892-016-0770-2

[49] Quinn JL, Axtell RC. Emerging role of follicular T helper cells in multiple sclerosis and experimental autoimmune encephalomyelitis. Int J Mol Sci. 2018;19(10). DOI: 10.3390/ijms19103233

[50] Song W, Craft J. T follicular helper cell heterogeneity: Time, space, and function. Vol. 288, Immunological Reviews. 2019. p. 85-96. DOI: 10.1111/imr.12740

[51] Romme Christensen J, Börnsen L, Ratzer R, Piehl F, Khademi M, Olsson T, et al. Systemic inflammation in progressive multiple sclerosis involves follicular T-helper, Th17- and activated B-cells and correlates with progression. PLoS One. 2013 Jan [cited 2015 Jan 12];8(3):e57820. DOI: 10.1371/journal.pone.0057820

[52] Prinz I, Silva-Santos B, Pennington DJ. Functional development of γδ T cells. Eur J Immunol. 2013;43(8):1988-1994. DOI: 10.1002/eji.201343759

[53] Serre K, Silva-Santos B. Molecular mechanisms of differentiation of murine pro-inflammatory γδ T cell subsets. Front Immunol. 2013;4(DEC):1-7. DOI: 10.3389/fimmu.2013.00431

[54] Battistini L, Caccamo N, Borsellino G, Meraviglia S, Angelini DF, Dieli F, et al. Homing and memory patterns of human γδ T cells in physiopathological situations. Vol. 7, Microbes and Infection. 2005. p. 510-517. DOI: 10.1016/j.micinf.2004.12.008

[55] Blink SE, Miller SD. The contribution of gammadelta T cells to the pathogenesis of EAE and MS. Curr Mol Med. 2009;9(1):15-22. DOI: 10.2174/156652409787314516

[56] Monteiro A, Cruto C, Rosado P, Martinho A, Rosado L, Fonseca M, et al. Characterization of circulating gamma-delta T cells in relapsing vs. remission multiple sclerosis. J Neuroimmunol. 2018;318. DOI: 10.1016/j.jneuroim.2018.02.009

[57] Iarlori C, Reale M, Lugaresi A, De Luca G, Bonanni L, Di Iorio A, et al. RANTES production and expression is reduced in relapsing–remitting multiple sclerosis patients treated with interferon-β-1b. J Neuroimmunol. 2000;107(1):100-107. DOI: 10.1016/S0165-5728(00)00261-7

[58] Szczuciński A, Losy J. Chemokines and chemokine receptors in multiple sclerosis. Potential targets for new therapies. Acta Neurol Scand. 2007;115(3):137-146. DOI: 10.1111/j.1600-0404.2006.00749.x

[59] Lehmann-Horn K, Kronsbein HC, Weber MS. Targeting B cells in the

treatment of multiple sclerosis: Recent advances and remaining challenges. Ther Adv Neurol Disord. 2013 May [cited 2015 Jan 10];6(3):161-173. DOI: 10.1177/1756285612474333

[60] Michel L, Touil H, Pikor NB, Gommerman JL, Prat A, Bar-Or A. B Cells in the multiple sclerosis central nervous system: Trafficking and contribution to CNS-compartmentalized inflammation. Vol. 6, Frontiers in immunology. Frontiers Media S.a.; 2015. DOI: 10.3389/fimmu.2015.00636

[61] Sospedra M. B cells in multiple sclerosis. Vol. 31, Current Opinion in Neurology. Lippincott Williams and Wilkins; 2018. p. 256-262. DOI: 10.1097/WCO.000000000000563

[62] Li R, Patterson KR, Bar-Or A. Reassessing B Cell contributions in multiple sclerosis. Vol. 19, nature immunology. Nature Publishing Group; 2018. p. 696-707. DOI: 10.1038/s41590-018-0135-x

[63] Perez-Andres M, Paiva B, Nieto WG, Caraux a., Schmitz a., Almeida J, et al. Human peripheral blood B-Cell compartments: A crossroad in B-cell traffic. Cytom Part B - Clin Cytom. 2010 Jan [cited 2014 Nov 5];78(SUPPL. 1):S47-60. DOI: 10.1002/cyto.b.20547

[64] Schubert RD, Hu Y, Kumar G, Szeto S, Abraham P, Winderl J, et al. IFN-β treatment requires B cells for efficacy in Neuroautoimmunity. J Immunol. 2015 Mar 1;194(5):2110-2116. DOI: 10.4049/jimmunol.1402029

[65] Longbrake EE, Cross AH. Effect of multiple sclerosis disease-modifying therapies on b cells and humoral immunity. Vol. 73, JAMA neurology. American Medical Association; 2016. p. 219-225. DOI: 10.1001/jamaneurol.2015.3977

[66] Monteiro A, Cruto C, Rosado P, Rosado L, Fonseca AM, Paiva A. Interferon-beta treated-multiple sclerosis patients exhibit a decreased ratio between immature/transitional B cell subset and plasmablasts. J Neuroimmunol. 2019;326. DOI: 10.1016/j.jneuroim.2018.11.001

[67] Mitsdoerffer M, Peters a. tertiary lymphoid organs in central nervous system autoimmunity. Vol. 7, Frontiers in immunology. Frontiers Media S.a.; 2016. DOI: 10.3389/fimmu.2016.00451

[68] Kieseier BC. The mechanism of action of interferon-beta in relapsing multiple sclerosis. CNS Drugs. 2011;25(6):491-502. DOI: 10.2165/11591110-000000000-00000