2019

The Role of Interferon-β in the Treatment of Multiple Sclerosis

Cameron Young
Assumption College

The Role of Interferon-β in the Treatment of Multiple Sclerosis

By

Cameron Young

Faculty Supervisor: Laura Marcotte, Ph. D.

Natural Sciences

A Thesis Submitted to Fulfill the Requirements of the Honors Program at Assumption College

Spring 2019

Abstract:

Multiple sclerosis (MS) is a progressive neurodegenerative disorder that affects 140 out of every 100,000 individuals in North America. MS is believed to be mediated by T cells, in which the T cells directly attack or indirectly activate immune cells for attack on the myelin sheath of neurons, which can lead to blindness, paralysis, muscle weakness, and walking difficulties. Treatment of MS involves the use of immunomodulatory therapeutics, such as interferon-β (IFN-β), to decrease the progression of demyelination within the CNS. IFN-β has been linked to many different immunomodulatory roles in the pathology of MS. Of interest, IFN-β is believed to alter T-cell activation, cytokine production, and transmigration of immune cells. Studies have found that IFN-β increases co-stimulatory molecule production in dendritic cells and monocytes. IFN-β has also been found to alter various cytokine expressions within immune cells, such as T-cells and dendritic cells. In addition, IFN-β inhibits immune cell movement through the blood-brain barrier. These results taken together suggest a complex inhibitory system, propagated by IFN-β signaling, that may reduce the functionality of T cells and dendritic cells, such that T-cells have reduced ability to mediate neuronal attack in cases of MS.

Introduction:

Multiple sclerosis (MS) is a progressive neurodegenerative, autoimmune disease where an individual's immune system attacks the central nervous system (CNS), which may lead to muscle weakness, trouble swallowing, depression, blindness, and paralysis (Parham, 2015; National Multiple Sclerosis Society, 2018). MS affects 33 out of every 100,000 individuals worldwide, with greater prevalence in North America (Leray et al., 2016).

The symptoms of MS result from the demyelination of the axons of specialized cells of the central nervous system called neurons (Marieb et al., 2016). Neurons have a unique structure in which an axon extends from the cell body of the neuron and connects each neuron allowing for electrical signals to be sent through the nervous system (Marieb et al., 2016). The axon is surrounded by myelin which functions as an insulator for the axons (Marieb et al., 2016). Myelin is crucial for the fast transduction of the electrical signals down the axons of the neurons (Marieb et al., 2016). Demyelination is believed to be mediated by T cells. The T cells directly attack or activate immune cells for attack on the myelin of neurons (Garg et al., 2015). In the immunopathology of MS, there are three distinct phases in the process of demyelination: 1) activation and differentiation of T cells; 2) transmigration of immune cells; and 3) immune response to neurons of the CNS (Garg et al., 2015). This immunopathology is believed to prime T cells for distinct roles in the demyelination of neurons, such that the immune cells move to the CNS and enact distinct inflammatory and immune responses against the host's myelin (Garg et al., 2015).

The current treatments available for MS are not cures, but rather aim at reducing the progression of the disease. Many different therapeutics exist for the treatment of MS, including glatiramer acetate, teriflunomide, fingolimod, alemtuzumab, and ocrelizumab (National Multiple

Sclerosis Society, 2018). The most common treatment for MS prescribed is interferon-β. Interferon-β (IFN-β) is a protein made within the human body and synthetically that enacts many changes to the immune cells through cell signaling (Parham, 2015). Within the immune system, IFN-β acts by reducing viral infection (Parham, 2015). However, treatments with IFN-β to some individuals with MS have shown marked decreases to the progression of the disease (Rog et al., 2006). Studies have shown that treatment with IFN-β to individuals with MS leads to many different changes in the function of immune cells, especially T cells and dendritic cells, which may alter the progression of MS. Three areas of effect were studied within this review: 1) the effect of IFN-β on T cell activation; 2) the effect of IFN-β on T cell and dendritic cell cytokine production; and 3) IFN-β effect on immune cell transmigration.

In this review, inhibitory and regulatory effects of IFN-β will be discussed. The inhibitory and regulatory effects of IFN-β include the changes to cytokine expression in dendritic and T cells, the effect of IFN-β on costimulatory and coinhibitory molecules in T cells and dendritic cells, and the inhibition of immune cell movement. The analysis of the data of current studies can provide new insights into the function of IFN-β as a therapeutic and its effect on immune cells. These findings can reveal new hypotheses into the immunopathology of MS. Additionally, these studies' findings can show new insights into the effects of IFN-β treatment in MS patients.

Epidemiology and Etiology of MS:

The prevalence of MS in the world has been increasing over the years from 30/100,000 individuals in 2008 to 33/100,000 individuals in 2013 (Leray et al., 2016). However, in North

America, the prevalence is the highest with 140 individuals per every 100,000 individuals being affected by the disease (Leray et al., 2016). In addition, the prevalence of MS is higher in women than in men with the peak onset of the disease appearing between twenty and fifty-five years old (Garg and Smith, 2015). Roughly 2.5 million individuals are affected by the disease worldwide. About eight-five percent of these individuals have relapsing-remitting multiple sclerosis, which is a period of new symptoms (relapse) that are followed by remissions with no new symptoms occurring (stable state) (National MS Society, 2018). The individuals diagnosed with relapsing-remitting MS will eventually progress to secondary progressive MS, which is a period of progressive decrease to an individual's neurological functioning (National MS Society, 2018).

The causative factors of MS have not been completely elucidated. Many different hypotheses exist to explain the onset of MS, such as exposure to smoking in childhood, reduced vitamin D throughout life, and exposure to Epstein-Barr virus in childhood (Leray et al., 2016; Tullman, 2013). These factors have all been correlated to the increased incidence of MS (Leray et al., 2016; Tullman, 2013). In addition, these potential environmental causative factors occurred primarily in childhood (Leray et al., 2016). Interestingly, MS may have a strong genetic component in addition to the environmental components. This genetic component can be seen in the ratio of women affected by MS in comparison to men. It has been shown that MS affects about 3 women to every 1 man, suggesting a sex linkage to the incidence of MS (Harbo et al., 2013). Additionally, it has been found that the risk of MS increases the more immediate the familial connection, such that daughter of the mother may have a higher risk of MS than the cousin of the mother (Kantarci, 2008). This hereditary aspect of MS may be due to certain genetic components, such as the expression of human leukocyte antigen-DR15 (HLA-DR15) haplotype (Leray et al., 2016). HLA-DR15 is a molecule on the surface of antigen-presenting

cells that holds foreign peptide fragments for T cells to react (Scholz et al., 2017). It is suggested that the distinct phenotype of the HLA-DR15 haplotype may predispose individuals for MS because it can present autoantigens for the attack on the central nervous system (Scholz et al., 2017).

Interferon-β: Signaling Pathway and Relevance:

One of the most common therapeutics used in treatment for MS is interferon-β (IFN-β) (Figure 1). IFN-β is therapeutic that diminishes the effects of MS and reduces the progression of MS, but does not cure the disease. Studies show that IFN- β caused a 30% decrease in the relapse rate of patients and the decrease of new lesions formed within the brain (Rog and Mottershead, 2006). IFN-β is a protein that binds to receptors found within many different immune cells called interferon-alpha/β receptors (IFNARs) (Weerd and Nguyen, 2012). The effects of IFN-β are widespread because the interferon-β receptors have been isolated on many different immune cell surfaces, such as T cells, dendritic cells, and macrophages (Weerd and Nguyen, 2012). The normal function of IFN-β in the host immune system is to induce viral protection within cells (Parham, 2015). Interestingly, in the treatment of MS, the binding of IFN-β to its specific receptors on various immune cells can cause many changes to the immune cells, which may limit the immune cells' abilities to enact immune responses to the autoantigens within the host's central nervous system. Some of these inhibitory functions enacted by IFN-β signaling are the changes to cytokines present within the environment of the T cell, the inhibition of T cell activation, and the inhibition to immune cell movement.

These alterations to the behaviors of various immune cells is mediated through a specific intracellular pathway (Figure 2). IFN-β binds to interferon alpha/β receptors (IFNAR) present on many different immune cells, including T cell subtypes, macrophages, and antigen-presenting

dendritic cells. Upon binding to the IFNAR-1 or -2, IFNAR-1 and -2 complex form a dimer. The dimerization of IFNAR-1 and -2 leads to the auto-phosphorylation of associated kinases: Janus and tyrosine kinases (Ivashkiv et al., 2014). Janus-activated kinase-1 (JAK-1) is associated with IFNAR-2, and tyrosine kinase-2 (TYK-2) is associated with IFNAR1. Autophosphorylation of IFNAR-1 and -2 leads to the formation of docking sites on the receptors (Ivashkiv et al., 2014). These docking sites bind signal transducers and activators of transcription (STATs) (Ivashkiv et al., 2014). Activated STAT proteins can dimerize and complex with other proteins to form specific transcriptional modifying protein complexes (Ivashkiv et al., 2014). Of interest to IFN-β, JAK-1 and TYK-2 allow for the phosphorylation and activation of STAT-1 and STAT-2 (Ivashkiv et al., 2014). The activated STATs can dimerize to form either homodimers or heterodimers. Any STAT complex can translocate to the nucleus and induce modifications to transcription. IFN-β signaling can lead to the formation of a complex called the interferon stimulated gene factor 3 (ISGF3) (Ivashkiv et al., 2014). ISGF3 is formed by the complexing of STAT-1 and STAT-2 with a DNA binding protein, interferon regulatory factor 9 (IRF9) (Ivashkiv et al., 2014). Once the dimers and complexes form and travel to the nucleus, different actions occur. For example, the STAT1 homodimer can bind to the IFN-y-activated sequences (GAS), whereas the ISGF3 complex can bind to the IFN-stimulated response elements (ISREs) (Ivashkiv et al., 2014). Each of these DNA binding and transcriptional changes leads to different gene expression performed by the cells that has been correlated to reduced inflammatory response (Ivashkiv, et al., 2014). Thus, it is possible that the transcriptional changes occurring from the IFN-β signaling lead to the positive results found in some individuals with MS that use IFN-β as a therapeutic.

The Nervous System and Its Relation to MS:

The nervous system is the network of communication and control found within the body. The nervous system performs three functions: 1) sensory input, 2) integration of the sensory input, 3) motor output (Marieb et al.,2016). The CNS controls all of the integration of the sensory information received from neurons all over the body and produces an output to react to the sensory information (Marieb et al.,2016). Within MS pathology, the immune system attacks the neurons of the central nervous system (CNS), which comprises the brain and the spinal cord (Archelos et al., 2002).

Neurons have a unique structure (Figure 3). The neuron contains a cell body, which holds the nucleus for the cell. The cell body has many projecting branches called dendrites that receive signals from other neurons, allowing for the propagation of signals from one neuron to the next (Marieb et al., 2016). The neuron also contains a single axon that carries a signal to the next neuron (Marieb et al., 2016). The transduction of the signals from one neuron to the next occurs at the synapse (Marieb et al., 2016). The synapse is a microscopic gap between the axon of the neuron sending the signal and the dendrite of the neuron receiving the signal (Marieb et al., 2016). The signal is propagated from one neuron to the next by way of chemical signal. The transduction of electrical signal by the signal-sending neuron causes a release of chemicals across the synaptic gap (Marieb et al., 2016). These chemicals bind to corresponding receptors on the dendrite of the receiving neuron leading to a change in ion flux producing an electrical signal in the signal receiving neuron (Marieb et al., 2016).

The fast transduction of the electrical signal down an axon is essential for the healthy function of any human nervous system. This fast transduction can only occur with the assistance of an insulator around the axon. This insulator is called myelin (Marieb et al., 2016). Myelin is a

sheath that surrounds the exterior of the axon (Marieb et al., 2016). It consists of layers of proteins and lipids (Morell et al., 1999). Connected to the myelin in the CNS is an oligodendrocyte. An oligodendrocyte is a myelin-producing cell, which both repairs and restores the myelin sheath, thus producing both proteins and lipids that wrap around the axon (Salzer et al., 2016). These layers of protein and lipids function as insulators to the electrical signal being sent down the axon (Marieb et al., 2016). If the insulation was not present, the electrical signal would dissipate before reaching its target neuron, or the body would have to expend more energy in order to propagate the neuronal signal from one neuron to the next (Marieb et al., 2016).

Two proteins have been found within myelin that have a linkage to MS pathology. Myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) have been used to induce auto-reaction for myelin within the immune cells of patients with MS (Peschl et al., 2017; Beniac et al., 1997). Consequently, immune cells can be activated for attack on the myelin proteins, thus disrupting and degrading the structure of the myelin sheath (Archelos et al., 2002). Without the myelin sheath, the electrical signals may be carried from one neuron to the next because the signal will dissipate before reaching the next neuron (Marieb et al., 2016). In the case of MS, this loss of rapid signaling, and possible loss of signaling in general, can lead to paralysis or blindness, as well as muscle weakness and loss of sensation (National Multiple Sclerosis Society, 2018).

The Immune System and Its Role in MS:

MS is an autoimmune disease in which the immune system causes demyelination of the CNS. Many different parts of the immune system play a role in the immunopathogenesis of MS.

Thus, it is important to discuss the different cells that play a role in the immunopathogenesis of MS. The immune system consists of the lymphatic system that is a series of vessels carrying immune cells to and from the blood stream (Parham, 2015). The lymphatic system connects the lymphoid tissues, which hold or produce a majority of the immune cells, to the blood stream (Parham, 2015). The peripheral lymphatic tissues are the areas where the lymphocytes, such as T cells, are primed for a response against a specific antigen (Parham, 2015). Antigens are foreign proteins that are found on pathogens, such as viruses or bacteria, and are taken up by phagocytosis and presented to T cells on antigen-presenting cells (Parham, 2015; Goldsby et al., 2002). In the case of MS, the antigen presented on antigen-presenting cells is an auto-antigen. Auto-antigens are proteins derived from the cells of the host, such as MBP or MOG from myelin (Parham, 2015).

The process of priming T cells involves two types of cells: CD4+ T cells and antigen-presenting cells (Parham, 2015; Goldsby et al., 2002). T have various functions (Parham, 2015; Goldsby et al., 2002). For example, CD4+ T cells are a distinct type of T cell because they express the CD4 T cell receptor on their cell surfaces (Parham, 2015). When these cells are activated, they function by activating other immune cells and producing cytokine environments, inducing a specific immune response to the antigen (Parham, 2015; Goldsby et al., 2002). The CD4+ T cell associates with antigen-presenting cells, such as monocytes and dendritic cells. Monocytes are cells that are present within the blood, spleen, and bone marrow, but cannot proliferate (Geissman et al., 2010). These cells are unique because they can differentiate into dendritic cells (DCs) (Geismann et al., 2010). Dendritic cells are the main antigen-presenting arm of the immune system (Geissman et al., 2010). DCs are far more potent at activating T cells and are differentiated from monocytes via the cytokine environment present, such that if enough

of a specific cytokine signal enters the cell, then the monocyte will begin to differentiate into a dendritic cell (Geissman et al., 2010). Dendritic cells travel throughout the bloodstream and tissues, phagocytosing foreign molecules and presenting them on MHC molecules for T cell activation (Geissman et al., 2010).

Once primed by the antigen-presenting cells (DCs and monocytes), the primed CD4+ T cells are differentiated by cytokine signaling (Parham, 2015). Cytokines are proteins produced by immune cells, such as macrophages, T cells, and antigen-presenting cells (Parham, 2015; Goldsby et al., 2002). The cytokine signal induces transcriptional changes within primed CD4+ T cells, leading to distinct functional roles depending on the transcriptional factor present (Parham, 2015; Goldsby et al., 2002). Two effector CD4+ T cells play a role in the immunopathology of MS: T helper type 1 (T_H1) cells and T helper type 17 (T_H17) cells. T_H1 cells are distinct because they express the transcriptional factor T-bet (Parham, 2015; Goldsby et al., 2002). The T-bet expression in primed CD4+ T cells is in response to the cytokine signaling from interleukin-12 (IL-12) and interferon-γ (IFN-γ). T_H1 cells are believed to function by producing pro-inflammatory cytokines contributing to the inflammatory immune response (Parham, 2015; Goldsby et al., 2002). In addition, T_H1 cells activate macrophages and produce cytokines that assist macrophages in their phagocytic roles (Parham, 2015; Goldsby et al., 2002). T_H17 cells are distinct from T_H1 cells because their transcription factor is RORγT (Parham, 2015). The expression of RORγT in primed CD4+ T cell is induced by cytokine signaling of interleukin-6 (IL-6), interleukin-21 (IL-21), and tumor growth factor-β (TGF-β) (Parham, 2015; Martinez et al., 2008). T_H17 cells are believed to function by producing pro-inflammatory cytokines that contribute to the inflammatory immune response (Parham, 2015; Martinez et al., 2008). In addition, it has been shown that T_H17 cells secrete the cytokines IL-17 A and IL-17 F

that have been found to increase neutrophil attraction and proliferation, as well as upregulating pro-inflammatory cytokines (Parham, 2015). This increase of neutrophils and pro-inflammatory cytokines can degrade epithelial barriers because of the phagocytic and inflammatory properties of neutrophils (Parham, 2015).

These effector T cells can play a role in activating and inducing phagocytic cell function. Two phagocytic cells are linked to these effector T cells: macrophages and neutrophils. Macrophages are activated by T_H1 cells (Parham, 2015). The macrophages receive two signals from the T_H1 cells: interferon-γ (IFN-γ) signal and CD40 receptor signal (Parham, 2015; Goldsby et al., 2002). The activation of macrophages leads to the production of many pro-inflammatory cytokines that increase the vascular permeability of endothelial cells, such as those found along the blood-brain barrier (Parham, 2015; Goldsby et al., 2002). In addition, these cytokines are also chemoattractants for other immune cells, signaling other immune cells to the site of attack (Parham, 2015; Goldsby et al., 2002). In the case of MS, the increase of pro-inflammatory cytokines and chemoattractant cytokines leads to neuronal damage by inflammation (Parham, 2015). Neutrophils are one of the immune cells signaled by the cytokines released from macrophages and T_H17 cells (Parham, 2015). Neutrophils are another subset of phagocytic cells that function by entering the site of infection and engulfing the pathogens and breaking them down (Parham, 2015; Goldsby et al., 2002). Neutrophils make up a majority of the white blood cells in the blood, and, as such, neutrophils are the first cells to the site of infection (Parham, 2015). In the case of MS, the increased vascular permeability may contribute to the increase of neutrophils in the CNS. Neutrophils could phagocytose the myelin in individuals with MS.

The roles of effector CD4+ T cells can also be suppressive. In the case of MS, regulatory T cells (T_{reg}), a type of effector CD4+ T cell, may play a role in the suppression of autoreactive T cells, such as those cells primed for MOG or MBP. T_{reg} cells are upregulated by the interaction of primed CD4+ T cells with interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) (Parham, 2015). The TGF-β and IL-10 signaling induce expression of the transcription factor FOXP3 (Parham, 2015). These T_{reg} cells function by suppressing the proliferation and function of other effector CD4+ T cells, such as T_H1 and T_H17 (Parham, 2015). The process of inhibition is not yet fully understood. However, studies have shown that a distinct receptor, the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), decreases the expression of co-stimulatory molecules on antigen-presenting cells (Schmidt et al., 2012). Also, it was found that T_{reg} cells produce perforin and granzymes that can induce apoptosis in effector T cells (Schmidt et al., 2012). In addition, these cells produce an anti-inflammatory cytokine environment that counteracts the pro-inflammatory cytokine environments produced by other effector CD4+ T cells (Parham, 2015). Most importantly, these cells dampen the activity of autoreactive T cells, such as those primed for MOG or MBP in MS, suggesting reduced autoimmune response with increasing T_{reg} populations (Parham, 2015; Corthay, 2009; Danikowski et al., 2017).

Immunopathogenesis of MS:

The immunopathogenesis of MS involves the progression of demyelination and axonal damage of the neurons by the immune system (Archelos et al., 2003). The immunopathogenesis of MS is a process that occurs in three stages: 1) autoactivation, proliferation, and differentiation of T cells within the lymphoid tissues, 2) transmigration of immune cells across the blood-brain barrier (BBB) into the CNS, and 3) reactivation of T cells and targeted immune response within

the CNS, producing demyelination and axonal damage (Archelos et al., 2002; Garg et al., 2015) (Figure 4.).

Autoactivation, Proliferation, and Differentiation of T cells in the Lymphoid Tissues:

The immunopathogenesis of MS begins in the lymphoid tissues of the lymphatic system. The lymphoid tissues are connected to the bloodstream and skin and are tissues that allow the antigen-presenting cells (dendritic cells) to interact with lymphocytes and start the adaptive immune response (Parham, 2015; Garg and Smith, 2015). Critical to the adaptive immune response, which is a targeted immune response to a specific pathogen, are the T cells. T cells are lymphocytes of the immune system that form a specific targeted attack on the pathogen that they have been primed for (Parham, 2015). In the case of MS, the T cells are primed for the MBP and MOG in the CNS (Archelos et al., 2002).

In the immunopathology of MS, various types of effector CD4+ T cells have been implicated in the autoreactive immune response. The activation of any CD4+ T cell involves the interaction between the CD4+ T cell receptor and major histocompatibility (MHC) molecules presented on the dendritic cell and the interaction of the CD28 receptor and the B7 costimulatory molecule on the dendritic cell (Parham, 2015). The co-stimulation and T cell receptor interactions induce proliferation of the naïve T cells via the upregulation of multiple transcription factors (Parham, 2015) (Figure 5). Differentiation of activated CD4+ T cells is believed to occur from signaling through cytokine receptor-cytokine interactions. Activated CD4+ T cells can be differentiated into various effect CD4+ T cell types by the interaction of cytokines, such as

interleukin-12 (IL-12), interferon-gamma (IFN-γ), interleukin-6 (IL-6), interleukin-21 (IL-21), and interleukin-17 (IL-17), with T cell receptors (Parham, 2015).

Two effector CD4+ T cells play a role in the immunopathogenesis of MS: T helper type 1 (T_H1) cells and T helper type 17 (T_H17) cells. T_H1 cells function by producing inflammatory cytokines and inducing inflammatory responses near the site of infection, or near the myelin in the case of MS (Parham, 2015). Additionally, T_H1 cells activate macrophages for attack (Parham, 2015). In the case of MS, the macrophages can phagocytose parts of the myelin sheath and increase the inflammatory response on the myelin (Wolf et al., 1990). T_H17 cells play a role in the immunopathology of MS by inducing neutrophil attraction and neutrophil accumulation at a site of infection (Parham, 2015) (Figure 6).

Transmigration of T Cells across the BBB:

Once the T cells are primed for their respective antigen, the T cells leave the lymphoid tissues and enter the bloodstream. The movement of the T cells through the blood brain barrier (BBB) occurs in the immunopathology of MS to allow for the T cells to begin an adaptive immune response to the autoantigens (MBP and MOG) found within the CNS. The process of transmigration (movement of T cells through the BBB) occurs with the T cell binding to the endothelial cells of the BBB by the interaction of various cell-adhesion molecules found on both the endothelial cells and T cells (Pinheiro et al., 2015). The interaction between the T cell and the endothelial cell via these molecules allows for the T cell to be slowed within the bloodstream and adhere to the endothelial cells (Pinheiro et al., 2015). The slowing of the T cells allows for increased reactions between these adhesion molecules, leading to the changes in the endothelial

cell connections called tight junctions (Pinheiro et al., 2015). The reduction in the connections between the endothelial cells allows the T cell to move through the gap (Pinheiro et al., 2015) (Figure 7). The final layer of the BBB is the basement membrane, which consists of many different structural proteins (Yong et al., 1998). The T cells produce matrix metalloproteinases (MMPs) that disrupt the basement membrane, allowing for degradation of the membrane (Yong et al., 1998). The degradation of the membrane allows for the T cells to enter into the CNS.

Immune Response within the CNS:

Once within the CNS, the T cells can begin the adaptive immune response to the specific autoantigen for which they are primed for, which are the MBPs and MOGs. Lesions within the CNS for MS patients can be active or inactive (benign) (Lucchinetti et al., 2000). Lesions are areas of demyelination, such that there is scarring or degradation on a portion of the brain (Sethi et al., 2017) The difference between an active and an inactive lesion is the presence of demyelination (Lucchinetti et al., 2000). The presentation of demyelinating factors, such as pro-inflammatory cytokines and increased levels of active T cells, shows that the lesion is active, and conversely, the lack of demyelinating factors shows that the lesion is inactive (Kornek et al., 2000). Demyelination is a complex process within the immunopathology of MS that is not completely understood. It is speculated that the demyelinating process involves the activation of damaging immune cells, such as macrophages, by the CD4+ T cells primed for the MBPs or MOGs of the neurons (Archelos et al., 2002). This activation of immune cells causes the increase of inflammatory cytokines and the direct degradation of the myelin sheath in the CNS, increasing the extent of myelin damage and inflammation (Archelos et al., 2002). This direct degradation of

the myelin occurs from the macrophages activated by T cells that have entered the CNS, engulfing the portions of the myelin containing the antigen (MBP and MOG) (Parham, 2015).

In addition to the T_H1 activation of macrophages, T_H17 cells are known to enact the recruitment and accumulation of neutrophils in the site of infection via IL-17 secretion (Parham, 2015). Neutrophils, like all traveling immune cells, enter the infected tissue via the interaction of epithelial proteins and the movement along a chemokine gradient (Parham, 2015). Once inside the CNS, the neutrophil functions as a phagocytic cell (Parham, 2015). However, neutrophils possess greater variability in their phagocytosis, thus they may be able to degrade greater portions of myelin (Parham, 2015). The increase of both T_H17 and T_H1 cells can mediate a phagocytic attack on the myelin of the CNS, leading to lesion formation in MS patients.

IFN-β's Effect on T cell Activation:

A key part of the immunopathology of MS is the activation of T cells for a specific autoantigen. For the activation of T cells, two signals are necessary. The first signal for the activation of T cells occurs between the T cell's CD4 receptor and the major histocompatibility complex (MHC) molecule (Parham, 2015). MHC molecules function by displaying peptide fragments of antigens, which prime the T cell for attack on cells presenting the specific antigen (Parham, 2015). The second signal occurs between a CD28 receptor on the T cells and a costimulatory molecule (CD80, CD86, and CD40) present on dendritic cells (Goldsby et al., 2002; Parham, 2015).

In the case of MS, the results of many different studies' findings regarding the upregulation of costimulatory and coinhibitory molecules by IFN-β treatment suggest a possible

inhibitory mechanism for T-cell activation. Namely, constant stimulation of T cells by the upregulated costimulatory molecules could lead to partial T cell exhaustion (Attanasio et al., 2016). This T cell exhaustion leads to the upregulation of coinhibitory molecules on T cells (Attanasio et al., 2016; Carter et al., 2002). The signaling through the co-inhibitory molecules has been found to reduce the T cell proliferation and may induce apoptosis (Carter et al., 2002; Parham, 2015). From these findings, it could be hypothesized that the amount of active T-cells would be reduced by the treatment of IFN-β to individuals with MS. This limitation to activation of T cells may reduce the ability of T cells to mediate active attacks and indirect attacks on the CNS.

The treatment of monocytes with IFN-β was shown to increase the expression of CD80, CD86, CD40, and human leukocyte antigen-DR isotype (HLA-DR) both *in vivo* and *in vitro* (Wiesemann et al., 2008; Marckmann et al., 2004). Similar to the treatment of monocytes with IFN-β, dendritic cells have been shown to have increased expression of MHC I and II molecules, CD 80, CD86, and CD40 (Pennell et al., 2017). However, some discrepancy exists regarding the effect of IFN-β on costimulatory molecule expression. It has been found that the IFN-β treatment of DCs could lead to decreases in costimulatory molecules, such as CD40 and CD80 (McRae et al., 2000). In addition, McRae et al. show that there is diminished function by the dendritic cells to activate T cells when IFN-β is present (McRae et al., 2000). Interestingly, the controversial results of the effect of IFN-β may be due to the sample of dendritic cells and the donor used for the dendritic cells or monocytes. This discrepancy would yield varying results due to each cell's complexity and variation in gene expression. These results suggest that the activation of T cells was increased by the upregulation of costimulatory molecules in monocytes and dendritic cells and MHC molecules in dendritic cells.

The increase in costimulatory molecules conflicts with the therapeutic effects of IFN-β. However, IFN-β has also been found to increase coinhibitory molecules. Coinhibitory molecules are similar to costimulatory molecules, but they work in opposition. The coinhibitory pathway of interest involves programmed death 1 receptor (PD-1) and its ligands programmed death ligand 1 and 2 (PD-L1, PD-L2) (Parham, 2015). These molecules have been implicated in cell signaling that causes cytokine alterations, increased differentiation of T cells into T regulatory cells, and reductions in cytotoxic T cells via apoptotic signaling, as well as apoptotic signaling in dendritic cells (Parham, 2015). For monocytes, IFN-β presence in cell culture showed increased levels of PD-L1 and PD-L2 in both healthy donors and MS patients (Wiesemann et al., 2008; Schreiner et al., 2004). PD-L1 and PD-L2 expression was not an exclusive case found in only one patient, but multiple MS patients had increased net expression of the PD-L1 co-inhibitory molecule (Wiesemann et al., 2008). However, the PD-L2 net expression did not increase to the same extent as PD-L1 (Wiesemann et al., 2008). In dendritic cells, only DCs semi-matured in inflammatory cytokine conditions and DCs matured in the presence of a superantigen (lipopolysaccharide) showed marked increases in PD-L1 when cultured in the presence of IFN-β (Schreiner et al., 2004; Wang et al., 2014). In addition to IFN-β treatment, it has been demonstrated that increased stimulation to CD4+ T cells by costimulatory molecules leads to the upregulation of PD-1 receptor on T cells (Carter et al., 2002). These results taken together suggest a possible inhibitory mechanism in which the overexpression of costimulatory molecules on dendritic cells and monocytes leads to overstimulation of CD4+T cells. This overstimulation could lead to an upregulation of PD-1 receptors on CD4+ T cells. Upregulation of PD-1 receptors may lead to increased signaling through the PD-1, thus reducing the proliferation and activation of CD4+ T cells.

Effects of IFN-β on Dendritic Cell Cytokine Production:

Cytokines play a key role in the differentiation of CD4+ T cells into their effector types (Parham, 2015). DCs can produce many different cytokines. DCs are one of the primary contributors to the cytokine milieu that determines the effector T cells differentiated (Parham, 2015; Goldsby et al., 2002). IFN-β is known to alter the cytokine expression in DCs through the IFN-β signaling pathway. DCs are known to produce many different cytokines. Relevant to the immunopathology of MS, IFN-β has been shown to alter the expression of the following cytokines: interleukin-10 (IL-10), the interleukin-12 (IL-12) family, interleukin-6 (IL-6), and interferon-γ (IFN-γ). These alterations to cytokine expression could have various effects on the immunopathology of MS, such as the reduction in T_H1 cell differentiation, an increase in T regulatory cell (T_{reg}) differentiation, suppression of T_H17 cells, and the decrease of macrophage functionality. These possible effects linked to the alterations of cytokine expression could reduce the demyelination and autoimmune response to myelin in individuals that have MS.

IL-10 Expression in DCs and the Effect of IFN-β:

IFN-β treatment of DCs has been found to cause increases in the IL-10 expression in DCs. IL-10 has many different functions within the immune system. IL-10 is believed to be an anti-inflammatory cytokine. IL-10 is known, normally, to induce the differentiation of naïve T cells in to effector T regulatory cells (T_{reg}) and T helper type 2 cells (T_H2) (Hsu et al., 2015; Parham, 2015). In addition to increasing the expansion of T_{reg} populations, IL-10 has been implicated in the downregulation of IL-12 in dendritic cells and macrophages (Goldsby et al.,

2007). Thus, IL-10 production can lead to the inhibition of the activation of macrophages (O-Farrell et al., 1998; Parham, 2015). In the case of MS, IL-10 may present a novel mechanism of inhibition for T_H1 cell differentiation and macrophage function, such that T_H1 cells and macrophages cannot affect an autoimmune response to myelin.

Dendritic cell expression of IL-10 can be regulated and altered by the treatment of IFN-β. In mice DCs lacking IFN-β expression, the production of IL-10 was significantly reduced in comparison to the mice with the normal IFN-β production (Pennell et al., 2017). This result suggests that the presence of physiological levels of IFN-β is necessary for production of normal levels of IL-10. In addition, it has been found that a marked increase in IL-10 expression has been seen in the exogenous treatment of DCs isolated from healthy human donors (Huang et al., 2001). The IL-10 upregulation shown from the treatment of DCs with IFN-β is intriguing because the upregulation in DCs is not isolated to one location of the immune organs, such that bone marrow DCs, splenic DCs, lymph node DCs, and peripheral DCs are upregulating IL-10 (Yen et al., 2015; Huang et al., 2001; Pennell et al., 2017). Interestingly, IL-10 upregulation by IFN-β treatment is shown to be caused by the IFN-β signaling pathway. It has been demonstrated that mice lacking interferon-α/β receptors (IFNARs) cannot produce increased levels of IL-10 (Pennell et al., 2017; Yen et al., 2015). In addition, it has been found that one of the primary factors of IFN-β signaling the upregulation of IL-10 is the presence of signal transducer and activators of transcription 2 (STAT-2) (Yen et al., 2015). Thus, without STAT-2 expressed within mice, it was demonstrated that IFN-β had no effect on DC expression of IL-10 (Yen et al., 2015). These results show that IFN-β treatment induces IL-10 expression through a JAK-STAT pathway in DCs throughout the lymphatic system.

Upregulation of IL-10 could have many different positive effects within the immunopathology of MS. The expression of IL-10 has been shown to reduce pro-inflammatory cytokines, namely, IL-12 and IFN-γ (Huang et al., 2005, Yen et al., 2015). In addition, IL-10 is known to induce differentiation of primed CD4+ T cells to either T_H2 cells or T_{reg} cells (Hsu et al., 2015; Parham, 2015). These results may present a unique inhibitory mechanism. The down-regulation of IL-12 and IFN-γ could reduce the differentiation of primed CD4+ T cells into T_H1 cells (Parham, 2015). Additionally, the presence of increased IL-10 in the cytokine milieu throughout the lymphatic system may play a role in the increased differentiation of primed CD4+ T cells into T_H2 and T_{reg} cells, thus reducing the T_H1 cell population (Hsu et al., 2015; Parham, 2015). T_{reg} cells have also been found to regulate the autoreactive T cell populations by inducing apoptosis or rendering the autoreactive T cell anergic (Parham, 2015). T_H1 cell differentiation being inhibited could decrease macrophage activation and the autoimmune response on myelin in individuals with MS (Parham, 2015; Goldsby et al., 2002). Additionally, the possible increased differentiation of T_{reg} cells could reduce the number of autoreactive T cells, such as those primed for MOG or MBP, thus limiting the attack on myelin (Hsu et al., 2015).

The Effect of IFN-β on the Cytokines of IL-12 Family:

DCs are known to produce many members of the IL-12 family, such as IL-12, IL-23, and IL-27, which can contribute to the differentiation of primed CD4+ T cells into T_H1 cells (Nagai et al., 2003; Parham, 2015). IFN-β has been found to affect the expression of these cytokines. IFN-β has been shown to reduce the expression of IL-12 and IL-23 and increase the expression of IL-27 (Nagai et al., 2003; Yen et al., 2015; Huang et al., 2001; McRae et al., 2000). These three distinct cytokines have been implicated in the contribution to the increased differentiation

of CD4+ T cells: IL-12, IL-23, and IL-27 (Vignali et al., 2012). This differentiation of CD4+ T cells into T_H1 cells by the mediation of IL-12 family cytokines could be linked to an increase in demyelination in patients with MS, and, thus, increase their symptoms.

The three distinct cytokines each have varying structures. The cytokines are heterodimers (Cooper et al., 2007; Vignali et al., 2012). For IL-12, the cytokine consists of two subunits: IL-12 p35 and IL-12 p40 (Cooper et al., 2007). The IL-23 cytokine consists of the subunits IL-23 p19 and IL-12 p40 (Cooper et al., 2007). IL-27 consists of two subunits: Epstein-Barr virus induced 3 (EBI3) and IL-12 p 28 (Cooper et al., 2007). The varying structures of the IL-12 family members lead to different functions. IL-12 functions by upregulating IFN-γ expression in DCs and T cells (Cooper et al., 2007; Vignali et al., 2012). Both IL-12 and IFN-γ synergize to induce the differentiation of T_H1 cells (Parham, 2015). The role of IL-23 is to steady the differentiation of primed CD4+ T cells into T_H17 cells (Vignali et al., 2012). The IL-27 cytokine functions as a synergistic cytokine that, when in the presence of IL-2 and/or IL-12 induces IFN-γ production in T cells and increased proliferation of T cells (Vignali et al., 2012; Pflanz et al., 2002). In the immunopathology of MS, T_H1 cells and T_H17 cells may play a role in the demyelination of the CNS. Thus, the effects of the IL-12 family may increase the T_H1 and T_H17 differentiation and cell populations leading to greater demyelination.

IFN-β has been found to alter the IL-12 family cytokine expression in DCs. Many studies have shown that the treatment of DCs with IFN-β has led to the down-regulation of IL-12 p 40 subunit (Yen et al., 2015; Huang et al., 2005; Huang et al., 2001; Nagai et al., 2003; McRae et al., 2000). In addition, it has been demonstrated that the treatment of DCs with IFN-β caused a decrease to the expression of IL-12 p35 subunit (Yen et al., 2015). Interestingly, the downregulation of the IL-12 p35 subunit was found in both the treatment of mice *in vivo* and the

in vitro treatment of mice DCs with IFN-β (Yen et al., 2015). These results show that the IL-12 cytokine has limited expression when DCs are treated with IFN-β. This result could indicate reductions in IFN-γ production by various immune cells because IL-12 is known to be a catalyst for IFN-γ expression (Cooper et al., 2007; Vignali et al., 2012).

It is known that both the IL-12 cytokine and IL-23 cytokine share the IL-12 p40 subunit in common. Thus, the limitation of the IL-12 p40 expression in DCs treated with IFN-β may also reduce the amount of active IL-23 in the cytokine milieu (Yen et al., 2015; Huang et al., 2005; Huang et al., 2001; Nagai et al., 2003; McRae et al., 2000). In mice that lacked IFN-β expression, it was demonstrated that the IL-23 expression was significantly increased (Pennell et al., 2017). In addition, the treatment of mice DCs with IFN-β showed a decrease in IL-23 production (Yen et al., 2015). Additionally, it was demonstrated that DCs throughout the lymphatic system had down-regulated IL-23 expression (Yen et al., 2015). Conflicting results exist for IFN-β effect on IL-23 p19 expression; some studies have shown that IFN-β treatment of DCs leads to modest increase in IL-23 p19 subunit expression, while other studies demonstrated that the IFN-β treatment of DCs leads to a drastic decrease in the IL-23 p19 subunit production (Yen et al., 2015; Nagai et al., 2003). These differences in IL-23 p19 expression in DCs treated with IFN-β may be the result varying exposure times to IFN-β (Nagai et al., 2003). The results of these various studies have shown that IFN-β treatment of DCs leads to the downregulation of IL-23 expression throughout the lymphatic system. The reduction of IL-23 production could lead to the inability of CD4+ T cells to differentiate into T_H17 cells. With reduced T_H17 cell populations, the autoimmune response on myelin may not be as severe due to the decrease in the pro-inflammatory cytokines that T_H17 cells produce.

IL-27 is another cytokine of the IL-12 family. It has been found that the treatment of DCs with IFN-β led to the increase of IL-27 expression (Nagai et al., 2003). Additionally, the knockdown of endogenous IFN-β in mice showed marked decreases in the IL-27 concentration (Pennell et al., 2017). These changes in the expression of IL-27 by the treatment of DCs with IFN-β was suggested to be the effect of increased IL-27 p28 expression in DCs (Nagai et al., 2003). Interestingly, IFN-β treatment led to no significant alterations to the EBI3 expression in DCs (Nagai et al., 2003). This result suggests that the IL-27 p28 subunit is a limiting factor for the expression of IL-27. These various studies showed that IL-27 expression is increased by IFN-β treatment. IL-27 is known to induce proliferation of T cells, as well as increased IFN-γ when in the presence of IL-2 and/or IL-12 (Vignali et al., 2012). In MS immunopathology, the increased IL-27 may cause increased IFN-γ expression and increased T_H1 cell differentiation (Parham, 2015; Vignali et al., 2012). However, this hypothesis is only possible if both IL-12 and/or IL-2 are present. It has been shown that IL-12 expression is limited in DCs which may lead to the ineffectiveness of IL-27 (Yen et al., 2015; Huang et al., 2005; Huang et al., 2001; Nagai et al., 2003; McRae et al., 2000).

In all, the DC expression of IL-12 family cytokines (IL-12, IL-23, and IL-27) have been shown to be altered by IFN-β treatment. The IFN-β treatment reduces both IL-12 and IL-23 expression in DCs by down-regulating the subunits of each cytokine (Yen et al., 2015; Huang et al., 2005; Huang et al., 2001; Nagai et al., 2003; McRae et al., 2000). The treatment of IFN-β also led to an increase in IL-27 production in DCs (Nagai et al., 2003; Pennell et al., 2017). In the case of MS, these cytokine changes limit the differentiation of CD4+ T cells into T_H1 and T_H17 cells. With reduced T_H1 and T_H17 cell populations, the inflammatory autoimmune response would be reduced (Parham, 2015). With a reduced inflammatory immune response, there would

be less demyelination in the CNS. Consequently, it could be hypothesized that the IFN-β treatment limits IL-12 family cytokines reducing the effector T cells that play a role in the process of demyelination.

The Effect of IFN-β on the DC Expression of Cytokine IL-6:

IFN-β treatment of DCs has been found to produce increased expression of IL-6 (McRae et al., 2000; Pennell et al., 2017; Huang et al., 2005). IL-6 is a pro-inflammatory cytokine that plays a role in the differentiation of primed CD4+ T cells into T_H17 cells (Parham, 2015). IL-6 is believed to function by increasing vascular permeability and metabolism of fat and muscle cells, subsequently altering body temperatures (Parham, 2015; Goldsby et al., 2002). Expression of IL-6 may allow for increased permeability of immune cells through the blood-brain barrier (Parham, 2015). In addition, the presence of IL-6 could suggest increases in the T_H17 cell population that would lead to increased inflammatory response to myelin in the CNS (Parham, 2015; Goldsby et al., 2002).

DCs treated with IFN-β have shown varied effects in IL-6 cytokine expression. A murine model for MS has been used in many studies to evaluate the effect of IFN-β. The mice are induced with experimental autoimmune encephalomyelitis (EAE), which presents similar immunopathology and symptoms to MS (Pennell et al., 2017; Constantinescu et al., 2011). EAE is induced in the murine model by using T cells primed for distinct peptides in the myelin, such as myelin oligodendrocyte glycoprotein (Pennell et al., 2017). It has been shown that when endogenous IFN-β is knocked down in EAE induced mice, the expression of IL-6 was significantly increased in DCs (Pennell et al., 2017). In addition to the murine model, it has been

demonstrated that an increase in the IL-6 cytokine expression in human DCs occurred when IFN-β treatment was used (Huang et al., 2005). These results support the positive effect of IFN-β on IL-6 expression. Thus, it may be suggested that the IFN-β treatment induces increased differentiation of T_H17 cells, leading to a greater inflammatory autoimmune response to the myelin in the CNS of individuals with MS (Parham, 2015; Goldsby et al., 2002).

These increases in IL-6 cytokine expression may be linked to costimulatory molecule expression on DCs. It has been demonstrated that the CD40 receptor was upregulated on DCs following IFN-β treatment (Wiesemann et al., 2008; Marckmann et al., 2004). In addition, studies have found that the association of CD40 receptors with their ligand, found on T cells, led to the increase of IL-6 secretion in DCs (McRae et al., 2000). In cultures containing only DCs, the treatment of IFN-β led to no change in the expression of IL-6 (Mc Rae et al., 2000). These results have shown that IFN-β treatment may increase IL-6 concentration when in physiological conditions because DCs can associate with the CD40 ligand on T cells upregulating the IL-6 expression in DCs (Wiesemann et al., 2008; Marckmann et al., 2004; McRae et al., 2000).

These results taken together have shown that IFN-β treatment of DCs led to the upregulation of IL-6 expression (McRae et al., 2000; Pennell et al., 2017; Huang et al., 2005). These results have also shown a novel mechanism for the increase of IL-6 expression in DCs following IFN-β treatment. The increase in CD40 receptor expression on DCs, caused by IFN-β treatment, suggests increased CD40 signaling which has been found to increase IL-6 expression in DCs (McRae et al., 2000; Pennell et al., 2017; Huang et al., 2005; Wiesemann et al., 2008; Marckmann et al., 2004). Consequently, IFN-β may function through an indirect pathway, namely, the CD40 pathway, to increase the IL-6 expression. This result may lead to increased differentiation of primed CD4+ T cells into T_H17 cells and increases the vascular permeability to

immune cells (Parham, 2015; Goldsby et al., 2002). T_H17 cells produce chemicals that are chemoattracts to neutrophils, which are phagocytic cells (Parham, 2015; Goldsby et al., 2002). In the case of MS, the blood-brain barrier is impaired and increased permeability occurs, such that neutrophils can enter the CNS and break down the myelin of individuals with MS (Prinz et al., 2017).

DCs Expression of IFN-γ and the Effect of IFN-β:

IFN-β treatment of DCs has been found to decrease the expression of IFN-γ (Pennell et al., 2017). IFN-γ functions by activating signaling in macrophages for pro-inflammatory cytokines (Parham, 2015; Goldsby et al., 2002). In addition to increasing macrophage cytokine expression, IFN-γ signaling in macrophages induces increased phagocytic activities (Parham, 2015; Goldsby et al., 2002). IFN-γ is also known to increase the IL-12 cytokine expression in macrophages and natural killer cells (Parham, 2015; Goldsby et al., 2007). IFN-γ is one of the cytokines that differentiates primed CD4+ T cells into T_H1 cells (Parham, 2015). In MS immunopathology, IFN-γ expression may lead to increased T_H1 cell populations. Increased T_H1 cells could indicate increased macrophage activity and inflammatory response to myelin in the CNS (Parham, 2015; Goldsby et al., 2002).

IFN-β has been found to alter the expression of IFN-γ. In a murine model induced with EAE and lacking IFN-β expression, the expression of IFN-γ was reduced (Yen et al., 2015). In a healthy murine model, the treatment of DCs with IFN-β on human DCs has been found to decrease the expression of IFN-γ (Huang et al., 2001). It has also been demonstrated that the treatment of IFN-β in the murine model led to an increase in IFN-γ expression in peripheral

blood mononuclear cells, which include DCs, throughout the lymphatic system (Ozenci et al., 2000; Weyenbergh et al., 2000; Yen et al., 2015). Interestingly, there is a lack of data on the effects of IFN-β on human DCs. However, the studies on murine models have shown that IFN-β may have a direct effect on IFN-γ expression within DCs. The decrease to the expression of IFN-γ expression may limit the differentiation of primed CD4+ T cells into T_H1 cells, thus reducing the inflammatory response to myelin in the CNS of MS patients (Parham, 2015).

IFN-γ expression is known to be associated with IL-12 signaling. It has been demonstrated that IFN-β treatment of DCs decreases the subunits of IL-12 p35 and IL-12 p40 that are necessary for the expression of IL-12 (Yen et al., 2015; Huang et al., 2005; Huang et al., 2001; Nagai et al., 2003; McRae et al., 2000). One function of IL-12 is to induce expression of IFN-γ in immune cells (Vignali et al., 2012). Thus, the reduction of IL-12 expression in DCs by IFN-β could reduce the expression of IFN-γ in immune cells (Vignali et al., 2012). This result may lead to the reduction of T_H1 cell populations. The reduction of T_H1 cells could lead to reduced activation of macrophages and reduced production of pro-inflammatory cytokines. This series of events could reduce the inflammatory response enacted on myelin in the CNS in cases of MS.

Effects of IFN-β on Effector CD4+ T cells:

In addition to affecting DC cytokine expression, IFN-β has been demonstrated to affect many different types of effector CD4+ T cells. In general, CD4+ T cells have been isolated from MS patients treated with IFN-β and non-treated patients. It was demonstrated that these cells were reactive to myelin basic protein by the increase of proliferation of CD4+ T cells from

non-treated patients in response to the presence of myelin basic protein (Bornsen, 2015). However, in the CD4+ cells isolated from IFN-β treated MS patients, a decrease in the proliferation of these CD4+ T cells was observed (Bornsen, 2015). This result was similarly shown in another study in which MS patients were treated with IFN-β and their CD4+ T cells were isolated (Hallal-Longo et al., 2007). These CD4+ T cells were found to have reduced reactivity to myelin basic protein, such that these CD4+ T cells had reduced proliferation in comparison to the non-treated control patients with MS (Hallal-Longo et al., 2007).

T_H1, T_H17, and T_{reg} cells have been shown to have altered cytokine and receptor expression and proliferation from IFN-β treatment (Boivin et al., 2015; Bornsen, 2015; Hallal-Longo et al., 2015; Sweeney et al., 2011; Axtell et al., 2011; Tao et al., 2014; Zhang et al., 2011; Ramgolam et al., 2009). The alterations to these cells' cytokine production and proliferation can present novel mechanisms for reducing the demyelination in MS patients.

T_H1 cells and The Effect of IFN-β Treatment:

T_H1 cells are effector CD4+ T cells that function by producing IL-12 and IFN-γ and activating macrophages (Parham, 2015). These functions can increase the inflammatory response to myelin in patients with MS. IFN-β has been shown to decrease the proliferation of T_H1 cells and decrease IFN-γ and other cytokine production by T_H1 (Nagai et al., 2003; Boivin et al., 2015; Bornsen, 2015; Hallal-Longo et al., 2015). The results of IFN-β treatment on T_H1 cells may suggest a decrease in the growth of the T_H1 cell population. The decrease in T_H1 cell populations could indicate the reduction in macrophage activity, as well as reduced inflammatory response to myelin in the CNS.

The generalized results of the effect of IFN-β treatment on MS patients CD4+ T cells are conserved in the T_H1 cells. In a murine model induced with EAE, the T_H1 cell proliferation was reduced by IFN-β on the mice (Bornsen, 2015). Studies have found that the treatment of murine T_H1 cells in culture alone and in culture with DCs produced a decrease in the proliferation of the T_H1 cells (Boivin et al., 2015). Interestingly, the study showed that the inhibition to T_H1 cell proliferation was greater in the presence of DCs than in culture alone (Boivin et al., 2015). These results show that IFN-β treatment affects the populations of T_H1 cells. In addition, the results may present a novel mechanism where the interaction between the antigen-presenting cells and CD4+ T cells is inhibited by some action of IFN-β. Taken together, these results show that T_H1 cells have reduced proliferation and replication from IFN-β that would affect the total population of T_H1. A reduced population of T_H1 cells may reduce the inflammatory response to MS and the macrophage activity in MS, thus suggesting a reduction in the demyelination of patients with MS.

IFN-β treatment on T_H1 cells also causes alterations to cytokine expression. It has been demonstrated that the treatment of T_H1 cells with IFN-β in a murine model led to the decrease in the expression of IFN-γ and IL-2 (Boivin et al., 2015). In addition, it was shown that the treatment of human T_H1 cells in culture with DCs with IFN-β had decreased IFN-γ and pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) (Nagai et al., 2003). It has also been found that IFN-β treatment on T_H1 cells led to a decrease in the IL-12 p40 expression in these T cells (McRae et al., 1998). Interestingly, the cytokine expression of IL-10 in T_H1 cells was increased following the treatment of T_H1 cells with IFN-β (McRae et al., 1998). The reduction of IFN-γ and IL-12 p40 could reduce the differentiation of primed CD4+ T cells into T_H1; thus, the IFN-β may reduce the autocrine signaling that occurs in T cells (Parham, 2015). The results also

showed a decrease of IL-2 cytokine expression from T_H1 cells. IL-2 functions as a signaling molecule for proliferation in T cells (Parham, 2015). Thus, it can be hypothesized that the reduction of IL-2 cytokine expression in T_H1 cells by IFN-β reduces T_H1 cell proliferation. These results taken together suggest that IFN-β treatment acts on T_H1 cells, reducing their cytokine expression and population growth. Interestingly, a decrease in TNF-α expression was seen following the IFN-β treatment of T_H1 cells. The reduction in TNF-α could indicate decreased vascular permeability, thus limiting the movement of T cells and inflammatory cytokines into the CNS (Parham, 2015).

It has been demonstrated that IFN-β alters the expression of some receptors on T_H1 cells that can be correlated to the alterations in proliferation and cytokine production. The treatment of murine T_H1 cells with IFN-β led to the increase in the expression of PD-1 receptor on the surface of T_H1 (Boivin et al., 2015). In addition, murine T_H1 cells were shown to increase the expression of T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) (Boivin et al., 2015). TIM-3 is known to be expressed in cases where T-cell exhaustion is present (Liu et al., 2018). The increased expression of TIM-3 and TIM-3 signaling has been found to reduce the expression of IFN-γ and TNF-α in effector T cells (Liu et al., 2018; Yang et al., 2008). The increase in the PD-1 receptor could indicate increased PD-1 signaling in T_H1. It has been shown that increased PD-1 signaling in effector CD4+ T cells led to reductions in IL-2 and IFN-γ expression (Carter et al., 2002; Schreiner et al., 2004; Goods et al., 2017; Latchman et al., 2001). In addition, the signaling through the TIM-3 receptor may suggest decreased IFN-γ and TNF-α from T_H1 cells (Lieu et al., 2018). These results taken together suggest that the reduction of cytokines may be the result of TIM-3 and PD-1 signaling. The changes in IL-2 and IFN-γ have been shown to reduce the proliferation and differentiation of T_H1 cells. Thus, the T_H1 cell population would be decreased

leading to reduced effector function from these cells and, by extension, reduced demyelination in patients with MS.

IFN-β Treatment of T_H17 and Its Effects:

IFN-β treatment on T_H17 cells has been shown to affect their effector functions. T_H17 cells function by producing cytokines that attract neutrophils to the sites of infection (Parham, 2015). In the case of MS, these cells may function by attracting the neutrophils to the blood-brain barrier and into the CNS where their phagocytic properties. In addition, T_H17 cells produce many different pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1β (Parham, 2015; Bettelli et al., 2008). These cytokines can increase vascular permeability, suggesting greater infiltration of immune cells and pro-inflammatory cytokines into the CNS (Parham, 2015). IFN-β treatment of T_H17 cells has been shown to reduce the T_H17 cell differentiation and proliferation and alter the cytokine expression of T_H17 cells. These alterations to T_H17 cells caused by IFN-β treatment may be indicative of novel mechanisms for the inhibition of T_H17 cell function and T_H17 cell population growth. In addition, the changes to T_H17 cells may suggest reduced pro-inflammatory response in patients with MS and reduced immune cell movement into the CNS in patients with MS.

The treatment of IFN-β on T_H17 cells may affect the T_H17 population size. It has been observed that T_H17 cells treated with IFN-β have reduced thymidine uptake (Sweeney et al., 2011). In addition, it has been found that the treatment of IFN-β treatment on a murine model induced with EAE was shown to have reduced IL-17 producing CD4+ T cell density (Galligan et al., 2010). This reduction in T_H17 population size could be the result of alterations to primed CD4+ T cell differentiation. T_H17 cells are known to be differentiated by three cytokines: IL-6, IL-21, and IL-17 A and F (Parham, 2015; Bettelli et al., 2008). In general, it was demonstrated

that CD4+ T cells produced decreased IL-21 when treated with IFN-β (Tao et al., 2014). Specifically, in T_H17 cells of a murine model, it was found that IFN-β treatment reduced the expression of both IL-17 A and IL-17 F in these T cells (Tao et al., 2014; Axtell et al., 2011; Tao et al., 2015; Zhang et al., 2011; Ramgolam et al., 2009). However, it is important to note that some discrepancy surrounds the effect of IFN-β on IL-17 A and F cytokines. Studies have found that the reductions in IL-17 A and F cytokine production in T_H17 cells of mice required the presence of IFN-β and IFN-γ in culture (Axtell et al., 2011). In contrast other studies have shown that IFN-γ was not required to induce the changes in IL-17 A and F expression in T_H17 (Tao et al., 2014; Rangolam et al., 2009; Zhang et al., 2011). Additionally, it has been demonstrated that the treatment of IFN-β on T_H17 cells led to the downregulation of the T_H17 cell transcription factor RORγT (Tao et al., 2014; Axtell et al., 2011). The downregulation of RORγT could be indicative of reduced IL-17 A and F expression and T_H17 expansion. These results may suggest one hypothesis for the reduction in the T_H17 cell density. The reduction in differentiating cytokines, namely, decreases in IL-21 and IL-17 A and F from IFN-β treatment on T cells, may cause reduced differentiation of T_H17 cells which could explain the decrease in T_H17 proliferation and density. In the context of MS, the decreased T_H17 populations could indicate decreased vascular permeability and inflammatory response, as well as reduced neutrophil response and attraction to the CNS, thus limiting the demyelination within the CNS.

Though inhibition of T_H17 cells by IFN-β has been reported by many studies, it is important to note that key discrepancies exist in the overall effectiveness of IFN-β on T_H17 cell populations. Most notably, it was previously described that upregulation of IL-6 cytokine expression in DCs was shown in the treatment of DCs with IFN-β (Pennell et al., 2017, Huang et al., 2005). It has been demonstrated that the IL-17 A decreased expression was mitigated only by

IFN-β's and IFN-γ's presence in culture with T_H17 (Axtell et al., 2011). However, IL-17F was shown to be increased when T_H17 cells were treated with IFN-β (Axtell et al., 2011). This finding was coupled with a result that showed mice induced with EAE had increased severity of EAE when T_H17 cells were the primary effector T cell and IFN-β treatment occurred (Axtell et al., 2011). These results taken together suggest that IFN-β treatment increases T_H17 cell expansion and cytokine production. In the context of MS, the findings hypothesize a possible mechanism in which IFN-β may not be an effective treatment in MS mediated primarily by T_H17 cells (Axtell et al., 2011).

The Effects of IFN-β Treatment on T_{reg} Cells:

IFN-β treatment has been found to increase the expansion of T_{reg} cells and the expression of T_{reg} cytokines. T_{reg} cells function by producing anti-inflammatory cytokines that reduce the inflammatory response of effector T cells, such as T_H1 and T_H17 cells (Parham, 2015; Corthay, 2009). T_{reg} cells also act as suppressors of effector T cells by downregulating costimulatory molecules on antigen-presenting cells and reducing the concentration of IL-2 in the surrounding environment (Schmidt et al., 2012). These functions can reduce the populations of autoreactive T_H1 and T_H17 cells. This reduction could suggest a decrease in the demyelination of the CNS because the inflammatory response and phagocytic cell activation is reduced from the inhibitory effect on effector CD4+ T cells. In the context of MS, it has been found that MS patients T_{reg} cells have reduced suppressive function in comparison to healthy controls (Venken et al., 2008). In addition, it was demonstrated that patients with MS have a lack of naïve CD4+ T cells with requisite markers for T_{reg} differentiation (Haas et al., 2007). These findings coupled with the

presentation of increased T_{reg} cell populations when MS patients are in relapse suggest the importance of T_{reg} cells in the immunopathogenesis of MS (Dalla-Libera et al., 2011).

T_{reg} cells have increased proliferation and differentiation when treated with IFN-β. The proliferation of T_{reg} cells has been correlated to the signaling through the glucocorticoid-induced tumor necrosis factor receptor (GITR) found on the T_{reg} cells (Cheng et al., 2015). It has been shown that the expression of GITR was increased in T_{reg} cells following the treatment with IFN-β (Cheng et al., 2015). In addition, it was demonstrated that the expression of the GITR ligand was upregulated on DCs by IFN-β treatment (Cheng et al., 2015). In MS patients treated with IFN-β, the percentage of T_{reg} cells was increased in comparison to untreated MS patients (Korporal et al., 2008). These results taken together showed that the treatment of IFN-β on T_{reg} cells led to increased T_{reg} cell proliferation. The increase in T_{reg} cell proliferation could indicate increased T_{reg} cell population. With greater numbers of T_{reg} cells, the increased T_{reg} population could have greater suppressive effects of the T_{reg} cells on effector T cells. In addition, there may be greater anti-inflammatory cytokines, such as IL-10, produced because there are greater numbers of T_{reg} cells.

In the context of MS, the T_{reg} cell suppressive function has been found to be reduced in patients with MS (Venken et al., 2008; Korporal et al., 2008). The suppressive functions of T_{reg} cells are known to be related to the expression of the T_{reg} transcription factor, FOXP3 (Fu et al., 2004; Tai et al., 2012; Parham, 2015). Studies have found that FOXP3 is upregulated by the treatment of IFN-β on T_{reg} cells (Venken et al., 2008; Cheng et al., 2015) It has been demonstrated that the suppressive functions of T_{reg} cells were increased when MS patients were treated with IFN-β (Korporal et al., 2008). The increased suppressive function coupled with the increased T_{reg} cell population could indicate a greater regulation of the autoreactive effector T

cells in MS. Greater regulation of these effector T cells would reduce the demyelination of the CNS of MS patients.

The possible mechanisms of suppression from T_{reg} cells involve CTLA-4 signaling. The upregulation of FOXP3 transcription factor has been found to induce the expression of CTLA-4 receptors on T_{reg} cells (Cheng et al., 2015; Schreiner et al., 2004; Marckmann et al., 2008). CTLA-4 associates with CD80 and CD86 located on DCs (Parham, 2015; Schreiner et al., 2004; Marckmann et al., 2008). Both CD80 and CD86 were found to be upregulated by IFN-β treatment on DCs (Wiesemann et al., 2008; Marckmann et al., 2004). In addition, CTLA-4 has been shown to have a nearly 10-fold greater affinity for CD80 and CD86 in comparison to the CD28 receptor (Parham, 2015). These results have shown that IFN-β increased expression of FOXP3 that upregulates the expression of CTLA-4 (Cheng et al., 2015; Fu et al., 2004; Tai et al., 2012). The upregulation of CTLA-4 coupled with its increased affinity for CD80 and CD86 may suggest a novel mechanism where naïve T cells cannot associate with the DCs and become activated. This result would increase the T_{reg} cell population in comparison to other effector T cells. With reduced effector T cells, the demyelination of the CNS would be reduced.

The signaling through the CTLA-4 has been shown to induce inhibitory pathways. CTLA-4 signaling was found to induce the expression of indolamine-2,3-dioxygenase in DCs (Fallarino et al., 2003). Indolamine-2,3-dioxygenase (IDO) functions by degrading tryptophan (Falllarino et al., 2003). It has been shown that IDO's reduction of tryptophan led to the inhibition of T cell proliferation (Mellor et al., 2003; Fallarino et al., 2003). In the context of MS, the increase of T_{reg} cells may increase the amount of CTLA-4 signaling in DCs. This increased signaling may induce an upregulation of IDO and increased IDO function. The effect of IDO has been shown to decrease the proliferation of T cells, such as the effector T cells. Reduced T_H1 and

T_H17 proliferation would reduce the demyelination within the CNS by diminishing the inflammatory response in the CNS.

In addition to the receptor inhibitory mechanisms, T_{reg} cells are known to produce anti-inflammatory cytokines, such as IL-10 (Parham, 2015). The upregulation of FOXP3 and increased T_{reg} cell population indicated that IL-10 was upregulated (Cheng et al., 2015; Parham, 2015). The increased IL-10 cytokines have shown to reduce the activation of macrophages and limit the differentiation of T_H17 and T_H1 cells (Parham, 2015; Hsu et al., 2015; Goldsby et al., 2002). The reduction of these effector T cells and the limitation to macrophage activation could indicate a decreased inflammatory response to the myelin in the CNS. This result would reduce the demyelination of the CNS in patients with MS.

IFN-β Modulation of Immune Cell Transmigration:

The movement of immune cells into the CNS is essential to the immunopathogenesis of MS. The immune cells are located within the lymphatic system and move into the CNS by a process called transmigration (Garg et al., 2015; Parham, 2015; Pinheiro et al., 2015). The CNS is protected by the blood-brain barrier (BBB) that is highly regulated controlling the amount of infiltrates into the CNS (Pinheiro et al., 2015). The BBB consists of three layers: the endothelial cells, the endothelial basement membrane, and the glial membrane (Pinheiro et al., 2015). The endothelial cells are connected by tight junctions that limit the movement of any vascular fluid or cells into the CNS (Pinheiro et al., 2015).

The process of transmigration is one of great complexity. It involves three distinct phases: 1) attraction to the site of transmigration by chemokines, 2) adhesion of immune cell to

the endothelial cell, 3) production of matrix metalloproteinases (MMPs) to allow movement through the endothelial basement and glial membrane layers (Pinheiro et al., 2015; Parham, 2015). The attraction of immune cells to the site of transmigration involves the presence of chemokines along the endothelial cell layer (Parham, 2015). Chemokines can be produced by many immune cells, including T cells, dendritic cells, macrophages, and neutrophils (Parham, 2015). Immune cells express chemokine receptors which bind to the chemokines located on the endothelial barrier (Parham, 2015). The chemokines on the endothelial cell layer form a concentration gradient on the endothelial cells (Parham, 2015). This increased concentration on the endothelial cells attracts the immune cells expressing the chemokine receptor that associates with the chemokine present on the endothelial cells (Parham, 2015).

The association with the chemokine by the immune cell involves a process by which the immune cell rolls along the endothelial cells (Parham, 2015). As the immune cell rolls, it associates with cell-adhesion molecules expressed on the endothelial cells (Parham, 2015). The cell-adhesion molecules that are present on the endothelial cells, include vascular cell-adhesion molecule 1 (VCAM-1), intercellular cell-adhesion molecule 1 (ICAM-1), P-selectin glycoprotein ligand 1 (PSGL-1) and intracellular cellular adhesion molecules 2 and 3 (Sagar et al., 2011). The association of these cell-adhesion molecules from the immune cell and the endothelial cell lead to the adherence of the immune cell to the endothelial cell layer (Parham, 2015). The association of the cell-adhesion molecules of the immune cell and the endothelial cell has been found to alter the tight junctions leading to the movement of the immune cell through the endothelial cell layer, which is also called diapedesis (Parham, 2015; Pinheiro et al., 2015).The final phase of the transmigration process that is specific to the movement through the BBB is the expression of MMPs (Pinheiro et al., 2015). Many different MMPs can be expressed by immune cells, but their

function is redundant. MMPs function by cleaving the glial and endothelial basement layer (Pinheiro et al., 2015). This function allows for the immune cell to move into the CNS.

IFN-β has been found to alter the chemokine and chemokine receptor expression, cell-adhesion molecule expression, and MMP expression of DCs and CD4+ T cells. These changes to the different components of the transmigration process by IFN-β may suggest novel mechanisms that reduce the movement of DCs and CD4+ T cells into the CNS. In the context of MS, this reduced movement could indicate decreased inflammatory response to myelin and immune cell attack and function within the CNS.

Interferon-β and Dendritic Cell Transmigration:

Dendritic cells are known to migrate through many tissues of the body carrying antigens to the lymphatic system from the site of antigen. In the context of MS, the antigens are auto-antigens, such as MOG or MBP (Peschl et al., 2017; Beniac et al., 1997). It has been demonstrated in a murine model of EAE that the increased movement of DCs into the CNS was linked to the increased severity of EAE (Sagar et al., 2012). IFN-β treatment of DCs has been shown to cause changes to chemokine receptor expression, cell-adhesion molecule expression, and MMP expression by DCs.

IFN-β treatment plays a role in the alteration of DCs expression of chemokines and chemokine receptors. DCs have been found to express chemokine receptors CXCR5, CXCR4, CCR4, and CCR7 (Sallusto et al., 1999). The treatment of IFN-β *in vivo* in mice and *in vitro* on human DCs have shown a down-regulation of CCR7 expression in DCs (Yen et al., 2010; Pennell et al., 2017). The CCR7 receptor attaches to chemokines CCL21 and CCL19 (Parham,

2015). These chemokines attract DCs to lymph nodes where they can prime T cells for attack against the myelin (Parham, 2015). The chemokines can also attract the DCs to the BBB, allowing for their phagocytosis and processing of auto-antigens for presentation in MHC molecules (Parham, 2015). The decrease to the expression of CCR7 on DCs could indicate the decreased chemotaxis of DCs to and from the lymph nodes and the CNS. This reduced chemotaxis may suggest reduced movement of DCs into the CNS, thus limiting the presentation of auto-antigens to T cells and the priming of autoreactive T cells.

In addition to the reduction of CCR7 expression in DCs, IFN-β treatment has been shown to affect the expression of cell-adhesion molecules in DCs. Various cell-adhesion molecules have been found to play a role in the adhesion of DCs to endothelial cells, including very-late antigen 4 (VLA-4), lymphocyte function associated antigen 1 (LFA-1) P or E selectin, and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DCSIGN) (Sagar et al., 2011). Studies have shown that the blockage of expression of VCAM-1, ICAM-1, and ICAM-2 limited the adhesion of DCs to human micro-vessel endothelial cells (Arjmandi et al., 2009). IFN-β has been shown to indirectly affect the expression of the cell-adhesion molecules on endothelial cells. For example, the downregulation of pro-inflammatory cytokines TNF-α and IL-1β has been found to reduce the expression of VCAM-1, ICAM-1, and P- and E-selectin in endothelial cells (Raab et al., 2002). IFN-β treatment on naïve CD4+ T cells and effector CD4+ T cells has demonstrated that IL-17 cytokine expression was decreased (Tao et al., 2014; Axtell et al., 2011; Tao et al., 2015; Zhang et al., 2011; Ramgolam et al., 2009). It has been found that reductions to IL-17 signaling decreases TNF-α and IL-1β expression (Bettelli et al., 2008). In addition, IFN-γ signaling in endothelial cells has been correlated with increases in the expression of ICAM-1 in endothelial cells (Chen et al., 2001; Raab et al., 2002). Studies have shown that the

treatment of IFN-β on DCs reduced the expression of IFN-γ in DCs (Nagai et al., 2003; Yen et al., 2015). The reduction of IFN-γ production may suggest a down-regulation of ICAM-1 on endothelial cells. These results taken together suggest indirect inhibition of cell-adhesion molecule expression on endothelial cells by IFN-β treatment. The reduction of endothelial cells by IFN-β would decrease the adhesion of DCs to the endothelial cells, and, thus, reduce the transmigration of DCs into the CNS or lymph nodes.

IFN-β treatment has been demonstrated to reduce the permeability of a bovine endothelial cell layer in inflammatory conditions that induce increased permeability (Kraus et al., 2008). Many possible hypotheses exist for this reduced permeability of the endothelial cell layer. One such hypothesis involves ICAM-1 signaling. Cell-adhesion molecules have been found to play a role in the alterations of tight junctions between endothelial cells. The adhesion of immune cells to the endothelial cell layer led to a distinct arrangement ICAM-1 along the endothelial cell membrane (Pinheiro et al., 2015; Greenwood et al., 2011). The arrangement of ICAM-1 caused distinct ICAM-1 signaling that induces changes to the tight junctions of endothelial cells, thus allowing the immune cell to move through (Pinheiro et al., 2015; Greenwood et al., 2011). The decreased expression of pro-inflammatory cytokines TNF-α, IL-1β, and IFN-γ as an effect of IFN-β have been shown to limit the expression of ICAM-1 in endothelial cells (Raab et al., 2002; Chen et al., 2001). Interestingly, ICAM-1 on the endothelial cell associates with LFA-1 on the DC (Parham, 2015; Sagar et al., 2011). LFA-1 expression on leukocytes was reported to be down-regulated in MS patients that underwent IFN-β treatment (Muraro et al., 2004). The reductions to ICAM-1 and LFA-1 may limit the movement of immune cells, such as DCs, through tight junctions by down-regulating ICAM-1 signaling.

Another possible hypothesis for the decreased permeability of the endothelial cell layer in inflammatory conditions involves the cell-adhesion molecules and their associations with tight junctions. Tight junctions consist of interlocking proteins, such as junctional adhesion molecule A (JAM-A) and platelet endothelial cell-adhesion molecule (PECAM) (Saga et al., 2011). Studies found that the cell-adhesion molecule LFA-1 has affinity for the JAM-A tight junction protein (Wojcikiewics et al., 2009). The association of LFA-1 present on DCs may disrupt the tight junction association of JAM-A and another cell-adhesion molecule, thus allowing for a decrease in the stability of the tight junction. LFA-1 expression on leukocytes, which include DCs and CD4+ cells, was shown to be reduced in MS patients receiving IFN-β treatment (Muraro et al., 2004). With reduced LFA-1 expression on DCs, the immune cell movement through the endothelial cell layer may be inhibited because there is less disruption of the tight junctions.

The final phase of transmigration involves breakdown of the glial and endothelial basement membrane layer by MMPs (Pinheiro et al., 2015). IFN-β treatment of DCs has been shown to alter the expression of MMPs by DCs both directly and indirectly. DCs produce MMP-9, MMP-12, and MMP2 (Toth et al., 2013; Zozulya et al., 2007). The exposure of DCs to inflammatory conditions in which prostaglandin E2 (PGE2), TNF-α, IL-1β, and granulocyte-macrophage colony-stimulating factor (GMCSF) are present led to the upregulation of MMP-12 and MMP-9 (Toth et al., 2013). The production of IL-17 has been found to induce the expression of PGE2, TNF-α, and IL-1β in immune cells (Fossiez et al., 1996). IFN-β treatment has been demonstrated to reduce the expression of IL-17 by immune cells (Tao et al., 2014; Axtell et al., 2011; Tao et al., 2015; Zhang et al., 2011; Ramgolam et al., 2009). In addition, IFN-β treatment on DCs led to the decrease of MMP-9 expression by DCs (Yen et al., 2010; Toth et al., 2013).

These results together showed that MMPs were down-regulated by the treatment of IFN-β. The inhibition of inflammatory cytokines by down-regulation of IL-17 expression could indicate the reduced production of MMP-9 and MMP-12 by DCs. This indirect inhibition coupled with the direct effect of IFN-β on DCs to down-regulate MMP-9 may suggest reduced ability of DCs to breakdown the glial and endothelial basement membrane layers of the BBB. This decrease in transmigration function may hinder the DCs entrance into the CNS. The decrease of DCs within the CNS may reduce the severity of MS.

The Effect of Interferon-β on T Cell Transmigration:

T cells transmigrate through the same process as DCs. However, differences in cell type lead to differences in the chemokine receptor, chemokines, and MMPs produced. In a murine model of MS, reduced T cell transmigration was shown following IFN-β treatment (Chen et al., 2015). T cells express many different chemokine receptors that associate with many different chemokines (Parham, 2015; Cheng et al., 2015). The effects of IFN-β on cell-adhesion molecules and MMPs in T cells may suggest hypotheses as to why T cell transmigration is altered

It is important to note that IFN-β treatment has been shown to affect both chemokines and chemokine receptors. For example, in MS patients that are untreated, the expression of chemokine receptors CCR4, CCR5, and CCR7 are down-regulated (Krakauer et al., 2006; Cheng et al., 2015). Following treatment with IFN-β, the expression of these chemokine receptors was increased in the CD4+ T cells of these patients (Krakauer et al., 2006). In contrast, the expression of the chemokine receptor CXCR3 that is highly expressed on T_H1 cells was decreased by IFN-β treatment (Sorensen et al., 2002; Groom et al., 2011). For chemokines, MS patients treated with

IFN-β showed increased expression of many chemokines including CCL1, CCL2, CCL7, CXCL9, CXCL10, CXCL11, and CXCL12 in CD4+ T cells (Cepok et al., 2009). However, studies have found that IFN-β treatment in patients with MS reduced the expression of chemokines, such as CCL3, CCL5, and CXCL10 (Cheng et al., 2015). In all, these results have shown conflicting findings. These findings suggest that a better understanding of the chemokine receptors and chemokines in CD4+ T cells may be necessary the understand IFN-β's effect.

CD4+ T cells are known to express various cytokines that can be affected by IFN-β treatment. T_H1 cells have decreased expression of IFN-γ following IFN-β treatment (Boivin et al., 2015; Nagai et al., 2003). T_H17 cells, when treated with IFN-β, were shown to have reduced expression of IL-17 cytokines (Tao et al., 2014; Axtell et al., 2011; Tao et al., 2015; Zhang et al., 2011; Ramgolam et al., 2009). The reduction to IFN-γ and IL-17 has been found to reduce the cell-adhesion molecules ICAM-1, VCAM-1, and P- and E-selectin by reducing the pro-inflammatory cytokine expression (Raab et al., 2002; Chen et al., 2001). The reduction in the cell-adhesion molecules present on the endothelium could suggest reduced adherence of CD4+ T cells on the endothelial cell layer. CD4+ T cells are also known to express the LFA-1 cell-adhesion molecule similar to DCs (Parham, 2015; Pinheiro et al., 2015). Similar to the hypotheses found in DC transmigration studies, the LFA-1 molecules have been shown to be reduced in expression MS patient leukocytes treated with IFN-β (Muraro et al., 2004). The reduction of LFA-1 cell-adhesion molecules in CD4+ T cells could suggest that tight junctions would not be disrupted, leading to decreased transmigration of CD4+ T cells into the CNS.

CD4+ T cell expression of MMPs has been shown to be affected by IFN-β treatment. In cases of MS, it has been demonstrated that MS patients have increased ratios between MMPs and tissue inhibitor of metalloproteinases (TIMPs) (Avolio et al., 2005). When these ratios are

increased, there is an increased movement of immune cells through the cell layers (Avoloi et al., 2005). Studies have shown that there is variation between the cell types for the MMPs produced. In the case of T_H1 cells, high levels of MMP2 and MMP9 have been reported, whereas in T_H17 cells, MMP3 and MMP13 was found to be highly expressed (Ovieda-Orta et al., 2008; Park et al., 2005). IFN-β treatment in MS patients has been shown to reduce the expression of MMP-8 and MMP-9 in the blood serum of MS patients (Alexander et al., 2010). Additionally, studies have reported that the treatment of IFN-β in MS patients reduced their MMP to TIMP ratio by increasing TIMP-1 and decreasing MMP-9 (Avolio et al., 2005). These results taken together suggest that IFN-β may reduce MMP function by increasing its inhibitor (TIMP). Additionally, IFN-β treatment has been shown to reduce MMPs directly, thus decreasing the ability of T cells to transmigrate into the CNS.

Concluding Remarks:

The role of IFN-β in the treatment of MS is one of increasing complexity. Studies have reported that IFN-β can affect multiple cell types that are involved in the complex immunopathogenesis of MS. In general, IFN-β treatment has been shown to reduce the inflammatory response by limiting the pro-inflammatory cytokine production by effector CD4+ T cells and DCs. In addition, IFN-β treatment has been correlated to a shift of cytokines from pro-inflammatory cytokines, such as IL-17, IFN-γ, IL-12, and TNF-α, to anti-inflammatory cytokines, such as IL-10 and IL-27. The shift from pro-inflammatory cytokines to anti-inflammatory cytokines has been shown to reduce the cell populations of T_H1 and T_H17 cells. Additionally, IFN-β treatment has been shown to inhibit T_H1 and T_H17 cell proliferation. These shifts in the cytokine profile have also been shown to induce the growth of T_{reg} cell populations.

Overall, these shifts in cytokine production may induce greater regulation of autoreactive effector T cells and reduce the progression of MS by limiting the effector T cell functions and population growth. In addition, these cytokines have been found to affect the expression of cell-adhesion molecules on endothelial cells inhibiting the movement of immune cells. IFN-β treatment has also been found to reduce some cell-adhesion molecules on leukocytes. In total, IFN-β treatment in MS patients suggests a complex system where pro-inflammatory response to myelin is reduced by changing the expression of cytokines, receptors, and cell-adhesion molecules effector CD4+ T cells and DCs (Table 1.).

The many effects of IFN-β on effector CD4+ T cells and DCs have shown to alter the protein expression of these immune cells. These alterations have been suggested to be used as measurements of effectiveness in MS patients receiving IFN-β. For example, the upregulation of CD80 and CD86 molecules on DCs has been suggested to be used as a measure of IFN-β treatment effectiveness (Wiesemann et al., 2008; Marckmann et al., 2004). This measurement of effectiveness could show earlier effectiveness of IFN-β treatment in MS patients. Another example is that in the murine model of EAE induced by T_H17 cells, IFN-β treatment was shown to exacerbate the symptoms in mice (Axtell et al., 2011). Reduced effectiveness of IFN-β treatment in MS patients presenting with an high T_H17 cell population may allow for better treatment options to be given to MS patients with high T_H17 populations; thus giving a marker for proper IFN-β treatment in MS patients (Axtell et al., 2011). The analysis of IFN-β's roles in the treatment of MS and its effects on immune cells provides better understanding to the cases of MS where IFN-β treatment is most beneficial for the patient.

References:

Archelos J. J., Hartung H.-P., Hemmer, B (2002). New Concepts in the Immunopathegenesis of Multiple Sclerosis. Neuroscience. Apr.; 3 (2): 291-301.

Parham P. (2015). The Immune System: Fourth Edition. *Garland Science*. 4th ed. Chapters 1-17.

Garg, Neeta, Smith, Thomas W (2015). An update on immunopathogenesis, diagnosis, and treatment of multiple sclerosis. Brain and Behavior. September; 5(9): e00362.

Lopes Pinheiro M. A., Kooij G., Mizee M. R., Kamermans A., Enzmann G., Lyck R., Schwaninger M., Engelhardt B., de Vries H. E. (2016). Immune cell trafficking across the barriers of the central nervous system in multiple sclerosis and stroke. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. Mar.; 1862(3): 461-471.

Leray E., Moreau T., Fromont A., Edan G. (2016). Epidemiology of multiple sclerosis. *Revue Neurologique*. Jan. ;172 (1): 3-13.

National Multiple Sclerosis Society (2018). MS symptoms. *National Multiple Sclerosis Society*. Retrieve from: https://www.nationalmssociety.org/Symptoms-Diagnosis/MS-Symptoms

National Multiple Sclerosis Society (2018). Relapsing Remitting MS. *National Multiple Sclerosis Society*. Retrieved from: https://www.nationalmssociety.org/What-is-MS/Types-of-MS/Relapsing-remitting-MS

Morell P, Quarles RH. Characteristic Composition of Myelin. In: Siegel GJ, Agranoff BW, Albers RW et al., editors. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. 6th edition. Philadelphia: Lippincott-Raven; 1999. Retrieved from: https://www.ncbi.nlm.nih.gov/books/NBK28221/

Peschl, P., Bradl, M., Höftberger, R., Berger, T., Reindl, M. (2017). Myelin Oligodendrocyte Glycoprotein: Deciphering a Target in Inflammatory Demyelinating Diseases. *Frontiers in Immunology*. May 8; *8*: 529.

Beniac D. R., Luckevich M.D., Czarnota G. J., Tompkins T. A., Ridsdale R.A., Ottensmeyer F. P., Moscarello M. A., Harauz G. (1997). Three-dimesional Structure of Myelin Basic Protein. *The Journal of Biological Chemistry*. Feb. 14; 272 (7): 4261-4268.

Yong V. W., Chabot S., Stuve O., Williams G. (1998). Interferon β in the treatment of multiple sclerosis. *American Academy of Neurology*. Sept. 1; 51 (3).

Wiesemann E., Deb M., Trebst C., Hemmer B., Stangel M., Windhagen A. (2008). Effects of interferon-β on co-signaling molecules: upregulation of CD40, CD86, and PD-L2 on monocytes in relation to clinical response to interferon-β treatment in patients with multiple sclerosis. *Multiple Sclerosis*. May 30; 14: 166-176.

Hamamcioglu K., Reder A. T. (2007). Interferon-β regulates cytokines and BDFN: greater effect in relapsing than in progressive multiple sclerosis. *Multiple Sclerosis.* Jul. 13; 13: 459-470.

De Weerd N.A., Nguyen T. (2012). The interferons and their receptors—distribution and regulation. *Immunology and Cell Biology*. Feb. 12; 1-9.

Markowitz C. E. (2007). Interferon-β: Mechanism of Action and dosing issues. *American Academy of Neurology*. Jun. 12; 68 (24), Suppl. 4: S8-S11.

Yen, J.-H., Kong, W., Ganea, D. (2010). Interferon B inhibits Dendritic cell migration through STAT-1 Mediated Transcriptional Suppression of CCR7 and Metalloproteinase 9.. *Journal of Immunology* (Baltimore, Md. : 1950). Apr. 1; 184(7): 3478–3486.

Rog D. J., Mottershead J. P. (2006). The role of interferon-β in multiple sclerosis management. *Future Prescriber*. 7(3): 15-19.

Kornek, B., Storch, M. K., Weissert, R., Wallstroem, E., Stefferl, A., Olsson, T., Linington C., Schmidbauer M., Lassmann, H. (2000). Multiple Sclerosis and Chronic Autoimmune Encephalomyelitis: A Comparative Quantitative Study of Axonal Injury in Active, Inactive, and Remyelinated Lesions. *The American Journal of Pathology* Jul; *157*(1): 267–276.

Lucchinetti C., Brück W. , Parisi J., Scheithauer B. , Rodriguez M., Lassmann H. (1999); A quantitative analysis of oligodendrocytes in multiple sclerosis lesions: A study of 113 cases. *Brain*. Dec 1; 122(12): 2279–2295

Goverman, J. (2009). Autoimmune T cell responses in the central nervous system. *Nature Reviews Immunology*. Jun.; *9*(6): 393.

Hoehn K., Marieb E.N. (2016) *Human Anatomy and Physiology: Tenth Edition*. *Pearson Education Inc*. 390-395.

Tullman, M. J. (2013). Overview of the Epidemiology, Diagnosis, and Disease Progression Associated with Multiple Sclerosis. *Am. J. Manag. Care*. Feb. 25; 19: S15-S20.

Ivashkiv, L. B., & Donlin, L. T. (2014). Regulation of type I interferon responses. *Nature reviews. Immunology*. Jan. 1; *14*(1): 36-49.

Huh, J. R., & Littman, D. R. (2012). Small molecule inhibitors of RORγt: targeting Th17 cells and other applications. *European journal of immunology*. Sept.; *42*(9): 2232-2237.

Ariga, H., Shimohakamada, Y., Nakada, M., Tokunaga, T., Kikuchi, T., Kariyone, A., Tamura, T., & Takatsu, K. (2007). Instruction of naive CD4+ T-cell fate to T-bet expression and T helper 1 development: roles of T-cell receptor-mediated signals. *Immunology*. Oct.; *122*(2): 210-221.

Ouyang, W., Kolls, J. K., & Zheng, Y. (2008). The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity*. Apr; 28(4): 454-467.

Hsu, P., Santner-Nanan, B., Hu, M., Skarratt, K., Lee, C. H., Stormon, M., Wong, M., Fuller, S. J., & Nanan, R. (2015). IL-10 Potentiates Differentiation of Human Induced Regulatory T Cells via STAT3 Foxo1. The Journal of Immunology. Oct. 15; 195 (8): 3665-3674.

Yen, J. H., Kong, W., Hooper, K. M., Emig, F., Rahbari, K. M., Kuo, P. C., Scofield, B. A., & Ganea, D. (2015). Differential effects of IFN-β on IL-12, IL-23, and IL-10 expression in TLR-stimulated dendritic cells. *Journal of leukocyte biology*. Nov.; *98*(5): 689-702.

Lee, S. W., Hong, Y. S., Chun, C. M., Moon, J. D., Kim, S. J., Jung, I. C., Yoon, Y. H., Lee, B. A., Moon, S. W., Choi, S. H., & Moon, C. K. (2002). Anti-inflammatory effects of IL-4 and IL-10 on human polymorphonuclear leukocytes. *Journal of Korean medical science*. Feb.; 17(1): 7-14.

Pennell, L. M. & Fish, E. N. (2017). Interferon-β regulates dendritic activation and migration in experimental autoimmune encephalomyelitis. *Immunology*. Jun. 24; 152(3).

Nagai, T., Devergne, O., Mueller, T. F., Perkins, D. L., van Seventer, J. M., & van Seventer G. A. (2003). Timing of IFN-β Exposure during Human Dendritic Cell Maturation and Naïve Th Cell Stimulation has Contrasting Effects on Th1 Subset Generation: A Role for IFN-β-mediated Regulation of IL-12 Family Cytokines and IL-18 in Naïve Th Cell Differentiation. *The Journal of Immunology*. 171: 5233-5243.

Liu, Z., Pelfrey, C. M., Cotleur, A., Lee, J. C., & Rudick, R. A. (2001). Immunomodulatory effects of interferon-β 1a in multiple sclerosis. *Journal of Neuroimmunology*. Jan.; 112(1-2): 153-162.

Huang, Y. M., Hussien, Y., Yarilin, D., Xiao, B. G., Liu, Y. J., & Link, H. (2001). Interferon-β induces the development of type 2 dendritic cells. *Cytokine*. Mar.; 13(5): 264-271.

Pflanz, S., Timans, J. C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., Blumenschein, W. M., Mattson, J. D., Wagner, J. L., To, W., Zurawski, S., McClanahan, T. K., Gorman, D. M., Bazan, J. F., Malefyt, R. D. W., Rennick, D., & Kastelein, R. A. (2002). IL-27, a Heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naïve CD4+ T cells. *Immunity*. Jun.; 16 (6): 779-790.

Cooper, A. M. & Khader, S. A. (2007). IL-12p40: an inherently agonistic cytokine. *Trends in Immunology*. Jan.; 28 (1): 33-38

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., Weiner, H. L., & Kuchroo V. K. (2006). Reciprocal developmental pathways for the generation of the pathogenic effector Th17 and regulatory T cells. *Nature*. May 11; 441: 235-238.

Vignali, D. A., & Kuchroo, V. K. (2012). IL-12 family cytokines: immunological playmakers. *Nature immunology*. Aug.; 13(8): 722-728.

Vanknin-Dembinsky, A., Balashov, K., & Weiner, H. L. (2006). IL-23 Increase in Dendritic cells in Multiple sclerosis and Down-regulation of IL-23 by antisense oligos increases dendritic cells IL-10 production. *The Journal of Immunology*. 176: 7768-7774.

McRae, B. L., Beilfuss, B. A., & van Seventer, G. A. (2000). IFN-β differentially regulates CD40-Induced cytokine secretion by human dendritic cells. *The Journal of Immunology*. Jan. 1; 164 (1): 23-28.

Serada, S., Fujimoto, M., Mihara, M., Koike, N., Ohsugi, Y., Nomura, S., Yoshida, H., Nishikawa, T., Terabe, F., Ohkawara, T., Takahashi, T., Ripley, B., Kimura, A., Kishimoto, T., & Naka, T. (2008). IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental autoimmune encephalomyelitis. *PNAS*. Jul. 1; 105 (26): 9041-9046.

Ozenci, V., Kouwenhoven, M., Teleshova, N., Pashenkov, M., Fredikson, S., & Link, H. (2000). Multiple Sclerosis: Pro- and anti-inflammatory cytokiens and metalloproteinases are affected differentially by treatment with IFN-β. *Journal of Neuroimmunology*. Aug.; 108 (1-2): 236-243.

Huang, Y. M., Adikari, S., Bave U., Sanna, A., & Alm, G. (2005). Multiple Sclerosis: interferon-β induces CD123+ BDCA2- dendritic cells that produce IL-6 and IL-10 and have no enhanced type I interferon production. *Journal of Neuroimmunology*. Jan.; 158 (1-2): 204-212.

Wolf, J. E. & Massof, S. E. (1990) In vivo Activation of Macrophage Oxidative burst activity by cytokines and Amphotericin B. *Infection and Immunity*. Feb.; 58 (5): 1296-1300.

Hassanzadeh-Kiabi, N., Yanez, A., Dang, I., Martins, G. A., Underhill, D. M., & Goodridge, H. S. (2017). Autocrine type I IFN signaling in Dendritic Cells stimulated with Fungal β-glucans or Lipopolysaccharide promotes CD8 T cell activation. *The Journal of Immunology*. Jan. 1; 198 (1): 375-382.

Geissmann, F., Manz, M. G., Jung, S., Sieweke, M. H., Merad, M., & Ley, K. (2010). Development of Monocytes, Macrophages, and Dendritic Cells. *Science*. Feb. 05; 327 (5966): 665-661.

Wang, F., Wang, Y. Y., Li, J., You, X., Qiu, X. H., Wang, Y. N., & Gao, F. G. (2014). Increased antigen presentation but impaired T cell priming after upregulation of interferon-β induced lipopolysaccharide mediated by upregulation of B7H1 and GITRL. *PLoS ONE*. Aug. 21; 9 (8): e105636.

Brown, J. A., Dorfman, D. M., Ma, F. R., Sullivan, E. L., Munoz, O., Wood, C. R., Greenfield, E. A., & Freeman, G. J. (2003) Blockade of Programmed Death-1 ligands on dendritic cells enhances T cell activation and cytokine production. The Journal of Immunology. Feb. 1; 170 (3): 1257-1266.

McRae, B. L., Nagai, T., Semnani, R. T., van Seventer, J. M., & van Seventer, G. A. (2000). Interferon-alpha and -β inhibit the in vitro differentiation of immunocompetent dendritic cells from CD14+ precursors. *Blood*. 96: 210-217.

Goods, B. A., Hernandez, A. L., Lowther, D. E., Lucca, L. E., Lerner, B. A., Gunel, M., Raddassi, K., Coric, V., Hafler, D. A., & Love, J. C. (2017). Functional differences

between PD-1+ and PD-1- CD4+ effector T cells in healthy donors and patients with glioblastoma multiforme. *PLoS ONE*. Sept. 7; 12 (9): e0181538.

Latchman, Y., Wood, C. R., Chernova, T., Chaudhary, D., Borde, M., Chernova, I., Iwai, Y., Long, A. J., Brown, J. A., Nunes, R., Greenfield, E. A., Bourque, K., Boussiotis, V. A., Carter, L. L., Carreno, B. M., Malenkovich, N., Nishimura, H., Okazaki, T., Honjo, T., Sharpe, A. H., Freeman, G. J. (2001). PD-2 is a second ligand for PD-1 and inhibits T cell activation. *Nature Immunology*. Apr.; 2 (3): 261-268.

Francisco, L. M., Salinas, V. H., Brown, K. E., Vanguri, V. K., Freeman, G. J., Kuchroo, V. K., & Sharpe, A. H. (2009). PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *The Journal of Experimental Medicine*. Dec. 21; *206*(13): 3015-3029.

Wiesemann, E., Deb, M., Trebst, C., Hemmer, B., Stangel, M., & Windhagen, A. (2008). Effect of interferon-β on co-signaling molecules: upregulation of CD40, CD86, and PD-L2 on monocytes in relation to clinical response to interferon-β treatment in patients with multiple sclerosis. *Multiple Sclerosis*. 14: 166-176.

Marckmann, S., Wiesemann, E., Hilse, R., Trebst, C., Stangel, M., & Windhagen, A. (2004). Interferon-β up-regulates the expression of co-stimulatory molecules CD80, CD86 and CD40 on monocytes: significance for treatment of multiple sclerosis. *Clinical and Experimental Immunology*. Dec.; 138(3), 499-506.

Schreiner, B., Mitsdoerffer, M., Kieseier, B. C., Chen, L., Hartung, H. P., Weller, M., & Wiendl, H. (2004). Interferon-β enhances monocyte and dendritic cell expression of B7-H1 (PD-L1), a strong inhibitor of autologous T-cell activation: relevance for the immune

modulatory effect in multiple sclerosis. *Journal of Neuroimmunology*. Oct.; 155 (1-2): 172-182.

Corthay, A. (2009). How do Regulatory T Cells Work?. *Scandinavian Journal of Immunology*. Oct.; 70 (4): 326-336.

Korporal, M., Haas, J., Balint, B., Fritzsching, B., Schwarz, A., Moeller, S., Fritz, B., Suri-Payer, E., Wildemann, B. (2008). Interferon-β-induced restoration of regulatory T-cell function in Multiple Sclerosis is prompted by an increase in newly generated naïve regulatory T cells. *Arch. Neurol.* Nov.; 65 (11): 1434-1439.

Chen, M., Chen, G., Deng, S., Liu, X., Hutton, G. J., & Hong, J. (2011). Interferon-β induces the proliferation of CD4+ CD25+ FoxP3+ regulatory T cells through upregulation of GITRL on dendritic cells in the treatment of multiple sclerosis. Journal of Neuroimmunology. Jan. 18; 242 (1-2): 39-46.

Wang, D., Ghosh, D., Touhidul-Islam, S. M., Moorman, C. D., Thomason, A. E., Wilkinson, D. S., & Mannie, M. D. (2016). Interferon-β facilitates neuroantigen-dependent induction of CD25+ FOXP3+ regulatory T cells that suppress experimental autoimmune encephalomyelitis. *The Journal of Immunology*. 197: 2992-3007.

Fu, S., Zhang, N., Yopp, A. C., Chen, D., Mao, M., Chen, D., Zhang, H., Ding, Y., & Bromberg, J. S. (2004). TGF-β induces Foxp3+ T-regulatory Cells from CD4+ CD25- Precursors. *American Journal of Transplantation*. Jul. 14; 4 (10).

Thornton, A. M., & Shevach, E. M. (1998). CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *The Journal of Experimental Medicine*. Jul. 20; 188(2): 287-96.

Fallarino, F., Grohmann, U., Hwang, K. W., Orabona, C., Vacca, C., Bianchi, R., Belladonna, M. L., Fioretti, M. C., Alegre, M. L., & Puccetti, P. (2003). Modulation of tryptophan catabolism by regulatory T cells. *Nature Immunology*. Dec.; 4 (12): 1206-1212.

Venken, K., Hellings, N., Thewissen, M., Somers, V., Hensen, K., Rummens, J. L., Medaer, R., Hupperts, R., & Stinissen, P. (2008). Compromised CD4+ CD25(high) regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. *Immunology*. Jan.; 123(1): 79-89.

Mellor, A. L., & Munn, D. H. (2003). Tryptophan Catabolism and Regulation of Adaptive Immunity. *The Journal of Immunology*. Jun. 15; 170 (12): 5809-5813.

Dieckmann, D., Plottner, H., Berchtold, S., Berger, T., & Schuler, G. (2001). Ex vivo isolation and characterization of CD4(+) CD25(+) T cells with regulatory properties from human blood. *The Journal of Experimental Medicine*. Jun. 4; 193(11), 1303-1310.

Tai, X., Van Laethem, F., Pobezinsky, L., Guinter, T., Sharrow, S. O., Adams, A., Granger, L., Kruhlak, M., Lindsten, T., Thompson, C. B., Feigenbaum, L., & Singer, A. (2012). Basis of CTLA-4 function in regulatory and conventional CD4(+) T cells. *Blood*. May 31; 119(22): 5155-63.

Danikowski, K. M., Jayaraman, S., & Prabhakar, B. S. (2017). Regulatory T cells in multiple sclerosis and myasthenia gravis. *Journal of Neuroinflammation*. 14 (117): 1-16.

Nelson, B. H. (2004). IL-2, Regulatory T cells, and Tolerance. *The Journal of Immunology*. Apr 1; 172 (7): 3983-3988.

Yang, Li, Anderson, D. E., Kuchroo, J., & Hafler, D. A. (2008). Lack of TIM-3 Immunoregulation in Multiple Sclerosis. *The Journal of Immunology*. Apr. 1; 180 (7): 4409-4414.

Boivin N, Baillargeon J, Doss PMIA, Roy A-P, & Rangachari M. (2015) Interferon-β Suppresses Murine Th1 Cell Function in the Absence of Antigen-Presenting Cells. *PLoS ONE*. 10(4): e0124802.

McRae, B. L., Semnani, R. T., Hayes, M. P., & van Seventer, G. A. (1998). Type I IFNs inhibit Human dendritic cell IL-12 production and Th1 Cell Development. *The Journal of Immunology*. 160: 4298-4304.

Hallal-Longo, D. E. M., Mirandola, S. R., Oliveira, E. C., Farias, A. S., Pereira, F. G., Metze, I. L., Brandao, C. O., Ruocco, H. H., Damasceno, B. P., & Santos, L. M. B. (2007). Diminished Myelin-Specific T cell Activation Associated with increase in CTLA-4 and Fas molecules in Multiple Sclerosis Patients treated with Interferon-β. *Journal of Interferon & Cytokine Research*. 27: 865-873.

Huh, J. R., & Littman, D. R. (2012). Small molecule inhibitors of RORγt: targeting Th17 cells and other applications. *European Journal of Immunology*. Sep.; 42(9): 2232-2237.

Kavrochorianou, N., Evangelidou, M., Markogiannaki, M., Tovey, M., Thyphronitis, G., & Haralambous, S. (2015). IFNAR signaling directly modulates T lymphocyte activity, resulting in milder experimental autoimmune encephalomyelitis development. *Journal of Leukocyte Biology*. Jul. 31; 99(1): 175-88.

Tao, Y., Zhang, X., Chopra, M., Kim, M. J., Buch, K. R., Kong, D., Jin, J., Tang, Y., Zhu, H., Jewells, V., & Markovic-Plese, S. (2014). The role of endogenous interferon-β in the

regulation of Th17 responses in patients with relapsing-remitting multiple sclerosis. *The Journal of Immunology*. Jun. 15; 192 (12): 5610-5617.

Zhang, L., Yuan, S., Cheng, G., & Guo, B. (2011). Type I IFN promotes IL-10 production from T cells to suppress Th17 cells and Th17-associated autoimmune inflammation. *PLoS ONE*. 6(12): e28432.

Börnsen L, Romme Christensen J, Ratzer R, Hedegaard C, Søndergaard HB, Krakauer M, Hesse D., Nielsen, C. H., Sorensen, P. S., Sellebjerg, F. (2015) Endogenous Interferon-β-Inducible Gene Expression and Interferon-β-Treatment Are Associated with Reduced T Cell Responses to Myelin Basic Protein in Multiple Sclerosis. *PLoS ONE*. 10(3): e0118830.

Sweeney, C. M., Lonergan, R., Basdeo, S. A., Kinsella, K., Dungan, L. S., Higgins, S. C., Kelly, P. J., Costelloe, L., Tubridy, N., Mills, K. H. G., Fletcher, J. M. (2011). IL-27 mediates the response to IFN-β therapy in multiple sclerosis patients by inhibiting Th17 cells. Brain, Behavior, and Immunity. Aug.; 25 (6): 1170-1181.

Axtell, R. C., de Jong, B. A., Boniface, K., van der Voort, L. F., Bhat, R., De Sarno, P., Naves, R., Han, M., Zhong, F., Castellanos, J. G., Mair, R., Christakos, A., Kolkowitz, I., Katz, L., Killestein, J., Polman, C. H., de Waal Malefyt, R., Steinman, L., & Raman, C. (2010). T helper type 1 and 17 cells determine efficacy of interferon-β in multiple sclerosis and experimental encephalomyelitis. *Nature Medicine*. Apr; 16(4): 406-12.

Bettelli, E., Korn, T., Oukka, M., & Kuchroo, V. K. (2008). Induction and effector functions of Th17 cells. *Nature*. Jun. 19; 453: 1051-1057.

Dong C. (2008). Regulation and pro-inflammatory function of interleukin-17 family cytokines. *Immunological reviews*. Dec.; *226*: 80-86.

Ramgolam, V. S., Sha, Y., Jin, J., Zhang, X., Markovic-Plese, S. (2009). Interferon-β inhibits human Th17 cell differentiation. *The Journal of Immunology*. Oct. 15; 183 (8): 5418-5427.

Yao, Z.,Fanslow, W. C., Seldin, M. F., Rousseau, A. M., Painter, S. L., Comeau, M. R., Cohen, J. I., & Spriggs, M. K. (1995). Herpesvirus Samiri endcodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity*. Dec.; 3: 811-821.

Wojcikiewicz, E. P., Koenen, R. R., Fraemohs, L., Minkiewicz, J., Azad, H., Weber, C., & Moy, V. T. (2008). LFA-1 binding destabilizes the JAM-A homophilic interaction during leukocyte transmigration. *Biophysical journal.,*Jan. 7; *96*(1): 285-93.

Arjmandi, A., Liu, K., & Dorovini-Zis, K. (2009). Dendritic cell adhesion to cerebral endothelium: role of endothelial cell-adhesion molecules and their ligands. Journal of Neuropathology & Experimental Neurology. Mar. 1; 68 (3): 300-313.

Kraus, J., Voigt, K., Schuler, A. M., Scholz, M., Kim, K. S., Schilling, M., Schabitz, W. R., Oschmann, P., & Engelhardt B. (2008). Interferon-β stabilizes barrier characteristics of the blood-brain barrier in four different species in vitro. *Multiple Sclerosis*. 14: 843-852.

Sagar, D., Foss, C., El Baz, R., Pomper, M. G., Khan, Z. K., & Jain, P. (2011). Mechanisms of dendritic cell trafficking across the blood-brain barrier. *Journal of Neuroimmune Pharmacology : The Official Journal of the Society on NeuroImmune Pharmacology*. Aug. 6; *7*(1): 74-94.

Yen, J. H., Kocieda, V. P., Jing, H., & Ganea, D. (2011). Prostaglandin E2 induces matrix metalloproteinase 9 expression in dendritic cells through two independent signaling pathways leading to activator protein 1 (AP-1) activation. *Journal of Biological Chemistry*. Nov. 11; 286: 38913-38923.

Sallusto, F., Palermo, B., Lenig, D., Miettinen, M., Matikainen, S., Julkunen, I., Forster, R., Burgstahler, R., Lipp, M., & Lanzavecchia, A. (1999). Distinct patterns and kinetics of chemokine production regulate dendritic cell function. *European Journal of Immunology*. Feb. 2; 29: 1617-1625.

Fossiez, F., Djossou, O., Chomarat, P., Flores-Romo, L., Ait-Yahia, S., Maat, C., Pin, J. J., Garrone, P., Garcia, E., Saeland, S., Blanchard, D., Gaillard, C., Das Mahapatra, B., Rouvier, E., Golstein, P., Banchereau, J., & Lebecque, S. (1996). T cell interleukin-17 induces stromal cells to produce pro-inflammatory and hematopoietic cytokines. *The Journal of Experimental Medicine*. Jun. 1; *183*(6): 2593-603.

Cheng, W., & Chen, G. (2014). Chemokines and chemokine receptors in multiple sclerosis. *Mediators of inflammation*, *2014*. 659206.

Raab, M., Daxecker, H., Markovic, S., Karimi, A., Griesmacher, A., & Mueller, M. M. (2002). Variation of adhesion molecule expression on human umbilical vein endothelial ccells upon multiple cytokine application. *Clinica Chimica Acta*. Jul.; 321 (1-2): 11-16.

Kis-Toth, K., Bacskai, I., Gogolak, P., Mazlo, A., Szatmari, I., & Rajnavolgyi, E. (2013). Monocyte-derived dendritic cell subpopulations use different types of matrix mettaloproteinases inhibited by GM6001. *Immunobiology*. Nov.; 218 (11): 1361-1369.

Yen, J. H., Kong, W., & Ganea, D. (2010). IFN-β inhibits dendritic cell migration through STAT-1-mediated transcriptional suppression of CCR7 and matrix metalloproteinase 9. *Journal of Immunology (Baltimore, Md. : 1950)*. Apr. 1; *184*(7): 3478-86.

Zozulya, A. L., Reinke, E., Baiu, D. C., Karman, J., Sandor, M., & Fabry, Z. (2007). Dendritic cell transmigration through brain microvessel endothelium is regulated by MIP-1 alpha chemokine and matrix metalloproteinases. *The Journal of Immunology*. 178: 520-529.

Chang, Y. J., Holtzman, M. J., & Chen, C. C. (2001). Interferon-y-induced epithelial ICAM-1 expression and monocyte adhesion: Involvement of protein kinase C-dependent c-Src tyrosine kinase activation pathway. *Journal of Biological Chemistry*. Dec. 20; 277: 7118-7126.

Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 21.1, Overview of Neuron Structure and Function. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21535/

Aung, L. L., Fitzgerald-Bocarsly, P., Dhib-Jalbut, S., & Balashov, K. (2010). Plasmacytoid dendritic cells in multiple sclerosis: chemokine and chemokine receptor modulation by interferon-β. *Journal of Neuroimmunology*. Sept. 14; *226*(1-2): 158-64.

Park, H., Li, Z., Yang, X. O., Chang, S. H., Nurieva, R., Wang, Y. H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., & Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nature immunology*. Oct. 22; *6*(11): 1133-41.

Yong, V. W., Zabad, R. K., Agrawal, S., DaSilva, A. G., & Metz, L. M. (2006). Elevation of matrix metalloproteinases (MMPs) in multiple sclerosis and impact of

immunomodulators. *Journal of the Neurological Sciences*. Nov.; doi: 10.1016/j.jns.2006.11.021

Krakauer, M., Sorensen, P. S., Khademi, M., Olsson, T., & Sellebjerg, F. (2006). Dynamic T-lymphocyte Chemokine receptor expression induced by Interferon-β therapy in Multiple Sclerosis. *Scandinavian Journal of Immunology*. May 10; 64: 155-163.

Cepok, S., Schreiber, H., Hoffman, S., Zhou, D., Neuhaus, O., von Geldern, G., Hochgesand, S., Nessler, S., Rothhammer, V., Lang, M., Hartung, H. P., & Hemmer, B. (2009). Enhancement of Chemokine expression by Interferon-β therapy in patients with multiple sclerosis. *Arch. Neurol.* Oct.; 66 (1): 1216-1223.

Sorensen, T. L., & Sellebjerg, F. (2002). Selective suppression of chemokine receptor CXCR3 expression by interferon- β 1a in multiple sclerosis. *Multiple Sclerosis*. 8: 104-107.

Groom, J. R., & Luster, A. D. (2011). CXCR3 in T cell function. *Experimental Cell Research*, *317*(5), 620-31.

Oviedo-Orta, E., Bermudez-Fajardo, A., Karanam, S., Benbow, U., & Newby, A. C. (2008). Comparison of MMP-2 and MMP-9 secretion from T helper 0, 1 and 2 lymphocytes alone and in coculture with macrophages. *Immunology*. May; *124*(1): 42-50.

Alexander, J. S., Harris, M. K., Wells, S. R., Mills, G., Chalamidas, K., Ganta, V. C., McGee, J., Jenning, M. H., Gonzalez-Toledo, E., & Minagar, A. (2010). Alterations in serum MMP-8, MMp-9, IL-12p40, and IL-23 in multiple sclerosis patients treated with interferon-β 1b. *Multiple Sclerosis*. Jul. 9; 16 (7): 801-809.

Cheng, W. C., Zhao, Q., Xi, Y., Li, C., Xu, Y., Wang, L., Niu, X., Wang, Z., & Chen, G. (2015). IFN-β inhibits T cells accumulation in the central nervous system by reducing the expression and activity of chemokines in experimental autoimmune encephalomyelitis. *Molecular Immunology*. Mar.; 64 (1): 152-162.

Avolio, C., Filippi, M., Tortorella, C., Rocca, M. A., Ruggieri M., Agosta, F., Tomassini, V., Pozzilli, C., Stecchi, S., Giaquinto, P., Livrea, P., & Trojano, M. (2005). Serum MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios in multiple sclerosis: relationships with different magnetic resonance imaging measures of disease activity during IFN-β-1a treatment. *Multiple Sclerosis*. 11: 441-446.

Kantarci, O. H. (2008). Genetics and Natural History of Multiple Sclerosis. *Seminars in Neurology*. 28 (1): 7-16.

Tullman, M. J. (2013). Overview of the Epidemiology, Diagnosis, and Disease Progression Associated with Multiple Sclerosis. *American Journal of Management Care*. Feb. 25; 19: S15-S20.

Harbo, H. F., Gold, R., and Tintoré, M. (2013). Sex and gender issues in multiple sclerosis. *Therapeutic Advances in Neurological Disorders*. Jul.; 6(4): 237–248.

Salzer, J. L. and Zalc, B. (2016). Myelination. *Current Biology*. Oct. 24; 26 (20): R971-R975.

Geissmann, F., Manz, M. G., Jung, S., Sieweke, M. H., Merad, M., and Ley, K. (2010). Development of monocytes, macrophages, and dendritic cells. *Science (New York, N.Y.)*. Feb. 5; 327(5966): 656–661.

Martinez, G. J., Nurieva, R. I., Yang, X. O., and Dong, C. (2008). Regulation and function of proinflammatory TH17 cells. *Annals of the New York Academy of Sciences*. Nov.; 1143: 188–211.

Scholz, E. M., Marcilla, M., Daura, X., Arribas-Layton, D., James, E. A., and Alvarez, I. (2017). Human Leukocyte Antigen (HLA)-DRB1*15:01 and HLA-DRB5*01:01 Present Complementary Peptide Repertoires. *Frontiers in Immunology*. Aug; 8: 984.

Sethi, V., Nair, G., Absinta, M., Sati, P., Venkataraman, A., Ohayon, J., and Reich, D. S. (2016). Slowly eroding lesions in multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England).* Jul. 2016; 23(3): 464–472.

Attanasio, J., and Wherry, E. J. (2016). Costimulatory and Coinhibitory Receptor Pathways in Infectious Disease. *Immunity*. May 17; 44(5): 1052–1068.

Carter, L. L., Fouser, L. A., Jussif, J., Fitz, L., Deng, B., Wood, C. R., Collins, M., Honjo, T., Freeman, G. J., and Carreno, B. M. (2002). PD-1: PD-L1 inhibitory pathway affects both CD4+ and CD8+ T cells and is overcome by IL-2. *European Journal of Immunology*. Feb. 12; 32 (3).

Constantinescu, C. S., Farooqi, N., O'Brien, K., and Gran, B. (2011). Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *British Journal of Pharmacology*. Oct; 164(4): 1079–1106.

Prinz, M. and Priller, J. (2017). The role of peripheral immune cells in the CNS in steady state and disease. *Nature Neuroscience*. Jan. 16; 20: 136-144.

Van Weyenbergh, J., da Purificacao, M., Silva, P., Bafica, A., Cardoso, S., Wietzerbin, J., Barral-Netto, M. (2001). IFN-β and TGF-β differentially regulate IL-12 activity in human peripheral blood mononuclear cells. *Immunology Letter*. Sept. 18; 75: 117-122.

Liu, J. F., Wu, L., Yang, L. L., Deng, W. W., Mao, L., Wu, H., and Sun, Z. J. (2018). Blockade of TIM3 relieves immunosuppression through reducing regulatory T cells in head and neck cancer. *Journal of Experimental & Clinical Cancer Research: CR*. Mar 5; 37(1): 44.

Schmidt, A., Oberle, N., and Krammer, P. H. (2012). Molecular mechanisms of Treg-mediated T cell suppression. *Frontiers in Immunology*. 3, 51.

Haas, J., Fritzsching, B., Trubswetter, P., Korporal, M., Milkova, L., Fritz, B., Vobis, D., Krammer, P. H., Suri-Prayer, E., and Wildemann, B. (2007). Prevalence of Newly Generated Regulatory T cells (T_{reg}) is Critical for T_{reg} Suppressive Function and Determines T_{reg} Dysfunction in Multiple Sclerosis. *The Journal of Immunology*. Jul. 15; 179 (2): 1322-1330.

Dalla Libera, D., Di Mitri, D., Bergami, A., Centonze, D., Gasperini, C., Grasso, M. G., and Furlan, R. (2011). T regulatory cells are markers of disease activity in multiple sclerosis patients. *PloS one*. Jun. 24; 6(6): e21386.

Fu, S., Zhang, N., Yopp, A . C., Chen, D., Mao, M., Chen, D., Zhang, H., Ding, Y., and Bromberg, J. S. (2004). TGF-βInduces Foxp3+ T-Regulatory Cells from CD4+ CD25- Precursors. *American Journal of Transplantation*. Jul. 14; 4(10).

Greenwood, J., Heasman, S. J., Alvarez, J. I., Prat, A., Lyck, R., and Engelhardt, B. (2011). Review: Leucocyte-endothelial cell crosstalk at the blood-brain barrier: A prerequisite for

successful immune cell entry to the brain. *Neuropathology and Applied Neurobiology*. Oct. 14; 37 (1).

Leung, S., Liu, X., Fang, L., Chen, X., Guo, T., & Zhang, J. (2010). The cytokine milieu in the interplay of pathogenic Th1/Th17 cells and regulatory T cells in autoimmune disease. *Cellular & Molecular Immunology*. Apr. 12; 7(3): 182–189.