Universitat Autònoma de Barcelona

NOVEL EXPERIMENTAL STRATEGIES TO TREAT MULTIPLE SCLEROSIS

Alba Sánchez Fernández

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ACADEMIC DISSERTATION

To obtain the degree of PhD in Neuroscience

by the Universitat Autònoma de Barcelona

(2019)

NeuroPlasticity & Regeneration
INc Institut de Neurociències
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SUMMARY

Despite multiple sclerosis was described by Dr. Jean-Martin Charcot in the 19th century, it is currently a challenging neurological disorder. The etiology of this pathology is not fully understood but the degeneration that occurs in myelin sheaths and neurons in individual suffering from multiple sclerosis is linked to aberrant presence of immune cells in the lesioned central nervous system. For this reason, in the present thesis we propose to modulate the inflammatory response to ameliorate neurological decline and demyelination in experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis.

The data shown here reveals that the administration of anti-inflammatory approaches, such as OLT1177 and IL-37, or specialized pro-resolving mediators, such as Resolvin-D1 or Maresin-1, has therapeutic effects in mice with experimental autoimmune encephalomyelitis, even if these treatments are initiated at the onset of the disease. OLT1177, IL-37 and Maresin-1 exerts beneficial effects by reducing some common aspects of inflammation, such as the production pro-inflammatory cytokines and the accumulation of immune cells. IL-37 and Maresin-1 also modulate the inflammatory response in the periphery and alter the polarization of lymphocyte and macrophage towards a more anti-inflammatory or regulatory phenotype. Collectively, the results presented here provide novel data that could pave the way to develop more effective strategies to treat multiple sclerosis.

INTRODUCTION

1. MULTIPLE SCLEROSIS

More than 100 years has passed since Charcot, Carswell, Cruveilhier and others described the clinical and pathological characteristics of multiple sclerosis (MS). This disease has originally been defined as a chronic inflammatory disease of the central nervous system (CNS), leading to focal plaques of primary demyelination and axonal damage in the white matter of the brain and spinal cord. It manifests clinically as neurological deficit (Charcot, 1880). 40 years later it was categorized as an autoimmune disease (Wu and Alvarez, 2011; Baecher-Allan et al., 2018). However, the aetiology and the pathogenesis of this disease are not yet conclusively known (Reviewed at Dyment et al., 2004; Trapp and Nave, 2008; Wu and Alvarez, 2011; Tselis, 2012; Didonna and Oksenberg, 2017; Baecher-Allan et al., 2018). This enigmatic, relapsing and often eventually progressive disorder of the white matter of the CNS continues to challenge researchers trying to understand the pathogenesis of the disease and prevent its progression.

MS is a chronic, slowly progressive, inflammatory, demyelinating and degenerative disease of the CNS characterized by an autoimmune inflammation(Malpass, 2012; Grigoriadis and van Pesch, 2015; Bjelobaba et al., 2017). The symptoms vary widely, and affected individuals can experience one or more effects of nervous system damage This pathology affects about 2.5 million people worldwide, ranking as one of the most prevalent neurodegenerative diseases (Wekerle, 2008). MS affects 50000 people in Spain, being the second cause of disability in our country after traumatic lesions (Spanish Society of Neurology 2015). MS typically presents in adults aged 20-50 years, with a peak of age onset at 30 years old. Women are more commonly affected with MS than men, with a ratio of 3:1 (female:male) (Reviewed at Harbo et al., 2013).

Aetiology and epidemiology

Although the cause of MS is unknown, it is quite clear that complex interactions between genetic background and environmental factors result in MS presentations. Thus, a myriad of factors may increase the risk of developing MS: age, sex, family history, infections, race, climate, some autoimmune diseases or smoking (Correale et al., 2013; Jörg et al., 2016; Kamińska et al., 2017).

Results from adoptions studies clearly show that adoptive relatives were no more likely to develop MS than expected from general population (lifetime risk of 0.2%). Consequently, genetic variants influencing susceptibility from MS have been explored in studies of siblings or twin concordance. The data from experiments with siblings of affected individuals (first degree or even dizygotic twins) have shown that they have a 10- to 20-fold higher risk (2–4%), with monozygotic twins having an even higher recurrence risk (25%) (Dyment, Ebers and Sadovnick, 2004).

There are about 100 genes or gene loci associated with MS, but the strongest genetic association resides within the major histocompatibility complex (*MHC*) in chromosome 6p21.3. This region contains approximately 160 closely linked genes. About half of these genes have important roles in the regulation of the immune system, including the classical human leukocyte antigen (*HLA*) genes—the class I genes *HLA-A, HLA-B*, and *HLA-C*, and the class II genes *HLA-DPB1, HLA-DQB1*, and *HLA-DRB1.* Other candidates that link genetics with MS are some polymorphisms in *T-cell receptor beta*, *CTLA4*, *ICAM1*, *SH2D2A*, *IL-2RA* or *IL7R*, between others *(Genome-wide association studies; GAWSs)* (Dyment et al., 2004; Baranzini and Oksenberg, 2017; Didonna and Oksenberg, 2017).

Nevertheless, the large amount of data on genetic predisposition for autoimmune diseases can, in most of the cases, only explain a part of the disease risk, supporting the view that the increasing prevalence of MS is triggered in addition by environmental factors. For instance, Epstein–Barr virus infection (EBV), smoking, dietary habits or vitamin D status may all contribute to explain differences in disease prevalence and incidence of MS (Milo and Kahana, 2010; Correale et al., 2013; Jörg et al., 2016). Indeed, epidemiological studies have shown a striking trend of MS toward higher prevalence with increasing latitude and a growing in disease

incidence in developed countries (Figure 1) (Milo and Kahana, 2010; Simpson et al., 2011).

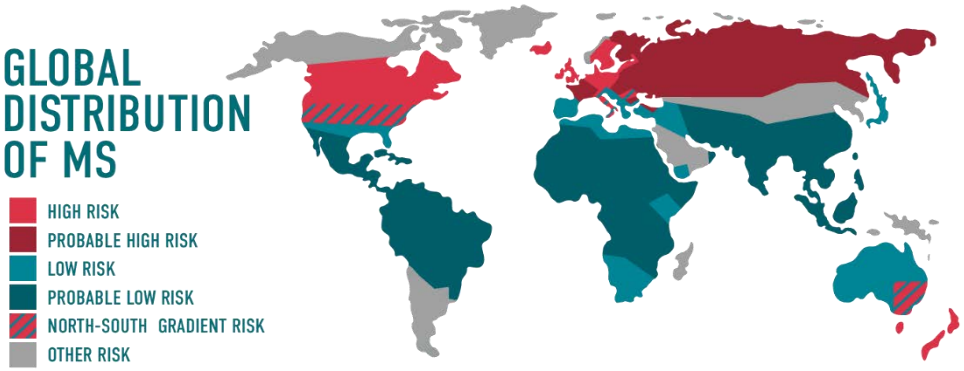

Figure 1. Global distribution of MS. There is a greater prevalence in higher latitudes (Extracted from www.multiplesclerosis.net).

On the one hand, it seems clear that EBV infection plays some role in MS pathogenesis since the evidence for prior infection in adult-onset MS is essentially 100% and the MS risk is 10 times higher in EBV-positive individuals. Hence, EBV is the strongest infectious risk factor in MS (Tselis, 2012).

On the other hand, increasing research efforts indicate that nutritional factors have the capability to potently modulate autoimmune responses and inflammation. Recent studies present further evidence that the gut microbiome may also play a role in diseases affecting the CNS, such as MS. The impact of gut microbiota and its metabolites on the mucosal immune system was shown to modulate extra-intestinal immune responses by influencing the balance of pro- and anti-inflammatory immune cell subsets (Jörg et al., 2016; Kirby and Ochoa-Repáraz, 2018).

In addition, there is compelling epidemiological evidence that the risk of developing MS is increased in association with low levels of sun exposure, possibly because this is associated with low vitamin D status. Therefore, there is a marked season of birth effect in MS, consistent with increased risk associated with lower sun exposure/vitamin D in the late first trimester of pregnancy (Fernandes De Abreu et al., 2011). This raises the possibility that early life (in utero) sun exposure can alter susceptibility to MS development as an adult. The link between vitamin D and MS risk seems evident since the active form of vitamin D plays an essential role in lymphocyte activation and proliferation, T-helper cell differentiation, tissue-specific

lymphocyte homing, production of specific antibodies and, ultimately, regulation of the immune response (Sintzel et al., 2017).

It is also interesting to explore the role of environment in the unequal gender distribution of MS. Some studies demonstrated that sex hormones may play an important role in MS pathogenesis. For instance, experimental data from animal models and clinical observations in humans are consistent with the hypothesis that estrogen may influence vitamin D metabolism, and consequently its anti-inflammatory and neuroprotective functions in the CNS (Correale et al., 2013; Harbo et al., 2013).

Classification and clinical features

According to the Overcoming Multiple Sclerosis (OMS) organization, MS is typically classified in five different types (Figure 2):

- ***Relapse-Remitting MS (RRMS):*** is the most common form of MS (65-80% of people with MS) and typically occurs in young people. In this form of MS there are unpredictable relapses (exacerbations or attacks) during which new symptoms appear or existing symptoms become more severe. Relapses can last for from days to months and after that there is partial or total recovery. However, relapses become less frequent over the years, but the recovery is lower after each one. The disease may be inactive for months or years.

- ***Secondary progressive MS (SPMS):*** most patients suffer RRMS before it progresses to SPMS after some years (about 40% of diagnosed patients and 80% of people with RRMS). Relapses give way to steady decline in mobility and progression to disability, usually without discrete relapses.

- ***Primary progressive MS (PPMS):*** this form is less frequent than the previous ones (about 10-15% of diagnosed patients) and it is more common in middle-aged men. It is characterized by the lack of distinct attacks, but with slow onset and steadily worsening symptoms. Thus,

there is an accumulation of deficits and disability that may level off at some point or continue over months and years.

- ***Progressive-Relapsing MS (PRMS):*** it is an uncommon form of MS (5% of diagnosed patients). People experience both progressive neurological decline and attacks. It is often misdiagnosed as PPMS until relapses start.

- ***Clinical isolated syndrome (CIS):*** it is an unusual form of MS (5-20% of diagnosed people). People suffer one or two attacks with complete recovery, they do not get worse with time and there is no permanent disability. If someone with MS has remained mostly functional for 15 years, they are generally considered to have benign MS.

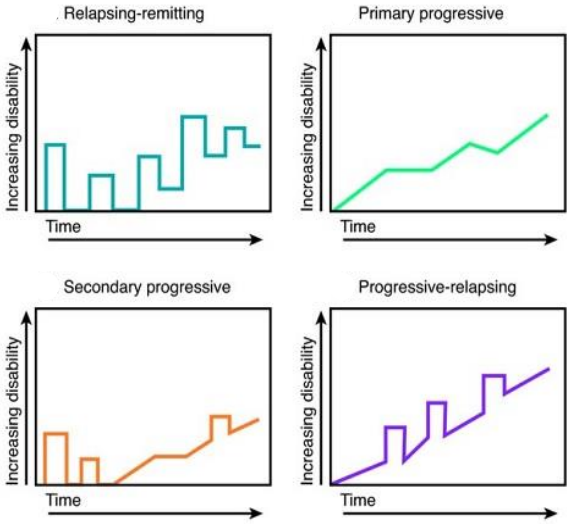

Figure 2. Types and courses of MS. RRMS is characterized by clearly defined acute attacks with full recovery. PPMS is characterized by disease showing progression of disability from onset, without plateaus or remissions or with occasional plateaus and temporary minor improvements. SPMS is the progression of RRMS in most patients. The disability increases without relapses. PRMS is characterized by sequelae and residual deficit upon recovery (Lublin and Reingold, 1996).

Since MS is an immune-mediated disease, symptoms are variable and unpredictable. Symptoms can be different in each type of MS or even vary from person to person. However, some of them are commonly associated with all types of MS: numbness or tingling (63.5%) and walking difficulties (50%) are the most common affectations. People with MS also use to have vision problems (40%), fatigue (40%), weakness (25%), dizziness (23%), neuropathic pain (20%), muscle spasms (17%), depression (15%) or cognitive dysfunction (13%). In less proportion, patients could have

bladder (11%) and bowel (6%) dysfunctions (OMS organization) (Ghasemi et al., 2017).

Pathophysiology

Despite de fact that the aetiology of MS remains elusive, clues to its pathogenesis have been derived from the basic pathologic characterization of CNS tissues of death MS patients (Frohman et al., 2006; Bo, 2009). Thus, the MS pathological process includes breakdown of the blood-brain barrier (BBB), multifocal inflammation, demyelination, oligodendrocyte loss, reactive gliosis and axonal degeneration (Trapp and Nave, 2008; Bhise and Dhib-Jalbut, 2016).

a. Central nervous system injuries

MS has been defined as an inflammatory demyelinating disease, indicating that the formation of brain and spinal cord lesions is driven by an inflammatory process (Pérez-Cerdá et al., 2016; Bjelobaba et al., 2017). Focal plaques of primary demyelination, reflected by complete loss of myelin (Figure 3), but a partial preservation of axons and neurons, are the hallmark of MS pathology (Ludwin, 1978; Kuhlmann et al., 2002; Bo, 2009).

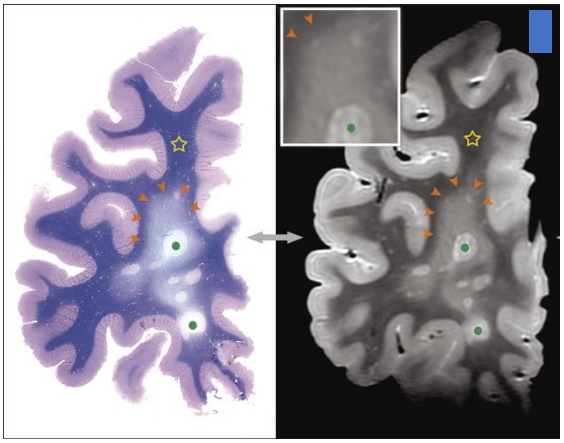

Figure 3. Brain undergoing demyelination in a MS patient. Yellow stars indicate normal-appearing white matter, green dots indicate focal plaques of demyelination and orange arrows diffuse changes (Seewan et al. 2009).

Demyelinating plaques are present not only in the white matter, but they are also in the grey matter, such us the cerebral and cerebellar cortex and the basal ganglia, the thalamus and the hypothalamus (Bo, 2009). Besides, there are diffuse changes in the brain (Figure 3), consistent of small perivascular demyelination lesions, axonal injury and neurodegeneration, microglia activation and astrocytic scar formation in the entire white and grey matter of the brain and spinal cord. All these events lead

to tissue loss and atrophy in the CNS (David, 2015; Grigoriadis and van Pesch, 2015; Pérez-Cerdá et al., 2016).

Traditionally, MS plaque classification has been based on temporal progression of inflammatory destruction of nervous tissue. Thus, acute, chronic active and chronic silent lesions are thought to occur along a continuous timeline:

The *acute MS plaque* represents the earliest stage of lesion formation. They are characterized by robust inflammatory infiltration and demyelination throughout the lesion. These lesions have diffuse margins of myelin loss, infiltration of immune cells and parenchymal edema. The degree of oligodendrocyte loss within active lesions can be variable. Axonal injury can be extensive in acute lesions and glial reactivity, particularly hypertrophic astrocytes, is noted within the lesions. Dense glial scarring is not typical of the acute plaque (Genain et al., 1999).

The *chronic active lesions* are characterized by a region of hypocellularity with loss of myelin and glial scarring. Lesions borders of chronic plaques are more sharped that those of acute lesions. Inflammation continues along the outer border, as it occurs in acute lesions. Thus, borders are populated with activated microglia and macrophages, vessels demonstrating perivascular cuffing and reactive astrocytes. Areas of remyelination are often observed on the edge of lesions. Besides, the core of chronic lesions is typically hypocellular and contains naked damaged axons (Genain et al., 1999).

Finally, *chronic silent lesions* are characterized by loss of the inflammatory traits. Remyelination and the presence of oligodendrocyte progenitors are uncommon. Moreover, there is a complete loss of oligodendrocytes and a variable reduction of axonal density (Prayson, 2012).

Overall, the gross and histologic features of the MS plaque imply a complex progression of inflammatory damage culmination in a scarred region of demyelination (Bo, 2009; Wu and Alvarez, 2011; Pérez-Cerdá et al., 2016).

Importantly, histopathological examination of normal apparent white matter (NAWM) (Figure 3) support the concept that areas outside of plaques also have pathologic changes: microglial activation, T cells infiltration and perivascular

cuffing. These changes may be also associated with the progression of the disease (Allen et al., 2001).

b. Axonal and neuronal damage

Inflammatory CNS injury in MS has been also associated with axonal damage. Although MS has classically been described as a disease marked by the loss of myelin in greater proportion to the loss of axons, axonal damage was noted in the earliest stages of MS lesions (Charcot, 1880). The active areas of MS lesions were found to contain more transected axons than inactive areas. Moreover, comparisons of biopsy and autopsy samples from patients with RRMS, SPMS and PPMS suggest that axonal pathology is greatest within the first year of disease onset. Thus, axonal damage occurs more likely in areas of active inflammation and early during the disease (Kuhlmann et al., 2002). Furthermore, patients with higher levels of motor disability have fewer surviving corticospinal axons travelling through their spinal cord, demonstrating a direct correlation of axonal damage and disease progression (Trapp et al., 1998). The mechanisms involved in axonal damage in MS are still under investigation.

Neuronal loss in MS can be severe and occurs more frequently within the brain. Neuronal loss in the range of 18–35% has been reported in the cortex, hippocampus, thalamus and spinal cord (Bo, 2009) caused by the direct immune injury to the grey matter.

Further, axonal and neuronal survival is directly linked to the trophic support provided by myelin, which may be particularly relevant during a high metabolic demand state of neurons exposed to inflammatory stressors (Trapp and Nave, 2008).

2. THE INFLAMMATORY RESPONSE IN MULTIPLE SCLEROSIS

Since inflammation is the driving force of tissue injury in MS, understanding its immunological mechanisms is essential for the development of effective therapeutic strategies. Despite there is an ongoing debate that lingers over the autoimmunity nature of MS, it is well stablished that the immune system directly participates in the destruction of myelin and nervous cells (Wu and Alvarez, 2011; Dendrou et al., 2015; Pérez-Cerdá et al., 2016).

T cells in MS

MS is believed to be caused by the activation of peripheral autoreactive CD4 T cells that migrate into the CNS and initiate the disease process. Once in the CNS, autoreactive effector CD4 T cells are locally reactivated by APCs and recruit additional T cells and macrophages to establish the inflammatory lesion (Malpass, 2012).

MS lesions also contain CD8 T cells, which are mostly found at the edges of lesions, and CD4 T cells, which are deeper in the lesions, and, they are also present in the CSF of patients with MS (Traugott et al., 1983). These cells cause the hallmarks of MS: myelin loss, oligodendrocyte destruction and axonal damage, leading to neurologic dysfunction. In parallel, immune-modulatory networks are triggered to limit inflammation and to initiate repair, which results in, at least, partial remyelination, associated with clinical remission (Baecher-Allan et al., 2018).

IFNγ, produced by Th1 cells, and IL-17, secreted by Th17 cells, are believed to be pathogenic initiators of MS (Kallaur et al., 2013; David, 2015). In addition, Th17 cells express IL-21, IL-22 and TNF-α, which regulate B cell activation and plasma cells generation, augment inflammation via Signal transducer and activator of transcription 3 (STAT3) activation. Importantly, IL-17-producing T cells are also believed to co-produce IFNγ and IL-17, which may function in disease to activate resident microglia (Murphy et al., 2010). On the other hand, IL-17 can also be secreted by non-T cells, such NK cells and astrocytes. Besides, it is believed that IL-

17 plays a crucial role in the breakdown of the BBB by inducing ROS within endothelial cells (Huppert et al., 2010).

Although much research has focused on CD4 T cells in MS, it appears that CD8 T cells also play a significant role in human MS (Traugott et al., 1983). CD8 T cells isolated from the CNS, CSF and blood of patients exhibit evidence of oligoclonal expansion, indicating that these oligoclonal cells have been amplified via antigen-specific responses (Zang et al., 2004). The cytotoxic function of effector CD8 T cells might play a central role in axonal damage, oligodendrocyte death and neuronal damage. Pathogenic CD8 T cells might also contribute to pathology by secreting IFNγ, IL-17 and granzymes (reviewed at Baecher-Allan et al., 2018).

On the other hand, subsets of T cells that modulate immune activation and control the development of autoimmunity have been identified. At least, two subsets of CD4+ regulatory cells (Tregs) have been identified and studied in the context of MS: T regs that express FoxP3 (classic Tregs) and surface molecules that contribute to their capacity to supress other immune cells proliferation and activation (Lu and Rudensky, 2009) and Tr1 regulatory cells, that express CD49b+ (non-classic Tregs) and inhibit cell proliferation primarily via the secretion of IL-10 (Gagliani et al., 2013; Fan et al., 2018).

B cells in MS

Clonally expanded B cells are found in the brain parenchyma, meninges and CSF of MS patients. These cells are present in the CNS at greater frequency earlier in the disease. Increased B cell numbers in the CSF correlates with a faster disease progression. Beyond their potential ability to produce autoantibodies, B cells in the CNS could act in MS by secreting chemokines/cytokines and by presenting antigens to T cells. Importantly, B cells can cross the BBB and become long-term CNS residents in lymph-node-like follicles found in the meninges, which are often adjacent to cortical lesions. On the other hand, in MS memory cells exhibit enhanced pro-inflammatory properties and have capacity to drive T cells activation (Reviewed at Grigoriadis and van Pesch, 2015; Baecher-Allan et al., 2018).

Macrophages and microglia in MS

Reactive macrophages/microglia can mediate cell damage or neuroprotective effects in MS. This characteristic may contribute to the relapsing-remitting feature of MS. During the acute phase, microglia and macrophages become classically activated M1 after activation and they potentially release a large amount of protein inflammatory cytokines that induce the damage in the CNS. During the second phase, after the peak, M2 cells are predominant in the CNS, where the release anti-inflammatory cytokines, implicated in resolving inflammation and tissue repair. In addition, M2 macrophages can promote the differentiation of Th2 cells and Treg cells as well as damper Th1 cell activity and pro-inflammatory cytokine release (Reviewed at Chu et al., 2018).

Astrocytes in MS

Astrocytes play an instrumental role in the formation of MS lesions through a multitude of functional changes associated with their activation. Astrocytes are early responders in nascent white matter lesions, are the main recruiters of lymphocytes and act themselves as immunocompetent cells that contribute to innate immunity (Reviewed at Nair et al., 2008; Correale and Farez, 2015). Moreover, astrocytes not only can adopt a neurotoxic phenotype (A1), but also confine inflammation through scar formation and can promote neuroprotection and tissue repair. Astrocytic dysfunction associated with a genetic MS risk variant further suggests that astrocyte-mediated processes are causative in lesion pathology. Thus, while MS is driven by dysfunction of the peripheral immune system, CNS cells such as astrocytes may contribute to MS pathology by targeting dysregulated immune responses to the CNS (Reviewed at Ponath et al., 2018).

Of note, whereas peripheral adaptive immune system (T cells) drives RRMS, the innate immune system (microglia and astrocytes) with B cells is the primary driver of progressive forms of MS (Reviewed at Baecher-Allan et al., 2018).

3. ANIMAL MODELS OF MULTIPLE SLCEROSIS

Since MS is a complex disease, there is no a single animal model that can capture the entire spectrum of heterogeneity of human MS. However, the main positive aspect is that they can surely serve as a testing tool to study disease development and for novel therapeutic approaches. Thus, there are several established experimental demyelination models that reflect the heterogeneity of MS and are therefore seen as suitable to study MS pathogenesis. These models include toxin-induced models, virus-induced and immune-mediated (Reviewed at Bjelobaba et al., 2018).

Toxin-induced animal models

There are several agents known to generate demyelination using stereotactic injections of gliotoxins in the white matter, such as ethidium bromide and lysolecithin, or systematically administered toxins, such as cuprizone.

Ethidium bromide is an intercalating agent that is toxic for all cells with nuclei and can be used to create a focal demyelination lesion. Ethidium bromide injection preferentially compromises mitochondrial DNA in glial cells rather than neurons and endothelial cells. Ethidium bromide induce apoptosis within the first week after injection and remyelination starts after six weeks after injection. In this model there is also an inflammatory response, probably because astrocyte death compromises the BBB, leading to the infiltration of peripheral inflammatory cells. This model has been carried out extensively in rats as a demyelinating model (Blakemore, 2005; Merrill, 2009).

Lysolecithin has detergent-like agent activity, so it can solubilize membranes. Interestingly, this compound is selective for myelin-producing cells (Hall, 1972). In this model remyelination is faster, starting 3 weeks after injection. Lysolecithin is considered a useful model to study demyelination and remyelination in the CNS (Jeffery and Blakemore, 1995; Bieber et al., 2003).

Cuprizone (oxalic acid) is a well-known copper-chelating agent that is known to be toxic to myelin sheet. Cuprizone-induced demyelination is a straight forward model used to investigate brain-intrinsic inflammatory responses, together with

demyelination/remyelination processes (Carlton, 1967; Ludwin, 1978). The hallmark of the lesions that cuprizone elicits is oligodendrocyte dysfunction and apoptosis. Activation of microglia starts 2 weeks after treatment and gradually decreases with the remyelination process. Astrocyte activation persist throughout the disease. Interestingly, spontaneous remyelination occurs after cessation of intoxication (Blakemore, 1972).

Virus-induced animal models

Considering the relevance of infectious factors contributing to MS, suitable animal models that involve virus-induced demyelination were developed. The best characterized virus-induced demyelination model is Theiler's murine encephalomyelitis (TMEV). TMEV infection induces axonal damage that precede demyelination. Then, axonal damage aggravates immune cells to recruit pro-inflammatory mediators to generate demyelination. The pitfall of this model is that experiments are time-consuming and demyelination and remyelination occur simultaneously (Tsunoda et al., 2016).

Immune mediated animal models

The most studied animal model of MS is the experimental autoimmune encephalomyelitis (EAE), in which autoimmunity to CNS components is induced in susceptible mice through immunization with self-antigens derived from myelin proteins.

The model was firstly described in monkeys (Rivers et al., 1933). They were immunized with rabbit brain extracts and developed paralysis associated to perivascular infiltrates and demyelination in the brain and spinal cord. Experiments were also performed in other animal species, but mice resulted the best model to evaluate monophasic, RRMS and progressive forms of MS. Moreover, depending on mice strain, age and gender the development of the disease is different (Reviewed at Procaccini et al., 2015).

EAE can be induced through two different approaches: active immunization with myelin peptides or passively transferred encephalitogenic T cells.

a. Active EAE

In actively induced EAE, susceptible strains of mice (SJL/J, C57Bl/o or NOD) are subcutaneously immunized with a myelin-related antigen or peptide emulsified in complete Freund's adjuvant (CFA), a mineral oil-based adjuvant supplemented with heat-inactivated mycobacteria, or incomplete Freund's adjuvant (IFA), without mycobacteria supplementation. The ratio antigen/adjuvant is of crucial importance for the EAE development. CFA promotes Th1 immune response while IFA induces Th2-dominated response. CFA also increases BBB permeability (Reviewed at Constantinescu et al., 2011; Procaccini et al., 2015; Bjelobaba et al., 2018).

To generate disease, mice require additional injections of pertussis toxin, a virulence factor of *Bordetella pertussis*. This toxin is thought to facilitate immune cell entry to the CNS, as well as to promote proliferation and cytokine production by T cells and break T cell tolerance, but the mechanisms are unknown (Bittner et al., 2014). Immunization leads to the priming of myelin-specific T cells in the secondary lymphoid organs. Activated T cells undergo maturation and clonal expansion, thus forming a large pool of myelin-specific CD4+ T cells in the periphery. Later, they differentiate into effector cells and leave the lymphoid organs to pass into the blood circulation. The entry of those cells to the CNS is accompanied by adhesion molecules, cytokines and chemokines, together with their receptors. All this leads to disintegration of BBB. Within the CNS, effector cells become reactivated after they recognize antigens or APCs (microglia/macrophages, astrocytes). These events are followed by secretion of pro-inflammatory mediators attracting huge numbers of effectors T cells and mononuclear cells in the CNS, resulting in gliosis, demyelination and axonal loss, mainly seen at the peak of the disease. The most pronounced changes are observed in the spinal cord, while only sparse inflammation is seen in the brain. Functional deficits, such as tail and hind limb paralysis, occurs (Reviewed at Constantinescu et al., 2011; Procaccini et al., 2015; Bjelobaba et al., 2018).

b. Passive EAE

Another way to induce EAE is accomplished with the adoptive transfer by inoculating naïve mice with activated myelin antigen-specific T cells harvested from the periphery (lymph nodes and/or spleen) of active EAE-immunized mice. Passive EAE proves to be valuable in evaluating the central role of CD4+ T cells during disease. This model is also useful for studying the effector phase of the disease in the absence of adjuvant. EAE develops faster and more homogeneously, which has questionable relevance to MS. Moreover, it is possible to manipulate the T cells *in vitro* with a variety of inflammatory molecules prior to transfer, which allows studying the influence of different subtypes of T cells involved in the disease (Reviewed at Constantinescu et al., 2011; Procaccini et al., 2015; Bjelobaba et al., 2018).

4. CURRENT THERAPIES FOR MULTIPLE SCLEROSIS

The current US Food and Drug administration (FDA) and European Medicines Agency (EMA) approved some treatments for MS. The main goal of MS therapy is the reduction of clinical relapses and the decreased accumulation of CNS lesions, as well as to delay the evolution from RRMS to PPMS. To fulfil this aim, a two-fold strategy has been widely adopted: (i) a short-term treatment with glucocorticoids to reduce the accumulation of disease burden after a relapse, and (ii) a long-term treatment with disease-modifying drugs (DMDs), which prevent relapses slowing MS progression. However, due to the heterogenous patterns of MS, most of these treatments result ineffective in an elevated number of RRMS patients and all them have powerful side-effects (Maker-Clark and Patel, 2013; Wagner and Goverman, 2015). Indeed, there is basically any treatment for the progressive phenotypes of MS (Reviewed at Thompson, 2017; Tur and Montalban, 2017).

Glucocorticoids

Glucocorticoids have short-term effects on the speed of functional recovery in patients with acute attacks of MS. Thus, these molecules are used in the treatment of acute exacerbations in RRMS (Sloka and Stefanelli, 2005; Lattanzi et al., 2017).

Glucocorticoids have a wide variety of immune modulatory mechanisms of action: inhibition of antigen presentation, anti-inflammatory effects (reduction of edema and arachidonic acid metabolites), a decrease in the pro-inflammatory cytokine expression and the inhibition of lymphocyte proliferation. Indeed, the high doses needed, and the prolonged use of glucocorticoids carry side effects that include osteoporosis, metabolic syndrome, stomach ulcers, cataracts and muscle weakness (Sloka and Stefanelli, 2005; Lattanzi et al., 2017).

Disease-modifying drugs

In a simplified scenario, approved long-term MS therapies can be grouped in four main categories: immunomodulators (IFN-β, Glatimer acetate, Dimethyl fumarate

and Daclizumab), inhibitors of immune cell trafficking (Natalizumab and Fingolimod), inhibitors of cell replication (Teriflunomide, Mitoxantrone) and promotors of immune cell depletion (Alemtuzumab, Ocrelizumab and Cladribine) (Reviewed at Baecher-Allan et al., 2018).

All these drugs have demonstrated the reduction in annualized relapse rate in RRMS (Tramacere et al., 2015; Filippini, 2017; Hauser et al., 2017). Unfortunately, such meaningful advances are still lacking for those suffering from progressive forms of MS. Just the Ocrelizumab may cause a reduced disease progression in PPMS (Montalban et al., 2017; Thompson, 2017; Tur and Montalban, 2017).

Table 2. Disease-modifying drugs for MS. All these drugs have been approved by the FDA and EMA (modified from Baecher-Allan et al. 2018).

DRUG (Approval)	**TARGET**	**MECHANISM OF ACTION**	**SIDE EFFECTS**
IFN-β (1993)	Binds to type I IFN receptor on human cells	Inhibits T cell division, matrix metallo-proteinase, BBB migration and pro-inflammatory cytokines. Induces T and B regulatory cells	Flu-like symptoms, liver toxicity and depression
Glatimer acetate (1997)	Random polymers of glutamic acid, lysine, alanine and tyrosine bind to MHC	Competes with peptide binding to MHC, increases IL-10, IL-4 and TGFβ. Induces CD8 and T regs and causes Th1 to Th2 shift	Idiosyncratic reactions, lymphadenopathy
Mitoxantrone (2000)	Interferes with DNA repair	Causes nucleotide crosslinking and DNA strand breaks. Inhibits lymphocyte and monocyte migration, B cell function and secretion of TNFα, IL-2 and IFNγ	Bone marrow suppression, cardiomyopathy and leukaemia
Natalizumab (2004)	CD49d, the α4 subunit of VLA4 integrin	Blocks B and T cell migration into the CNS. Blocks VLA4 binding to VCAM-1 and fibronectin	Reactivation of the JC virus in the CNS, infusion reactions
Fingolimod (2010)	Sphinosine-1-phosphate receptor	Sequesters lymphocytes in lymph nodes by inhibiting lymphocyte egress. Inhibits the migration of dendritic cells to secondary lymphoid organs	Bradycardia, infection, macular edema
Teriflunomide (2012)	Inhibits dihydroorotate dehydrogenase	Inhibits pyrimidine synthesis, secretion of pro-inflammatory cytokines and T cell activation	Hair thinning, liver toxicity, teratogenesis

(Continues Table 2)

Dimethyl fumarate (2013)	Nrf2 pathway	Activates the Nrf2 transcriptional pathway	Flushing, lymphopenia
Alemtuzumab (2014)	CD52 on T and B cells (humanized mAb)	Depletes B and T cells	Autoimmune diseases
Daclizumab (2016)	CD25, anti-IL-2R (humanized mAb)	Prevents IL-2 signalling through the high affinity to IL-2R. Augments NK cell activity	Liver toxicity, skin reactions
Ocrelizumab (2017)	CD20+ B cells (humanized mAb)	Depletes CD20+ B cells. Reduces pathogenic B cell antigen presentation	Infusion reactions, risk of breast cancer
Cladribine (2017)	Adenosine deaminase	Depletes immune cells by inducing lymphocyte apoptosis. Sustained reduction in CD4 and CD8 T cells and transient reduction of B cells	Infection, lymphopenia
Siponimod (2019)	Sphingosin-1-phospahte receptor	Prevents leukocyte infiltration in the CNS. Binds to oligodendrocytes and astrocytes modulating damaging cell activity	Headache, slow heart rate, dizziness, nose or throat infections

Thus, enormous progress has been made in the understanding of MS and it has allowed to uncover treatments for MS, especially in the relapsing and inflammatory stages. However, there are two current keys unanswered in MS: how to avoid the side effects of DMDs and how to treat and better understand the progressive form of the disease. For that reason, further investigation is needed in this field.

5. INTERLEUKIN-1 FAMILY

There are 11 members of the IL-1 family (IL-1F) and 10 members of receptors. Among the 11 members, 7 members have pro-inflammatory actions (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β and IL-36γ); three cytokines act as a receptor antagonist (IL-1Ra, IL-36Ra and IL-38) to block the pro-inflammatory signalling of IL-1α , IL-1β and IL-36; and finally, only one IL-1F family cytokine, IL-37, is known to act as an anti-inflammatory agonist with critical functions to contain inflammation (Garlanda et al., 2013).

In this thesis, we focused our attention in IL-1β, IL-18 and IL-37.

INTERLEUKIN-1β

IL-1 was the first interleukin to be identified. It was purified in 1977 by Charles Dinarello, but it was not until 1984 when was discovered that IL-1 consists in two different proteins: IL-1α and IL-1β.

IL-1β is the most important mediator in the inflammatory response due to its potent pro-inflammatory activity. It is also known to be involved in a variety of cellular activities, such as cell proliferation, differentiation and apoptosis (Dinarello, 1997). Subsequent studies have allowed to demonstrate the contribution of IL-1β to the course of many diseases, such as MS (Reviewed at Gabay et al., 2010).

Human IL-1β, as other eight in IL-1F, is located on chromosome 2 at the band of q13 (Lafage et al., 1989). IL-1β is not constitutively expressed but its expression is induced upon stimulation by inflammatory signals. IL-1β is expressed in the cytoplasm as 31 kDa pro-form and is then processed and released from cells by a mechanism involving caspase-1 (see below "NLRP3 inflammasome-dependent secretion of IL-1β and IL-18"). Thus, IL-1β is biologically inactive and must be converted to the mature 17 kDa IL-1β to acquire the ability to bind its receptors (Reviewed at Krumm, Xiang and Deng, 2014).

Despite IL-1α and IL-1β are encoded by different genes, they bind to the same receptor, which is IL-1 receptor type 1 (IL-1R1). After binding to IL-1β, the receptor

associates with the co-receptor IL-1RAP forming a high affinity complex that mediates the innate immunity reactions (Arend et al., 1998). In response to this binding, a complex sequence of combinatorial phosphorylation and ubiquitination events results in activation of NF-κβ signalling and the JNK and p38 mitogen-activated protein kinase pathways, which, cooperatively, induce the expression of canonical IL-1 target genes (such as IL-6, IL-8, MCP-1, COX-2, MAPK) by transcriptional and post-transcriptional mechanisms (Weber et al., 2010).

INTERLEUKIN-18

IL-18 was firstly described in 1989 as "INFγ-inducing factor" (Nakamura et al., 1989). IL-18 also plays an important role in host innate and acquired immune defense, with its activity being modulated in vivo by its naturally occurring antagonist IL-18 binding protein (IL-18BP) (Dinarello, 1999).

The gene of IL-18 is located on chromosome 11, unlike IL-1β. However, IL-18 is closely related to IL-1β in that both are first synthetized as inactive precursors and IL-18 also required caspase-1 to be processed as a 18kDa mature protein and bind to its receptors (see below "NLRP3 inflammasome-dependent secretion of IL-1β and IL-18"). However, unlike IL-1β, IL-18 precursor is constitutively expressed in nearly all cells in healthy humans and animals (Lee et al., 2004). Thus, the activity of IL-18 is balanced by the presence of the high affinity protein IL-18BP (reviwed at Dinarello, 2007). The imbalance between IL-18 and IL-18BP has been related to many diseases. Besides, the biology of IL-18 is greatly similar to IL-1β (Dinarello, 1999).

IL-18 forms a signalling complex by binding to IL-18Rα chain, which is the ligand binding chain for mature IL-18. Then, this association recruits the coreceptor IL-18Rβ, triggering the signalling pathway that includes the degradation of IκB and the release of NFkB (Born et al., 1998; Dinarello, 1999; Wu et al., 2003).

The NLRP3 inflammasome-dependent secretion of IL-1β and IL-18

Inflammasomes are a group of cytosolic protein complex that are formed to mediate host immune response to microbial infection a cellular damage. Assembly of an inflammasome triggers proteolytic cleavage of procaspase-1 into active caspase-1, that converts the precursors of IL-1β and IL-18 into mature biologically active proteins (Reviewed at Shao et al., 2015; He et al., 2016).

Among inflammasomes, the NLRP3 inflammasome has been in the spotlight due to its implication in several human diseases, such as MS and EAE (Inoue and Shinohara, 2013). Besides, the NLRP3 is currently the most fully characterised and it is known that compromises three proteins: the NLRP3, the adapter protein apoptosis-associated speck-like protein (ASC) and pro-caspase 1 (Figure 4). The NLRP3 inflammasome is activated by a wide variety of particles, crystals, bacterial toxins, ATP, mitochondrial damage, ROS, as well as virus, bacteria, and fungi (Reviewed at Shao *et al.*, 2015).

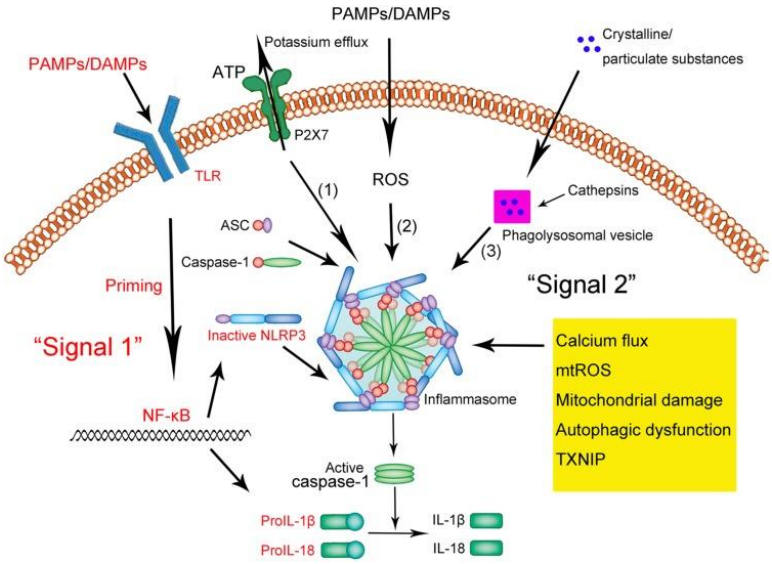

Figure 4. NLRP3 inflammasome activation. NLRP3 assembly allows the autocatalytic activation of caspase-1, necessary for the activation and release of IL-1β and IL-18 (Shao *et al.,* 2015)

INTERLEUKIN-37

IL-37 was first discovered and identified through computational sequence analysis (Kumar et al., 2000). Then, IL-37 was classified as the 7th cytokine of IL-1 family (IL-1F) (Dunn et al., 2001). More recently, other studies led by professor Charles A. Dinarello found that IL-37 can suppress both innate (Nold et al., 2010a) and adaptive immunity (Luo et al., 2014). Thus, in the recent years IL-37 has been found

to play an important regulatory role in the development of a variety of inflammatory diseases and tumours (Reviewed at Cavalli and Dinarello, 2018).

Structure and types of IL-37

Human IL-37 gene is located on chromosome 2 with a length of 3.617 kb and it is a part of the large IL-1 gene cluster, in remarkably proximity to the regulatory regions for IL-1α and IL-1β genes (Busfield et al., 2000; Kumar et al., 2000; Pan et al., 2001). This specific location is likely critical to the role of IL-37 as a master regulator of inflammatory responses: IL-37 could be simultaneously transcribed that IL-1α and IL-1β genes limiting the detrimental consequences of inflammation (Sharaf et al., 2014).

The structure of IL-37 is similar to all IL-1F members and consist of 12β tubular lines. The six exons encode five isoforms (IL-37a-e) (Figure 5), but the differences in the activity and relative abundance of each one remain poorly understood. IL-37 is an immature precursor peptide and each isoform is converted from an inactive precursor state to an active state by the cleavage of caspase-1 during expression. All subtypes regulate each other to form relatively stable state. IL-37b is the most complete of these isoforms (exons 1, 2, 4, 5 and 6) and it is also the most abundant and studied. IL-37b has an intact exon end with the largest molecular weight and the most complex biological functions (Busfield et al., 2000; Kumar et al., 2000; Pan et al., 2001).

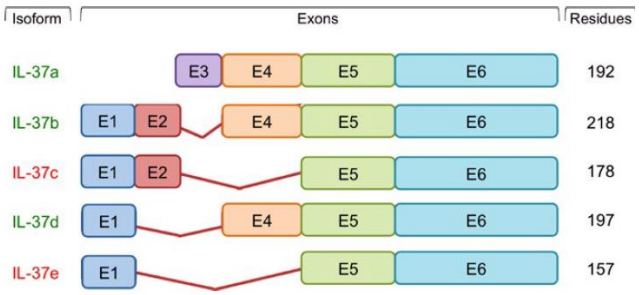

Figure 5. Human *IL-37* transcripts and IL-37 isoforms (Cavalli & Dinarello 2018)

It is important to highlight that no mouse gene homologue to human *IL-37* has been identified. However, the first exon of *IL-37* is present in mouse, but there is no open reading frame. Divergent evolutionary pathways due to selective pressure from divergent pathogens may account for the absence of this gene. Therefore, it was necessary to generate a transgenic mouse expressing the human *IL-37* (hIL-37tg) to facilitate the studies in pathological murine models (Nold et al., 2010a).

Production of IL-37

IL-37 expression has been described across several human tissues and cell lines. Among immune cells, IL-37 is expressed mainly in monocytes and macrophages (81%-91% of IL-37+ PBMCs) but also in T cells, B cells, plasma cells (6%-8%) and DCs (1%-2%). IL-37 was also detected in human tissues such as liver, lung, thymus, bone marrow, lymph nodes, placenta, testis, uterus and tumour tissue (reviewed at Jia, Liu and Han, 2018). However, the detection of IL-37 at protein levels is almost impossible so far in hIL-37tg mice due to the high homology with others in IL-1F.

However, *IL-37* expression is very low in healthy human tissues because its mRNA is quite unstable under normal conditions and can be easily degraded. Interestingly, stability of *IL-37* mRNA is significantly enhanced under severe inflammatory conditions (Bufler et al., 2004).

Mechanism of action and receptors

The studies of Bulau *et al.*, 2014 and Nold-Petry *et al.*, 2015 have uncovered that IL-37 can exert its anti-inflammatory activity via two different mechanisms: extracellular (receptor-mediated) and intracellular (nuclear function) pathways.

a. Intracellular pathway of IL-37: translocation to the nucleus

Although it is assumed that IL-37 acts primarily as a secreted mediator by binding to extracellular receptors, as occurs with the members of IL-1 family including IL-1α, IL-1β IL-33 or IL-36, IL-37 can be also translocated to the nucleus.

IL-37b has one cleavage site located in the exon 1, between amino acids D20 (aspartic acid) and E21 (glutamic acid), which is recognized by caspase-1. Upon caspase-1 cleavage, IL-37 translocates to the nucleus. Indeed, around 20% of the total IL-37 protein translocates to the nucleus where interacts with phospo-SMAD3 and suppresses the transcription of pro-inflammatory cytokines without altering the expression of those that exert anti-inflammatory effects (Figure 6)(Bulau et al., 2014; Li et al., 2019).

b. Extracellular pathway of IL-37: binding to IL-18Rα-SIGIRR complex

In addition, the precursor of IL-37 can be externalized. IL-37 has a second cleavage site located in the exon 2 between amino acids F45 (phenylalanine) and V46 (valine). The presence of this second cleavage site suggests that other proteases may process IL-37. This hypothesis is supported by the fact that the cell lines overexpressing the complete form of IL-37b yield a soluble cytokine starting at V46. In contrast with the first cleavage site, caspase-1 inhibitors do not prevent the generation of this second processed IL-37 form. Indeed, IL-37 can be externalized in both forms, pro- (full length) or mature (cleaved at V46), and both forms binds to extracellular receptors (Nold-Petry et al., 2015b).

In general, cytokine receptors have at least two receptor chains that oligomerize during ligand-induced signalling. Although initial experiments suggested that IL-37 may act as an antagonist of IL-18Rα, this was then discarded due to low binding affinity of this cytokine with IL-18Rα. Now it is known that mature IL-37 binds to the IL-18Rα and this complex recruits the co-receptor single immunoglobulin IL-1 receptor related protein (SIGIRR; also known as IL-1R8) which was previously considered an orphan receptor (Figure 6) (Nold-Petry et al., 2015a). Unlike other IL-1 receptor family members, SIGIRR has a non-canonical function: it inhibits innate immunity by reducing IL-1 and toll like receptor-dependent inflammation trough different mechanisms (Reviewed at Cavalli and Dinarello, 2018). The interaction of IL-37 with IL-18Rα-SIGIRR complex dampens intracellular pro-inflammatory pathways, such as inhibition of kinases such as the MAPK (MAPK), JNKs and NF-κβ pathway, and stimulates activation of the anti-inflammatory STAT3, Mer and PTEN and starvational effects on the mTOR pathway (Nold-Petry et al., 2015a).

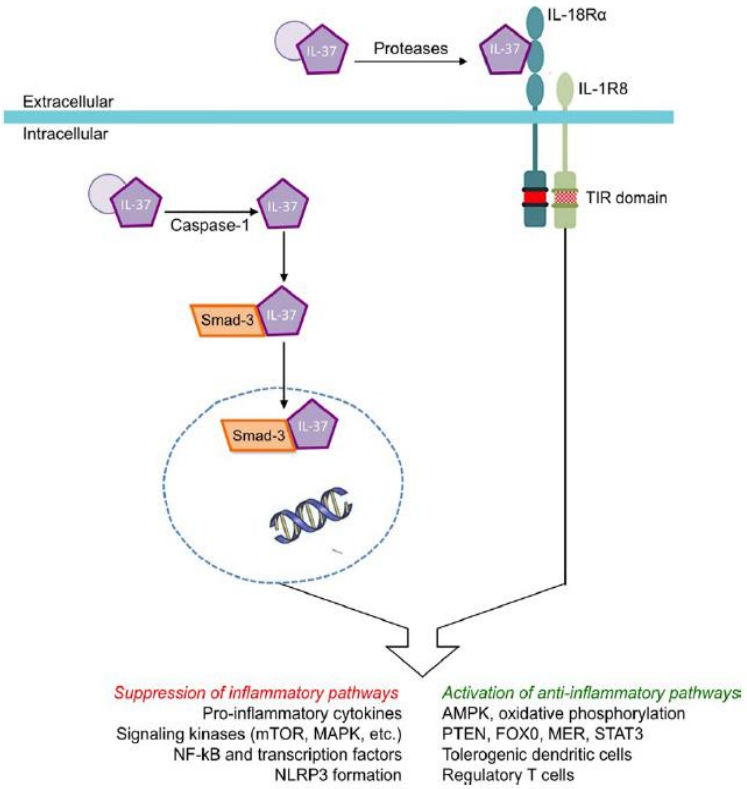

Figure 6. Mechanism of action of IL-37 (Cavalli and Dinarello 2018).

Effects of IL-37

Several studies have uncovered that the expression of IL-37 modulates the activation of many signalling phosphokinases and triggers the balance towards anti-inflammatory pathways (Reviewed at Cavalli and Dinarello, 2018).

On the one hand, there are many mediators that transduce pro-inflammatory signals whose activation is remarkably reduced by IL-37 (up to 80%): FAK, STAT3, mTOR, p53, STAT1, p38, paxillin, Syk, SHP-2 and PKB. On the other hand, experiments in hIL-37tg mice have revealed the induction of anti-inflammatory mediators such as the phosphatase PTEN, which inhibits the PK3 kinase, mTOR, MAPK, and FADK pathways. Those findings showed that IL-37 can supress innate immunity by reducing the production of pro-inflammatory cytokines such as IL-1α, IL-1β, IL-8, IL-17, IL-23, TNFα or IFNγ and chemokines such as MIP-2/CXCL2, CCL12/MCP-5 and BCA-1/CXCL13. IL-37 also inhibits the expression of M-GSF and GM-CSF but increase the production of TGFβ1, which is an immunosuppressive factor. IL-37 can

also supress the innate immunity by inhibiting the activation of NLRP3 (Reviewed at Cavalli and Dinarello, 2018).

Regarding to the adaptive immunity, IL-37 can reduce the expression of MHC-II and costimulatory molecules such as CD40 on the surface of DCs, decreasing their ability to activate CD4+ and CD8+ T cells, but enhancing the differentiation of Treg cells (Luo et al., 2014).

IL-37 in disease

Researchers have described several human pathological conditions in which the levels of IL-37 were increased in an attempt to suppress the inflammatory response, such as rheumatoid arthritis (serum), chronic obstructive pulmonary disease, systemic lupus erythematosus or hepatitis B or C infection, between others. However, the levels of IL-37 in these pathologies are probably insufficient to greatly improve the symptoms. In contrast, lower levels of IL-37 compare to healthy subjects characterize patients with a wide range of inflammatory conditions such as psoriasis, asthma, rheumatoid arthritis (synovia) or periodontal disease, reflecting again a failure to induce sufficient levels to mitigate disease severity (Reviewed at Jia et al., 2018).

Nonetheless, it has been widely reported that IL-37 has a protective role in several experimental conditions such as colitis (McNamee et al., 2011a), diabetes type 2 (Cavalli et al., 2017), invasive pulmonary aspergillosis (Moretti et al., 2014), oncogenesis (Henry et al., 2015), arthritis (Ye et al., 2015; Cavalli et al., 2016), calcific aortic valve disease (Zeng et al., 2017) and spinal cord injury (Coll-Miró et al., 2016).

6. RESOLVING THE INFLAMMATION

In response to injury or infection, specialized leukocytes migrate to infected/damaged sites to neutralize and eliminate the injury stimuli. This requirement is perhaps the most obvious but undeniably critical one for acute inflammation to resolve. For many years, resolution of inflammation was considered a passive phenomenon, purely associated with the removal of inflammatory stimuli, end of chemoattractant production and dilution of chemokine gradients over time, leading to the egress of white blood cells and re-establishment of tissue function (Robbins and Cotran, 1979). However, several studies from Serhan's lab at Harvard University showed that the resolution of inflammation is an active process encoded at the onset of inflammation and brought about by the biosynthesis of active mediators, which act on key events of inflammation to promote the return to homeostasis. Hence, resolution of inflammation involves a program of unique pathways, mediators and cell subtypes (Serhan et al., 2010).

Therefore, resolution is defined as the interval from maximum leukocyte infiltration to the point when they are cleared from the tissue (Serhan et al., 2007) (Figure 7).

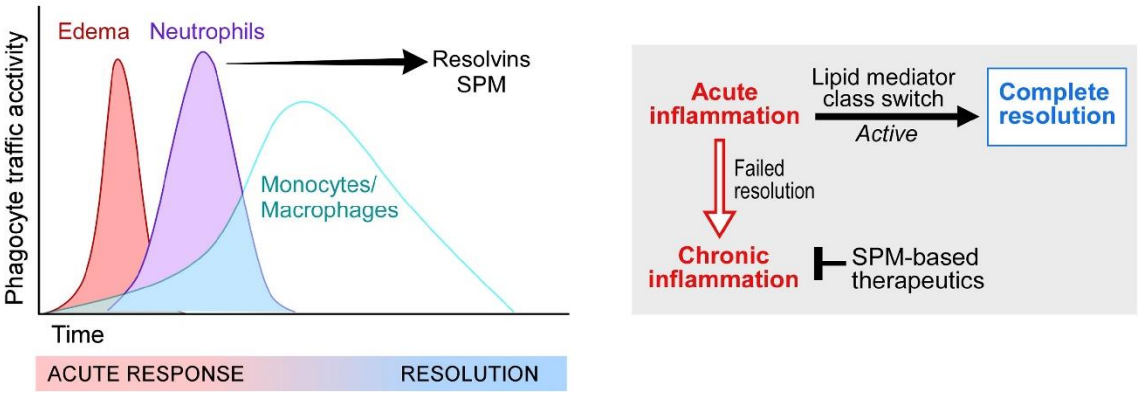

Figure 7. Function of SPMs in acute inflammation resolution. The interval from maximum leukocyte infiltration to the point when they are cleared from the tissue is known as "resolution of inflammation". The lipid mediator switch is necessary to go over acute inflammation to resolution phase (Serhan *et al.,* 2007).

Evidence that the resolution of inflammation is, in fact, an active process came from studying acute self-limiting responses. In resolving inflammatory exudates (e.g. pus), cell-cell interaction lead to the generation of active signals that limit neutrophil

recruitment to the tissue and enhance the engulfment of neutrophils by macrophages, promoting the return to tissue homeostasis (Serhan et al., 2007).

The most important point in this active resolution process is the lipid-mediator class-switching in exudates (Figure 7). By this process, prostaglandins (PGs) and leukotrienes (LTs), that are pro-inflammatory molecules involved in the initiation phase of inflammation, activate the translation of mRNAs encoding enzymes that are necessary to produce immunoresolvents during resolution phase. These PGs are important in upregulating the expression of 15-lipoxygenase type 1 (15-LOX-1), the initiating enzyme in the lipid mediator biosynthesis (Figure 6) (Levy et al., 2001).

Active resolution also evokes specialized signalling pathways, including NF-κβ and ATP metabolism. In addition to local processes, systemic mechanisms, for example the release of anti-inflammatory hormones and the induction of neural mechanisms, such as the cholinergic anti-inflammatory pathway, have been demonstrated to have a key role in actively suppressing inflammation (Buckley et al., 2013).

In short, there are some key steps in the resolution of inflammation (Figure 8):

a. Clearance of the inciting stimuli and normalisation of chemokine gradients.
b. Silencing of intracellular pro-inflammatory signalling pathways (NF-κβ or AP-1) and suppressing the expression of pro-inflammatory cytokines (e.g. IL-1β, iNOS or IL-6).
c. Release of pro-resolving mediators: overexpression of anti-inflammatory cytokines (IL-4 and IL-10) and a shift in lipid mediator biosynthesis from pro-inflammatory to specialized pro-resolving lipid mediators (SPMs).
d. Apoptosis of PMNs and their efferocytosis by tissue and monocyte-derived macrophages.
e. Regulation of edema.
f. Induction of exit phagocytes: incorporation of these myeloid cells into the local population or their recirculation via lymphatic system or blood.
g. Initiation of healing processes: culmination of resolution is the return to tissue homeostasis.

Failure of resolution has been defined in terms of insufficiency or inadequacy leading to chronic inflammation, excess tissue damage and dysregulation of tissue healing, including fibrosis. Nowadays, excessive inflammation is widely appreciated as a unifying component in many chronic diseases, such as vascular diseases, metabolic syndrome, neurological diseases and many others, so it becomes as a public health concern (Buckley et al., 2013). For that reason, understanding endogenous control points within the inflammatory resolution may give new views on disease pathogenesis and treatment approaches.

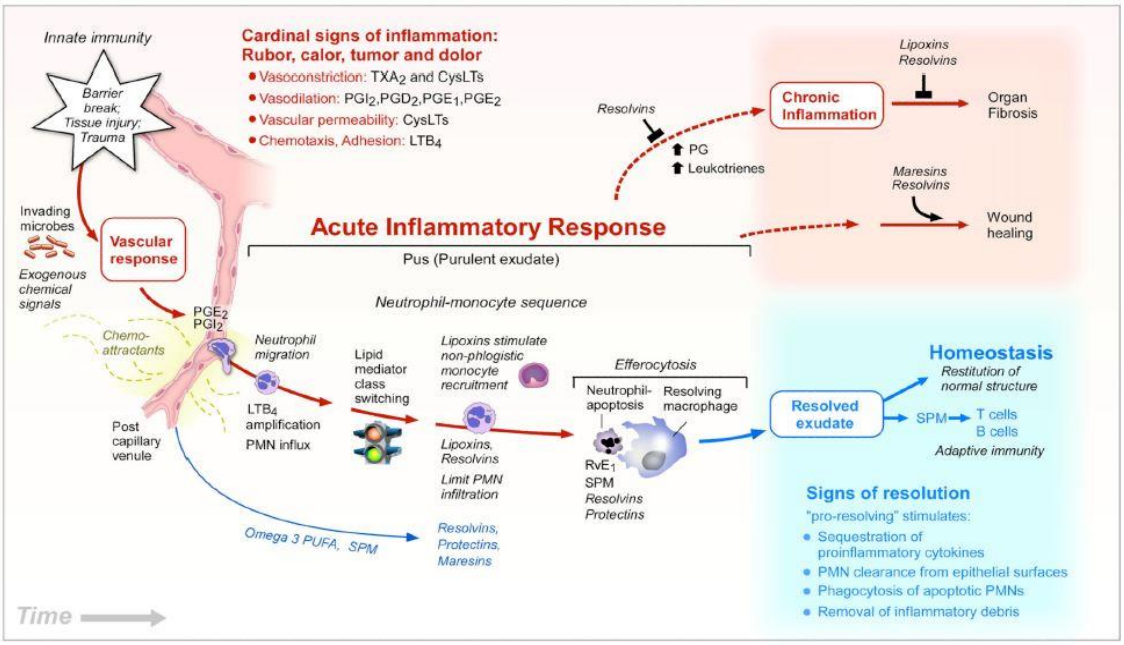

Figure 8. Lipid mediators in the acute response and resolution. Lipid mediators play pivotal roles in the vascular response and leukocyte trafficking, from initiation to resolution. SPM stimulate cellular events that counter-regulate pro-inflammatory mediators and regulate PMN response, leading to resolution (Serhan, 2014).

Lipid mediator class switching

In a classical acute inflammatory response, proinflammatory lipid mediators, such as eicoisanoids (PGs and LTs), are generated during the initial phase of the inflammatory response through enzymatic modification of arachidonic acid (AA) (omega-6 fatty acid) by cyclooxygenases 1 or 2 (COX-1, COX-2) and lipoxygenase 5 (5-LOX) (Figure 8). These pro-inflammatory molecules have important roles in initiating leukocyte trafficking and stimulating blood flow changes as well as increasing vasopermeability an edema, all these facts leading to neutrophil influx to

the site of inflammation. Indeed, PGs and LTB4 are involved in the initiating steps that permit leukocytes to leave the vessels and get in to the damaged tissue via diapedesis (Figure 8).

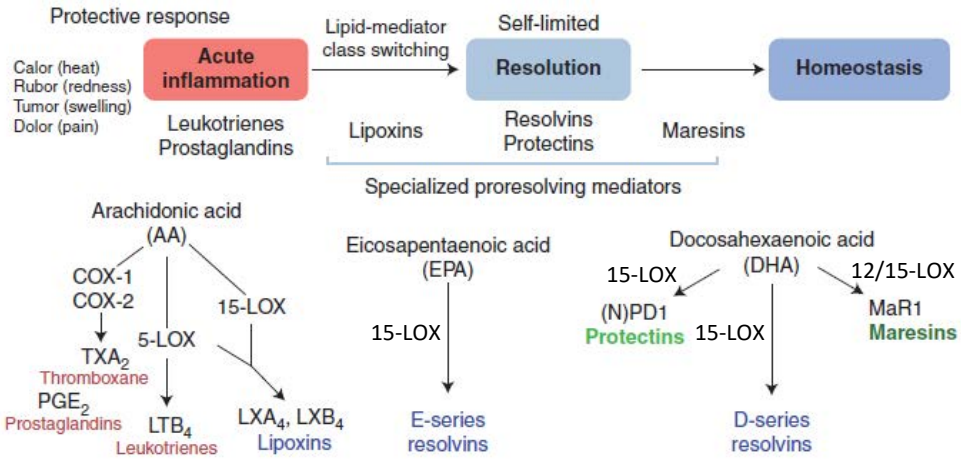

Figure 9. Lipid mediator biosynthesis in resolution of acute inflammation. 5-LOX and COX-1/2 convert AA to PGs or LTs, 12/15-LOX converts EPA and DHA into SPMs. AA can also be converted directly in an SPM due the synthetase activity of 12/15-LOX (Serhan *et al.,* 2007).

Thereby, the biosynthesis of the resolution-phase mediators in *sensu stricto* is initiated during lipid-mediator class switching, in which PGs and LTs switch to produce SPMs. In this process, the omega-3 fatty acids [ecoisapentaenoic acid (EPA) and docosahexaenoic acid (DHA)], enriched in marine oils ingested by diet, compete with AA reducing pro-inflammatory eicosanoids. It means that 12/15-LOX converts EPA and DHA into SPMs instead of 5-LOX and COX-1/2 converting AA to PGs or LTs (Figure 8) (Levy et al., 2001). Moreover, AA can also be converted directly in an SPM due the synthetase activity of 12/15-LOX (Figure 9) (Serhan and Sheppard, 1990).

In the other hand, some SPMs antagonize the signalling of LT by acting as a partial agonist/antagonist to their receptors (Colas et al., 2016). For that reason, omega-3 fatty acids have long been held to display anti-inflammatory properties (Levy et al., 2001).

Of note, inhibition of PG biosynthesis is linked to a reduction of SPMs and a delay in the termination of inflammation in experimental systems (Fukunaga et al., 2005). Thus, these studies demonstrated that the acute inflammatory response is a

coordinated process with the initiation and termination phases being intricately linked.

Specialized pro-resolving lipid mediators

The identification of signals that control the termination of inflammation have uncovered a genus of mediators that are produced via the conversion of essential fatty acids (omega-3 and omega-6). Given their potent actions and exclusive chemical structures these mediators are known as SPMs (Reviewed at Serhan, 2014; Serhan et al., 2015).

This super family of lipid mediators is composed of four main families: **Lipoxins (LX)** (derived from AA: C20:4n6), **resolvins (Rv)** (derived from docosapentaenoic acid (DPA): C22:5n3, DHA: C22:6n3 or EPA: C20:5n3), **protectins (PD)** (derived from DPA or DHA) and **maresins (MaR)** (derived from DPA or DHA) (Reviewed at Serhan, 2014; Serhan et al., 2015).

There are some important points to distinguish a pro-resolving mediator from an anti-inflammatory one (Reviewed at Serhan, 2014; Serhan et al., 2015):

1. Counter-regulates mediators that summon leukocytes to inflamed site.
2. Dampen pain.
3. Stimulate monocyte recruitment.
4. Activate macrophages in a manner that enhance efferocytosis of apoptotic granulocytes and promote the clearance of tissue debris.

Lipoxins

LXs are a series of trihidroxytetraene-containing bioactive eicosanoids that were first isolated from human leukocytes in the mid-1980s. Two LXs have been identified so far: **LXA4** and **LXB4** (Serhan, 1997).

These lipid mediators are generated through cell-cell interactions by a process known as transcellular biosynthesis. During the first biosynthetic step of LX

biosynthesis, the enzyme LOX inserts molecular oxygen into AA. This can be achieved by three different routes, as it is showed in figure 10 (Edenius et al., 1990; Levy et al., 1993). The first pathway in figure 10 is mediated by the exogenous administration of aspirin, which irreversible acetylates COX-2. The LXs resulted after the addition of aspirin are known as aspirin-triggered LXs (ATLs) (Clària and Serhan, 1995).

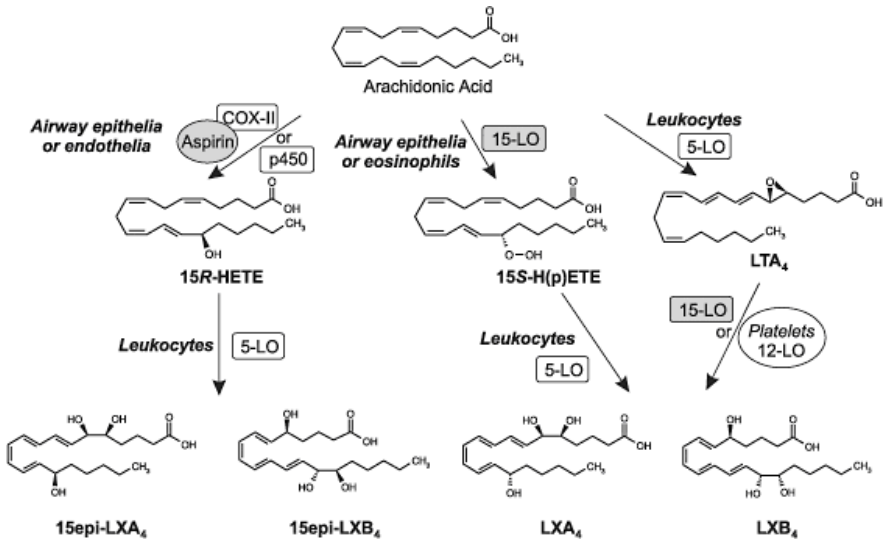

Figure 10. Routes of lipoxins synthesis. In the first pathway, arachidonic acid is converted to ATLs (15-epi-LXA_4 and 15-epi-LBA_4 via the action of epithelial or endothelial COX-2 in the presence of aspirin, which is followed by the action of 5-LO. Moreover, LXA_4 and LXB_4 are generated from AA in a transcellular manner involving neutrophils, platelets, and resident tissue cells, such as epithelial cells. AA is transformed by the sequential action of LOX into LXA 4 or LXB 4 with 15-hydroxyeicosatetraneoic acid (15-HETE) as the intermediate (Brick et al. 2003).

LXA4 and ATLs elicit their multicellular responses via receptor of LXA4 (ALX), which was first identified due to the sequence homology with the formyl peptide receptor-like-1 (FPRL1). The ALX is a specific G-protein couple receptor isolated and cloned in human, mouse, and rat tissues. This receptor has been recently coined ALX/FPR2 by the international nomenclature committee. The human ALX/FPR2 is highly expressed in myeloid cells and at a lower manner in lymphocytes, dendritic cell and resident cells (Reviewed at Bennett and Gilroy, 2016). LXB4 has not been identified yet.

LXs are anti-inflammatory at nanomolar concentrations, controlling neutrophil, eosinophil and monocyte entry to sites of inflammation. LXs also stimulate macrophages to ingest and clear neutrophils, elevate the levels of some anti-inflammatory cytokines (such as TGF-β1), which downregulates many pro-

inflammatory pathways. LXs counteract the fibrotic response, improving tissue remodelling (Reviewed at Bennett and Gilroy, 2016).

E-resolvins

E-resolvins is a family of SPMs constituted by three members produced from EPA: **resolvin E1** (RvE1: 5S,12R,18R-trihydroxy-EPA), **resolvin E2** (RvE2: 5S,18R-dihydroxy-EPA) and **resolvin E3** (RvE3: 17R,18R/S-dihydroxy-EPA) (Figure 11) (Reviewed at Chiang and Serhan, 2017).

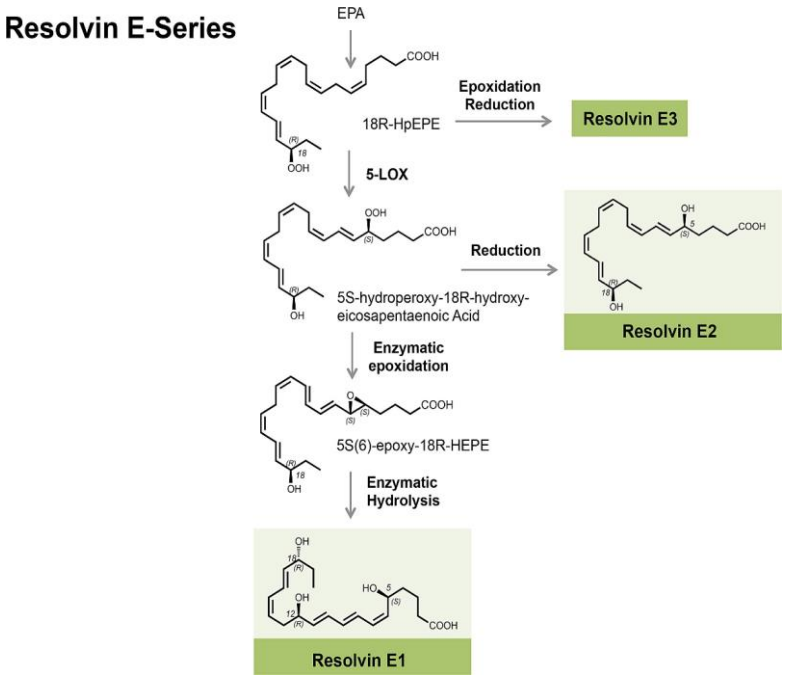

Figure 11. Resolvins E biosynthetic pathway. Biosynthesis of E-series resolvins is initiated with molecular oxygen insertion at carbon-18 position of EPA, which is converted to bioactive E-series members resolvin E1, resolvin E2 and resolvin E3 (Chiang and Serhan 2017).

Biosynthesis of E-series resolvins is initiated with molecular oxygen insertion at carbon-18 position of EPA, resulting first in some intermediates and finally in RvE1, RvE2 or RvE3, as it is illustrated in figure 11. Animal studies have revealed that RvE1, RvE2, and RvE3 are synthesized by PMN leukocytes and cause the inhibition of leukocyte/neutrophil migration associated with a reduction in the release of pro-inflammatory cytokines and an increase in phagocytic activity of macrophages, which in turn cause tissue cleaning (Reviewed at Chiang and Serhan, 2017).

It has been shown that the anti-inflammatory effects of RvE1 and RvE2 could be attributed to their ability to bind to the GPCR ChemR23, and LTB4 receptor 1, which is a receptor of the pro-inflammatory LTB4 (Arita et al., 2005; Schwab et al., 2007).

D-resolvins

The biosynthetic conversion of DHA to D-series resolvins (RvD1–RvD6) involves different lipoxygenation steps. The first step is the conversion of DHA to 1-hydroperoxydocosahexaenoic acid (17S-H(p)DHA) by 15-LOX. This intermediate can be converted in several compounds through different pathways (Figure 12) (Reviewed at Chiang and Serhan, 2017b). Besides, an aspirin-triggered D-series resolvins were also identified (Reviewed at Chiang and Serhan, 2017b; Dalli and Serhan, 2018).

RvD1 is the best characterized of this family. It is produced by neutrophils and has a potent anti-inflammatory and pro-resolutory effect. RvD1 stops neutrophils infiltration to lesioned site and enhance macrophage efferocytosis in the later stages of inflammatory response, as well as it has been described that RvD1 is able to reduce pain. Likewise, RvD2 is known to modulated leukocyte-endothelium interactions, to modify cytokine and eicosanoid profiles (reduce IL-17, PGE2 and LTB4) and also to reduce pain (Reviewed at Ji et al., 2011; Dalli and Serhan, 2018). However, how each RvD exert its biological actions is still poorly understood, but it seems that many of them act through G-protein couple receptors (Reviewet at Recchiuti, 2013).

It is known that the ALX/FPR2 receptor mediates the actions of the RvD1, RvD3 and their aspirin triggered epimers. This receptor is expressed in leucocytes, microglia, endothelial cells and epithelial cells. In addition, activation of the orphan receptor GPR32 (present in PMNs, monocytes and vascular endothelial cells) by RvD1 leads to regulation of many miRNA involved in the orchestration of acute inflammation and it also mediates the expression of pro-inflammatory cytokines, such as IL-1β or IL-18. Additionally, this receptor is involved in regulating macrophage phenotype and function. GPR32 activation also mediates biological actions of RvD3, RvD5 and their epimers. GPR32 is usually activated by RvD5 in the context of bacterial

infections and it leads to enhanced phagocytosis in human macrophages and downregulation of several pro-inflammatory genes, including NF-κβ, TGF-β and TNF-α. GPR18 is a receptor for RvD2; its activation leads to an increase in intracellular cAMP as well as CREB, ERK1/2 and STAT3 phosphorylation (Reviewed at Krishnamoorthy et al., 2012; Recchiuti, 2013; Dalli and Serhan, 2018).

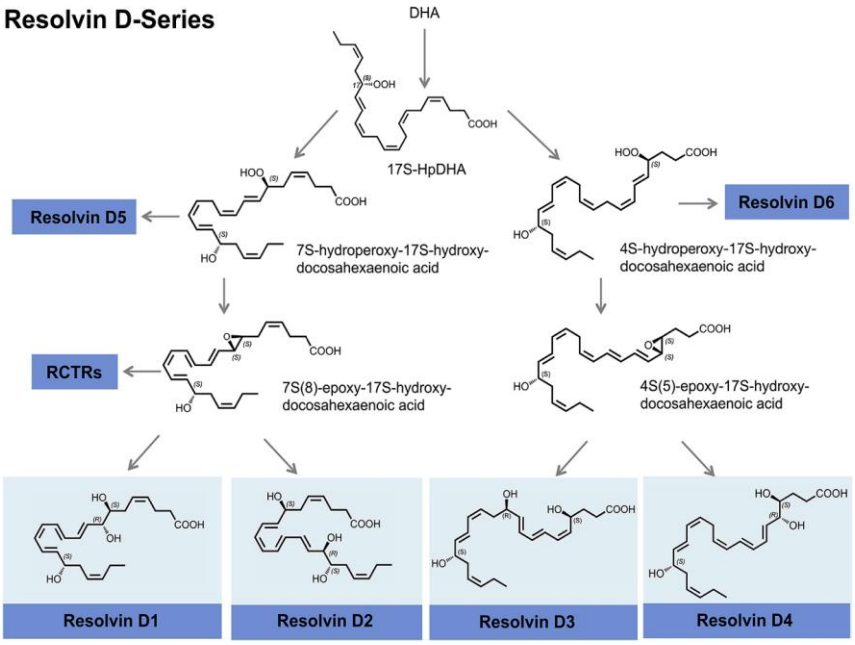

Figure 12. Resolvin D series biosynthetic pathway. Resolution metabolome activates 17-lipoxygenation of DHA; *17S*-HpDHA is converted to resolving-epoxide intermediates by 5-LOX, that are transformed to D-series resolvins, which each carry potent actions (Chiang and Serhan 2017).

Protectins

DHA also serves as a precursor for the biosynthesis of PDs enzymatically converted following the steps showed in figure 13 (Reviewed at Serhan et al., 2006; Chiang and Serhan, 2017b; Dalli and Serhan, 2018).

One specific DHA-derived lipid mediator, 10,17S-docosatriene, was termed protectin D1 (PD1). When generated in neural tissue, however, this compound is called neuroprotection D1 (NPD1) (Bazan, 2006). PDs are distinguished by their potent bioactivity; PD1 exhibits tissue-specific bioactivity, as in humans is synthesised by peripheral blood mononuclear cells and Th2 $CD4^+$ T cells, microglial cells and peripheral blood cells, while in mice it has been isolated from exudates and

brain cells (Hong et al., 2003; and reviewed at Chiang and Serhan, 2017b; Dalli and Serhan, 2018).

Same than RvDs, PD1 exerts potent immunomodulatory effects that include inhibiting neutrophil migration and Toll-like receptor mediated activation, suppression of Th2 inflammatory cytokines and eicosanoids, as well as it blocks T-cell migration *in vivo* and promotes T-cell apoptosis and neuroprotection (Mukherjee et al., 2004; Ariel et al., 2005; and reviewed at Chiang and Serhan, 2017b; Dalli and Serhan, 2018).

Maresins

Maresins (*ma*crophage mediators in *re*solving *in*flammation) are derived from DHA. These SPMs are produced by macrophages and include three different families: maresin 1 (MaR1), maresin 2 (MaR2) and maresin conjugate in tissue regeneration (MCTR), depending on the enzymatical pathway (Figure 13) (Serhan et al., 2015b; Chiang and Serhan, 2017a; Ohuchi et al., 2018).

MaR1 appears in later stages of the inflammatory response and it is released by resolutory macrophages. MaR1 has potent bioactions limiting PMN infiltration and enhancing macrophage phagocytosis and efferocytosis (Serhan et al., 2012; Dalli et al., 2013). It is also known that MaR1 is involved in shorten resolution interval and supresses oxidative stress (Sun et al., 2017). Similarly to other SPMs, MaR1 counterregulates proinflammatory chemical mediators, controls pain and enhances tissue regeneration (Reviewed at Serhan et al., 2006; Chiang and Serhan, 2017b; Dalli and Serhan, 2018). Besides, the 13S,14S-epoxy-maresin intermediate possess regulatory functions and stimulates the pro-inflammatory to anti-inflammatory switch of macrophages and inhibits the production of LTs (Dalli et al., 2013).

MCTRs have recently been uncovered as a new series of bioactive peptide-lipid-conjugated mediators (Figure 13) that are produced during the later stages of self-resolving infections. Researchers identified these mediators from human milk, mouse exudates and human macrophages (Reviewed at Dalli et al., 2016; Chiang and Serhan, 2017b).

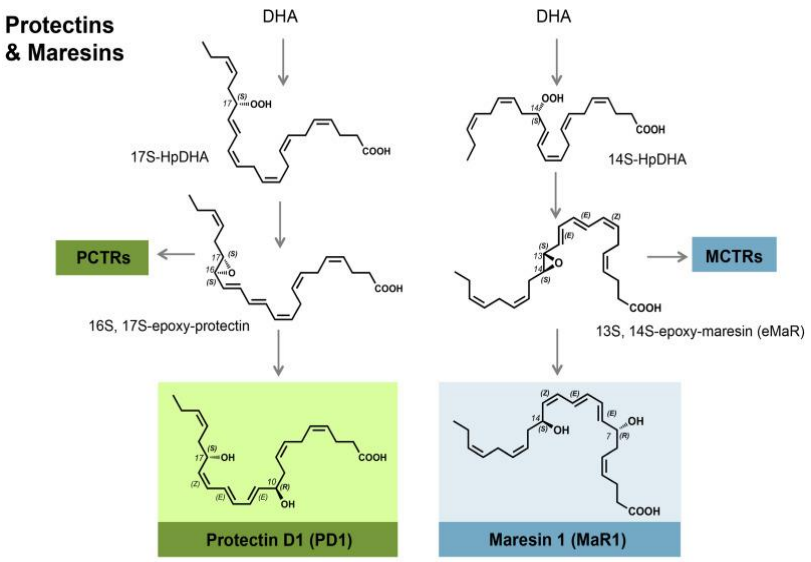

Figure 13. Protectins and maresins biosynthetic pathway. 17-HpDHA is precursor to 16,17-epoxide-protectin intermediate that is converted to PD1 and related protectins. The maresins pathway is initiated by the lipoxygenation of DHA to yield 13S,14S-epoxy-maresin. This intermediate is then enzymatically hydrolyzed to maresin 1 or via a soluble epoxide hydrolase to maresin 2. 13S,14S-epoxy-maresin is also a substrate for GSTM4 and LTC4S yielding MCTR1, which is then converted to MCTR2 and to MCTR3 (Chiang and Serhan 2017).

The production of all these SPMs families is regulated in a time, organ and stimulus dependent manner, and their relative concentration to classic eicosanoids are also dependent on these factors. For example, in cerebrospinal fluid from patients with MS, PGE2 and RvD1 are present in similar concentrations (~1pg/mL). In experimental model of eye infections, LXA4 concentrations were between 2- and 10-fold higher than those of PGE2. The production of eicosanoids and SPM is also regulated in a sex-dependent manner: LXA4 concentration in females was elevated when compared to males during experimental eye infections, whereas RvD and RvE are elevated during experimental inflammation in humans (Reviewed at Dalli and Serhan, 2018).

To sum up, in figure 14 there is a summary of SPMs precursors, biological actions and the targeted cell types.

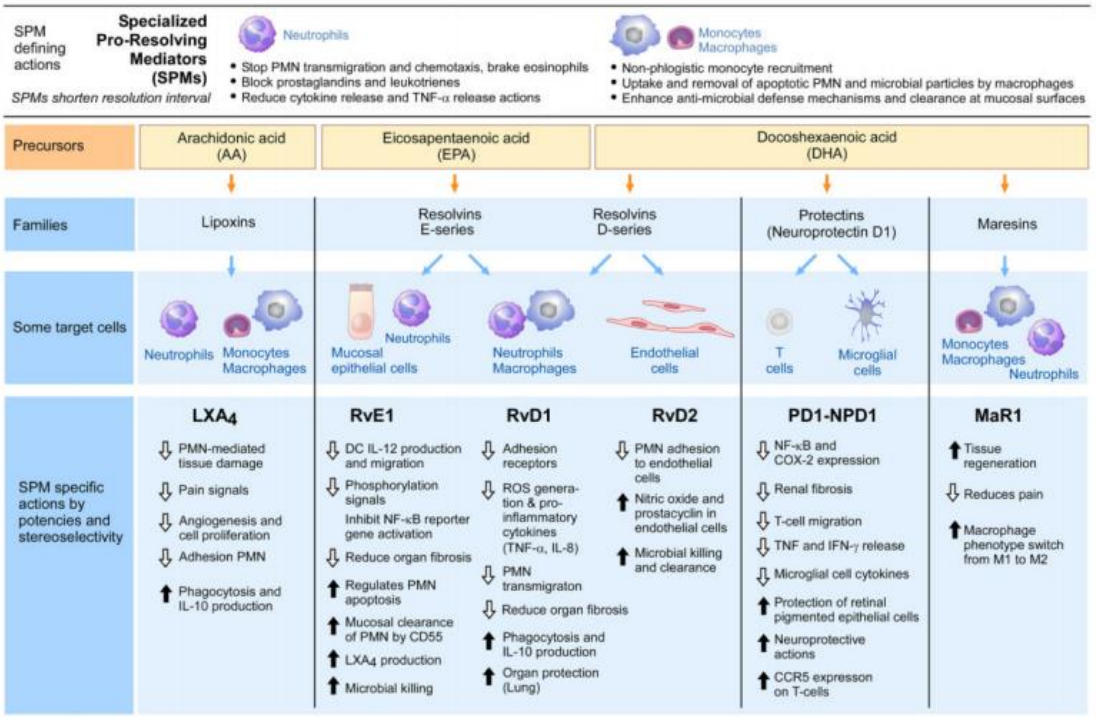

Figure 14. SPMs precursors, actions and targeted cell types. The precursors essential polyunsaturated fatty acids (AA, EPA, DHA) are converted by leukocytes to separate chemically distinct families of mediators that stimulate active resolution responses of different cell types and tissues (Buckley et al., 2014).

Defective resolution programs and CNS disease

Since it has been identified that the induction of the synthesis of SPMs is crucial for an efficient resolution of the inflammatory response (Reviewed at Serhan, 2014; Chiang and Serhan, 2017b; Dalli and Serhan, 2018), previous studies in our laboratory were focus on the assessment of the SPMs levels after spinal cord injury (Francos-Quijorna et al., 2017). In this study has been shown that the CNS milieu is characterized by a defective and delayed induction of SPMs, involving those derived from AA, DHA and EPA pathways. This inability to generate a resolution conducive environment is contrasted by a high expression of PGE2, a hall mark of pro-inflammatory activity. This data indicates that the lipid mediator class switching from pro-inflammatory to pro-resolution mediators does not occurs in the injured spinal cord. Furthermore, they found that MaR1 administration significantly improved locomotor recovery and mitigated secondary injury progression in a clinical relevant model of spinal cord injury (Francos-Quijorna et al., 2017). In the same line, in our laboratory has been demonstrated that amyotrophic lateral

sclerosis patients have increased levels of PGs and LTs and also a defective synthesis of SPMs in the spinal cord as compared to controls (unpublished data).

Related to MS, there is a study demonstrating that SPMs pathways were regulated differentially in the cerebrospinal fluid of MS patients, depending on MS severity. They found that patients with highly active MS had increased 15-HETE and PGE_2 levels, which are products of 15-LOX and COX-2. However, LXA_4 levels were not increased in these patients (Prüss et al., 2013).

Anti-inflammatory vs. pro-resolution therapies

Agents that target pro-inflammatory mediators have dominated the pharmacopoeia for inflammatory diseases for more than 100 years. Their aim is to limit the recruitment of leukocytes into the damaged tissue. Although many existing anti-inflammatory therapies are very useful to alleviate inflammation, they evoke some unwanted effects. For example, they are quiet immunosuppressant and are associated with increased susceptibility to infections (Reviewed at Vandewalle et al., 2018). Importantly, although anti-inflammatory drugs are able to minimize the infiltration of leukocytes, they fail to stimulate their clearance from the tissue. This is a striking difference with the pro-resolutory therapies, since SPM do not only act as anti-inflammatory agents, but also, they stimulate the clearance of accumulated leukocytes from the challenged tissue. Highlight that most of the anti-inflammatory therapies block, directly or indirectly, the expression or activity of lipoxygenases. This is critical since these are key enzymes involved in the production of SPMs. Therefore, although anti-inflammatory agents can dampen the inflammatory response in the acute phase, they can also hamper the resolution response at later stages, and thus, further chronifying the immune response (Schwab et al., 2007; and reviewed at Chiang and Serhan, 2017b; Dalli and Serhan, 2018).

HYPHOTESIS AND OBJECTIVES

In this thesis we hypothesized that the modulation of the inflammatory response will ameliorate the neurological decline, clinical signs and tissue damage in experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis.

Therefore, the main goal of the present thesis is to investigate whether administration of anti-inflammatory molecules or resolution agonists ameliorates the pathogenesis of experimental autoimmune encephalomyelitis in mice. To address this general aim, the thesis has been divided in three chapters with the following specific objectives:

Chapter 1. OLT1177 (Dapansutrile), an NLRP3 inflammasome inhibitor, ameliorates experimental autoimmune encephalomyelitis.

- To study whether the prophylactic inhibition of NLRP3, with an OLT1177-enriched diet, prevents functional deficits and demyelination in the spinal cord of mice with experimental autoimmune encephalomyelitis.
- To assess the effects of prophylactic treatment with an OLT1177-enriched food on cytokine levels in plasma and spinal cord after in mice suffering from experimental autoimmune encephalomyelitis.
- To study whether mice fed the prophylactic OLT1177-enriched diet show reduced immune cell counts in the spinal cord and blood of experimental autoimmune encephalomyelitis mice.
- To investigate whether therapeutic administration of OLT1177 by oral gavage exerts ameliorates symptomatology of mice with experimental autoimmune encephalomyelitis.

Chapter 2. IL-37 exerts therapeutics effects in experimental autoimmune encephalomyelitis.

- To assess whether *IL-37* expression is induced in the central nervous system of transgenic mice carrying human *IL-37b* isoform after experimental autoimmune encephalomyelitis.

- To evaluate whether the transgenic expression of human *IL-37* decreases functional deficits and demyelination after experimental autoimmune encephalomyelitis
- To study the effects of transgenic expression of *IL-37* on inflammation in mice with experimental autoimmune encephalomyelitis
- To assess whether the components of the IL-37 extracellular receptor IL-18Rα/SIGIRR are expressed in the mouse spinal cord under physiological conditions and after induction of experimental autoimmune encephalomyelitis
- To study the importance of the extracellular function of IL-37 to experimental autoimmune encephalomyelitis.
- To investigate whether the extracellular function of IL-37 is induced in MS individuals.
- To address whether exogenous administration of recombinant human IL-37 protein mediates therapeutic effects in experimental autoimmune encephalomyelitis.

Chapter 3. Administration of Maresin-1 ameliorates the physiopathology of experimental autoimmune encephalomyelitis.

- To analyse whether the enzymes involved in the synthesis of specialized pro-resolving lipid mediators are induced in multiple sclerosis individuals and experimental autoimmune encephalomyelitis mice.
- To assess whether exogenous administration of specialized pro-resolving lipid mediators (Maresin-1, Resolvin-D1 or Resolvin-E1) minimizes the symptomatology of experimental autoimmune encephalomyelitis in mice.
- To study the effects of exogenous delivery of MaR1 on inflammation in mice with experimental autoimmune encephalomyelitis

MATERIALS AND METHODS

All experimental procedures were approved by the Universitat Autònoma de Barcelona Animal Experimentation Ethical Committee (CEEAH 2878) and followed the European Communities Council Directive 2010/63/EU, and the methods were carried out in accordance with the approved guidelines.

Human Samples

Human samples from MS individuals and healthy donors were gently provided by the tissue bank of the Research Center of the University of Montreal Hospital Center-Multiple Sclerosis Clinic (Canada).

Brain normal appearance white matter (NAWM) regions and active lesions tissue samples were obtained from 6 SPMS and 1 RRMS individuals (Table 3). The median age at death was 50 years (range from 26 to 65 years). Regions of interest for RNA isolation were dissected manually from 50 µm cryosections per block.

Table 3. Human brain active lesions and NAWM samples.

ID Sample	Sex	Age	Type of MS
AB50	F	55	SPMS
AB56	F	50	SPMS
AB103	M	48	SPMS
AB172	F	60	SPMS
AB154	F	49	SPMS
AB184	M	65	SPMS
AB187	M	26	RRMS

In addition, blood samples were collected from 11 selected patients with clinically definite MS characterized by RRMS (7 patients), SPMS (3 patients) and CIS (1 patient) (Table 4). Their ages ranged from 27 to 65 years (mean age: 43 years). At time of investigation no patient was on treatment with steroids or immunosuppressive substances. 7 healthy volunteers were included as controls (HC); their ages ranged from 24 to 42 years (mean age: 35 years). A written

informed consent was obtained from patients and healthy donors in accordance with the local ethics committee. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples collected in EDTA-coated Vacutainer tubes (BD Biosciences, Oakville, ON, Canada) using a Ficoll density gradient (Amersham Biosciences) as described previously (Arbour et al., 2003).

Table 4. Human PBMCs samples.

ID Sample	Sex	Age	Type of MS
MS-SS	F	63	RRMS
MS-ND	F	45	RRMS
MS-MB	F	36	RRMS
MS-GH	M	65	SPMS
MS-PD	M	31	RRMS
MS-LD	F	45	SPMS
MS-NV	F	44	RRMS
MS-SF	F	60	SPMS
MS-MP	F	34	CIS
MS-MAL	M	27	RRMS
MS-NP	F	27	RRMS
HC-JD	M	25	HC
HC-JL	M	44	HC
HC-XA	M	37	HC
HC-CH	F	30	HC
HC-MC	M	27	HC
HC-BoA	F	42	HC
HC-HX	F	37	HC

Mice

Experiments were performed in adult (8-10 weeks) females or males C57Bl/6J mice (Charles River), as well as in transgenic mice expressing human IL-37b in homozygosis (hIL-37tg; gently provided by Professor Charles Dinarello, University of Colorado, USA), *sigirr* knockout (*sigirr* KO; kindly provided by Professor Cecilia Garlanda from Humanitas University, Italy), or double transgenic hIL-37tg x *sigirr* KO), and their respective wildtype littermate mice. Mice were randomly distributed in groups of 5-6 mice per cage.

All the mice were housed with food and water *ad libitum* at room temperature of 22±2ºC under 12:12h light-dark cycle.

Experimental Autoimmune Encephalomyelitis Induction

Mice (8-10 weeks old; Charles River Laboratories) were sedated with intramuscular injection of a mixture of ketamine (22mg/kg) (Imalgen 1000, Merial) and xylazine (2.5mg/kg) (Rompun, Bayer). EAE was actively induced by subcutaneously immunization with 300µg of myelin oligodendrocyte glycoprotein peptide 35-55 (MOG35-55 MEVGWYRSPFSRVVHLYRNGK, Thermo Fisher Scientific, MA, USA) in 200µl Complete Freund's Adjuvant (CFA) (Difco, MI, USA) supplemented with 4mg/mL of heat inactivated Mycobacterium tuberculosis (Difco, MI, USA) solved. Intraperitoneal (i.p.) injections of 400ng of pertussis toxin (Sigma-Aldrich, ON, USA) in 100µl sterile saline were administered at the time of induction and again 48 h later.

Experimental Autoimmune Encephalomyelitis Clinical Signs Evaluation

Mice were daily scored from day 0 to day 21 after induction of EAE. The researcher was blind to experimental groups during the functional evaluation. A 6-point scale (Table 5) was used to evaluate the clinical signs of EAE:

Table 5. EAE scoring scale.

0	normal walking, upright tail.
0.5	partially paralyzed tail, no other symptoms.
1	fully paralyzed tail, no other symptoms.
2	mild hind limb weakness, quick righting reflex (<5 seconds), paralyzed tail. Hindlimbs will be weight-bearing and the gait will be minimally affected.
3	severe hind limb weakness, slow righting reflex (>5-10 seconds) or inability to right. Hindlimbs will be splayed laterally, gait affected and unable to bear weight.
3.5	severe hind limb weakness and partial paralysis of hind limb.
4	complete paralysis of at least one hind limb.
4.5	complete paralysis of one or both hind limbs and trunk weakness. Mice are usually found in a curled position lying on their side.
5	complete paralysis of one or both hind limbs, forelimb weakness or paralysis.
6	mouse is found death by EAE.

Once mice reach grade 3, food was placed inside the cage and long nozzles were attached to the water bottles. Mice at grade 5 were sacrificed if they remained at this clinical for more than 24 hours.

Drug administration

EAE-induced mice were randomly assigned to the treatment and control experimental groups.

OLT1177 (Dapansutrile)

Oral OLT117 administration

EAE-mice were fed either a OLT1177-enriched diet or standard food diet from the day same of the EAE induction. The composition of the food was identical, except that OLT1177 enriched food contained either 3.75 g or 7.5 g of OLT1177 per kilogram of food. Standard and OLT1177 enriched food were prepared by Research Diets (New Brunswick, NJ, USA). Food and water were provided *ad libitum* for the entire length of the study 23 days post EAE induction.

Intraperitoneal OLT1177 administration

OLT1177 solubilized with sterile saline and administered intraperitoneally (i.p.), daily until the end of the study (21 days). Two different administration protocols were tested: (i) 200 mg/kg of OLT1177 once per day starting at the day of the EAE immunization; (ii) the 60 mg/kg dose of OLT177, twice a day, starting at the day of the EAE induction. Control mice were administered saline at the days

Oral gavage OLT1177 administration

60 mg/kg of OLT1177 solubilized with distilled water and were administered daily, twice per day, by oral gavage. Treatment was initiated on the first day the animals displayed the first signs of EAE until the end of the follow up. Control mice were administered distilled water at the same days.

Recombinant human IL-37 protein

Mice were daily treated i.p. with 1 μg of native$^{46\text{-}218}$ IL-37 protein, or with the mutant monomeric forms IL-37^{D73K}, IL-37^{Y85A} in 200μl of sterile saline (0.9% NaCl) or vehicle (0.9% NaCl) starting from the first day of the clinical signs until the end of the study 21 days after induction. The dose was stablished according previous studies in the lab (Coll-Miró et al., 2016).

Specialized pro-resolving mediators

Mice were randomly assigned to the treatment and control experimental groups. Daily i.p. injections of 1 μg of either RvD1, RvE1 or MaR1 in 200μl of sterile saline (0.9% NaCl) or vehicle (0.9% NaCl + 10% EtOH) were done starting from the first day of the clinical signs until the end of the study 21 days after induction. The dose was stablished according previous studies in the lab (Francos-Quijorna et al., 2017).

Real-time Quantitative PCR Assay (qPCR)

Total RNA was isolated from postmortem human brain active lesion and normal appearance white matter (NAWM), as well as, from spinal cords harvested of EAE mice harvested at different stages of the disease, using the RNeasy Lipid Tissue Mini Kit (Qiagen) following the manufacturer's procedures. Total RNA was also extracted from PBMCs collected from MS patients and healthy donors using the QIAamp RNA Blood Tissue Mini Kit (Qiagen) according to the guide protocol.

RNA was retro-transcribed using the cDNA Reverse Transcription Kit (Applied Biosystems). cDNA human libraries were analysed using a Bio-Rad CFX384 (CFX Manager V3.1) The expression of human *IL-37* and murine *gapdh* (table 7) was analysed using SYBR Green III technology (Bio-Rad) and the custom design primers. The expression of mouse and human genes (*sigirr, il-18rα, 12-lox, 15-lox* and *gapdh)* (table 8) was analysed using a Bio-Rad CFX384 (CFX Manager V3.1).

All data ware analysed using the $2^{\Delta\Delta}$Ct method.

Table 6. TaqMan specific primers for human samples.

Gene	Reference
IL-37	Hs00367201_m1
IL-1β	Hs01555410_m1
SIGIRR	Hs00222347_m1
IL-18Rα	Hs00977691_m1
15-LOX	Hs00993765_g1
GAPDH	Hs02786624_g1

Table 7. SYBR Green specific primers.

Gene	Forward primer	Reverse primer
Human *IL-37*	5' CTT AGA AGA CCC GGC TGG AAG 3'	5' TGT GAT CCT GGT CAT GAA TGC T 3'
Murine *gapdh*	5' TCA ACA GCA ACT CCC ACT CTT CCA 3'	5' ACC CTG TTG CTG TAG CCG TAT TCA 3'

Table 8. TaqMan specific primers for mice samples.

Gene	Reference
sigirr	Mm01275624_g1
il-18rα	Mm00515178_m1
12/15-lox	Hs00993765_g1
gapdh	Hs02786624_g1

Histological analysis

EAE mice were euthanised 21 days post-immunization with an overdose of pentobarbital sodium (Dolethal) and transcardially perfused with 4% paraphormaldehyde (PFA) in 0.1M of phosphate buffer (PB). Then, lumbar segments of spinal cords were harvested, post-fixed in 4% PFA for 2 hours in ice and cryoprotected in 30% sucrose in 0.1M PB at 4ºC for at least 48 hours. Then, spinal cords were embedded in TissueTek OCT (Sakura), cut in transversal sections (15µm-thick) with a cryostat (Leica) between L3 and L5 segments and serially picked up on gelatine-coated glass slides. Samples were stored at –20ºC.

Sections were stained with Luxol Fast Blue (LFB) (Sigma Aldrich) to assess the myelin loss. Briefly, after a graded dehydration, sections were placed in 1mg/mL of LFB solution in 96% ethanol and 0.05% acetic acid overnight at 37ºC and protected from light. Then, slides were washed with 96% ethanol, rehydrated with distilled water and placed in a 0.5mg/mL Li_2CO_3 solution in distilled water for 3-5 minutes at room temperature. Finally, sections were washed in distilled water, dehydrated serially again and mounted in DPX mounting medium (Sigma Aldrich). To assess the demyelinated area in the spinal, 6 random images per mice were captured at 10X magnification with an Olympus BX51 and the attached Olympus DP73 Camera. The total demyelinated area within the spinal cord was measured with Image J software.

Cytokine Protein Expression Assessment

C57Bl/6 and EAE mice were euthanatized with an overdose of pentobarbital sodium (Dolethal) and transcardially perfused with 60 mL of sterile saline (0.9% NaCl). Then, spinal cords were harvested and rapidly frozen with liquid nitrogen. Protein isolation from the spinal cord samples and cytokine quantification was performed as we described previously (Amo-Aparicio *et al.*, 2018). Briefly, tissue was homogenized mechanically, and total protein was isolated using a specific protein extraction buffer (25 mM HEPES, IGEPAL 1%, 0.1 $MgCl_2$, 0.1M EDTA pH=8, 0.1M EGTA pH=8, 0.1 PMSF, 10µl/ml of Protease Inhibitor cocktail (Sigma) and 1 pill of PhosphoStop). Then, protein concentration was determined with a DC Protein Assay (Bio-Rad). Protein homogenates were concentrated to 4µg/µl using MicroCon Centrifugation Filters (Millipore) and finally, protein levels of customer-designed mix of cytokines were analysed using a custom-designed Milliplex Cytokine Magnetic Bead Panel on a MAGPIX system (EMD Millipore).

Fluorescence Activated Cell Sorting (FACS)

Female hIL-37tg mice and WT littermates were euthanised at peak of EAE with and overdose of pentobarbital sodium (Dolethal). To analyse the different immune cells, the spinal cord and lymph nodes tissues processed as we previously described in

Amo-Aparicio et al. 2018. Briefly, these tissues were cut in small pieces and enzymatically dissociated incubating them 30 minutes at 37ºC in 1mL of HBSS without Ca^{2+}/Mg^{2+} and 0.1% collagenase and 0.1% DNase. Then, samples were mechanically disintegrated by passing them with DMEM-10% FBS through a 70μm cell strainer. After two washes, cell suspension was split into different 1.5mL microcentrifuge tubes according to the number of antibody combinations. For extracellular staining, samples were incubated with the primary antibodies (table 9) for 1h at 4ºC in soft agitation, washed with 10% FBS-DMEM and centrifuged twice at 300g for 10 min at 4ºC to remove debris.

For extracellular staining, samples were incubated with the primary antibodies (Table 9) for 1h at 4ºC in soft agitation.

Table 9. FACS primary extracellular antibodies.

Epitope	Fluorochrome	Dilution	Manufacturer
CD45	PerCP	1:300	BioLegend
CD11b	PE, PE-Cy7	1:300	BioLegend
CD11c	488	1:300	BioLengend
F4/80	PE, APC	1:300	eBioscience
Ly6C	FITC	1:300	BD Bioscience
Ly6G	PE	1:300	BD Bioscience
CD3	FITC, APC, PerCP	1:300	eBioscience
CD4	APC-Cy7	1:300	eBioscience
CD8	APC	1:300	eBioscience
CD49b	PE	1:300	BD Bioscience
CD24	PE	1:300	Invitrogen
CD16/32	PE	1:300	BioLegend

Samples were then fixed with 1% PFA. For intracellular staining, cells were fixed with 1% PFA and permeabilizated with Permeabilization Wash Buffer (Biolegend), except for those stained against FOXP3, which were permeabilizated using FoxP3 Transcription Factor Staining Buffer Set (eBioscience). Samples were them immunostained with the intracellular antibodies (table 10) for 1h at 4ºC and incubated for 45 minutes

Table 10. FACS primary intracellular antibodies.

Epitope	Fluorochrome	Dilution	Manufacturer
FoxP3	PE-Cy7	1:400	eBioscience
tBet	PerCP	1:400	eBioscience
RORγ	APC	1:400	eBioscience
GATA3	PE	1:400	eBioscience
IFNγ	488	1:200	eBioscience
IL-17A	488	1:200	eBioscience
IL-10	488	1:200	eBioscience
IL-4	PE-Cy7	1:200	Biolegend
iNOS	Alexa 647	1:400	Abcam

Isotype-matched samples were always used to avoid nonspecific binding signals.

Samples were analysed on a FACS Canto Flow Cytometer (BD Bioscience). Immune cell populations were defined according to the expression of different markers (table 11) (Coll-Miró *et al.*, 2016; Francos-Quijorna *et al.*, 2016, 2017; Amo-Aparicio *et al.*, 2018) using FlowJo® software V.10.

Table 11. Definition of immune cell populations.

IMMUNE CELL POPULATION	ANTIBODY COMBINATION
Macrophages	$CD45^{high}$, $CD11b^{+}$, $F4/80^{+}$
Microglia	$CD45^{low}$, $CD11b^{+}$, $F4/80^{+}$
Anti-inflammatory macrophages/microglia	$CD45^{+}$, $CD11b^{+}$, $F4/80^{+}$, $Ly6C^{neg}$
Pro-inflammatory macrophages/microglia	$CD45^{+}$, $CD11b^{+}$, $F4/80^{+}$, $Ly6C^{high}$ or $CD45^{+}$, $CD11b^{+}$, $F4/80^{+}$, $CD16/32^{+}$, $iNOS^{+}$
Neutrophils	$CD45^{high}$, $CD11b^{+}$, $F4/80^{-}$, $Ly6G^{+}$
Lymphocytes	$CD45^{high}$, $CD11b^{-}$, $CD3^{+}$
T helper cells	$CD3^{+}$, $CD4^{+}$
T cytotoxic cells	$CD3^{+}$, $CD8^{+}$
B cells	$CD45^{high}$, $CD11b^{-}$, $CD3^{-}$, $CD24^{+}$
Classic T regulatory cells	$CD3^{+}$, $CD4^{+}$, $FOxP3^{+}$
Non-classic T regulatory cells	$CD3^{+}$, $CD4^{+}$, $CD49b^{+}$
Th1 cells	$CD3^{+}$, $CD4^{+}$, $tBet^{+}$, ($IFNy^{+}$)
Th2 cells	$CD3^{+}$, $CD4^{+}$, $GATA3^{+}$, ($IL\text{-}4^{+}$/$IL\text{-}10^{+}$)
Th17 cells	$CD3^{+}$, $CD4^{+}$, $RORy^{+}$, ($IL\text{-}17A^{+}$)

Statistical Analysis

Data are shown as mean ± standard error of the mean (SEM). The Kolmogorov-Smirnov test was used to check normality. Paired or unpaired t test was used for the comparison between two different groups (gene expression, histological analysis or FACS analysis). One-way ANOVA was used for the comparison of three different groups with *post-hoc* Bonferroni's test for multiple comparisons (cytokine assay, FACS analysis in chapter 1). EAE clinical score was analysed by using repeated measures two-way ANOVA with *post-hoc* Bonferroni's test for multiple comparisons. Differences were considered significant at $p<0.05$.

RESULTS

CHAPTER 1

OLT1177 (Depansutrile), a selective NLRP3 inflammasome inhibitor, ameliorates experimental autoimmune encephalomyelitis pathogenesis

OLT1177 (Depansutrile), a selective NLRP3 inflammasome inhibitor, ameliorates the pathogenesis of experimental autoimmune encephalomyelitis

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ABSTRACT

IL-1β and IL-18 are pro-inflammatory cytokines that are linked to inflammation. Activation of the NOD-like receptor protein 3 (NLRP3) inflammasome is required for the maturation and secretion of IL-1β and IL-18 and, thus, plays a key role in the pathogenesis of many inflammatory conditions, including multiple sclerosis (MS). OLT1177™ (dapansutrile) is a newly developed drug that is safe in humans and inhibits specifically the NLRP3 inflammasome. In the present study, we investigated whether OLT1177 exerts therapeutic effects in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. We found that EAE mice fed an OLT1177-enriched diet prophylactically were significantly protected against functional deficits and demyelination. We also demonstrated that prophylactic administration of OLT1177 led to marked reduction (~2-3 fold) in the protein levels of IL-1β and IL-18, as well as, IL-6 and TNFα, in the spinal cord of EAE mice. Moreover, prophylactic treatment with OLT1177 significantly attenuated the infiltration of CD4 T cells and macrophages in the spinal cord. We also demonstrated that oral administration of OLT1177, starting at disease onset, resulted in significant amelioration of the clinical signs of EAE. Overall, these first data suggest that OLT1177 could have clinical benefit for the treatment of MS in humans.

INTRODUCTION

Cytokines are critically involved in the course of a myriad of inflammatory diseases, such as MS (Becher et al., 2017). MS is a chronic, neuroinflammatory and demyelinating disease of the central nervous system (CNS) that affects around 2.5 million people worldwide. The etiology of MS is still poorly understood, but it is well established that demyelination and further neurodegeneration are linked to neuroinflammation since infiltrated and activated resident immune cells are present in all stages of MS and in all patients (Lassmann et al., 2007; Wu and Alvarez, 2011; Dendrou et al., 2015; Popescu and Lucchinetti, 2016).

Clinical studies have shown that progression of MS correlates with the dysregulation of some cytokine networks within the CNS (Becher et al., 2017; Palle et al., 2017). IL-1β and IL-18 are two members of the IL-1 family with broad pro-inflammatory actions (Dinarello et al., 2012; Hewett et al., 2012) and both are significantly elevated in MS patients (Losy and Niezgoda, 2001; Nicoletti et al., 2001; Heidary et al., 2014; Rossi et al., 2014a). Pharmacological blockade or genetic removal of IL-1β or IL-18 resulted in protection against the development of experimental autoimmune encephalomyelitis (EAE), the murine model of MS (Symons et al. 1987; Baker et al. 1991; Chung et al. 2009; Lévesque et al. 2016).

IL-1β and IL-18 are produced as inactive precursors and require the cleavage by caspase-1 to become active (Yang et al., 2019). The activation of caspase-1 is mediated by the oligomerization of multi-protein complexes, known as inflammasomes (Martinon et al., 2002; Franchi et al., 2009). To date, several inflammasomes have been described. Nonetheless, the NLRP3 is the most extensively studied.

The contribution of the NLRP3 inflammasome to human diseases has been demonstrated by studies revealing constitutively active forms of NLRP3 by mutations within the Nlrp3 locus. The mutations correlate to autoinflammatory syndromes, such as Muckle-Wells syndrome (MWS), cryopyrin-associated periodic syndrome and familial cold autoinflammatory syndrome (Feldmann et al., 2002; Ting et al., 2006). NLRP3 inflammasome has been related to many human diseases such as gout and type II diabetes, among others (Conforti-Andreoni et al., 2011;

Menu and Vince, 2011; Strowig et al., 2012). NLRP3 inflammasome also play a critical role in EAE pathogenesis since *Nlpr3*-deficient mice undergo milder EAE severity (Gris et al., 2010; Inoue and Shinohara, 2013). Similarly, administration of MCC950, a small NLRP3 inflammasome inhibitor, has shown efficacy in a relapsing-remitting EAE mouse model (Coll et al., 2015a; Khan et al., 2018), suggesting the NLRP3 inflammasome as a new potential target for the treatment of MS.

OLT1177 (dapansutrile) is a specific inhibitor of the NLRP3 inflammasome. OLT1177 is active *in vivo* and limits the severity of endotoxin-induced inflammation and joint arthritis (Marchetti et al., 2018b). This drug was initially formulated as a candidate for the topical treatment of degenerative arthritis and subsequently the oral form was developed. Just as the topical gel, the oral capsules also are demonstrating that dapansutrile is safe and well-tolerated in humans (Marchetti et al., 2018b, 2018a). In the current study, we assessed whether OLT1177 exerts therapeutic effects in a chronic model of EAE. We revealed that oral administration of OLT1177 mediated marked anti-inflammatory actions and ameliorated EAE severity in mice.

MATERIALS AND METHODS

Chronic experimental Autoimmune Encephalomyelitis

All experimental procedures were approved by the Universitat Autònoma de Barcelona Animal Experimentation Ethical Committee (CEEAH 2878) and followed the European Communities Council Directive 2010/63/EU, and the methods were carried out in accordance with the approved guidelines.

Female adult C57BL/6 (8-10 weeks old; Charles River Laboratories) were sedated with intramuscular injection of a mixture of ketamine (22mg/kg) (Imalgen 1000, Merial) and xylazine (2.5mg/kg) (Rompun, Bayer). Experimental autoimmune encephalomyelitis (EAE) was actively induced by subcutaneous immunization with 300µg of myelin oligodendrocyte glycoprotein peptide 35-55 (MOG_{35-55} MEVGWYRSPFSRVVHLYRNGK, Thermo Fisher Scientific, MA, USA) in 200µl Complete Freund's Adjuvant (CFA) (Difco, MI, USA) supplemented with 4mg/mL of heat inactivated *Mycobacterium tuberculosis* (Difco, MI, USA). Intraperitoneal (i.p.) injections of 400ng of pertussis toxin (Sigma-Aldrich, ON, USA) in 100µl sterile saline were also administered at the day of induction and again 48 h later. All the mice were housed with food and water *ad libitum* at a room temperature of 22±2ºC under 12:12h light-dark cycle.

Drug administration

EAE-induced mice were randomly assigned to the OLT1177 treatment and control experimental groups. OLT1177 was administered orally or intraperitoneally.

Oral OLT117 administration

EAE-mice were fed either an OLT1177-enriched diet or standard food diet from the day same of the EAE induction. The composition of the food was identical, except that OLT1177-enriched food contained either 3.75 g or 7.5 g of OLT1177 per kilogram of food. Standard and OLT1177-enriched food were prepared by Research

Diets (New Brunswick, NJ, USA). Food and water were provided *ad libitum* for the entire length of the study, or 23 days post EAE induction.

Intraperitoneal OLT1177 administration

OLT1177 solubilized with sterile saline and administered intraperitoneally (i.p.), daily until the end of the study (21 days). Two different administration protocols were tested: (i) 200 mg/kg OLT1177 injected once per day starting on the day of the EAE immunization; and (ii) 60 mg/kg OLT177 injected twice a day starting on the day of the EAE induction. Control mice were administered saline on the same days.

Oral gavage OLT1177 administration

OLT1177 was solubilized with distilled water and administered daily (60 mg/kg), twice per day, by oral gavage. Treatment was initiated on the first day the animals displayed the first signs of EAE until the end of the follow up. Control mice were administered distilled water on the same days.

Functional Evaluation

Mice were scored daily from day 0 to day 21 after induction of EAE. The researcher was blind to the experimental groups during the functional evaluation. A 6-point scale was used to evaluate the clinical signs of EAE: 0=normal walking, 0.5=partially paralyzed tail, 1=fully paralyzed tail, 2=mild hind limb weakness, quick righting reflex, 3=severe hind limb weakness, slow righting reflex, unable to bear weight, 3.5=severe hind limb weakness and partial paralysis of hind limb, 4=complete paralysis of at least one hind limb, 4.5=complete paralysis of one or both hind limbs and trunk weakness, 5=complete paralysis of one or both hind limbs, forelimb weakness or paralysis, 6=mouse is found death by EAE.

Histological analysis

EAE mice were euthanised at either day 21 or 23 post-immunization with an overdose of pentobarbital sodium (Dolethal) and transcardially perfused with 4%

paraformaldehyde (PFA) in 0.1M phosphate buffer (PB). Lumbar segments of spinal cords were harvested, post-fixed in 4% PFA for 2 hours and cryoprotected in 30% sucrose in 0.1M at 4ºC for at least 48 hours. Spinal cords were embedded in TissueTek OCT (Sakura), cut in transversal sections (15µm-thick) with a cryostat (Leica) between L3 and L5 segments and serially picked up on gelatine-coated glass slides. Samples were stored at –20ºC.

Sections were stained with Luxol Fast Blue (LFB) (Sigma Aldrich). Briefly, after a graded dehydration, sections were placed in 1mg/mL of LFB solution in 96% EtOH and 0.05% acetic acid overnight at 37ºC and protected from light. Then, slides were washed with 96% EtOH, rehydrated in distilled water and placed in a 0.5mg/mL Li_2CO_3 solution for 3-5 minutes at room temperature. Finally, sections were washed in distilled water, dehydrated again in 100% EtOH and mounted in DPX (Sigma Aldrich). To assess the demyelinated area in the spinal cord, 6 random images per mice were captured at 10X magnification with an Olympus BX51 and the attached Olympus DP73 Camera. The total demyelinated area within the spinal cord was measured with Image J image analysis software.

Cytokine Protein Expression

EAE mice were euthanised 3 days after EAE onset with an overdose of pentobarbital sodium (Dolethal). Blood (500 µl) was obtained by cardiac puncture and centrifuged at 25000g for 10 min at room temperature to collect plasma separately from blood cells. Then, mice were transcardially perfused with 60 mL of sterile saline (0.9% NaCl). Spinal cords were harvested and rapidly frozen in liquid nitrogen. Protein isolation from the spinal cord samples and cytokine quantification was performed as we described previously (Amo-Aparicio et al., 2018). Protein levels of 6 cytokines (IL-1β, IL-18, IL-6, KC, TNFα and IL-10) were analysed using a custom-designed Cytokine Magnetic Bead Panel (Invitrogen) on a MAGPIX system (Millipore).

Fluorescence Activated Cell Sorting (FACS)

Immune cell infiltration was determined in the spinal cord of EAE mice at disease peak. Mice were euthanised with an overdose of pentobarbital sodium (Dolethal) and transcardially perfused with 60 mL of sterile saline (0.9% NaCl). Spinal cords were collected, cut in small pieces and enzymatically dissociated in 1mL of Hank's Balanced Salt Solution (*HBSS*) without Ca^{2+}/Mg^{2+} containing 0.1% collagenase and 0.1% DNase for 30 minutes at 37ºC. Then spinal cords were mechanically disintegrated by passing it with Dulbecco's Modified Eagle Medium (DMEM)-10% fetal bovine serum (FBS) through a 70µm cell strainer to obtain a cell suspension (Amo-Aparicio et al., 2018).

Cell suspensions were split into different 1.5mL microcentrifuge tubes according to the number of antibody combinations. Cells alone and isotype-matched control samples were generated to control for nonspecific binding of antibodies and for autofluorescence. For extracellular staining, the following antibodies from eBioscience were used at a 1:300 concentration: CD45-PerCP, CD11b-PE or -PE-Cy7, F4/80-PE or -APC, Ly6C-FITC, Ly6G-PE, CD3-FITC-APC-PerCP; CD4-APC-Cy7, CD8-APC, CD49b-PE, CD24-PE. Samples were incubated with the primary antibodies for 1h at 4ºC in soft agitation, washed with DMEM-10% FBS, centrifuged twice at 300g for 10 min at 4ºC to remove debris and then fixed with 1% PFA. For intracellular staining, the following antibodies from eBioscience were also used at 1:300 concetration: FoxP3- PE-Cy7, tBet-PerCP, RORγ-APC and GATA3-PE. After extracellular staining, cells were fixed and permeabilizated using FoxP3 Transcription Factor Staining Buffer Set (eBioscience) for 40 min at 4ºC. Then, samples were immunostained with the intracellular antibodies over nigh at 4ºC. Finally, stained cells were washed with PBS twice and fixed with 1% PFA.

Samples were analysed on a FACS Canto Flow Cytometer (BD Bioscience) and all data were processed using FlowJo® software V.10.

Statistical analyses

Data are shown as mean ± standard error of the mean (SEM). The Kolmogorov-Smirnov test was used to check normality. Two tailed Student's t test was used for

the comparison between two different groups (histological analysis). One-way ANOVA was used for the comparison of two different doses and the control condition with *post-hoc* Bonferroni's test for multiple comparisons (cytokine assay, FACS analysis). EAE clinical score was analysed by using two-way repeated measures ANOVA with *post-hoc* Bonferroni's test for multiple comparisons. Differences were considered significant at $p<0.05$.

RESULTS

OLT1177 administration in daily diet ameliorates neurological decline and nervous tissue damage in EAE mice

We first aimed at investigating whether oral prophylactic treatment with OLT1177 led to beneficial effect in EAE. For this purpose, mice were fed a standard or OLT1177-enriched diet at two doses (3.75 g or 7.5 g of OLT1177 per kg of food) from the day of the immunization until the end of the experiment. We found that both OLT1177 doses ameliorated the neurological deficits of EAE disease, although only the low OLT1177 dose reached statistical significance (Figure 1A, B). We also observed that EAE mice fed the OLT1177 diet tended to show reduced weight loss, a feature associated with the disease progression. However, this effect was not statistically significant or believed to be an effect of the OLT1177(Figure 1C).

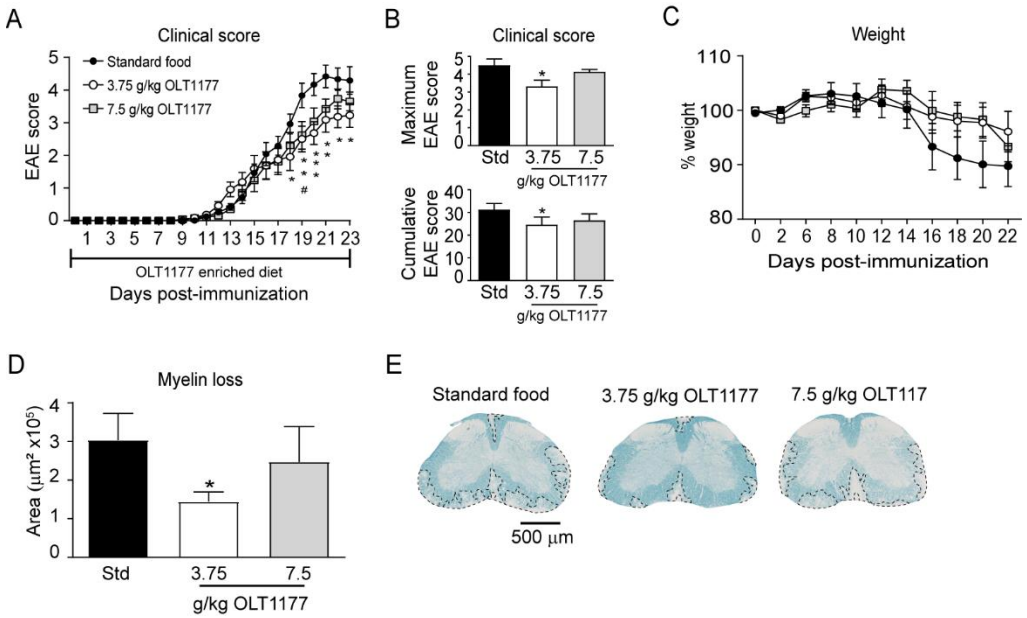


Figure 1. OLT1177-enriched food enhances functional and histological outcomes in EAE. (A-B) Graph showing the evolution of the EAE clinical score **(A)**, as well as, the cumulative and maximum EAE score **(B)** of mice fed a standard diet or a diet supplemented with 3.75 g or 7.5 g of OLT1177 per kg of food. (**C)** Plot showing the percent of weight of mice fed the standard and OLT1177-enriched food (3.75 g/kg or 7.5 g/kg). (**D-E)** Graph showing the quantification of demyelination in the lumbar spinal cord of mice fed the standard food, 3.75 g/kg or 7.5 g/kg OLT1177 at 23 days after induction. **(D)** Representative spinal cord sections from each experimental group **(E).** #p<0.05 7.5g/kg vs standard diet; *p<0.05; **p<0.01; ***p<0.001 3.75g/kg vs. Standard food. Two-way RM ANOVA, Bonferroni's *post hoc* test in **A** and **C** (n=11 per group); One-way ANOVA, Bonferroni's *post hoc* test in **B** (n=11 per group) and D (n=8 for Standard food and n=11 for 3.75 g/kg and 7.5 g/kg OLT1177). Data shown as mean±sem.

Next, we studied whether OLT1177 protected against demyelination. In line with functional outcomes, histological analysis revealed that spinal cords from mice fed the lower OLT1177 dose diet had ~2-fold reduced demyelination than mice receiving the standard food (Figure 1D-E). The higher OLT1177 dose also led to slight protection against myelin loss, but this effect was not statistically significant (Figure 1D-E).

OLT1177-enriched diet modulates the inflammatory response in the spinal cord of EAE mice

We next sought to investigate whether OLT1177 attenuated inflammation in EAE mice. With this aim, we first studied whether mice fed the OLT1177-enriched food (3.75 g or 7.5 g of OLT1177 per kg of food) reduced cytokine levels in the blood plasma and spinal cord of EAE mice at 3 days after disease onset. In the spinal cord, protein levels for IL-1β and IL-18 in the spinal cord were significantly reduced in mice fed the low OLT1177 diet compared with mice fed the standard diet (Figure 2B), corroborating the NLRP3 inhibition. As expected, in plasma, these analyses revealed that OLT1177 no change in the concentration of any of the 6 cytokines assessed samples (Figure 2A). Moreover, the lower dose of OLT1177 also reduced the levels of TNFα and IL-6 but did not affect the levels of the anti-inflammatory, IL-10 (Figure 2A). The high dose of OLT1177 also significantly decreased the protein levels of IL-1β and TNFα in the spinal cord but did not attenuate the levels of IL-18 or IL-6 (Figure 2B). These data suggest that OLT1177 mediates anti-inflammatory actions in the CNS of EAE mice.

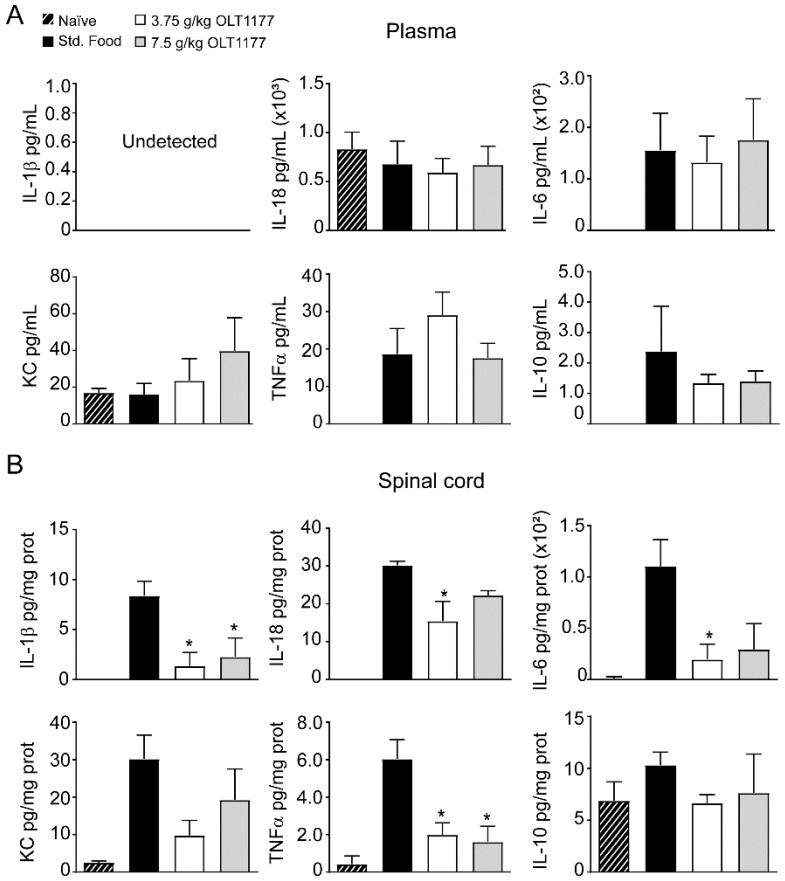


Figure 2. OLT1177-enriched food attenuates the protein levels of pro-inflammatory cytokines in the spinal cord of EAE mice. A-B Protein levels of IL-1β, IL-18, IL-6, KC, TNFα and IL-10 in the plasma **(A)** and in the spinal cord **(B)** in EAE mice fed standard food, 3.75 g/kg or 7.5 g/kg OLT1177. *p<0.05; **p<0.01; ***p<0.001 vs. Standard food. One-way ANOVA, Bonferroni's *post hoc* test (n=4 per group). Data shown as mean ±sem.

We then studied whether the reduction of cytokines in the spinal cord of EAE mice mediated by OLT1177 attenuated the accumulation of immune cells in the CNS at the peak of the disease or altered their phenotype. Flow cytometry analysis revealed that mice fed the low OLT1177-enriched diet showed ~2-fold reduction in the infiltration of T cells, which was due to decreased counts of CD4 rather than CD8 T cells. The low OLT1177 dose also reduced the accumulation of macrophages, but did not significantly decreased the counts of microglia, neutrophils or B cells (Figure 3A, Supplementary figure S1A-F). Mice fed the high OLT1177-enriched diet also attenuated the accumulation of CD4 T cells, but in contrast to the low dose, it did not reduce the number of macrophages in the spinal cord (Figure 3A, Supplementary figure S1A-F). None of the two doses of OLT1177 tested led to alteration in the

polarization of CD4 T cells, as revealed by the expression of the transcription factors that identify Th1 (tBet), Th2 (GATA3), Th17 (RORγ) and the classical (FoxP3) and non-classical (CD49b) regulatory CD4 T cells (Figure 3B, Supplementary figure S2A). Similarly, OLT1177 did not modify the proportion of pro-inflammatory (Ly6C^{high}) and anti-inflammatory (Ly6C^{low}) macrophages (Figure 3C, Supplementary figure S2B).

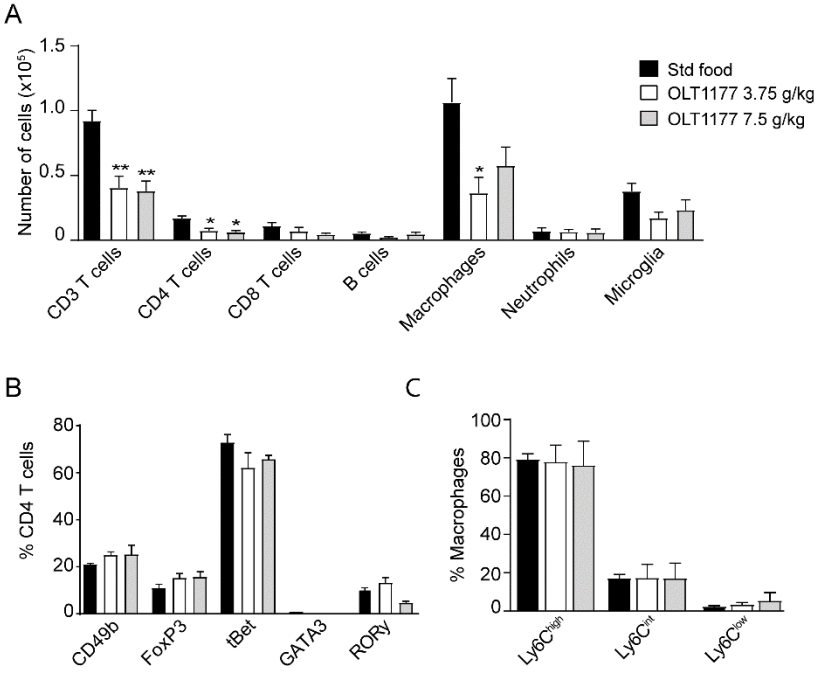


Figure 3. OLT1177-enriched food reduces the accumulation of immune cells in the spinal cord of mice at the peak of EAE. A-C Graphs showing the counts of different immune cell subsets in the spinal cord **(A)**, the percent of CD4+ T cells expressing the transcription factors CD49b, FoxP3, tBet, GATA3 or RORγ **(B)** and the percent of different macrophage subsets according to Ly6C expression **(C)** in the spinal cord of mice fed standard food, 3.75 g/kg or 7.5 g/kg OLT1177 at the peak of EAE. **(C)**. *p<0.05; **p<0.01 vs. Standard food. One-way ANOVA, Bonferroni's *post hoc* test (n=4 per group). Data shown as mean±sem.

Effects of therapeutic administration of OLT1177 in EAE mice

We next sought to investigate whether OLT1177 mediates beneficial effects when administrated in EAE mice after disease onset. Since EAE mice undergo weight loss after disease onset due to reduced food intake, we aimed at delivering the OLT1177 by oral gavage once mice showed the first signs of the disease. For this purpose, and since the above described data reveal that OLT1177 losses efficacy at high doses, we first tested the effectivity of the prophylactic effects of two different OLT1177

administration regime in EAE: (i) single daily administration of OLT1177 (200mg/kg mouse) from the day of the immunization; (ii) twice-daily administration of OLT1177 (60mg/kg mouse) from the day of the induction. These experiments revealed that, similar to the OLT1177-enriched food data, the high dose (200 mg/kg; i.p.) of OLT1177 did not prevent neurological decline of EAE, while the low dose of OLT1177 (60mg/kg; i.p) given twice a day ameliorated functional deficits and demyelination (Figure 4B-D).

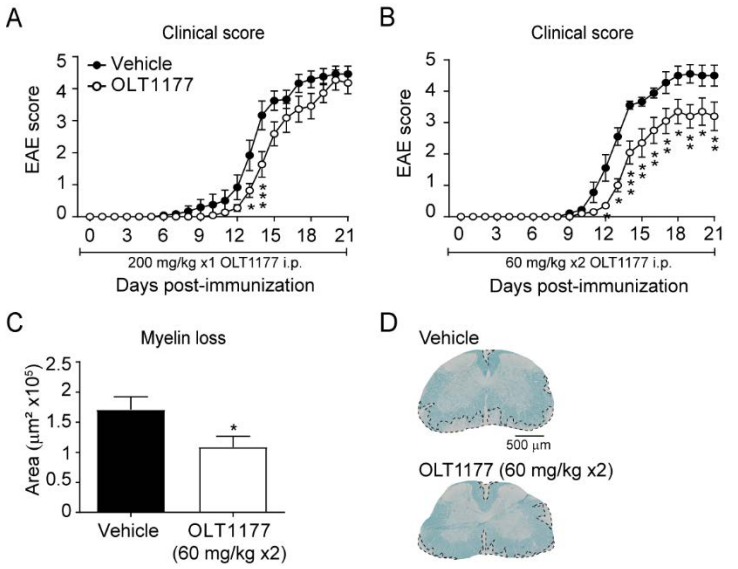


Figure 4. Effects of prophylactic treatment of OLT1177 EAE mice. A-B Average EAE clinical score of mice treated with single-daily i.p. administration of 200 mg/kg **(A)** or 60 twice-daily administration of 60 mg/kg OLT1177 **(B). C-D,** Graph showing the quantification of demyelination in the lumbar spinal cord of mice treated with both OLT1177 doses. **(C)** Representative images of lumbar spinal cord tissue sections stained against LFB from EAE treated with vehicle, single-daily 200 mg/kg and twice-daily 60 mg/kg of OLT **(D)**. *p<0.05; **p<0.01; ***p<0.001 vs. Vehicle. Two-way RM ANOVA with Bonferroni's *post hoc* test in **A** (n=12 in vehicle and n=10 in OLT1177) and **B** (n=9 in vehicle and n=10 in OLT1177); Unpaired t-test in **C** (n=9 in vehicle and n=10 in OLT1177). Data shown as mean±sem.

We then tested whether the twice-daily oral gavage (60mg/kg mouse) of OLT1177, starting at disease onset, attenuated the clinical signs of EAE. We observed that mice treated with OLT1177 showed reduced neurological deficits despite treatment was initiated at disease onset. Indeed, mice treated with OLT1177 showed a reduction in ~1 point in the EAE score compared to mice given with vehicle (Figure 5A). Furthermore, OLT1177 also significantly reduced the cumulative and maximum clinical score in EAE (Figure 5B). In accordance to functional data, we also observed

that OLT1177 protected against demyelination as revealed the histological analysis of LFB stained spinal cords (Figure 5C-D). These data support the beneficial effects of the therapeutic administration of OLT1177 in EAE.

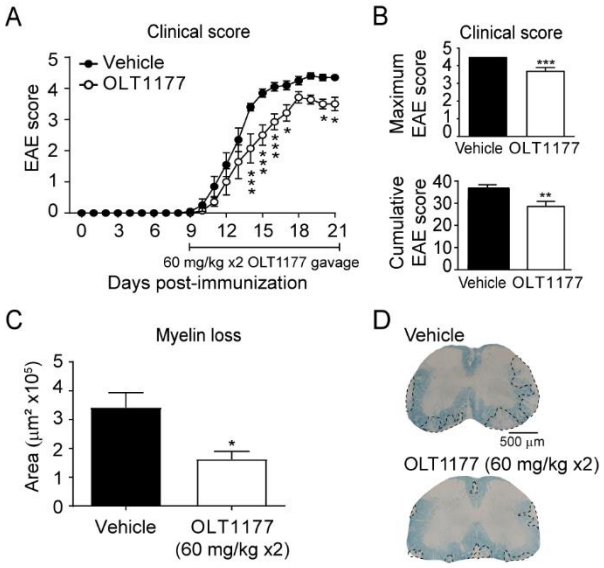


Figure 5. Oral administration of OLT1177 mediate therapeutic effects in EAE. (A-B) Plot showing the course of the EAE clinical **(A)** and cumulative and maximum clinical EAE score **(B)** of mice treated with twice-daily oral gavage of OLT1177 (60 mg/kg) or vehicle from disease onset. (**C)** Graph showing the quantification of demyelinating in the spinal cord of vehicle- or OLT1177- treated mice. **(D)** Representative LFB stained lumbar spinal cord sections from OLT1177- and vehicle-treated mice at 21 days post-immunization. *p<0.05; **p<0.01; ***p<0.001 vs. vehicle. Two-way RM ANOVA with Bonferroni's *post hoc* test in A n=10 for vehicle and n=7 for OLT1177). Unpaired t-test in B (n=10 for vehicle and n=7 for OLT1177) and C (n=9 in vehicle and n=7 in OLT1177). Data shown as mean±sem.

DISCUSSION

MS is a highly disabling disease of the CNS that affects approximately 2.5 million people worldwide (Dendrou et al., 2015; Bhise and Dhib-Jalbut, 2016; Popescu and Lucchinetti, 2016). There are currently some available therapies in the clinic for the treatment of MS. However, most of them show poor efficacy, especially in individuals who suffer from progressive MS. Moreover, they also have several side effects (Wagner and Goverman, 2015; Pérez-Cerdá et al., 2016).

Although the aetiology of MS is still unknown, inflammation has a key implication in its physiopathology (Dendrou et al., 2015; Grigoriadis and van Pesch, 2015). Pro-inflammatory cytokines, such as IL-1β or IL-18, have been extensively reported in mouse models to contribute to the pathogenesis of MS and EAE (Symons et al., 1987; Shi et al., 2000; Losy and Niezgoda, 2001; Nicoletti et al., 2001; Chung et al., 2009; Heidary et al., 2014; Rossi et al., 2014b; Lévesque et al., 2016). In this scenario, OLT1177 may emerge as an effective and safe candidate to treat this disease. OLT1177 is an active moiety discovered during the investigation of synthetic reactions containing chlorinating agents and methionine. OLT1177 inhibits the NLRP3 inflammasome, a macromolecular structure needed for the processing and release of IL-1β and IL-18, and has been shown in Phase 1 and 2 clinical trials to be safe when administered orally (and topically) in humans (Marchetti et al., 2018a; Yang et al., 2019).

In the current study, we reported that both oral an intraperitoneal OLT1177 administration exerted protective effects on functional and histological outcomes in EAE mice when given prophylactically. We uncovered that oral administration of OLT1177 to EAE mice decreased the concentration of pro-inflammatory cytokines in the spinal cord and the infiltration of immune cells. Importantly, therapeutic treatment of OLT1177 resulted in protection against neurological decline and demyelination when administered orally.

Previous reports revealed that OLT1177 inhibits specifically the NLRP3 inflammasome, and thus, prevents the autoproteolytic activation of caspase-1, needed for the processing and release of IL-1β and IL-18 (Marchetti et al., 2018a). Caspase-1, together with IL-1β and IL-18, are increased in MS patients (Losy and

Niezgoda, 2001; Nicoletti et al., 2001; Ming et al., 2002; Heidary et al., 2014; Rossi et al., 2014b). Pharmacological or genetic inhibition of either IL-1β or IL-18 protected against the pathogenicity of EAE (Symons et al., 1987; Baker et al., 1991; Shi et al., 2000; Chung et al., 2009; Lévesque et al., 2016) suggesting an important role of NLRP3 in the pathogenesis of EAE. This is further supported by experiments using NLPR3-deficient mice, which displayed reduced EAE severity (Gris et al., 2010). To our knowledge, there are two previous studies demonstrating that the pharmacological inhibition of the NLRP3 inflammasome, using a small molecule tool compound, MCC950, reduced functional impairments in a model of relapsing-remitting EAE when given prophylactically (Coll et al., 2015b; Khan et al., 2018). To this extent, we also showed the efficacy of the prophylactic inhibition of the NLRP3 inflammasome by OLT1177 in a model of chronic EAE, which is a more challenging condition (Thompson, 2017; Tur and Montalban, 2017).

A previous report demonstrated that mice fed an OLT1177-enriched diet reduced the clinical signs of arthritis (Marchetti et al., 2018b). Here, we revealed that EAE mice fed a low dose of OLT1177-enriched food (3.75 g/kg food) displayed enhanced functional and histopathological outcomes in a chronic model of EAE. These effects were likely due to the ability of this drug to reduce NLRP3 inflammasome activation, as revealed the lower protein levels of IL-1β and IL-18 in the spinal cord of EAE mice. These data are consistent with the previous studies demonstrating the ability of this drug to reduce IL-1β and IL-6 levels in human monocyte-derived macrophages stimulated with LPS but also in animal models of arthritis (Marchetti et al., 2018a, 2018b). The low dose of OLT1177 showed greater efficacy in reducing EAE pathogenesis than the high dose when given in the diet and intraperitoneally. However, until the pharmacokinetics results have confirmed exposure levels between the low and high doses of OLT1177, no conclusions can be made regarding a dose response. Notwithstanding, mice fed with low OLT1177 dose in the diet significantly decreased the protein levels of both IL-1β and IL-18 in the spinal cord of EAE mice while the high OLT1177 dose reduced only IL-1β, but not IL-18. We also found that both doses of OLT1177 in the diet led to marked attenuation of the levels of TNFα in the spinal cord parenchyma of EAE mice, but only the low dose reduced significantly the protein levels of IL-6. OLT1177 seems to mediate its helpful effects

by acting preferably in the CNS of EAE mice, since it did not alter cytokine concentrations in the blood. However, until the pharmacokinetics results have confirmed exposure levels between the low and high doses of OLT1177, no conclusions can be made regarding a dose response. Importantly, OLT1177 did not reduced the protein levels of IL-10 in the spinal cord or blood of EAE mice, indicating that this drug is not altering the natural anti-inflammatory mechanisms to contain inflammation.

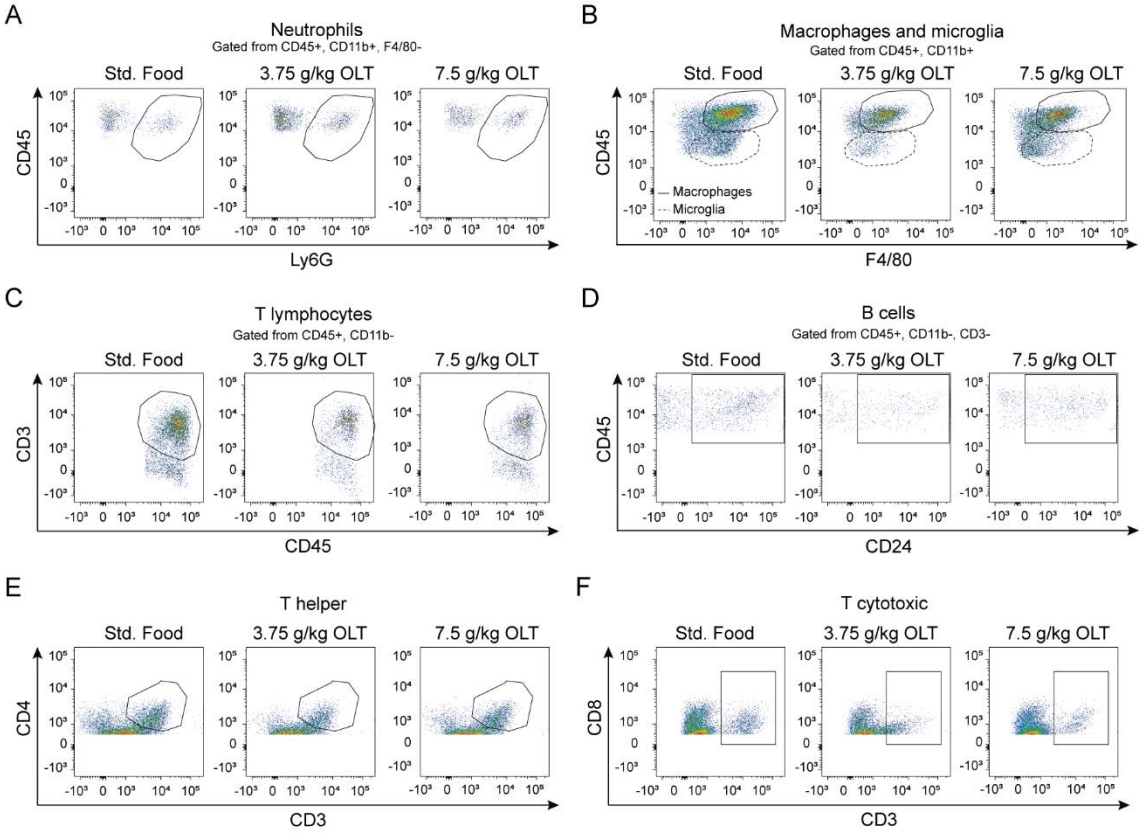
The modulation of the pro-inflammatory milieu in the spinal cord of EAE mice has remarkable outcomes since cytokines play a critical role in the mobilization of immune cells from the periphery, as well as in altering their phenotype (Becher et al., 2017; Caravagna et al., 2018). Indeed, we found a link between the reduction of pro-inflammatory cytokines and decreased infiltration of immune cells into the spinal cord of EAE mice fed the OLT1177-enriched food. CD4 T cells and macrophages populations were specially reduced in the spinal cord by OLT1177. Other immune cell subsets were also affected, such as T cytotoxic lymphocytes, B cells or microglial cells, albeit the reduction was not statistically significant. However, OLT1177-enriched diet did not altered the infiltration of neutrophils, contrary to previous reports on joint inflammation (Marchetti et al., 2018b), and did not modify the phenotype of CD4 T cell or macrophage. In line with our functional and histological results, the dose of 3.75 g of OLT1177 per kg of food resulted in more pronounced effects on the infiltration of immune cells than the higher OLT1177 dose (7.5 g/kg food), which may be directly related to the cytokine levels in the spinal cord. However, until the pharmacokinetics results have confirmed exposure levels between the low and high doses of OLT1177, no conclusions can be made regarding a dose response.

Importantly, we also showed that oral administration of OL1177 reduced the clinical severity of EAE when treatment was initiated after disease onset. In these experiments, OLT1177 was administered by gavage and not directly in the diet because EAE mice show reduced food intake as a consequence of the disease. To our knowledge, this is the first study demonstrating the efficacy of therapeutic treatment in EAE with a selective NLRP3 inhibitor in clinical development, i.e., OLT1177. OLT1177 could have clinical relevance since it mimics a more suitable

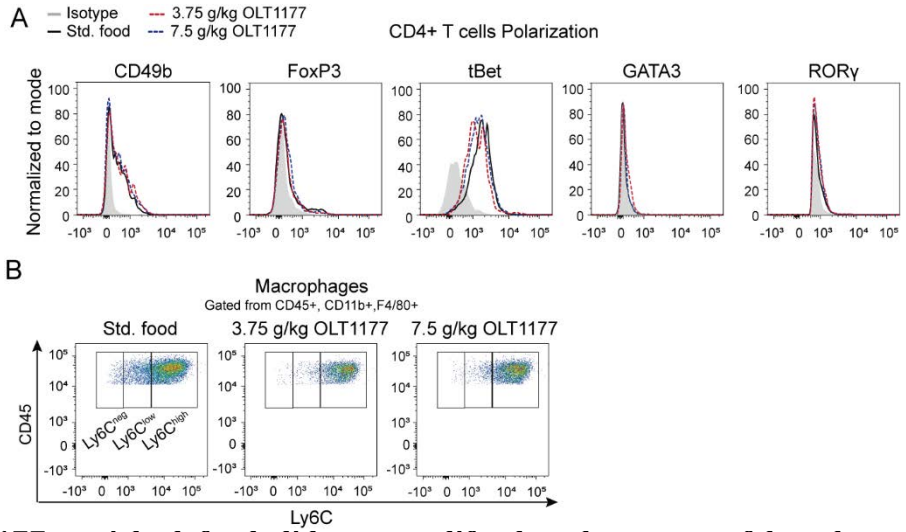
clinical scenario for MS patients. Importantly, oral OLT1177 has satisfactorily overcome a phase I safety trial in humans (Marchetti et al., 2018a) and it is currently in a phase II clinical trials for acute gout flare (Jansen et al., 2019), heart failure and rare disease which could be paving the way to clinical translation.

Overall, our data provide clear evidences that oral administration of OLT1177 exerts potent anti-inflammatory effects in EAE mice by inhibiting NLRP3 inflammasome and mediates beneficial effects when administered prophylactically and therapeutically in this model. This study therefore suggests that OLT1177 may constitute a novel safe and effective approach for the treatment of MS in humans.

SUPLEMMENTARY FIGURES



S1. OLT1177 enriched food reduces the accumulation of immune cells in the spinal cord of mice at the peak of EAE. A-F, Representative dot plots showing neutrophils **(A)**, macrophages and microglia **(B),** T lymphocytes **(C),** B cells **(D),** T helper cells **(E)** and T cytotoxic cells **(F)** in the spinal cord of mice fed the standard food or OLT117 enriched diet (3.75 g/kg or 7.5 g/kg food)



S2. OLT1177 enriched food did not modify the phenotype of lymphocytes nor macrophages in the spinal cord of EAE mice. (A) Representative flow cytometry histograms characterising the expression CD4 T cells polarization markers in the spinal cord of mice fed the standard food or OLT117 enriched diet (3.75 g/kg or 7.5 g/kg food) at EAE disease peak. **(B)** Representative dot plots showing different macrophage subsets in the spinal cord from the same animals based on the expression of Ly6C.

REFERENCES

Amo-Aparicio J, Martínez-Muriana A, Sánchez-Fernández A, López-Vales R (2018) Neuroinflammation Quantification for Spinal Cord Injury. Curr Protoc Immunol:1–15.

Baker D, O'Neill JK, Turk JL (1991) Cytokines in the central nervous system of mice during chronic relapsing experimental allergic encephalomyelitis. Cell Immunol 134:505–510 Available at: http://www.ncbi.nlm.nih.gov/pubmed/1902401 [Accessed February 21, 2019].

Becher B, Spath S, Goverman J (2017) Cytokine networks in neuroinflammation. Nat Rev Immunol 17:49–59 Available at: http://www.nature.com/articles/nri.2016.123 [Accessed February 21, 2019].

Bhise V, Dhib-Jalbut S (2016) Further understanding of the immunopathology of multiple sclerosis: impact on future treatments. Expert Rev Clin Immunol 12:1069–1089 Available at: https://www.tandfonline.com/doi/full/10.1080/1744666X.2016.1191351 [Accessed May 14, 2019].

Caravagna C, Jaouën A, Desplat-Jégo S, Fenrich KK, Bergot E, Luche H, Grenot P, Rougon G, Malissen M, Debarbieux F (2018) Diversity of innate immune cell subsets across spatial and temporal scales in an EAE mouse model. Sci Rep 8:5146 Available at: http://www.nature.com/articles/s41598-018-22872-y [Accessed April 12, 2019].

Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, Ma L, Watowich SS, Jetten AM, Tian Q, Dong C (2009) Critical Regulation of Early Th17 Cell Differentiation by Interleukin-1 Signaling. Immunity 30:576–587 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19362022 [Accessed April 12, 2019].

Coll RC et al. (2015a) A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat Med 21:248–255 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25686105 [Accessed May 14, 2019].

Coll RC et al. (2015b) A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat Med 21:248–255 Available at: http://www.nature.com/articles/nm.3806 [Accessed May 17, 2019].

Conforti-Andreoni C, Ricciardi-Castagnoli P, Mortellaro A (2011) The inflammasomes in health and disease: from genetics to molecular mechanisms of autoinflammation and beyond. Cell Mol Immunol 8:135–145 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21258359 [Accessed May 28, 2019].

Dendrou CA, Fugger L, Friese MA (2015) Immunopathology of multiple sclerosis. Nat Rev Immunol 15:545–558 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26250739 [Accessed May 1, 2019].

Dinarello CA, Simon A, van der Meer JWM (2012) Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov 11:633–652 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22850787 [Accessed May 14, 2019].

Feldmann J, Prieur A-M, Quartier P, Berquin P, Certain S, Cortis E, Teillac-Hamel D, Fischer A, Basile G de Saint (2002) Chronic Infantile Neurological Cutaneous and Articular Syndrome Is Caused by Mutations in CIAS1, a Gene Highly Expressed in Polymorphonuclear Cells and Chondrocytes. Am J Hum Genet 71:198–203 Available at: http://www.ncbi.nlm.nih.gov/pubmed/12032915 [Accessed May 28, 2019].

Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G (2009) The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol 10:241–247 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19221555 [Accessed May 10, 2019].

Grigoriadis N, van Pesch V (2015) A basic overview of multiple sclerosis immunopathology. Eur J Neurol 22:3–13.

Gris D, Ye Z, Iocca HA, Wen H, Craven RR, Gris P, Huang M, Schneider M, Miller SD, Ting JP-Y (2010) NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses. J Immunol 185:974–981 Available at: http://www.ncbi.nlm.nih.gov/pubmed/20574004 [Accessed May 17, 2019].

Heidary M, Rakhshi N, Pahlevan Kakhki M, Behmanesh M, Sanati MH, Sanadgol N, Kamaladini H, Nikravesh A (2014) The analysis of correlation between IL-1B gene expression and genotyping in multiple sclerosis patients. J Neurol Sci 343:41–45 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24867167 [Accessed May 10, 2019].

Hewett SJ, Jackman NA, Claycomb RJ (2012) Interleukin-1β in Central Nervous System Injury and Repair. Eur J Neurodegener Dis 1:195 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4465544/ [Accessed May 14, 2019].

Inoue M, Shinohara ML (2013) NLRP3 Inflammasome and MS/EAE. Autoimmune Dis 2013:859145 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23365725 [Accessed April 17, 2019].

Jansen T, Klück V, Janssen M, Comarniceanu A, Efdé M, Scribner C, Barrow R, Skouras D, Dinarello C, Joosten L (2019) P160 The first phase 2A proof-of-concept study of a selective NLRP3 inflammasome inhibitor, dapansutrile™ (OLT1177™), in acute gout. Ann Rheum Dis 78:A70–A71 Available at: https://ard.bmj.com/content/78/Suppl_1/A70 [Accessed May 13, 2019].

Khan N, Kuo A, Brockman DA, Cooper MA, Smith MT (2018) Pharmacological inhibition of the NLRP3 inflammasome as a potential target for multiple sclerosis induced central neuropathic pain. Inflammopharmacology 26:77–86 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28965161 [Accessed May 10, 2019].

Lassmann H, Brück W, Lucchinetti CF (2007) The immunopathology of multiple sclerosis: An overview. Brain Pathol 17:210–218.

Lévesque SA, Paré A, Mailhot B, Bellver-Landete V, Kébir H, Lécuyer M-A, Alvarez JI, Prat A, Vaccari JP de R, Keane RW, Lacroix S (2016) Myeloid cell transmigration across the CNS vasculature triggers IL-1β–driven neuroinflammation during autoimmune encephalomyelitis in mice. J Exp Med 213:929–949 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27139491 [Accessed February 21, 2019].

Losy J, Niezgoda A (2001) IL-18 in patients with multiple sclerosis. Acta Neurol Scand 104:171–173 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11551238 [Accessed May 10, 2019].

Marchetti C et al. (2018a) OLT1177, a β-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation. Proc Natl Acad Sci 115:E1530–E1539 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29378952 [Accessed May 13, 2019].

Marchetti C, Swartzwelter B, Koenders MI, Azam T, Tengesdal IW, Powers N, de Graaf DM, Dinarello CA, Joosten LAB (2018b) NLRP3 inflammasome inhibitor OLT1177 suppresses joint inflammation in murine models of acute arthritis. Arthritis Res Ther 20:169 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30075804 [Accessed May 13, 2019].

Martinon F, Burns K, Tschopp J (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 10:417–426 Available at: http://www.ncbi.nlm.nih.gov/pubmed/12191486 [Accessed May 14, 2019].

Menu P, Vince JE (2011) The NLRP3 inflammasome in health and disease: the good, the bad and the ugly. Clin Exp Immunol 166:1–15 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21762124 [Accessed May 28, 2019].

Ming X, Li W, Maeda Y, Blumberg B, Raval S, Cook SD, Dowling PC (2002) Caspase-1 expression in multiple sclerosis plaques and cultured glial cells. J Neurol Sci 197:9–18 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11997061 [Accessed May 17, 2019].

Nicoletti F, Di Marco R, Mangano K, Patti F, Reggio E, Nicoletti A, Bendtzen K, Reggio A (2001) Increased serum levels of interleukin-18 in patients with multiple sclerosis. Neurology 57:342–344 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11468327 [Accessed May 10, 2019].

Palle P, Monaghan KL, Milne SM, Wan ECK (2017) Cytokine Signaling in Multiple Sclerosis and Its Therapeutic Applications. Med Sci 5 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29099039 [Accessed May 14, 2019].

Pérez-Cerdá F, Sánchez-Gómez MV, Matute C (2016) The link of inflammation and neurodegeneration in progressive multiple sclerosis. Mult Scler Demyelinating Disord 1:9 Available at: http://msddjournal.biomedcentral.com/articles/10.1186/s40893-016-0012-0 [Accessed May 2, 2019].

Popescu BFG, Lucchinetti CF (2016) Immunopathology. In: Handbook of clinical neurology, pp 95–106 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27112673 [Accessed May 14, 2019].

Rossi S, Motta C, Studer V, Macchiarulo G, Volpe E, Barbieri F, Ruocco G, Buttari F, Finardi A, Mancino R, Weiss S, Battistini L, Martino G, Furlan R, Drulovic J, Centonze D (2014a) Interleukin-1β causes excitotoxic neurodegeneration and multiple sclerosis disease progression by activating the apoptotic protein p53. Mol Neurodegener 9:56 Available at: http://molecularneurodegeneration.biomedcentral.com/articles/10.1186/1750-1326-9-56 [Accessed February 21, 2019].

Rossi S, Motta C, Studer V, Macchiarulo G, Volpe E, Barbieri F, Ruocco G, Buttari F, Finardi A, Mancino R, Weiss S, Battistini L, Martino G, Furlan R, Drulovic J, Centonze D (2014b) Interleukin-1β causes excitotoxic neurodegeneration and multiple sclerosis disease progression by activating the apoptotic protein p53. Mol Neurodegener 9:56 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25495224 [Accessed April 13, 2019].

Shi F-D, Takeda K, Akira S, Sarvetnick N, Ljunggren H-G (2000) The Journal of Immunology. J Immunol 157:3223–3227 Available at: http://www.jimmunol.org/content/165/6/3099 [Accessed May 10, 2019].

Strowig T, Henao-Mejia J, Elinav E, Flavell R (2012) Inflammasomes in health and disease. Nature 481:278–286 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22258606 [Accessed May 14, 2019].

Symons JA, Bundick R V, Suckling AJ, Rumsby MG (1987) Cerebrospinal fluid interleukin 1 like activity during chronic relapsing experimental allergic encephalomyelitis. Clin Exp Immunol 68:648–654 Available at: http://www.ncbi.nlm.nih.gov/pubmed/3498582 [Accessed February 21, 2019].

Ting JP-Y, Kastner DL, Hoffman HM (2006) CATERPILLERs, pyrin and hereditary immunological disorders. Nat Rev Immunol 6:183–195 Available at: http://www.ncbi.nlm.nih.gov/pubmed/16498449 [Accessed May 28, 2019].

Wagner CA, Goverman JM (2015) Novel Insights and Therapeutics in Multiple Sclerosis. F1000Research 4:517 Available at: /pmc/articles/PMC4544373/?report=abstract.

Wu GF, Alvarez E (2011) The immunopathophysiology of multiple sclerosis. Neurol Clin 29:257–278 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21439440 [Accessed February 22, 2018].

Yang Y, Wang H, Kouadir M, Song H, Shi F (2019) Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis 10:128 Available at: http://www.nature.com/articles/s41419-019-1413-8 [Accessed May 10, 2019].

CHAPTER 2

IL-37 exerts therapeutic effects in experimental autoimmune encephalomyelitis

IL-37 exerts therapeutic effects in experimental autoimmune encephalomyelitis

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ABSTRACT

Interleukin 37 (IL-37) is one of the eleven members of the IL-1 family and broadly suppresses innate inflammation and acquired immunity. IL-37 mediates its biological effects by acting as dual-function cytokine in that IL-37 signals via the extracellular receptor complex IL-18Rα/SIGIRR, but also translocates to the nucleus. We recently demonstrated that IL-37 exerts potent anti-inflammatory actions in the spinal cord after traumatic injury and ameliorates functional deficits. However, whether IL-37 has similar beneficial actions in neuroinflammatory diseases remains to be elucidated. Here, we investigated the role of IL-37 in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS). We demonstrated that transgenic expression of IL-37 protected against neurological deficits and myelin loss, and markedly reduced the inflammatory response. We also found that the components of the extracellular receptor for IL-37 were expressed in the spinal cord in physiological conditions and during EAE, and that the beneficial effects of IL-37 in EAE were completely lost in the lack of its extracellular activity. We also uncovered that the IL-37 surface receptors were expressed in peripheral blood mononuclear cells and in the brain white matter from MS patients but IL-37 was not efficiently induced. Remarkably, we demonstrated that administration of various forms of recombinant human IL-37 protein exerted potent therapeutic actions in EAE mice when treatment was initiated at disease onset. Taking together, this study presents novel data indicating that IL-37 exert anti-inflammatory and therapeutic actions in EAE by functioning as a extracellular mediator, and that this natural mechanism to prevent inflammation is defective in MS patients.

INTRODUCTION

MS is the most common demyelinating disease of the CNS and affects more than 2.5 million individuals worldwide (Wekerle, 2008). The etiology of MS has not been defined yet, but it is thought to be autoimmune due to the presence of T cells directed against antigens detected in the myelin sheaths. Moreover, the presence of autoantibodies to myelin antigens in the cerebrospinal fluid of MS patients suggests an autoimmune ethiology (Noseworthy et al., 2000).

IL-1 family includes some prototypic cytokines that promote acute and chronic inflammation in a broad spectrum of diseases (Dinarello et al., 2012; Hewett et al., 2012). The IL-1 family consists of 11 members: 7 of them have pro-inflammatory properties (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β and IL-36γ) and 3 more members act as receptors antagonists, blocking the pro-inflammatory signalling of IL-1 and IL-36 (Dinarello, 2018). The last member, IL-37, is the only cytokine from this family that exerts potent suppressive actions on innate (Nold et al., 2010a) and adaptive immunity (Luo 2014). IL-37 exert anti-inflammatory actions by acting as a ligand for the extracellular receptor complex IL-18Rα/SIGIRR (Nold-Petry et al., 2015a), as well as, by translocating to the nucleus via caspase-1 cleavage and binding to SMAD3 (Bulau et al., 2014; Li et al., 2019).

Unlike other members of the IL-1 family, the open read frame for the IL-37 homolog is lacking in the mouse, and thus, it was necessary to generate a knock in mouse expressing human *IL-37b* (hIL-37tg) to investigate the role IL-37 in animal models of disease. hIL-37tg mice have shown resistant against many pathological conditions, such as colitis (McNamee et al., 2011a), obesity-induced inflammation (Ballak et al., 2014) or candidiasis (van de Veerdonk et al., 2015). Besides, treatment with recombinant human IL-37 protein showed also anti-inflammatory actions after aspergillosis (Moretti et al., 2014) or rheumatoid arthritis (Moretti et al., 2014; Cavalli et al., 2016).

Little is known about the role of IL-37 in the central nervous system. We previously reported that IL-37 exerted potent anti-inflammatory action after spinal cord contusion injury in mice. Importantly, hIL-37tg mice displayed significant resistance against functional deficits and demyelination after spinal cord injury (Coll-Miró et

al., 2016). However, whether IL-37 has similar therapeutic effects in neuroinflammatory conditions, such as MS, has not been addressed yet.

In the present study, we investigated whether IL-37 mediates anti-inflammatory and therapeutic actions in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. We demonstrated that transgenic expression of IL-37 mediated potent anti-inflammatory actions in EAE and protected mice from functional deficits and demyelination and that the beneficial effects of IL-37 in EAE are dependent on its extracellular function. We also revealed that this natural anti-inflammatory mechanism is dysregulated in MS patients. Importantly, administration of recombinant human IL-37 protein markedly reduced EAE pathogenesis. These findings revealed that IL-37 could be employed as a new approach for the treatment of MS.

MATERIALS AND METHODS

Human Samples

To assess gene expression, we used post-mortem MS patients fresh-frozen brain shaves from active lesions and normal apparent white matter (NAWM).

To analyse gene expression from human peripheral blood mononuclear cells (PBMCs) we used blood samples from MS patients and healthy controls.

All the human samples were kindly provided by Alexandre Prat.

Mice

All experimental procedures were approved by the Universitat Autònoma de Barcelona Animal Experimentation Ethical Committee (CEEAH 2878) and followed the European Communities Council Directive 2010/63/EU, and the methods were carried out in accordance with the approved guidelines.

Experiments were performed in adult (8-10 weeks) females or males C57Bl/6 mice (Charles River), transgenic mice expressing human *IL-37b*) in homozygosis (hIL-37tg), *sigirr* deficient mice (*sigirr* KO), hIL-37tg-sigirr KO mice, and their respective wildtype littermates. Mice were randomly distributed in groups of 5-6 mice per cage. All the mice were housed with food and water *ad libitum* at room temperature of 22±2ºC under 12:12h light-dark cycle.

EAE Induction

Mice were sedated with intramuscular injection of mixture of ketamine (22mg/kg) (Imalgen 1000, Merial) and xylazine (2.5mg/kg) (Rompun, Bayer). EAE was actively induced by subcutaneously injection in each flank at the base of the tail of 100µl of 3mg/mL of myelin oligodendrocyte glycoprotein peptide 35-55 (MOG_{35-55}) in Complete Freund's Adjuvant (CFA) (Difco, Detroit, MI, USA) supplemented with 4mg/mL of heat inactivated *Mycobacterium tuberculosis* (Difco, Detroit, MI, USA).

Immediately after induction, and on day 2, mice received an intraperitoneal (i.p.) injection of 400ng of pertussis toxin (PTX) in 100µl sterile saline (0.9% NaCl).

EAE Clinical Signs Evaluation

Mice were daily scored from day 0 to day 21 after induction of EAE. The researcher was blind to experimental groups during the functional evaluation. A 6-point scale was used to evaluate the clinical signs of EAE: 0=normal walking, 0.5=partially paralyzed tail, 1=fully paralyzed tail, 2=mild hind limb weakness, quick righting reflex, 3=severe hind limb weakness, slow righting reflex, unable to bear weight, 3.5=severe hind limb weakness and partial paralysis of hind limb, 4=complete paralysis of at least one hind limb, 4.5=complete paralysis of one or both hind limbs and trunk weakness, 5=complete paralysis of one or both hind limbs, forelimb weakness or paralysis, 6=mouse is found death by EAE.

Recombinant human IL-37 protein administration

Female C57Bl6/J mice were treated with daily injections (i.p) of 1µg of native IL-$37^{46\text{-}218}$ or the mutated monomeric forms IL-37^{D73K} or IL-37^{Y85A} in 200 µl of saline. The different recombinant human IL-37 protein forms were administered when mice showed the first signs of the EAE disease, and then daily until the end of the follow up. Control mice were treated with 200 µl of saline following the same protocol.

Real-time Quantitative PCR Assay (qPCR)

Total RNA was isolated from postmortem human brain active lesion and NAWM, as well as, from spinal cords harvested of EAE mice harvested at different stages of the disease, using the RNeasy Lipid Tissue Mini Kit (Qiagen) following the manufacturer's procedures. Total RNA was also extracted from PBMCs collected from MS patients and healthy donors using the QIAamp RNA Blood Tissue Mini Kit (Qiagen) according to the guide protocol.

RNA was retro-transcribed using the cDNA Reverse Transcription Kit (Applied Biosystems). cDNA human libraries were analysed using a Bio-Rad CFX384 (CFX Manager V3.1) and TaqMan-designed primers (ThermoFisher Scientific) for the following human and mouse genes: *IL-37b* (Hs00367201_m1), *SIGIRR* (Hs00222347_m1), *IL-18Rα* (Hs00187256_m1), *GAPDH* (Hs02786624_g1), *gapdh* (Mm03302249_g1), *sigirr* (Mm01275624_g1), *il-18rα* (Mm00516053_m1). GAPDH/*gapdh* was used as a housekeeping gene for human and mouse samples, respectively. All data were analysed using the $2^{\Delta\Delta}$Ct method.

Histological analysis

Female hIL-37tg mice and WT littermates were euthanised at 21 days post induction with and overdose of pentobarbital sodium (Dolethal) and, then, transcardially perfused with 4% paraphormaldehyde (PFA) in 0.1M of phosphate buffer (PB). Lumbar segments of spinal cord were harvested, post-fixed in 4% PFA for 2 hours in ice and cryoprotected in 30% sucrose in 0.1M PN at 4ºC for at least 48 hours. Then, spinal cords were embedded in Tissue-Tek®OTC (Sakura, Japan), cut in transversal sections (15μm-thick) with a cryostat (Leica) between L3 and L5 segments and serially picked up on gelatine-coated glass slides. Samples were stored at –20ºC.

Sections were stained with Luxol Fast Blue (LFB) (Sigma Aldrich). Briefly, after a graded dehydration, sections were placed in 1mg/mL of LFB solution in 96% ethanol and 0.05% acetic acid overnight at 37ºC and protected from light. Then, slides were washed with 96% ethanol, rehydrated with distilled water and placed in a 0.5mg/mL Li_2CO_3 solution in distilled water for 3-5 minutes at room temperature. Finally, sections were washed in distilled water, dehydrated again and mounted in DPX mounting medium (Sigma Aldrich). To assess the demyelinated area in the spinal cords, 6 random images per spinal cord were captured at 10X magnification with an Olympus BX51 and the attached Olympus DP73 Camera. The total demyelinated area in the spinal cord was measured with Image J image analysis software.

Cytokine Protein Expression Assessment

Female hIL-37tg mice and WT littermates were euthanised 3 days after the onset of EAE with an overdose of pentobarbital sodium (Dolethal) and transcardially perfused with 60 mL of sterile saline (0.9% NaCl). Then, spinal cords were harvested and rapidly freeze to −80ºC with liquid nitrogen. To assess the protein levels of cytokines, spinal cord were processed as it is detailed in Amo-Aparicio et al. 2018. Briefly, tissue was homogenized in HEPES and then protein concentration was determined with a DC Protein Assay (Bio-Rad). Protein homogenates were concentrated to 4μg/μl and finally, protein levels of IL-4, IL-10, IL-1α, IL-1β, IL-6, TNF-α, IFNγ, IL-17A, CSF-3, CCL-5, CCL-2, CXCL-2 and CXCL-10 were analysed using a custom-designed Milliplex Cytokine Magnetic Bead Panel on a MAGPIX system (Affymetrix, eBioscience).

Fluorescence Activated Cell Sorting (FACS)

Female hIL-37tg mice and WT littermates were euthanised at peak of EAE with and overdose of pentobarbital sodium (Dolethal). To analyze the different immune cells, the spinal cord and lymph nodes tissues processed as we previously described in Amo-Aparicio et al. 2018. Briefly, these tissues were cut in small pieces and enzymatically dissociated incubating them 30 minutes at 37ºC in 1mL of HBSS without Ca^{2+}/Mg^{2+} and 0.1% collagenase and 0.1% DNase. Then, samples were mechanically disintegrated by passing them with DMEM-10% FBS through a 70μm cell strainer. After two washes, cell suspension was split into different 1.5mL microcentrifuge tubes according to the number of antibody combinations. For extracellular staining, samples were incubated with the following primary antibodies from eBioscience for 1h at 4ºC in soft agitation at a 1:300 concentration: CD45-PerCP, CD11b-PE or PE-Cy7, F4/80-PE or -APC, Ly6C-FITC, Ly6G-PE, CD3-FITC-APC-PerCP; CD4-APC-Cy7, CD8-APC, CD49b-PE, CD24-PE. Samples were then fixed with 1% PFA. For intracellular staining, cells were fixed with 1% PFA and permeabilizated with Permeabilization Wash Buffer (Biolegend), except for those stained against FOXP3, which were permeabilizated using FoxP3 Transcription Factor Staining Buffer Set (eBioscience). Samples were them immunostained with

the intracellular antibodies (eBioscience) for 1h at 4ºC and incubated for 45 minutes. FoxP3- PE-Cy7, tBet-PerCP, RORγ-APC, GATA3-PE. Then these samples were stained at room temperature with secondary antibodies as described above. Samples were finally analysed on a FACS Canto Flow Cytometer (BD Bioscience) and all data were processed using FlowJo® software V.10.

Statistical analyses

Data are shown as mean ± standard error of the mean (SEM). Paired or unpaired Student's t test was used for the comparison between two different groups (human qPCR, histological analysis, cytokine levels, accumulation of immune cells). Mean clinical score follow-up was analysed by using two-way ANOVA repeated measures with *post-hoc* Bonferroni's test for multiple comparisons. Differences were considered significant at $p<0.05$.

RESULTS

Transgenic expression of IL-37 reduces neurological deficits and protects against demyelination in the spinal cord of EAE mice.

Since the role of IL-37 in demyelinating diseases of the CNS is still poorly understood, we first sought to explore whether this cytokine exerted beneficial effects in EAE mice. Since the open frame of the IL-37 orthologue is not present in rodents, we induced EAE in hIL-37tg mice and wildtype littermates (WT). We found that IL-37 transcripts were detected at very low levels in the spinal cord of hIL-37tg at physiological conditions, but they were significantly increased (~7 fold) at the peak and chronic phase of the disease, but not at the onset (Fig. 1A).

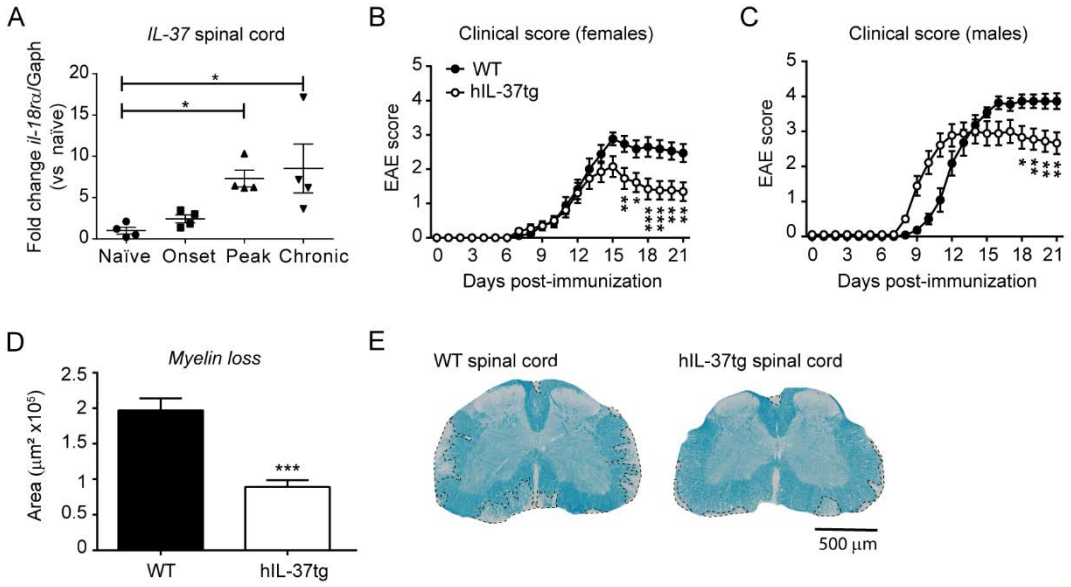


Figure 1. The transgenic expression of *IL-37* protects against functional deficits and myelin loss in the spinal cord of mice with EAE. (A) Graph showing the levels of *IL-37* mRNA in the spinal cord of hIL-37tg mice at different phase of EAE disease**. (B)** Clinical score of females and **(C)** male hIL-37tg mice and WT littermates. **(D)** Graph showing the quantification of the area of demyelination in the spinal cord of hIL-37tg and WT littermates at 21 days post induction. **(E)** Representative images of lumbar spinal cord from hIL-37tg mice and WT littermates at day 21 after EAE induction. *p<0.05; **p<0.01; ***p<0.001 vs. control. One-way ANOVA with Bonferroni *post hoc* test in **A** (n=4 per time point); Two-way repeated measures ANOVA with Bonferroni's *post hoc* test in **B** (n=15 WT; n=14 hIL-37tg) and **C** (n=9 per group); Unpaired t-test in **D** (n=15 WT; n=14 hIL-37tg). Data shown as mean±sem.

Knowing that the expression of IL-37 was successfully induced in the spinal cord upon EAE induction, we next evaluated its contribution to disease. We demonstrated that female hIL-37tg mice showed significant reduced functional

deficits compared to WT mice (Fig. 1B). Indeed. At the end of the follow up hIL-37tg female mice showed a reduction of about 1.5 points in the EAE clinical score. Similar beneficial effects were found in male hIL-37tg mice upon EAE induction. However, the onset of EAE disease occurred significantly earlier in male mice expressing IL-37 (Fig. 1C).

We then evaluated whether the enhancement in functional outcomes of female hIL-37tg mice after EAE induction was linked to myelin preservation. Histological analysis of lumbar spinal cords harvested at the end of the follow up (21 days post induction) revealed that the area of demyelination was decreased ~50% in the hIL-37tg mice (Fig 1D-E).

IL-37 attenuates inflammation in mice with EAE

We next studied whether IL-37 mediated suppressive actions on inflammation in EAE. For this purpose, we first measured the protein levels of several cytokines in the spinal cord at day three after disease onset. Luminex assay revealed that transgenic expression of IL-37 significantly reduced the protein levels of 5 pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, TNF-α and IFNγ), all of them linked to development of EAE pathophysiology (Figure 2A). Since cytokines coordinate the recruitment and activation of immune cells into the CNS parenchyma, we next evaluated whether transgenic expression of IL-37 reduced the accumulation of immune cells in the spinal cord at the disease peak. Flow cytometry analysis showed that hIL-37tg mice had significant lower counts of T cells (CD3+), including CD4 and CD8 T cells, macrophages (CD45high, CD11b+, F4/80+) and activated microglial cells (CD45low, CD11b+, F4/80+) than WT mice (Figure 2B, Supplementary figure S1A, S1C-E). IL-37, however, did not attenuated the number of B cells (CD45+, CD11b–, CD3–, CD24+) and neutrophils (CD45+, CD11b+, F4/80–, Ly6G+) (Figure 2B, Supplementary figure S1B, S1F). Highlight that transgenic expression of IL-37 reduced ~2 fold the proportion of pathogenic Th1 Cells (CD3+CD4+tBet+) and increased the proportion of classical regulatory CD4 cells (CD3+CD4+FoxP3+) in the spinal cord (Figure 2C, Supplementary figure S2A). Moreover, IL-37 also reduced

the proportion of CD16/32+ and iNOS+ macrophages ~20% (Figure 2D, Supplementary figure S2B), although it did not alter the expression of Ly6C in macrophages (Figure 2E, Supplementary figure S2C). These data suggest that IL-37 not only reduced the counts of immune cells in the spinal cord but also it modulated their phenotype towards a more anti-inflammatory state.

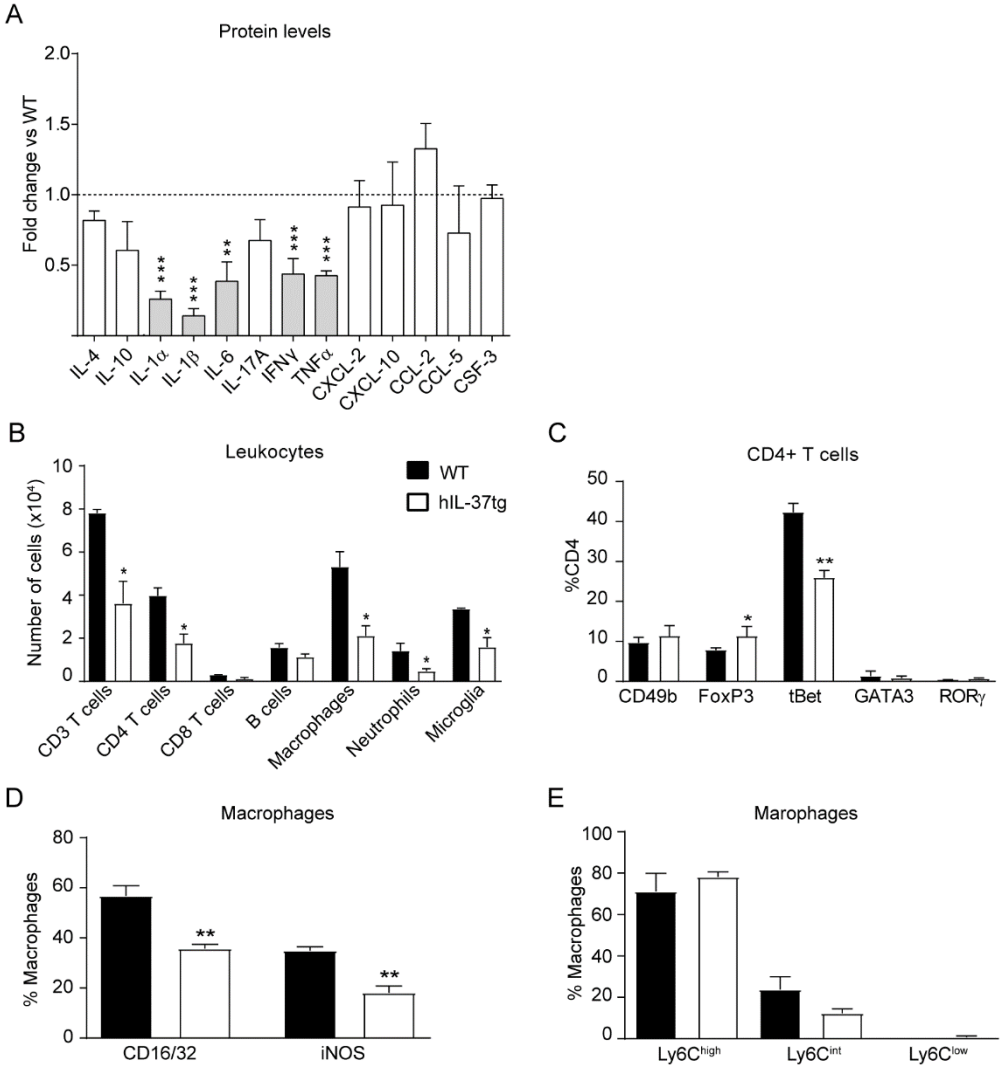


Figure 2. IL-37 reduces the inflammatory response in the spinal cord of mice at the peak of EAE. (A) Protein level profile of cytokines (IL-4, IL-10, IL-1α, IL-1β, IL-6, IL-17A, IFNγ, TNFα) and chemokines (CXCL-2, CXCL-10, CCL-2, CCL-5, CSF-3) in the spinal cord of hIL-37tg relative to WT at the peak EAE. **(B)** Number of infiltrated immune cells and **(C)** percentage of CD4+ T cells expressing the transcription factors CD49b, FoxP3, tBet, GATA3 or RORγ in the spinal cord of WT and hIL-37tg mice at the peak of EAE**. (D)** Percentage of macrophages expressing the pro-inflammatory markers CD16/32 and iNOS or **(E)** Ly6C in the spinal cord of WT and hIL-37tg mice at the peak of EAE. *p<0.05; **p<0.01; ***p<0.001 vs. WT. Unpaired t-test (n=4 per group). Data shown as mean±sem.

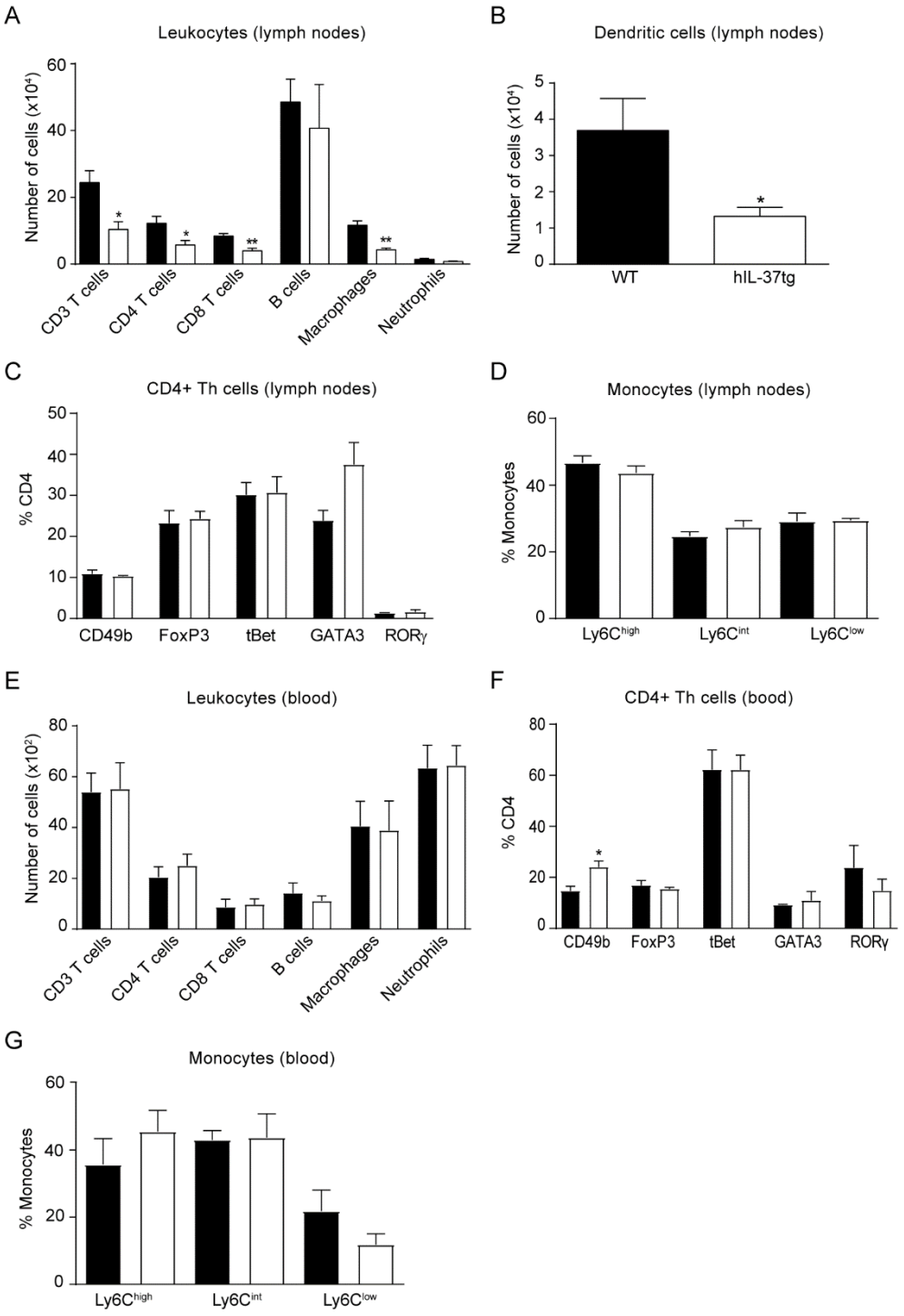


Figure 3. IL-37 attenuates the proliferation of immune cells in the lymph nodes of transgenic mice at the peak of EAE. (A) Number of immune cells in the lymph nodes and **(E)** blood of WT and hIL-37tg mice at the peak of EAE. **(B)** Number of dendritic cells in the lymph nodes. **(C)** Percentage of CD4+ T cells expressing the transcription factors CD49b, FoxP3, tBet, GATA3 or RORγ in lymph nodes and **(F)** blood. **(D)** Percentage of monocytes expressing Ly6C in lymph nodes and **(G)** blood. *p<0.05; **p<0.01; ***p<0.001 vs. WT. Unpaired t-test (n=4 per group). Data shown as mean±sem.

We then assessed whether the reduced accumulation of immune cells in the spinal cord of hIL-37tg mice in EAE was due to a central or peripheral effect of this cytokine. With this aim, we first assessed the immune cell counts in the lymph nodes and blood at EAE disease peak. Similar to the spinal cord, flow cytometry analysis demonstrated that hIL-37tg mice had significant reduced count of CD4 T cells in the lymph nodes (Figure 3A), suggesting that this cytokine attenuated the expansion of this T cell subset. However, IL-37 did not altered polarization of this CD4 T cells (Figure 3C). IL-37 also reduced the number of macrophages and dendritic cells (CD45+, CD11b+, CD11c+) (Figure 3B), which is compatible with reduce T cell priming observed in the spinal cord (Figure 2C). The proportion of monocytes expressing Ly6C remained unaltered in lymph nodes (Figure 3D). In the blood samples, we found that IL-37 did not decreased the numbers of the different leukocyte population (Figure 3E), but importantly, increased the proportion of non-classical regulatory CD4 T cells (Figure 3F). The proportion of monocytes expressing Ly6C remained unaltered in blood (Figure 3G).

Extracellular function of IL-37 is critical for mediating beneficial effects in EAE

As reported before, IL-37 is a dual-function cytokine that can mediate its biological effects by acting as a extracellular cytokine (Nold-Petry et al., 2015b) or by translocating into the nucleus (Nold et al., 2010a). Here, we the sought to uncover the importance of the extracellular function of IL-37 in EAE.

For this purpose, we firstly characterized the expression of IL-37 receptor complex in the spinal cord in different phases of EAE. We found that both components, *il-18rα* and *sigirr*, were expressed in the CNS at physiological conditions and over EAE disease progression (Figure 4A and 4B), although *il-18rα* transcripts significantly increased in the peak and chronic phase of EAE.

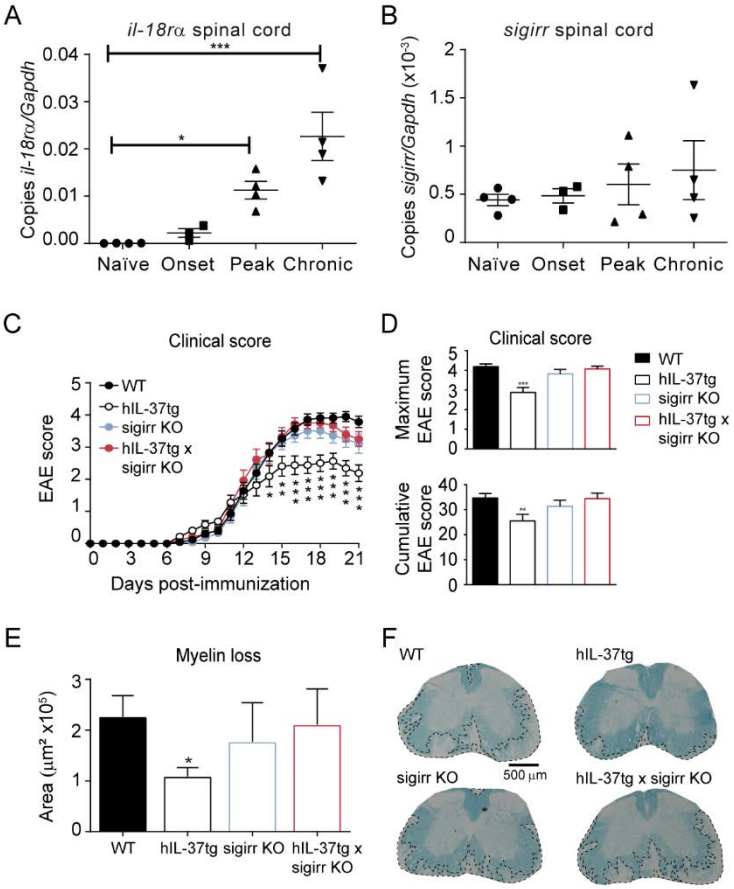


Figure 4. The protective role of IL-37 against EAE is mediated by its extracellular pathway. A-B, Graphs showing the relative expression the *il-18rα* **(A)** and *sigirr* **(B)** in the spinal cord of mice undergoing EAE compare to naïve. **C-D,** Clinical score of WT, hIL-37tg, *sigirr* KO and hIL-37tg x *sigirr* KO mice showed as mean clinical score **(C)** and as maximum and cumulative score **(D)**. **E-F,** Graph showing the quantification of demyelinating areas in the spinal cord of WT, hIL-37tg, *sigirr* KO and hIL-37tg x *sigirr* KO mice at 21 days post-induction **(E)** and representative images of these spinal cords **(F)**. *p<0.05; **p<0.01; ***p<0.001 vs. WT. One-way ANOVA in **A** and **B** (n=3 in onset and n=4 in the other groups). Two-way ANOVA with repeated measures, Bonferroni's *post hoc* test in **C** (n=21 in WT, n=16 in hIL-37tg, n=14, n=14 in *sigirr* KO and n=14 in hIL-37tg x *sigirr* KO). One-way ANOVA in **D** (n=21 in WT, n=16 in hIL-37tg, n=14 in *sigirr* KO and n=14 in hIL-37tg x *sigirr* KO) and **E** (n=20 in WT, n=16 in hIL-37tg, n=13 in *sigirr* KO and n=13 in hIL-37tg x *sigirr* KO). Data shown as mean±sem.

Knowing that the components of the IL-37 receptor complex are expressed in the spinal cord we aimed at evaluating to what degree the extracellular function of IL-37 is important to mediate its beneficial effects in EAE. For this purpose, we crossed hIL-37tg mice with *sigirr* KO mice. This double transgenic mouse expresses the human form of *IL-37b* in homozygosis, but this cytokine only has nuclear functions since it lacks one of the components of IL-37 receptor complex. EAE induction in *sigirr* KO mice revealed that the lack of this receptor does not alter EAE progression. However, the beneficial effects of IL-37 were completely lost in the lack *sigirr*

(Figure 4C-D). In line with functional data, histological analysis revealed that the protective actions of IL-37 against demyelination were abrogated in the absence of *sigirr* (Figure 4E-F). Thus, these experiments provide clear evidence that the extracellular function of IL-37 is crucial to confer protection against EAE.

IL-37 is not induced in MS patients

Since IL-37 plays a beneficial role in EAE, we next study whether this anti-inflammatory mechanism was present in MS patients. For this purpose, we assessed the expression of IL-37 and its extracellular receptors in PBMCs of MS patients and healthy controls, as well as, in active lesions of post-mortem MS patients brain samples.

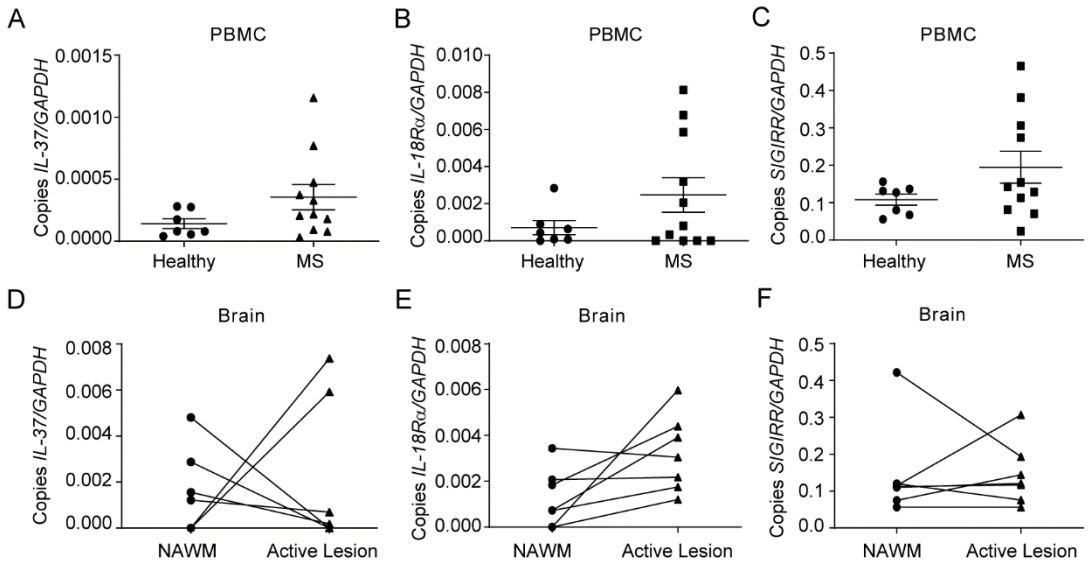


Figure 5. Although *IL-37* mRNA is barely detected in patients of MS, its extracellular receptors are expressed. (A-C) Graphs showing the expression of IL-37 and its extracellular receptors in PBMCs and **(D-F)** brain in patients of MS and healthy controls. Unpaired t-test in **A, B** and **C** (n=7 in healthy controls, n=11 in MS). Paired t-test in **C, D** and **E** (n=6 per group). Data shown as mean±sem.

qPCR analysis revealed that *IL-37* transcripts were barely detectable in PBMC from healthy controls, and that the levels of this anti-inflammatory cytokine were not increased in MS patients (Figure 5A), despite the components of its receptors were expressed (Figure 5B-C). Similarly, *IL-37* was detectable at very low levels in NAWM of MS brains. Strikingly, IL-37 levels tended to decrease in the brain active lesions,

except in two of the patients who showed a marked increase in the transcripts of this cytokine (Figure 5D). In line with the PBMC data, *IL-18Rα* and *SIGIRR* were also expressed in the NAWM of brain MS samples, and that the levels of *IL-18Rα* tended increased in active lesions (Figure 5E-F). These data reveal that the components of the IL-37 receptors are present in the PBMC and brain of MS individuals but IL-37 is deficiently induced.

Administration of recombinant human IL-37 protein reduces neurological deficits and demyelination in EAE mice

To study whether IL-37 may have therapeutic potential in MS, we administered recombinant human IL-37 protein in EAE mice, starting at disease onset. Since IL-37 can form dimers, which have reduced anti-inflammatory actions (Ellisdon et al., 2017; Eisenmesser et al., 2019), we tested the efficacy of the native IL-37 (IL-37^{46-218}) but also two mutant monomeric forms (IL-37^{D73K} and IL-37^{Y85A}) that cannot dimerize. We found that daily administration of 1μg of native IL-37^{46-218}, IL-37^{D73K} or IL-37^{Y85A} from the onset of EAE enhanced functional outcomes and protected against demyelination (Fig. 6A-D). Among the three recombinant IL-37 proteins, the mutant monomeric form IL-37^{D73K} showed the greatest efficacy at both, functional and histological level, although this did not reach statistical significance compared to IL-37^{46-218} and IL-37^{Y85A} (Fig. 6A-D). Overall, these data support the therapeutic actions of exogenous administration of recombinant human IL-37 protein in in EAE.

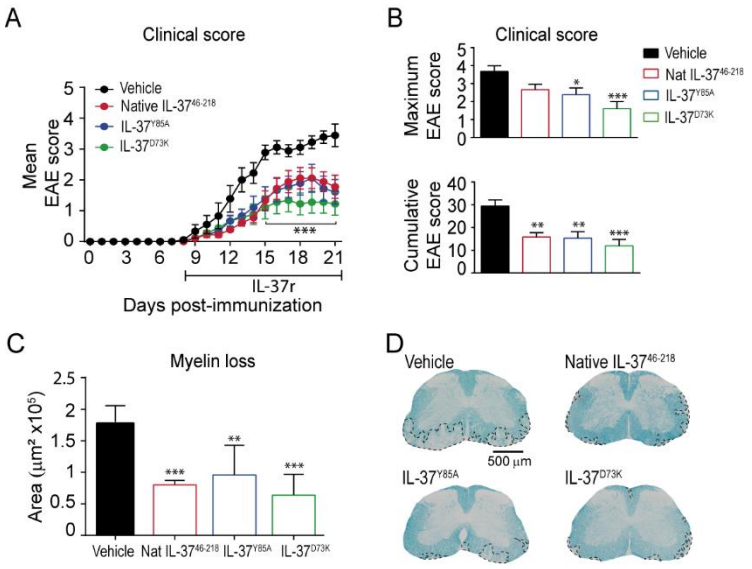


Figure 6. The administration of IL-37r protein protects against neurological impairments and myelin loss in the spinal cord in EAE mice. (A) Clinical score of mice treated with IL-37$^{46-218}$, IL-37^{D73K}, IL-37^{Y85A} or vehicle showed as mean clinical score and as **(B)** maximum and cumulative score. **(C)** Graph showing the quantification of demyelinating areas in the spinal cord of mice treated with native IL-37$^{46-218}$, IL-37^{D73K}, IL-37^{Y85A} or vehicle and **(D)** representative images of these spinal cords. *p<0.05; **p<0.01; ***p<0.001 vs. vehicle. Two-way ANOVA with repeated measures, Bonferroni's *post hoc* test in **A** (n= 9 per group in vehicle, IL-37^{D73K} and IL-37^{Y85A} and n=10 in native IL-37$^{46-218}$). One-way ANOVA in **B** and **C** (n= 9 per group in vehicle, IL-37^{D73K} and IL-37^{Y85A} and n=10 in native IL-37$^{46-218}$).

DISCUSSION

In this study we investigated whether the anti-inflammatory cytokine, IL-37, was able to enhance the physiopathology of EAE. We reported that IL-37 ameliorated the clinical signs of EAE and protected from demyelination. We also revealed that IL-37 suppressed the inflammatory response by reducing the protein levels of different pro-inflammatory cytokines in the spinal cord. Accordingly, IL-37 reduced the counts of CD4 T cells, macrophages and dendritic cells in the lymph nodes, and attenuated the accumulation of most of the immune cells populations into the CNS. Moreover, IL-37 switched the phenotype of CD4 T cells and macrophages in the CNS resulting in more regulatory and less inflammatory cells. We also described that the beneficial actions of IL-37 were mediated by signalling via the surface receptor complex *il-18rα-sigirr*. We demonstrated that this protective mechanism to attenuate inflammation is aberrant in MS patients. Importantly, we also provided clear evidence that administration of recombinant human IL-37 protein in EAE mice alleviated the symptomatology of the disease and protected against demyelination.

Central aspects concerning the pathogenesis of MS have become highly debated in recent times, but demyelination and further neurodegeneration have an undeniable link to inflammation, as it also occurs in EAE (Constantinescu et al., 2011; Pérez-Cerdá et al., 2016; Caravagna et al., 2018). Thus, braking the inflammation to avoid neurological damage is still in the spotlight for MS treatments.

IL-37 is a member of the IL-1 family that exert potent anti-inflammatory action (Nold et al., 2010b; Cavalli and Dinarello, 2018). Although this cytokine is not present in mice, the development of a transgenic mouse that expresses the human form of IL-37 and the use of recombinant IL-37 protein have consistently demonstrated its protective properties from a myriad of immunological challenges, such as colitis (McNamee et al., 2011b), metabolic syndrome and type 2 diabetes (Ballak et al., 2014), lung inflammation (Moretti et al., 2014), cancer (Henry et al., 2015), arthritis (Ye et al., 2015), calcific valve disease (Zeng et al., 2017) and spinal cord injury (Coll-Miró et al., 2016).

In hIL-37tg mice, IL-37 transcription is artificially regulated by a cytomegalovirus promoter, which is constitutively expressed in all the cells (Nold et al., 2010b).

However, IL-37 is not as broadly expressed as expected in the mouse due to the presence of an instability sequence in the mRNA that limits its half-life. Nonetheless, under inflammatory conditions the IL-37 mRNA is stabilized and, consequently, the translation of the protein is allowed (Bufler et al., 2004). In this line, and similar to a previous work (Coll-Miró et al., 2016), we found that IL-37 mRNA is present at very low levels in the spinal cord of hIL-37tg mouse at physiological conditions, but their levels are markedly upregulated in EAE. Indeed, IL-37 mRNA reached the maximum expression at the peak of EAE, when leukocytes are highly accumulated in the spinal cord (Caravagna et al., 2018). Highlight that IL-37 expression was associated to reduced neurological impairments and demyelination of EAE mice, supporting the beneficial action of IL-37 in neuroinflammatory conditions, as we previously demonstrated in spinal cord injury (Coll-Miró et al., 2016).

It is well stablished that the cytokines produced by the initial wave of CNS-infiltrating leukocytes are the dominant force that orchestrates the following inflammatory cascade in MS and EAE (Grigoriadis and van Pesch, 2015; Becher et al., 2017). These cytokines play a key role in the recruitment and activation of more immune cells from the circulation, as well as, in the triggering glial reactivity (Becher et al., 2017), and thus, are key contributors to MS pathology. In this line, cytokines appeared among the most associated risk factor genes for MS in genome-wide association study (Sawcer et al., 2011).

Here we report that transgenic expression of IL-37 silenced several pro-inflammatory cytokines in the spinal cord that have a key contribution to EAE and MS pathology (Symons et al., 1987; Baker et al., 1991; Schrijver et al., 2003; Rossi et al., 2014a; Lévesque et al., 2016). (Eugster et al., 1998) (Kallaur et al., 2013). The effects of IL-37 on cytokine levels was associated to reduced infiltration of immune cells into the spinal cord at the peak of the disease. This is quite relevant since leukocytes accumulate in demyelinating areas and their numbers correlate to tissue damage in MS patients and EAE mice (Ajami et al., 2011; Duffy et al., 2014).

A part from modulating the infiltration of leukocytes into tissues, cytokines are also responsible for the polarization of immune cells (Becher et al., 2017). For instance, CD4 T lymphocytes include four main phenotypes differentially involved in the physiopathology of MS and EAE: Th1 and Th17 cells produce pro-inflammatory

cytokines (Mosmann and Sad, 1996; Reboldi et al., 2009) that worsen the tissue damage in MS and EAE (Lovett-Racke et al., 2011). Th2 cells represent a protective anti-inflammatory subpopulation of T cells which produce anti-inflammatory cytokines (Mosmann and Sad, 1996) and they are believe to exert a suppressive role in EAE due to blockade of Th1 responses (Bitan et al., 2010). Finally, T regulatory cells are the most immunomodulators of the adaptive immune system, since they supress the action of T cells and maintain the immune homeostasis (Kronenberg and Rudensky, 2005). The increased proportion of T regulatory cells has been defined as a hallmark of the recovery phase in EAE mice (O'Connor and Anderton, 2015) , as well as, of the remission phase in RRMS individuals (Dalla Libera et al., 2011). In this line, transgenic expression of IL-37 reduced the percentage of pathogenic Th1 cells while increased the percent of T regulatory cells in the spinal cord at the peak of the EAE disease. Importantly, we found that the polarizing effects of IL-37 were not restricted to CD4 T cells, since hIL-37tg mice also showed lower proportion of macrophages expressing CD16/32 and iNOS, which have potent pro-inflammatory actions and contribute to EAE pathology (Liu et al., 2013). We also found decreased counts of various immune cell populations in the lymph nodes of EAE mice with transgenic IL-37 expression. This included ~50% reduction in the number of dendritic cells, which are potent antigen-presenting cells endowed with the ability to prime T-cell responses. Indeed, the number of MS plaques as well as the severity of EAE correlate with the presence and functional status of dendritic cells (Greter et al., 2005; Serafini et al., 2006). In this line, we observed that the counts of CD4 T cells were significantly reduced in the lymph nodes of hIL-37tg mice, suggesting that the expansion of this T cell subset was attenuated by IL-37. However, this cytokine did not alter the phenotype of CD4 T cells in the lymph nodes. Similarly, IL-37 did not impede the efflux of immune cells into the circulation since the number of the different immune cells analysed did not change after its transgenic expression. However, IL-37 favoured the mobilization of regulatory CD4 T cell, since they were increased in the hIL-37tg mice. These data therefore suggest that the reduced accumulation of the immune cells in the spinal cord in EAE mice with transgenic expression of IL-37 was likely due to the ability of this cytokine to reduce their infiltration into CNS rather than suppressing their mobilization into the circulation.

IL-37 is a dual-function cytokine that can exert its anti-inflammatory activity trough two different pathways: (i) the activation of the extracellular receptor complex IL-18Rα-SIGIRR or (ii) the translocation to the nucleus (Nold-Petry et al., 2015a). We demonstrated that the interaction of IL-37 with its extracellular receptor complex was totally necessary to mediate its protective effects in EAE, since the genetic deletion of *sigirr* abolished the beneficial actions of this cytokine, despite the intracellular function of IL-37 was not affected. However, we do not discard that the nuclear function of IL-37 could also promote some anti-inflammatory actions in EAE, as we recently observed under endotoxemia (Li et al., 2019).

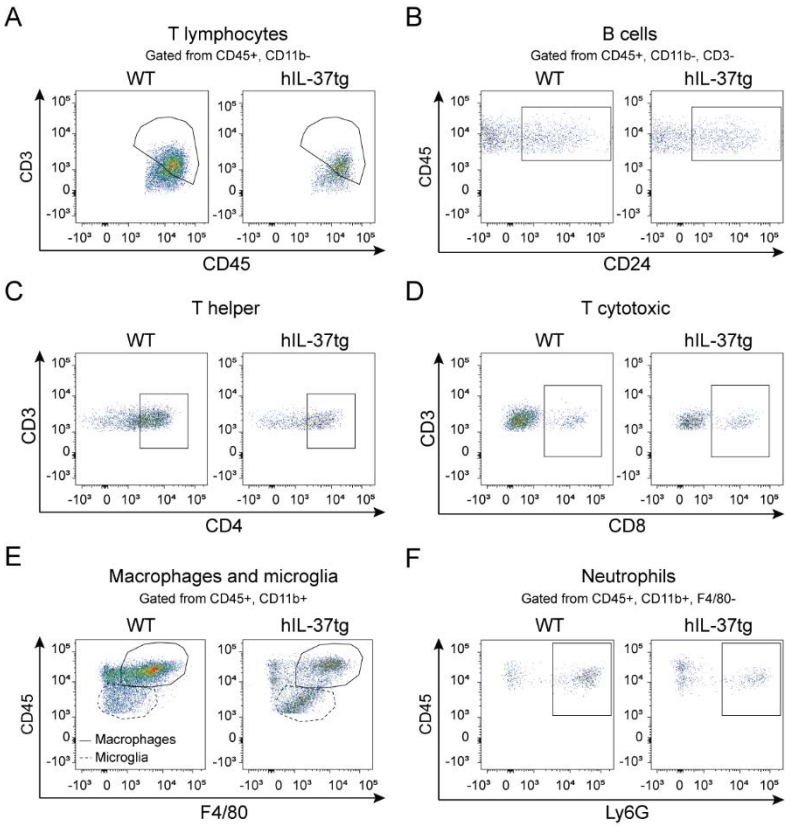
There is accumulated evidence corroborating that IL-37 is dysregulated in several pathological conditions, such as, systemic lupus erythematosus (Wu et al., 2016), psoriasis (Sehat et al., 2000), aortic valve lesion (Zeng et al., 2017) or hepatitis B and C (Li et al., 2013), In this line, we also observed that IL-37 transcripts were not increased or even decreased in PBMCs and brain active lesions of MS patients. Nonetheless, the components of the extracellular receptors for IL-37 we expressed in PBMS and brain samples of healthy donors and MS patients. These data suggest that although MS individuals have the machinery to activate this beneficial mechanism to contain inflammation, IL-37 signalling is not switched on due to the aberrant production of IL-37. A similar scenario also occurs in mice, since the complex *il-18rα/sigirr* is expressed in the spinal cord at physiological and pathological conditions but IL-37 is absent due to the lack of the open read frame for this cytokine. Importantly, we found that the exogenous administration of recombinant human IL-37 protein in EAE mice ameliorated the clinical and histopathological signs of the disease, suggesting the therapeutic potential of IL-37 in MS. Highlight that treatment with recombinant IL-37 protein was initiated once mice showed the first clinical signs of EAE, what makes these findings more clinically relevant.

Recent studies have been reported that IL-37 forms dimers with nanomolar affinity, resulting in limited bioactivity (Ellisdon et al., 2017). They uncovered that mutations in the dimer interface, such as IL-37^{D73K} and IL-37^{Y85A}, specifically disrupt dimer formation, resulting in stable monomers that provide greater suppression of inflammation compared with native IL-37, (Eisenmesser *et al.*, 2019). However, we

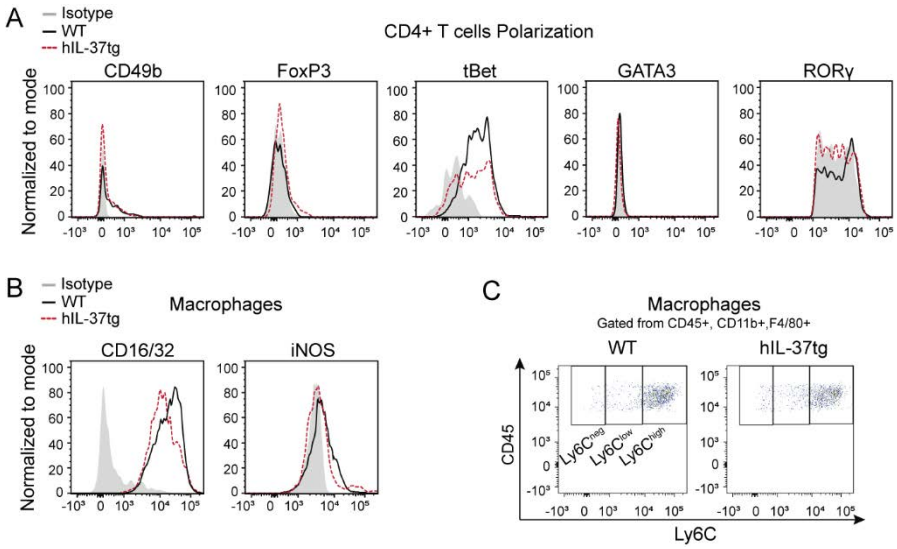
did not find any significant enhanced effect of the two mutant monomers IL-37^{D73K} and IL-37^{Y85A} compared to the native IL-37 form, although the monomer IL-37^{D73K} resulted in slight greater efficacy in EAE. This may indicate that the amount of IL-37 we administered was not high enough to favour the formation of dimers

Altogether, we provide clear evidence that IL-37 wields a beneficial effect in EAE by signalling through its surface receptor complex IL-18Rα/sigirr. Further studies are needed to elucidate the role of IL-37 in MS patients, but our data suggest that this cytokine could have therapeutic applications.

SUPLEMMENTARY FIGURES



S1. Transgenic expressing of IL-37 reduces the accumulation of immune cells in the spinal cord of mice at the peak of EAE. Representative dot plots showing different **(A)** T lymphocytes**, (B)** B cells, **(C)** T helper cells, **(D)** T cytotoxic cells, **(E)** macrophages and microglia and **(F)** neutrophils in the spinal cord at the peak of EAE in WT and hIL-37tg mice.



S2. IL-37 polarizes immune cells towards a more anti-inflammatory phenotype in the spinal cord of EAE mice. (A) Representative flow cytometry histograms characterising the expression of Th cells polarization markers and **(B-C)** macrophages in the spinal cord at the peak of EAE in WT and hIL-37tg mice.

REFERENCES

Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM V (2011) Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nat Neurosci 14:1142–1150 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21804537 [Accessed April 13, 2019].

Amo-Aparicio J, Martínez-Muriana A, Sánchez-Fernández A, López-Vales R (2018) Neuroinflammation Quantification for Spinal Cord Injury. Curr Protoc Immunol:1–15.

Baker D, O'Neill JK, Turk JL (1991) Cytokines in the central nervous system of mice during chronic relapsing experimental allergic encephalomyelitis. Cell Immunol 134:505–510 Available at: http://www.ncbi.nlm.nih.gov/pubmed/1902401 [Accessed February 21, 2019].

Ballak DB et al. (2014) IL-37 protects against obesity-induced inflammation and insulin resistance. Nat Commun 5:4711 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25182023 [Accessed August 10, 2018].

Becher B, Spath S, Goverman J (2017) Cytokine networks in neuroinflammation. Nat Rev Immunol 17:49–59 Available at: http://www.nature.com/articles/nri.2016.123 [Accessed February 21, 2019].

Bitan M, Weiss L, Reibstein I, Zeira M, Fellig Y, Slavin S, Zcharia E, Nagler A, Vlodavsky I (2010) Heparanase upregulates Th2 cytokines, ameliorating experimental autoimmune encephalitis. Mol Immunol 47:1890–1898 Available at: http://www.ncbi.nlm.nih.gov/pubmed/20399501 [Accessed February 25, 2019].

Bufler P, Gamboni-Robertson F, Azam T, Kim S-H, Dinarello CA (2004) Interleukin-1 homologues IL-1F7b and IL-18 contain functional mRNA instability elements within the coding region responsive to lipopolysaccharide. Biochem J 381:503–510 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15046617 [Accessed March 20, 2019].

Bulau A-M, Nold MF, Li S, Nold-Petry CA, Fink M, Mansell A, Schwerd T, Hong J, Rubartelli A, Dinarello CA, Bufler P (2014) Role of caspase-1 in nuclear translocation of IL-37, release of the cytokine, and IL-37 inhibition of innate immune responses. Proc Natl Acad Sci 111:2650–2655 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24481253 [Accessed August 9, 2018].

Caravagna C, Jaouën A, Desplat-Jégo S, Fenrich KK, Bergot E, Luche H, Grenot P, Rougon G, Malissen M, Debarbieux F (2018) Diversity of innate immune cell subsets across spatial and temporal scales in an EAE mouse model. Sci Rep 8:5146 Available at: http://www.nature.com/articles/s41598-018-22872-y [Accessed April 12, 2019].

Cavalli G, Dinarello CA (2018) Suppression of inflammation and acquired immunity by IL-37. Immunol Rev 281:179–190 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29247987 [Accessed August 9, 2018].

Cavalli G, Koenders M, Kalabokis V, Kim J, Tan AC, Garlanda C, Mantovani A, Dagna L, Joosten LAB, Dinarello CA (2016) Treating experimental arthritis with the innate immune inhibitor interleukin-37 reduces joint and systemic inflammation. Rheumatology 55:2220–2229 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27567100 [Accessed August 10, 2018].

Coll-Miró M, Francos-Quijorna I, Santos-Nogueira E, Torres-Espin A, Bufler P, Dinarello CA, López-Vales R (2016) Beneficial effects of IL-37 after spinal cord injury in mice. Proc Natl Acad Sci 113:1411–1416 Available at:

http://www.pnas.org/lookup/doi/10.1073/pnas.1523212113.

Constantinescu CS, Farooqi N, O'Brien K, Gran B (2011) Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol 164:1079–1106 Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3229753&tool=pmcentrez&rendertype=abstract.

Dalla Libera D, Di Mitri D, Bergami A, Centonze D, Gasperini C, Grasso MG, Galgani S, Martinelli V, Comi G, Avolio C, Martino G, Borsellino G, Sallusto F, Battistini L, Furlan R (2011) T regulatory cells are markers of disease activity in multiple sclerosis patients. PLoS One 6:e21386 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21731726 [Accessed February 25, 2019].

Dinarello CA (2018) Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev 281:8–27 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29247995 [Accessed May 24, 2019].

Dinarello CA, Simon A, van der Meer JWM (2012) Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov 11:633–652 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22850787 [Accessed May 14, 2019].

Duffy SS, Lees JG, Moalem-Taylor G (2014) The contribution of immune and glial cell types in experimental autoimmune encephalomyelitis and multiple sclerosis. Mult Scler Int 2014:285245 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25374694 [Accessed February 21, 2019].

Eisenmesser EZ, Gottschlich A, Redzic JS, Paukovich N, Nix JC, Azam T, Zhang L, Zhao R, Kieft JS, The E, Meng X, Dinarello CA (2019) Interleukin-37 monomer is the active form for reducing innate immunity. Proc Natl Acad Sci 116:201819672 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30819901 [Accessed March 28, 2019].

Ellisdon AM, Nold-Petry CA, D'Andrea L, Cho SX, Lao JC, Rudloff I, Ngo D, Lo CY, Soares da Costa TP, Perugini MA, Conroy PJ, Whisstock JC, Nold MF (2017) Homodimerization attenuates the anti-inflammatory activity of interleukin-37. Sci Immunol 2:eaaj1548 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28783685 [Accessed March 28, 2019].

Eugster H-P, Frei K, Kopf M, Lassmann H, Fontana A (1998) IL-6-deficient mice resist myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. Eur J Immunol 28:2178–2187 Available at: http://www.ncbi.nlm.nih.gov/pubmed/9692887 [Accessed April 12, 2019].

Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, Noelle RJ, Becher B (2005) Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nat Med 11:328–334 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15735653 [Accessed February 21, 2019].

Grigoriadis N, van Pesch V (2015) A basic overview of multiple sclerosis immunopathology. Eur J Neurol 22:3–13.

Henry CJ, Casás-Selves M, Kim J, Zaberezhnyy V, Aghili L, Daniel AE, Jimenez L, Azam T, McNamee EN, Clambey ET, Klawitter J, Serkova NJ, Tan AC, Dinarello CA, DeGregori J (2015) Aging-associated inflammation promotes selection for adaptive oncogenic events in B cell progenitors. J Clin Invest 125:4666–4680 Available at: https://www.jci.org/articles/view/83024 [Accessed August 10, 2018].

Hewett SJ, Jackman NA, Claycomb RJ (2012) Interleukin-1β in Central Nervous System Injury and Repair. Eur J Neurodegener Dis 1:195 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4465544/ [Accessed May 14, 2019].

Kallaur AP, Oliveira SR, Simao ANC, De Almeida ERD, Morimoto HK, Lopes J, De Carvalho Jennings Pereira WL, Andrade RM, Pelegrino LM, Borelli SD, Kaimen-Maciel DR, Reiche EMV (2013) Cytokine profile in relapsing-remitting Multiple sclerosis patients and the association between progression and activity of the disease. Mol Med Rep 7:1010–1020 Available at: https://www.spandidos-publications.com/10.3892/mmr.2013.1256 [Accessed February 21, 2019].

Kronenberg M, Rudensky A (2005) Regulation of immunity by self-reactive T cells. Nature 435:598–604 Available at: http://www.nature.com/articles/nature03725 [Accessed February 25, 2019].

Lévesque SA, Paré A, Mailhot B, Bellver-Landete V, Kébir H, Lécuyer M-A, Alvarez JI, Prat A, Vaccari JP de R, Keane RW, Lacroix S (2016) Myeloid cell transmigration across the CNS vasculature triggers IL-1β–driven neuroinflammation during autoimmune encephalomyelitis in mice. J Exp Med 213:929–949 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27139491 [Accessed February 21, 2019].

Li C, Ji H, Cai Y, Ayana DA, Lv P, Liu M, Jiang Y (2013) Serum interleukin-37 concentrations and HBeAg seroconversion in chronic HBV patients during telbivudine treatment. J Interferon Cytokine Res 33:612–618 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23697556 [Accessed April 10, 2019].

Li S, Amo-Aparicio J, Neff CP, Tengesdal IW, Azam T, Palmer BE, López-Vales R, Bufler P, Dinarello CA (2019) Role for nuclear interleukin-37 in the suppression of innate immunity [Immunology and Inflammation]. Proc Natl Acad Sci U S A 116:4456–4461 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30792349 [Accessed March 28, 2019].

Liu C, Li Y, Yu J, Feng L, Hou S, Liu Y, Guo M, Xie Y, Meng J, Zhang H, Xiao B, Ma C (2013) Targeting the Shift from M1 to M2 Macrophages in Experimental Autoimmune Encephalomyelitis Mice Treated with Fasudil Nataf S, ed. PLoS One 8:e54841 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23418431 [Accessed May 29, 2019].

Lovett-Racke AE, Yang Y, Racke MK (2011) Th1 Versus Th17: Are T Cell Cytokines Relevant in Multiple Sclerosis? Biochim Biophys Acta 1812:246 Available at: http://www.ncbi.nlm.nih.gov/pubmed/20600875 [Accessed February 25, 2019].

McNamee EN, Masterson JC, Jedlicka P, McManus M, Grenz A, Collins CB, Nold MF, Nold-Petry C, Bufler P, Dinarello CA, Rivera-Nieves J (2011a) Interleukin 37 expression protects mice from colitis. Proc Natl Acad Sci 108:16711–16716 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21873195 [Accessed August 10, 2018].

McNamee EN, Masterson JC, Jedlicka P, McManus M, Grenz A, Collins CB, Nold MF, Nold-Petry C, Bufler P, Dinarello CA, Rivera-Nieves J (2011b) Interleukin 37 expression protects mice from colitis. Proc Natl Acad Sci 108:16711–16716 Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.1111982108 [Accessed August 10, 2018].

Moretti S, Bozza S, Oikonomou V, Renga G, Casagrande A, Iannitti RG, Puccetti M, Garlanda C, Kim S, Li S, van de Veerdonk FL, Dinarello CA, Romani L (2014) IL-37 Inhibits Inflammasome Activation and Disease Severity in Murine Aspergillosis Klein BS, ed. PLoS Pathog 10:e1004462 Available at: http://dx.plos.org/10.1371/journal.ppat.1004462 [Accessed August 10, 2018].

Mosmann TR, Sad S (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 17:138–146 Available at: http://www.ncbi.nlm.nih.gov/pubmed/8820272 [Accessed February 25, 2019].

Nold-Petry C a et al. (2015a) IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. Nat Immunol 16:354–365 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25729923.

Nold-Petry CA et al. (2015b) IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. Nat Immunol 16:354–365 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25729923 [Accessed August 9, 2018].

Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA (2010a) IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol 11:1014–1022 Available at: http://www.ncbi.nlm.nih.gov/pubmed/20935647 [Accessed August 9, 2018].

Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA (2010b) IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol 11:1014–1022 Available at: http://www.nature.com/articles/ni.1944 [Accessed August 9, 2018].

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG (2000) Multiple Sclerosis. N Engl J Med 343:938–952 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11006371 [Accessed January 18, 2018].

O'Connor R a., Anderton SM (2015) Inflammation-associated genes: risks and benefits to Foxp3(+) regulatory T-cell function. Immunology 146:194–205 Available at: http://doi.wiley.com/10.1111/imm.12507%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/26190495%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4582961.

Pérez-Cerdá F, Sánchez-Gómez MV, Matute C (2016) The link of inflammation and neurodegeneration in progressive multiple sclerosis. Mult Scler Demyelinating Disord 1:9 Available at: http://msddjournal.biomedcentral.com/articles/10.1186/s40893-016-0012-0 [Accessed May 2, 2019].

Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, Uccelli A, Lanzavecchia A, Engelhardt B, Sallusto F (2009) C-C chemokine receptor 6–regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol 10:514–523 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19305396 [Accessed February 25, 2019].

Rossi S, Motta C, Studer V, Macchiarulo G, Volpe E, Barbieri F, Ruocco G, Buttari F, Finardi A, Mancino R, Weiss S, Battistini L, Martino G, Furlan R, Drulovic J, Centonze D (2014) Interleukin-1β causes excitotoxic neurodegeneration and multiple sclerosis disease progression by activating the apoptotic protein p53. Mol Neurodegener 9:56 Available at: http://molecularneurodegeneration.biomedcentral.com/articles/10.1186/1750-1326-9-56 [Accessed February 21, 2019].

Sawcer S et al. (2011) Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 476:214–219 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21833088 [Accessed April 12, 2019].

Schrijver HM, Van As J, Crusius JBA, Dijkstra CD, Uitdehaag BMJ (2003) Interleukin (IL)-1 gene polymorphisms: Relevance of disease severity associated alleles with IL-1β and IL-1ra production in multiple sclerosis. Mediators Inflamm 12:89–94 Available at: http://www.ncbi.nlm.nih.gov/pubmed/12775358 [Accessed February 21, 2019].

Sehat M, Talaei R, Dadgostar E, Nikoueinejad H, Akbari H (2000) Iranian journal of allergy, asthma and immunology. TUMS Publications. Available at: http://ijaai.tums.ac.ir/index.php/ijaai/article/view/1434 [Accessed April 10, 2019].

Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Capello E, Mancardi GL, Aloisi F (2006) Dendritic cells in multiple sclerosis lesions: Maturation stage, myelin uptake, and interaction with proliferating T cells. J Neuropathol Exp Neurol 65:124–141 Available at: https://academic.oup.com/jnen/article/2645241/Dendritic [Accessed February 21, 2019].

Symons JA, Bundick R V, Suckling AJ, Rumsby MG (1987) Cerebrospinal fluid interleukin 1 like activity during chronic relapsing experimental allergic encephalomyelitis. Clin Exp Immunol 68:648–654 Available at: http://www.ncbi.nlm.nih.gov/pubmed/3498582 [Accessed February 21, 2019].

van de Veerdonk FL, Gresnigt MS, Oosting M, van der Meer JWM, Joosten LAB, Netea MG, Dinarello CA (2015) Protective host defense against disseminated candidiasis is impaired in mice expressing human interleukin-37. Front Microbiol 5:762 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25620965 [Accessed November 29, 2018].

Wekerle H (2008) Lessons from multiple sclerosis: Models, concepts, observations. In: Annals of the Rheumatic Diseases, pp iii56-iii60 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19022815 [Accessed January 23, 2018].

Wu G-C, Li H-M, Wang J-B, Leng R-X, Wang D-G, Ye D-Q (2016) Elevated plasma interleukin-37 levels in systemic lupus erythematosus patients. Lupus 25:1377–1380 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27125292 [Accessed April 10, 2019].

Ye L, Jiang B, Deng J, Du J, Xiong W, Guan Y, Wen Z, Huang K, Huang Z (2015) IL-37 Alleviates Rheumatoid Arthritis by Suppressing IL-17 and IL-17–Triggering Cytokine Production and Limiting Th17 Cell Proliferation. J Immunol 194:5110–5119 Available at: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1401810 [Accessed August 10, 2018].

Zeng Q, Song R, Fullerton DA, Ao L, Zhai Y, Li S, Ballak DB, Cleveland JC, Reece TB, McKinsey TA, Xu D, Dinarello CA, Meng X (2017) Interleukin-37 suppresses the osteogenic responses of human aortic valve interstitial cells in vitro and alleviates valve lesions in mice. Proc Natl Acad Sci 114:1631–1636 Available at: http://www.pnas.org/lookup/doi/10.1073/pnas.1619667114 [Accessed August 10, 2018].

CHAPTER 3

Administration of Maresin-1 ameliorates the physiopathology of experimental autoimmune encephalomyelitis

Administration of Maresin-1 ameliorates the physiopathology of experimental autoimmune encephalomyelitis

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ABSTRACT

Resolution of inflammation is an active and regulated process that leads to the clearance of cell debris and immune cells from the challenged tissue, facilitating tissue homeostasis. This physiological response is coordinated by endogenous bioactive lipids known as specialized pro-resolution mediators (SPMs). When resolution fails, inflammation becomes uncontrolled leading chronic inflammation and tissue damage, as occurs in multiple sclerosis (MS). Here, we show that enzymes involved in SPM synthesis are not efficiently induced in MS patients and in mice with experimental autoimmune encephalomyelitis (EAE), the animal model of MS. We also demonstrate that the administration of the SPM maresin-1 (MaR1) modulated different key events of the inflammatory response in EAE mice. In particular, MaR1 suppressed the protein levels of various pro-inflammatory cytokines, reduced the accumulation of immune cells in the spinal cord. MaR1 also decreased the numbers of Th1 cells, increased the accumulation of regulatory T cells (Treg), drove macrophage polarization towards an anti-inflammatory phenotype, and modulated the efflux of different leukocyte subsets from immune organs. Importantly, we also provide clear evidence indicating that administration of MaR1 in mice with clinical signs of EAE enhances neurological outcomes and protects from demyelination. Overall, these data demonstrate that immunoresolvent therapies could be a novel avenue for the treatment of MS.

INTRODUCTION

Acute inflammation is a key physiological mechanism that promotes the repair of injured tissues and eliminates infectious organisms and toxic agents. This response is tightly controlled and finalizes with the elimination of the immune cells and cellular debris from the tissue, paving the way for the recovery of homeostasis (Russell, 2007; Serhan, 2014; Serhan et al., 2015). Contrary, uncontrolled inflammation, as it occurs when the resolution phase fails, becomes harmful to the tissue and it is a hallmark of a wide variety of pathological conditions, including in multiple sclerosis (MS) (Schwab et al., 2007; Prüss et al., 2013; Serhan, 2014; Francos-Quijorna et al., 2017).

For many years, termination of inflammation was believed to be a passive process triggered by the dilution of pro-inflammatory mediators at the challenged tissue thus halting the recruitment of leukocytes from circulation. However, it is now known that the resolution of inflammation is an active and coordinated event controlled by a genus of lipids known as SPMs (Schwab et al., 2007; Serhan, 2014). This bioactive lipid family includes lipoxins (LX), resolvin D series (RvD), resolvin E series, (RvE), protectins (PD) and maresins (Schwab et al., 2007; Serhan, 2014; Chiang and Serhan, 2017; Dalli and Serhan, 2018). SPMs are naturally synthesized by immune cells from omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) through lipoxygenases (12-LOX and 15-LOX) (Serhan et al., 2015; Chiang and Serhan, 2017; Dalli and Serhan, 2018). Failure to produce adequate amounts of SPMs has been associated with persistent inflammation in many inflammatory disorders such as asthma, atherosclerosis, ulcerative colitis, spinal cord injury and Alzheimer's disease, among others (Schwab et al., 2007; Serhan, 2014; Wang et al., 2015; Francos-Quijorna et al., 2017; Serhan, 2017). However, there are currently very few studies addressing the importance of the resolution of inflammation in MS.

MS is a chronic neuroinflammatory and demyelinating disease of the central nervous system (CNS) affecting around 2.5 million individuals worldwide (Dendrou et al., 2015; Bhise and Dhib-Jalbut, 2016; Popescu and Lucchinetti, 2016). Although the exact cause of MS is not yet known, a hallmark of the pathology is the presence of persistent numbers of immune cells in the white and gray matter of the CNS (Prat and Antel, 2005; Dendrou et al., 2015; Bhise and Dhib-Jalbut, 2016; Popescu and

Lucchinetti, 2016). The question whether neurodegeneration in MS is independent or related to inflammation has been long debated. However, neuropathological studies show that inflammation is present in all MS stages and that putative neurodegenerative lesions are likely driven by immune cells (Prineas et al., 2001; Lassmann et al., 2007; Popescu and Lucchinetti, 2016).

To our knowledge, there are currently two studies addressing the importance of the resolution of inflammation in MS, which suggest that this event is dysfunctional in the patients (Prüss et al., 2013; Poisson et al., 2015). The first report analyzed the levels of SPMs in the cerebrospinal fluid (CSF) of a small cohort of MS individual. This work suggested that the production of SPM in the CNS of MS individual was aberrant, especially in those with highly active MS (Prüss et al., 2013). The second study showed that metabolites of PUFAs, including omega-3 and omega-6 PUFAs, were decreased either in mouse models of MS and in patients with MS. Importantly, this work also revealed that administration of the SPM known as RvD1 ameliorated clinical signs of EAE in mice (Poisson et al., 2015). However, the causes underlying the inappropriate production of SPMs in MS patients, and whether the administration of other immunoresolvent lipids could mediated beneficial effects is currently unknown.

In the present work, we reveal that the expression of enzymes involved in the synthesis of SPMs is defective in active MS brain lesions and peripheral blood mononuclear cells (PBMC) of MS patients, as well as, in the CNS of mice undergoing EAE. We also reveal that the exogeneous administration of RvD1 and MaR1, but not RvE1 in mice after EAE onset, confers protection against neurological decline. MaR1 also has protective effects against myelin loss in the spinal cord and modulates different aspects of inflammation.

MATERIALS AND METHODS

Human Brain Samples

Human brain tissue was obtained from MS patients diagnosed with clinical and neuropathological MS diagnosis according to the revised 2010 McDonald's criteria1. Tissue samples were collected from MS patients with full ethical approval (BH07.001) and informed consent as approved by the local ethics committee (Table 1). Autopsy samples were cryopreserved and lesions classified using Luxol Fast Blue/Haematoxylin & Eosin staining and Oil Red O staining.

For this study, normal appearing white matter (NAWM) regions and 7 active lesions tissue samples representing 56 secondary-progressive MS (SPCMS) and 12 relapsing-remitting MS (RRMS) cases were used. The median age at death was 50 years (range from 26 to 65 years). Regions of interest (lesion or NAWM) for RNA isolation were dissected manually from several 5-6 50 µm cryosections per block. To analyse gene expression from human peripheral blood mononuclear cells (PBMCs) we used blood samples from MS patients and healthy controls.

MS and healthy donor PBMC isolation

Blood samples were collected from 7 patients with relapsing-remitting MS (RRMS), 3 with secondary progressive (SPMS) and 1 with clinically isolated syndrome (CIS), recruited at the MS Clinic at the Research Center of the University of Montreal Hospital Center. Their ages ranged from 27 to 65 years (mean age, 43 years). At time of blood sampling, none of the patients were receiving treatment with steroids or disease modifying drugs. 7 healthy volunteers were included as controls; their ages ranged from 24 to 42 years (mean age, 35 years). A written informed consent was obtained from patients and healthy donors in accordance with the local ethics committee (CRCHUM research ethic committee approval number BH07.001). PBMCs were isolated from blood samples collected in EDTA-coated Vacutainer tubes (BD Biosciences, Oakville, ON, Canada) using a Ficoll density gradient (Amersham Biosciences)

EAE Induction

Mice were sedated with intramuscular injection of mixture of ketamine (22mg/kg) (Imalgen 1000, Merial) and xylazine (2.5mg/kg) (Rompun, Bayer). EAE was actively induced by subcutaneously injection in each flank at the base of the tail of 100μl of 3mg/mL of myelin oligodendrocyte glycoprotein peptide 35-55 (MOG_{35-55}) in Complete Freund's Adjuvant (CFA) (Difco, Detroit, MI, USA) supplemented with 4mg/mL of heat inactivated *Mycobacterium tuberculosis* (Difco, Detroit, MI, USA). Immediately after induction, and on day 2, mice received an intraperitoneal (i.p.) injection of 400ng of pertussis toxin (PTX) in 100μl sterile saline (0.9% NaCl).

EAE Functional Evaluation

Mice were daily scored from day 0 to day 21 after induction of EAE. The researcher was blind to experimental groups during the functional evaluation. A 6-point scale was used to evaluate the clinical signs of EAE: 0=normal walking, 0.5=partially paralyzed tail, 1=fully paralyzed tail, 2=mild hind limb weakness, quick righting reflex, 3=severe hind limb weakness, slow righting reflex, unable to bear weight, 3.5=severe hind limb weakness and partial paralysis of hind limb, 4=complete paralysis of at least one hind limb, 4.5=complete paralysis of one or both hind limbs and trunk weakness, 5=complete paralysis of one or both hind limbs, forelimb weakness or paralysis, 6=mouse is found death by EAE.

Histological analysis

C57/Bl6 mice were euthanised at 21 days post induction with and overdose of pentobarbital sodium (Dolethal) and, then, transcardially perfused with 4% paraphormaldehyde (PFA) in 0.1M of phosphate buffer (PB). Lumbar segments of spinal cord were harvested, post-fixed in 4% PFA for 2 hours in ice and cryoprotected in 30% sucrose in 0.1M PN at 4ºC for at least 48 hours. Then, spinal cords were embedded in Tissue-Tek®OTC (Sakura, Japan), cut in transversal sections (15μm-thick) with a cryostat (Leica) between L3 and L5 segments and serially picked up on gelatine-coated glass slides. Samples were stored at –20ºC.

Sections were stained with Luxol Fast Blue (LFB) (Sigma Aldrich). Briefly, after a graded dehydration, sections were placed in 1mg/mL of LFB solution in 96% ethanol and 0.05% acetic acid overnight at 37ºC and protected from light. Then, slides were washed with 96% ethanol, rehydrated with distilled water and placed in a 0.5mg/mL Li_2CO_3 solution in distilled water for 3-5 minutes at room temperature. Finally, sections were washed in distilled water, dehydrated again and mounted in DPX mounting medium (Sigma Aldrich). To assess the demyelinated area in the spinal cords, 6 random images per spinal cord were captured at 10X magnification with an Olympus BX51 and the attached Olympus DP73 Camera. The total demyelinated area in the spinal cord was measured with Image J image analysis software.

Cytokine Protein Expression Assessment

C57/Bl6 mice were euthanised at the peak of EAE with an overdose of pentobarbital sodium (Dolethal) and transcardially perfused with 60 mL of sterile saline (0.9% NaCl). Then, spinal cords were harvested and rapidly freeze to –80ºC with liquid nitrogen. To assess the protein levels of cytokines, spinal cord were processed as it is detailed in Amo-Aparicio et al. 2018. Briefly, tissue was homogenized in HEPES and then protein concentration was determined with a DC Protein Assay (Bio-Rad). Protein homogenates were concentrated to 4μg/μl and finally, protein levels of IL-4, IL-10, IL-1α, IL-1β, IL-6, TNF-α, IFNγ, IL-17A, CSF-3, CCL-5, CCL-2, CXCL-2 and CXCL-10 were analysed using a custom-designed Milliplex Cytokine Magnetic Bead Panel on a MAGPIX system (Affymetrix, eBioscience).

Fluorescence Activated Cell Sorting (FACS)

Immune cell infiltration was determined from blood, lymph nodes and spinal cord of EAE mice at disease peak. Briefly, mice were euthanised with an overdose of pentobarbital sodium (Dolethal). 15μl of blood was collected from a cardiac puncture and stored in heparinized vials at 4ºC. Then, mice were transcardially perfused with 60 mL of sterile saline (0.9% NaCl) and the spinal cords and lymph nodes (cervical and inguinal) were collected.

Blood samples were incubated with red blood cell lysis buffer (BioLegend) according to manufactures' guide to obtain a cell suspension enriched in leukocytes. Spinal cords and lymph nodes were cut in small pieces and enzymatically dissociated in 1mL of HBSS without Ca^{2+}/Mg^{2+} containing 0.1% collagenase and 0.1% DNase for 30 minutes at 37ºC, and then, mechanically disintegrated through a 70µm cell strainer to obtain a cell suspension (Amo-Aparicio et al., 2018).

Cell suspensions were split into different 1.5mL microcentrifuge tubes according to the number of antibody combinations. For extracellular staining, samples were divided and unstained cells and isotype-matched control samples were generated to control for nonspecific binding of antibodies and for autofluorescence. For extracellular staining the following antibodies from eBioscience were used at a 1:300 concentration: CD45-PerCP, CD11b-PE or PE-Cy7, F4/80-PE or -APC, Ly6C-FITC, Ly6G-PE, CD3-FITC-APC-PerCP; CD4-APC-Cy7, CD8-APC, CD49b-PE, CD24-PE. Samples were incubated with the primary antibodies for 1h at 4ºC with gentle agitation, washed with DMEM-10% FBS and centrifuged twice at 300g for 10 min at 4ºC to remove debris and then fixed with 1% PFA. For intracellular staining, the following antibodies from eBioscience were also used at 1:300 concentration: FoxP3- PE-Cy7, tBet-PerCP, RORγ-APC, GATA3-PE, IFNy-Alexa488, IL-17A-Alexa488, IL-4-APC, IL-10-Alexa488. Cells were fixed with 1% PFA and permeabilized with Permeabilization Washing Buffer (Biolegend). Cells stained for FoxP3 were fixed and permeabilized using FoxP3 Transcription Factor Staining Buffer Set (eBioscience). Samples were immunostained with the intracellular antibodies for 1h at 4ºC. Finally, stained cells were washed with PBS twice and fixed with 1% PFA.

Samples were analysed on a FACS Canto Flow Cytometer (BD Bioscience) and all data were processed using FlowJo® software V.10.

Statistical analyses

Data are shown as mean ± standard error of the mean (SEM). Paired or unpaired Student's t test was used for the comparison between two different groups (human qPCR, histological analysis, cytokine levels, accumulation of immune cells). Mean clinical score follow-up was analysed by using two-way ANOVA repeated measures with *post-hoc* Bonferroni's test for multiple comparisons. Differences were considered significant at $p<0.05$.

RESULTS

MS patients have an impairment in the expression of the key enzymes involved in switching on the inflammatory resolution programs

A previous study assessing the levels of lipid mediators derived from AA and DHA in CSF samples suggested that resolution of inflammation was defective in MS patients (Prüss et al. 2013). To elucidate whether this is associated with a failure to induce the expression of the key enzymes involved in the synthesis of SPMs, we measured the expression of lipoxygenase-12 (A*LOX12)* and lipoxygenase-15 *(ALOX15),* in PBMCs from active MS patients and healthy donors, as well as, in active lesions in post-mortem brain samples. qPCR analysis revealed that mRNA levels of A*LOX12* and A*LOX15* were undetected or found at very low levels in PBMCs from healthy patients, and that these enzymes were not up-regulated in PBMCs from MS patients (Figure 1A-B). When assessing the expression of *ALO*X12 and *ALOX15* in post-mortem active MS brain samples, we found that, overall, there was no significant induction of *ALOX12* or *ALOX15* in active lesions compared to NAWM (Figure 1C-D). However, in 4 out of 7 MS patients, the levels of *ALOX12* were increased between 3.67 and 5.11- fold in active lesions, whereas the transcripts for *ALOX15* in active lesions was higher in only 2 out of 7 patients, although they were increased 14.32 and 24.39-fold (Figure 1C-D). This data reveals that the enzymes involved in the production of SPMs are not properly induced in all MS patients.

We then studied whether this phenomenon also occurred in EAE, one of the most widely used animal models of MS. For this purpose, we analysed the dynamics of *12/15-lox* expression in the spinal cord of EAE mice. 12/15-*lox* is the murine ortholog of human *LOX-15* and the initiating enzyme in the SPMs biosynthetic pathway. qPCR analysis revealed that, similar to MS patients, mRNA levels of *12/15-lox* were not upregulated in the CNS of EAE mice, although the transcripts of this enzyme tended to be higher at the chronic stage of the disease (Figure 1E).

Overall, these results demonstrate that the key enzymes involved in the production of SPMs are not efficiently induced in MS individuals and EAE mice.

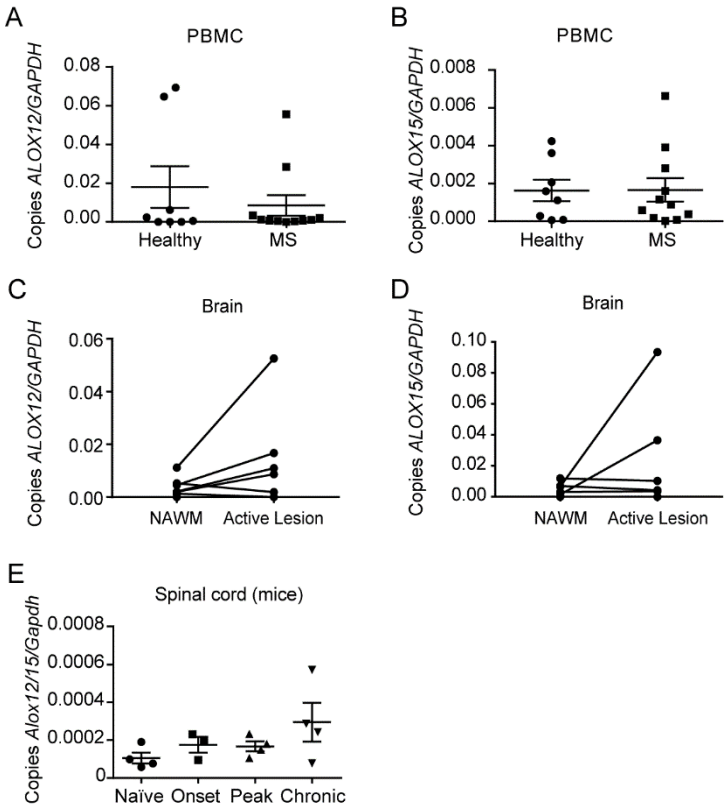


Figure 1. Expression of lipoxygenase 12 and 15 in MS patients and lipoxygenase 12/15 in EAE mice. A-D, Graphs showing the mRNA levels of *ALOX12* **(A, C)** and *ALOX15* **(B, D)** in PBMCs of MS individuals (n=11) and healthy donors (n=8) and in brain MS active lesions and NAWM **(C, D)** (n=7). Time course expression of of *Alox12/15* mRNA levels in the spinal cord of EAE mice over disease progression (n=3 for the onset and n=4 the other time points) **(E).** Unpaired t-test in **A-C**. Paired t-test in **C-D**. One-way ANOVA, *Bonferroni's post hoc* test in **E**. Data is shown as mean±SEM.

Systemic administration of SPMs ameliorates EAE outcomes

We then investigated whether exogenous administration of different SPMs derived from omega-3 fatty acids attenuated neurological impairments in EAE. For this purpose, we performed a pilot study to in which RvE1, RvD1 or MaR1 (1µg/mouse i.p.) from the disease onset. We found that the administration of RvD1 (Figure 2A), and MaR1 (Figure 2C-D) in a greater extent, conferred protection against functional loss, despite the treatment was initiated once the mice showed the first signs of the disease. However, the treatment with RvE1 did not ameliorated neurological deficits in EAE mice (Figure 2B).

Since MaR1 showed the greatest results on neurological outcomes, we then evaluated if the enhancement of the functional skills in EAE mice treated with MaR1

was related to preservation of myelin within the CNS. Histological analysis of spinal cord sections revealed that MaR1 protected against myelin loss (Figure 2E-F).

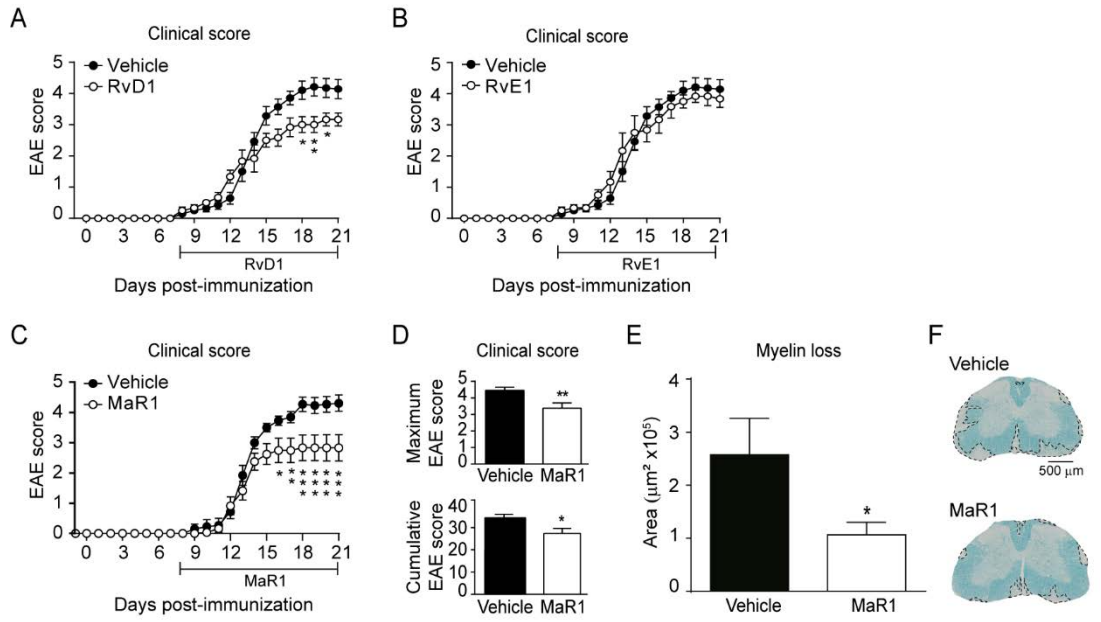


Figure 2. Effects of SPM administration on functional deficits and myelin loss in EAE mice. A-D, Graphs showing the clinical score of EAE mice treated with RvD1 **(A),** RvE1 **(B)** or MaR1 **(C-D)** compared with vehicle-treated mice over disease progression. **E-F,** Graph showing the quantification of myelin loss in the lumbar spinal cord of MaR1- or vehicle-treated mice at 21 days post induction **(E)** and representative histological spinal cord tissue sections stained with luxol fast blue from EAE mice treated with vehicle and MaR1 21 days post induction **(F).** *p<0.05; **p<0.01; ***p<0.001 vs. vehicle. Two-way ANOVA with repeated measures, Bonferroni's *post hoc* test in **A** (n=6 in RvD1 and n=14 in vehicle)**, B** (n=6 in RvE1 and n=14 in vehicle)**,** and **C** (n=12 per group). Unpaired t-test in **D** (n=12 per group) and **C** (n=9 in vehicle and n=11 in MaR1). Data is shown as mean±SEM.

MaR1 attenuates inflammation in mice with EAE

We next investigated whether the exogenous administration of MaR1 attenuates inflammation in mice undergoing EAE. With this aim, we assessed the protein levels of 14 cytokines in the spinal cord of vehicle- and MaR1-treated mice at the peak of EAE. Luminex assay revealed that MaR1 reduced significantly the levels of 5 out 9 pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, TNFα and IFNγ) and 3 out of the 4 chemokines measured (CCL-5, CCL-2 and CXCL-2) (Figure 3A) highlighting the potent actions of this SPM in attenuating the proinflammatory milieu within the spinal cord of EAE mice. However, MaR1 did not reduce the protein levels of the anti-inflammatory cytokine IL-10 (Figure 3A).

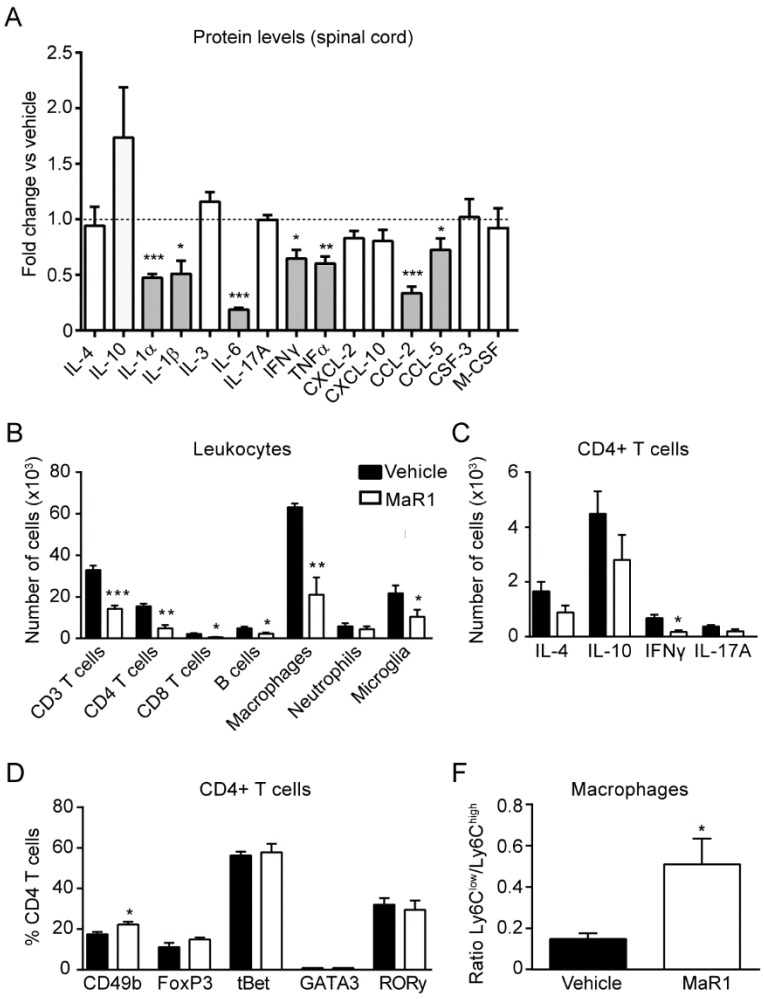


Figure 3. MaR1 attenuates inflammation in the spinal cord of mice undergoing EAE. Plot showing the fold change in cytokine protein levels of different cytokines in the spinal cord of EAE mice treated MaR1 relative to those treated with vehicle (dotted line) **(A)**. Grey bars highlight those cytokines that are significantly regulated by MaR1. **B-E**, Graph showing the quantification of different immune cell populations in the spinal cord **(B)**, the number of CD4+ T cells expressing IL-4, IL-10, IFNγ or IL-17A **(C)**, the percent of CD4+ T cells expressing the CD49b, FoxP3, tBet, GATA3 or RORγ **(D)** and ratio of $Ly6C^{low}/Ly6C^{high}$ macrophages **(E)** in the spinal cord after MaR1 or vehicle treatment. *p<0.05; **p<0.01; ***p<0.001 vs. vehicle. Unpaired t-test (n=4 per group). Data is shown as mean±SEM.

Since cytokines and chemokines coordinate the migration of immune cells in tissues, we studied whether MaR1 modulated the immune cell counts in the spinal cord of EAE mice at the peak of the disease. Flow cytometry experiments revealed that MaR1 strongly reduced the counts of T cells, including both Th cells (CD3+, CD4+) and T cytotoxic cells (CD3+, CD8+), and B cells (CD45+, CD11b–, CD3–, CD24+). This SPM also reduced the accumulation of macrophages (CD45high, CD11b+, F4/80+) and activated microglial cells (CD45low, CD11b+, F4/80+) but not neutrophils (Figure 3B; Supplementary figure S1).

Since cytokines and SPMs can modulate Th cell and macrophage responses (Chiurchiù et al. 2016; Francos-Quijorna et al. 2017), we investigated whether MaR1 affected Th cell polarization at the peak of EAE. We found that MaR1 reduced the number of CD4 T cells expressing IFNγ in the spinal cord of EAE mice suggesting that the number of pathogenic Th1 cells was reduced (Figure 3C; Supplementary figure S2). However, the proportion of Th cells expressing tBet, a transcription factor associated to Th1 responses, was not changed. MaR1 did not reduced the number of Th2 and Th17 since the expression of cytokines and their signature transcription factors (GATA3 and RORγ, respectively) were not affected (Figure 3D). MaR1 also increased the proportion of T regulatory cells expressing CD49b but not FoxP3, which play a crucial role in maintaining tolerance to self-antigens and prevent autoimmune disease (Figure 3D; Supplementary figure S2). Besides, MaR1 boosted the ratio of $Ly6C^{low}/Ly6C^{high}$ macrophages by ~3 fold (Figure 3E; Supplementary figure S2), suggesting that this SPM also drove macrophages towards a more anti-inflammatory phenotype.

We then investigated whether the reduction in the infiltration of leukocytes in the spinal cord of EAE mice was associated to the ability of MaR1 to attenuate the expansion of immune cells in the lymph nodes and/or to their migration to the blood stream. We found that MaR1 slightly reduced the number of the different lymphocyte populations in the lymph nodes, although it was not statistically significant (Figure 4A). Similarly, MaR1 did not modulate CD4 T cell polarization (Figure 4B). MaR1 also failed to modify the number of neutrophils, macrophages and their phenotype in the lymph nodes (Figure 4A, C). However, it reduced by ~2 fold the number of dendritic cells (Figure 4B), which have a decisive role in Th cell priming.

Despite the minimal actions of MaR1 on lymphocyte and monocyte counts in the lymph nodes, we observed that less CD4 T cells, B cells and monocytes trafficked from lymphatic organs and/or bone morrow to the blood stream (Figure 5A). MaR1 also led to changes in the phenotype of circulating CD4 T cells in EAE mice, increasing the proportion of CD49b+ non-classic T regulatory cells and reducing that of pathogenic Th1 cells (Figure 5B). However, MaR1 did not modify the phenotype of monocytes in the circulation, which had a predominant pro-

inflammatory state (Ly6C^{high}) (Figure 5C). These results suggested that some of immune cell responses observed in the spinal cord of EAE treated with MaR1 may be due, in part, to the actions of this SPM in the periphery.

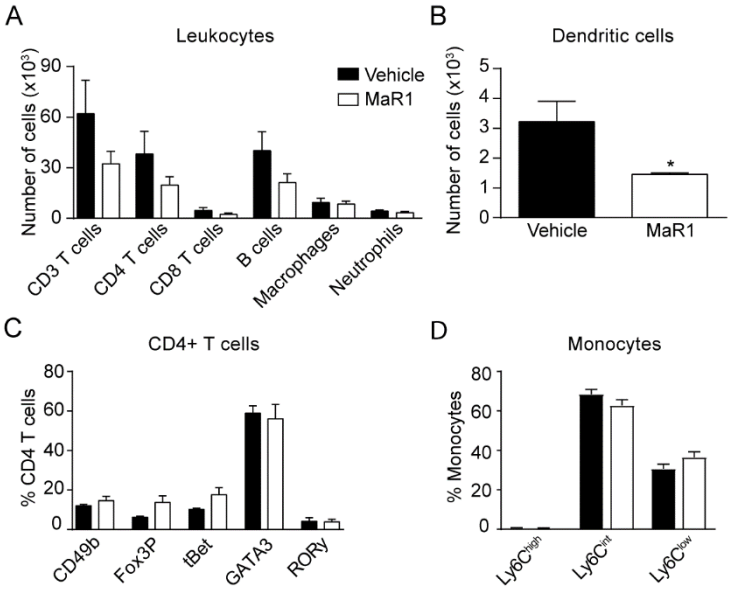


Figure 4. Effects of MaR1 on immune cells counts in the lymph nodes of EAE mice at disease peak. A-B, Graph showing the quantification of different leukocytes populations **(A)** and dendritic cells **(B)** in the lymph nodes of MaR1- or vehicle-treated mice. **C-D,** Plot showing the percent of CD4+ T cells expressing the transcription factors CD49b, FoxP3, tBet, GATA3 or RORγ **(C)** and the counts for the different macrophage subset according to Ly6C expression **(D)** in the lymph nodes of MaR1- or vehicle-treated mice at the peak of EAE. Unpaired t-test (n=4 per group). *p<0. vs. vehicle. Unpaired t-test (n=4 per group). Data is shown as mean±SEM.

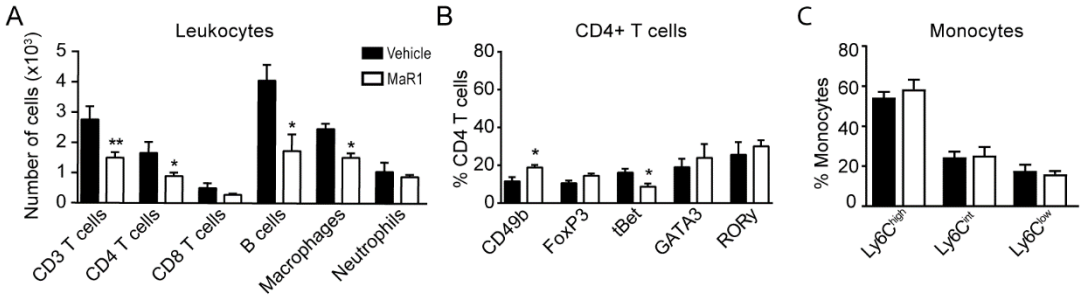


Figure 5. Effects of MaR1 on immune cells counts in the blood of EAE mice at disease peak. Graph showing the number of different leukocytes populations **(A),** plot showing the percent of CD4+ T cells expressing the transcription factors CD49b, FoxP3, tBet, GATA3 or RORγ **(B)** and the counts for different macrophage subset according to Ly6C expression **(C)** in blood of EAE mice treated with MaR1- or vehicle. Unpaired t-test (n=4 per group). Data shown as mean±sem.*p<0.05; **p<0.01 vs. vehicle. Unpaired t-test (n=4 per group). Data is shown as mean±SEM.

DISCUSSION

In the present study, we report that MS patients and EAE mice have aberrant induction of the key enzymes involved in the synthesis of SPMs. We reveal that the administration of the resolution agonists MaR1 and, in a lesser extent RvD1, conferred protection against neurological decline and myelin loss despite the treatment was initiated once the mice showed the first signs of the disease. Nonetheless, the treatment with RvE1 failed to protect against EAE outcomes. We also uncovered that the exogenous administration of MaR1 in EAE mice attenuates the pro-inflammatory environment of the CNS, modulates the polarization of Th cells and macrophages towards a more anti-inflammatory phenotype and reduces the trafficking of different leukocyte populations into the blood stream.

PUFAs play a critical role in the regulation of inflammation since they control several processes involved in the onset, but also, in the termination of this physiological response (Robinson, 1987; David et al., 2012; Serhan, 2014; Dalli and Serhan, 2018). In general, eicosanoids generated via the actions of cyclooxygenase 1 and 2 (COX-1 and 2) and 5-LOX from AA, such as prostaglandins, thromboxanes and leukotrienes, contribute to inflammation by promoting chemotaxis and activation of immune cells and increasing vascular permeability (Dennis and Norris, 2015; Belury and Harris, 2018). Nevertheless, not all the actions of eicosanoid are pro-inflammatory since a number of *in vivo* works reveal that 15d-Prostaglandin J2, for instance, attenuates inflammation and neurological decline in EAE and spinal cord injury (Diab et al., 2002; Kerr et al., 2008).

Contrary, SPMs are key players in the resolution of inflammation. Except for LXs, SPMs are derived from omega-3 PUFA, particularly from DHA and EPA. DHA can give rise to several resolution agonist including those of the RvD series, PD, and MaR. EPA can also yield some SPMs, namely RvE (Schwab et al., 2007; Buckley et al., 2014; Serhan, 2014; Chiang and Serhan, 2017; Dalli and Serhan, 2018).

Omega-3 PUFA are enriched in oils derived from fish and algae. Various epidemiological studies indicate that omega-3 PUFA dietary supplementation is associated with improved clinical improvement in various pathologies, including cardiovascular events or cancer (Siddiqui et al., 2004; Macsai, 2008; Marik and Varon, 2009). There are, however, conflicting results on the beneficial actions of omega-3 PUFA supplementation in MS, since several trials have failed to show efficacy despite the supplementation increased omega-3 PUFA levels in patients (Nordvik et al., 2000; Torkildsen et al., 2012; Jelinek et

al., 2013; Riccio et al., 2016). Our results suggest that this lack of efficacy is likely due to a failure of MS patient to produce adequate amounts of SPMs despite the bioavailability of omega-3 PUFA.

A previous report suggested that lipid mediator pathways are dysregulated in the CSF of MS patients (Prüss et al., 2013). Nonetheless, this phenomena is not unique of MS patients since this inappropriate production of SPM has been also observed in other CNS conditions such as Alzheimer's disease individuals (Wang et al., 2015) and in mice after contusion injury (Francos-Quijorna et al., 2017). In the present work, we provide evidence for the first time suggesting that the aberrant production of SPMs in MS patients is associated to an inappropriate induction of *LOX-12* and *LOX-15*. Our data reveal that the transcript of these two enzymes, which play a key role in the initiation of the SPMs biosynthetic pathway, are not induced in PBMC of MS patients. We also report that the levels of *LOX-12*, and *LOX-15* were only increased in active lesions of some MS individuals, whereas in others these transcripts remained unchanged or even down-regulated. Interestingly, we observed that *LOX-12* and *LOX-15*, were simultaneously increased in only 1 out of the 7 brain MS samples analysed, further indicating that the biosynthetic pathways of SPMs are likely to be impaired in the most MS patients. This dysregulation in the resolution pathways seems to be also present in EAE mice, since we uncovered that the transcripts of *Alox12/15* were not found in the spinal cord during EAE progression. In this line, a previous study revealed that RvD1 levels are markedly reduced in the plasma of EAE (Poisson et al., 2015). Importantly, administration of RvD1 in EAE attenuated the clinical signs of disease, suggesting that the exogenous administration of SPMs could revert of the pathological signs of the diseases (Poisson et al., 2015). Here we also demonstrate that RvD1 promotes therapeutic action in EAE mice.

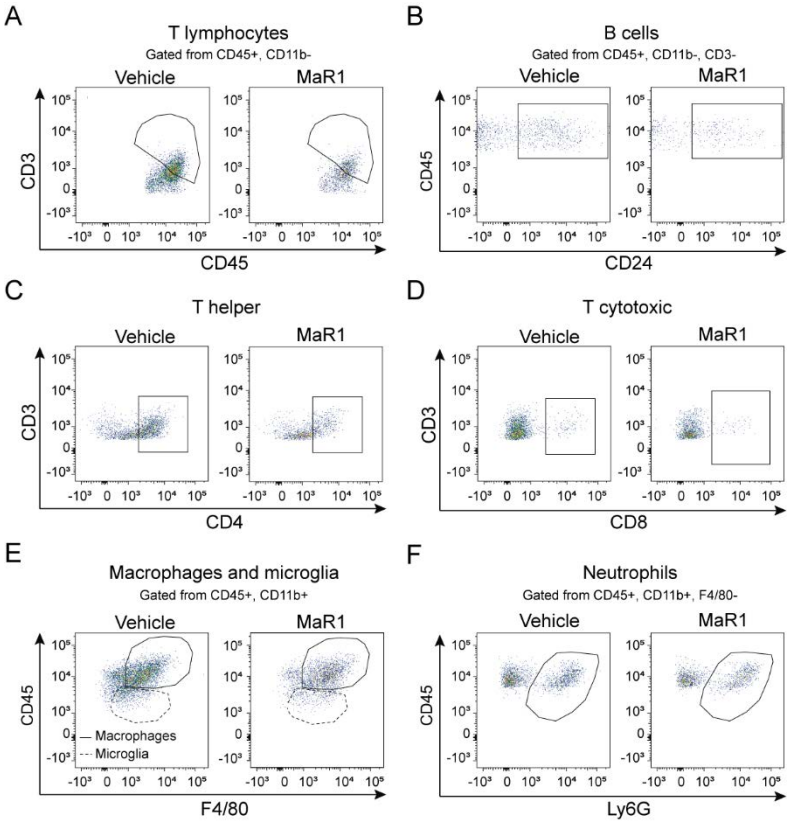
MaR1 is one of the members of SPMs less characterized. Recently, we have demonstrated that MaR1 enhanced inflammatory resolution after spinal cord injury and reduced myelin loss and neurological deficits, but also promote neuroprotection in spinal muscular atrophy (Francos-Quijorna et al., 2017). Our data reveals that administration of very low doses of MaR1 in EAE mice also conferred protection against functional deficits and myelin loss, despite MaR1 treatment was initiated after disease onset. Importantly, MaR1 exerted greater therapeutic actions than RvD1. We also found that MaR1 modulated different response associated to inflammation. First, MaR1 reduced the protein levels of different pro-inflammatory cytokines in the spinal cord of EAE mice, including IL-1α,

IL-1β, TNFα, IFNγ and IL-6, among others, but did not alter the amounts of anti-inflammatory/immunomodulatory cytokines, such as IL-4 or IL-10. These pro-inflammatory cytokines play a critical role in the development of EAE (Schrijver et al., 2003; Serada et al., 2008; Lévesque et al., 2016), and are associated to neurodegeneration and symptomatology progression in MS patients (Kallaur et al., 2013; Rossi et al., 2014), which may explains the therapeutic effect observed in EAE mice treated with MaR1. Second, MaR1 reduced the accumulation of most of the leukocyte population in the spinal cord of EAE mice, in exception of neutrophils. This is likely to be caused, at least in part, by the decreased levels of cytokines in the spinal cord, but also by the ability of MaR1 in hampering the efflux of these leukocyte population into the blood, since their counts were not attenuated in the lymph nodes but were reduced in the blood. Third, MaR1 modulated the polarization of macrophages and Th cells towards a more ant-inflammatory phenotype. Particularly, MaR1 reduced the pathogenic Th1 cells in the spinal cord and increased the number of non-classical T regulatory cells. Our data correlates with a previous study that revealed that resolution agonists, including MaR1, attenuates the activation of pathogenic Th1 and Th17 cells and their generation from naïve CD4 cells (Chiurchiù et al., 2016). Nonetheless, we did not observe any significant change in the counts of different Th cell populations in the lymph nodes, suggesting that MaR1 does not regulate the activation of Th cells in EAE, at least when MaR1 treatment is initiated after disease onset. However, MaR1 reduced the counts of Th1 cells in the blood and increased those for non-classical T regulatory cells, suggesting that MaR1 is selectively modulating the mobilization of these two Th cell subsets into the blood rather than their activation. Highlight, that similar to our previous studies in spinal cord injury (Francos-Quijorna et al., 2017), we observed that macrophages that infiltrated into the spinal cord of mice undergoing EAE adopted a more anti-inflammatory phenotype at the peak of the disease. This effect was observed in the CNS but not in the lymph nodes or blood, suggesting that the modulation of the inflammatory environment of the CNS by MaR1 is most likely to be involved in the polarizing effects on macrophages.

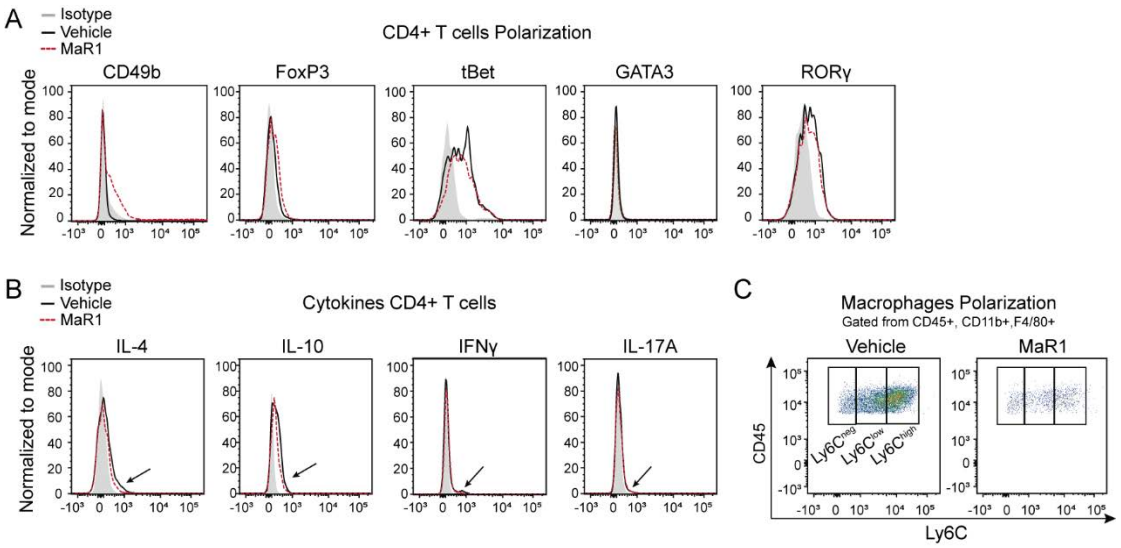
Overall, we provide clear evidence demonstrating that the enzymes involved in the biosynthetic pathways of pro-resolving lipid mediators are not efficiently turned on in the CNS and blood of MS patients. Our data indicate that exogenous administration of MaR1 confers protection against neurological decline and myelin loss by means of enhancing multiple mechanisms needed for preventing inflammation in EAE mice. This data

therefore indicate that MaR1 could open a novel therapeutic avenue for the treatment of MS patients.

SUPLEMMENTARY FIGURES



S1. MaR1 reduces the accumulation of immune cells in the spinal cord of mice at the peak of EAE. Representative dot plots showing different **(A)** T lymphocytes**, (B)** B cells, **(C)** T helper cells, **(D)** T cytotoxic cells, **(E)** macrophages and microglia and **(F)** neutrophils in the spinal cord at the peak of EAE in vehicle- and MaR1-treated mice.



S2. MaR1 polarizes immune cells towards a more anti-inflammatory phenotype in the spinal cord of EAE mice. Representative flow cytometry histograms characterising the expression of Th cells polarization markers **(A-B)** and macrophages **(C)** in the spinal cord at the peak of EAE in vehicle- and MaR1-treated mice.

REFERENCES

Belury MA, Harris WS (2018) Omega-6 fatty acids, inflammation and cardiometabolic health: Overview of supplementary issue. Prostaglandins, Leukot Essent Fat Acids 139:1–2 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30471767 [Accessed June 3, 2019].

Buckley CD, Gilroy DW, Serhan CN (2014) Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 40:315–327 Available at: http://linkinghub.elsevier.com/retrieve/pii/S1074761314000776 [Accessed July 23, 2018].

Chiang N, Serhan CN (2017) Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Aspects Med 58:114–129 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28336292 [Accessed June 21, 2018].

Chiurchiù V, Leuti A, Dalli J, Jacobsson A, Battistini L, Maccarrone M, Serhan CN (2016) Proresolving lipid mediators resolvin D1, resolvin D2, and maresin 1 are critical in modulating T cell responses. Sci Transl Med 8:353ra111-353ra111 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27559094 [Accessed February 25, 2019].

Dalli J, Serhan CN (2018) Identification and structure elucidation of the pro-resolving mediators provides novel leads for resolution pharmacology. Br J Pharmacol Available at: http://doi.wiley.com/10.1111/bph.14336 [Accessed July 19, 2018].

David S, Greenhalgh AD, López-Vales R (2012) Role of phospholipase A 2s and lipid mediators in secondary damage after spinal cord injury. Cell Tissue Res 349:249–267 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22581384 [Accessed February 20, 2019].

Dennis EA, Norris PC (2015) Eicosanoid storm in infection and inflammation. Nat Rev Immunol 15:511–523 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26139350 [Accessed June 3, 2019].

Diab A, Deng C, Smith JD, Hussain RZ, Phanavanh B, Lovett-Racke AE, Drew PD, Racke MK (2002) Peroxisome Proliferator-Activated Receptor- Agonist 15-Deoxy- 12,1412,14-Prostaglandin J2 Ameliorates Experimental Autoimmune Encephalomyelitis. J Immunol 168:2508–2515 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11859145 [Accessed June 3, 2019].

Francos-Quijorna I, Santos-Nogueira E, Gronert K, Sullivan AB, Kopp MA, Brommer B, David S, Schwab JM, López-Vales R (2017) Maresin 1 Promotes Inflammatory Resolution, Neuroprotection, and Functional Neurological Recovery After Spinal Cord Injury. J Neurosci 37:11731–11743 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29109234 [Accessed July 23, 2018].

Jelinek GA, Hadgkiss EJ, Weiland TJ, Pereira NG, Marck CH, van der Meer DM (2013) Association of fish consumption and omega 3 supplementation with quality of life, disability and disease activity in an international cohort of people with multiple sclerosis. Int J Neurosci 123:792–801 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23713615 [Accessed June 3, 2019].

Kallaur AP, Oliveira SR, Simao ANC, De Almeida ERD, Morimoto HK, Lopes J, De Carvalho Jennings Pereira WL, Andrade RM, Pelegrino LM, Borelli SD, Kaimen-Maciel DR, Reiche EMV (2013) Cytokine profile in relapsing-remitting Multiple sclerosis patients and the association between progression and activity of the disease. Mol Med Rep 7:1010–

1020 Available at: https://www.spandidos-publications.com/10.3892/mmr.2013.1256 [Accessed February 21, 2019].

Kerr BJ, Girolami EI, Ghasemlou N, Jeong SY, David S (2008) The protective effects of 15-deoxy-Δ-12,14-prostaglandin J2 in spinal cord injury. Glia 56:436–448 Available at: http://www.ncbi.nlm.nih.gov/pubmed/18205174 [Accessed June 3, 2019].

Lévesque SA, Paré A, Mailhot B, Bellver-Landete V, Kébir H, Lécuyer M-A, Alvarez JI, Prat A, Vaccari JP de R, Keane RW, Lacroix S (2016) Myeloid cell transmigration across the CNS vasculature triggers IL-1β–driven neuroinflammation during autoimmune encephalomyelitis in mice. J Exp Med 213:929–949 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27139491 [Accessed February 21, 2019].

Macsai MS (2008) The role of omega-3 dietary supplementation in blepharitis and meibomian gland dysfunction (an AOS thesis). Trans Am Ophthalmol Soc 106:336–356 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19277245 [Accessed June 3, 2019].

Marik PE, Varon J (2009) Omega-3 Dietary Supplements and the Risk of Cardiovascular Events: A Systematic Review. Clin Cardiol 32:365–372 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19609891 [Accessed June 3, 2019].

Nordvik I, Myhr K, Nyland H, Bjerve KS (2000) Effect of dietary advice and n-3 supplementation in newly diagnosed MS patients. Acta Neurol Scand 102:143–149 Available at: https://onlinelibrary.wiley.com/doi/abs/10.1034/j.1600-0404.2000.102003143.x?sid=nlm%3Apubmed [Accessed June 3, 2019].

Poisson LM, Suhail H, Singh J, Datta I, Denic A, Labuzek K, Hoda MN, Shankar A, Kumar A, Cerghet M, Elias S, Mohney RP, Rodriguez M, Rattan R, Mangalam AK, Giri S (2015) Untargeted Plasma Metabolomics Identifies Endogenous Metabolite with Drug-like Properties in Chronic Animal Model of Multiple Sclerosis. J Biol Chem 290:30697–30712 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26546682 [Accessed May 8, 2019].

Prüss H, Rosche B, Sullivan AB, Brommer B, Wengert O, Gronert K, Schwab JM (2013) Proresolution Lipid Mediators in Multiple Sclerosis — Differential, Disease Severity-Dependent Synthesis — A Clinical Pilot Trial Bozza PT, ed. PLoS One 8:e55859 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23409068 [Accessed July 23, 2018].

Riccio P, Rossano R, Larocca M, Trotta V, Mennella I, Vitaglione P, Ettorre M, Graverini A, De Santis A, Di Monte E, Coniglio MG (2016) Anti-inflammatory nutritional intervention in patients with relapsing-remitting and primary-progressive multiple sclerosis: A pilot study. Exp Biol Med 241:620–635 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26785711 [Accessed May 8, 2019].

Robinson DR (1987) Lipid mediators of inflammation. Rheum Dis Clin North Am 13:385–405 Available at: http://www.ncbi.nlm.nih.gov/pubmed/2827247 [Accessed February 20, 2019].

Rossi S, Motta C, Studer V, Macchiarulo G, Volpe E, Barbieri F, Ruocco G, Buttari F, Finardi A, Mancino R, Weiss S, Battistini L, Martino G, Furlan R, Drulovic J, Centonze D (2014) Interleukin-1β causes excitotoxic neurodegeneration and multiple sclerosis disease progression by activating the apoptotic protein p53. Mol Neurodegener 9:56 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25495224 [Accessed April 13, 2019].

Schrijver HM, Van As J, Crusius JBA, Dijkstra CD, Uitdehaag BMJ (2003) Interleukin (IL)-1 gene polymorphisms: Relevance of disease severity associated alleles with IL-1β and

IL-1ra production in multiple sclerosis. Mediators Inflamm 12:89–94 Available at: http://www.ncbi.nlm.nih.gov/pubmed/12775358 [Accessed February 21, 2019].

Schwab JM, Chiang N, Arita M, Serhan CN (2007) Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature 447:869–874 Available at: http://www.nature.com/articles/nature05877 [Accessed June 21, 2018].

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. Proc Natl Acad Sci 105:9041–9046 Available at: http://www.ncbi.nlm.nih.gov/pubmed/18577591 [Accessed May 8, 2019].

Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510:92–101 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24899309 [Accessed July 20, 2018].

Siddiqui RA, Shaikh SR, Sech LA, Yount HR, Stillwell W, Zaloga GP (2004) Omega 3-fatty acids: health benefits and cellular mechanisms of action. Mini Rev Med Chem 4:859–871 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15544547 [Accessed June 3, 2019].

Torkildsen Ø, Wergeland S, Bakke S, Beiske AG, Bjerve KS, Hovdal H, Midgard R, Lilleås F, Pedersen T, Bjørnarå B, Dalene F, Kleveland G, Schepel J, Olsen IC, Myhr K-M (2012) ω-3 Fatty Acid Treatment in Multiple Sclerosis (OFAMS Study). Arch Neurol 69:1044–1051 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22507886 [Accessed May 8, 2019].

Wang X, Zhu M, Hjorth E, Cortés-Toro V, Eyjolfsdottir H, Graff C, Nennesmo I, Palmblad J, Eriksdotter M, Sambamurti K, Fitzgerald JM, Serhan CN, Granholm AC, Schultzberg M (2015) Resolution of inflammation is altered in Alzheimer's disease. Alzheimer's Dement 11:40–50.e2 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24530025 [Accessed February 20, 2019].

GENERAL DISCUSSION

Although MS was described by Dr Jean-Martin Charcot 1868, it is still a challenging neurological disorder. There are several approved anti-inflammatory and immunomodulatory drugs to palliate the symptomatology, but unfortunately, all of them are highly ineffective, especially for those individuals suffering from PPMS and SPMS, the most aggressive forms of the pathology. Besides, all the approved drugs have powerful side-effects, worsening the quality of life in patients (Reviwed at Wagner and Goverman, 2015; Bjelobaba, Savic and Lavrnja, 2017; Thompson, 2017).

The cause of MS remains unknown, but it is widely accepted that demyelination and further neurodegeneration is indisputably linked to inflammation within the brain and spinal cord. In fact, MS is considered as an inflammatory disorder since the myelin sheaths are stripped off by infiltrated and resident inflammatory cells (Reviewed at Dendrou, Fugger, & Friese, 2015). Thus, inflammation is a double-edge sword. On one hand, under physiological conditions, the immune response plays an essential role after injury since it avoids infections, helps to clear cellular debris and initiates responses that are important for wound healing and for the restoration of homeostasis (Reviwed at Velnar et al. 2009). Wound healing and fight against infections are not simple phenomena but involve complex mechanisms with numerous cell types, cytokines, mediators and also the vascular system. After vasoconstriction and platelet aggregation, the initiation inflammatory phase that is characterised by an influx of inflammatory cells that release mediators and cytokines to promote angiogenesis, thrombosis and, finally, reepithelialisation (Reviwed Velnar et al. 2009). Inflammation is a finite process that resolves when damage or infection have been abated and the tissue has been repaired. Resolution of inflammation is an active and tightly regulated event that involves the clearance of activated inflammatory cells (Buckley et al., 2013; Serhan, 2014; Serhan et al., 2015). Unfortunately, many diseases are associated to altered inflammatory events. In some cases, these disorders consist of an inflammatory response triggered against an unidentified agent or even self-antigens, as it occurs in autoimmune disorders (Reviewed at Wang, Wang and Gershwin, 2015; Kapsogeorgou and

Tzioufas, 2016). In other cases, the inflammatory response is triggered when needed but it cannot be resolved and becomes chronic, what is also associated to a myriad of diseases (reviewed at Schett & Neurath 2018).

The self-antigen that triggers the inflammatory response in MS remains unknown, but depending on the clinical form, inflammation could be either episodic or persistent (Reviwed at Lassmann et al. 2007; Wu & Alvarez 2011). For instance, the RRMS form is characterized by clearly defined inflammatory attacks that produce new neurologic symptoms or worsen those that patients already had (Steinman, 2014). Nonetheless, in the progressive forms of MS, the inflammatory cells become trapped within the CNS behind an unaffected BBB forming lymph follicle-like aggregates in the meninges, which ends in accumulated neurologic decline (Pérez-Cerdá et al., 2016).

In the present thesis dissertation, we focused our interest in the modulation of the inflammatory response to investigate whether this strategy successfully prevent functional deficits and demyelination in EAE, the widest used murine model of MS. For this purpose, in the first chapter we attenuated inflammation by inhibiting the NLRP3 inflammasome, which mediates the conversion of the pro-inflammatory cytokines IL-1β and IL-18 into their forms. In the second chapter, we used IL-37, a new cytokine that has potent suppressive actions on immunity. Finally, in the third chapter, we activated of resolution programmes of the inflammation by using SPMs.

In chapter one, we focused our attention on IL-1β and IL-18, which belong to the IL-1 family (Garlanda et al., 2013; Keyel, 2014; Palomo et al., 2015). It has been widely reported that these cytokines are involved in the physiopathology of MS and EAE. IL-1β is increased in MS patients (Heidary et al. 2014; Rossi et al. 2014) and pharmacological treatments blocking IL-1β, as well as, mice lacking this cytokine resulted in protection against the development of EAE clinical sings (Symons et al. 1987; Baker et al. 1991; Chung et al. 2009; Lévesque et al. 2016). IL-18 is also increased in MS patients and EAE (Losy and Niezgoda, 2001; Nicoletti et al., 2001), and similar to IL-1β, knockout mice for IL-18 are protected against EAE (Shi et al., 2000). IL-1β and IL-18 are produced as an inactive precursor and require the maturation into an active cytokine by caspase-1 which, in turn, has to be activated by oligomerization of inflammasomes (Franchi et al., 2009). Many inflammasomes

have been currently described, but the most extensively characterized is the NLRP3 inflammasome (Reviewed at Yang *et al.*, 2019). Different pharmacological inhibitors have been generated for NLRP3 (Reviewed at Yang *et al.,* 2019). In fact, there are two study demonstrating that MCC950, a potent NLRP3 inhibitor, reduced neurological decline in a mouse model of RRMS (Khan et al., 2018). Here, we tested the effects of OLT1177, another specific inhibitor of NLRP3. This drug recently moved into phase I clinical trial conducted by Olatec Therapeutics and demonstrated that is safe in humans (Marchetti et al., 2018a). Moreover, OLT1177 has also provided favourable results in a phase II clinical trial with individuals suffering from gout (Jansen et al., 2019). In contrast to the two previous studies using the MCC950 compound in EAE, we conducted our studies in an chronic mouse model of EAE , which resembles more closely the progressive forms of MS, the most clinical challenging forms of MS since they lack any effective treatment (Thompson, 2017; Tur and Montalban, 2017). We demonstrated that OLT1177 could be a promising drug for MS. We showed that the delivery of this NLRP3 inhibitor in the food satisfactorily reduced the clinical signs of EAE when treatment was introduced on the day of the disease induction. Nonetheless, we found that the low OLT1177 dose (3.75 g/kg food), but not the high dose diet (7.5 g/kg food), protected against functional loss and demyelination. We then sought to investigate the mechanisms underlying the protective effects of this NLRP3 inhibitor in EAE, and, assessed the effects of this drug on cytokine levels in the spinal cord and blood.

Cytokines play a key role in the activation of resident glial cells and also in the recruitment of more immune cells from the circulation, that all together, contribute to EAE development (Reviewed at Becher et al. 2017). We found that the low dose of OLT1177 in the food showed a CNS-specific effect since the treatment reduced the protein levels of IL-1β, IL-18, IL-6 and TNFα in the spinal cord but not in the blood plasma. Similar to functional and histological outcomes, the high dose of OLT1177 in the food showed less effectivity in reducing cytokine concentration since it only decreased the levels of IL-1β and TNFα in the spinal cord of EAE mice.

IL-1β and IL-6 reduction by the low dose of OLT1177 was also reported previously in other tissues (Marchetti et al., 2018a, 2018b). However, OLT1177 failed to decrease the levels of TNFα in synovial tissue (Marchetti et al., 2018b), lung, liver,

spleen or muscle (Marchetti et al., 2018a). Since the low those of OLT1177 reduced dramatically the levels of TNFα in the spinal cord of EAE mice, this might be a specific effect of this drug in the CNS.

The reduction in cytokines levels by OLT1177 is likely to be crucial for its protective effects against EAE, since previous experiments demonstrated that mice lacking IL-1α, IL-1β or IL-6 are resistant to the development of EAE (Eugster et al., 1998; Chung et al., 2009). Besides, sustained TNFα production in the CNS also promotes EAE progression (Valentin-Torres et al., 2016), supporting the beneficial effects of this NLRP3 inhibitor.

The modulation of the pro-inflammatory milieu in the spinal cord of OLT1177-fed EAE mice was coupled to reduced accumulation of immune cells into the CNS, especially CD4 T cells and macrophages. This point might be essential for the protective actions of OLT1177 in this MS mouse model since the infiltration of immune cells from the periphery, and their accumulation in CNS, have been directly related to tissue damage and functional outcomes (Ajami et al., 2011; Duffy et al., 2014; Caravagna et al., 2018). Nonetheless, the functional phenotype of lymphocytes and macrophages remained unaltered by OLT1177, demonstrating that this drug has a specific effect on the infiltration but not on the polarization of immune cells. Again, the reduction of immune cells by OLT1177 was greater in the low dose (3.75 g/kg food) compared to the high dose (7.5 g/kg food), which is likely due the greater effectivity of this dose on reducing cytokine levels.

Contrary to our results, a direct correlation between increasing doses of OLT1177 and reduction of join inflammation was previously described (Marchetti et al., 2018a). Nevertheless, the pharmacokinetics and pharmacodynamics are currently under investigation and, probably, this drug may perform differently depending on the targeted tissue and/or disease.

We also assessed the therapeutic effects of OLT1177 in EAE physiopathology. For this purpose, we first defined an optimal dose of OLT1177 by based on two different prophylactic protocols: (i) single-daily administration (i.p.) of a high dose of OLT1177 (200 mg/kg); (ii) twice-daily administration (i.p.) of a low dose of OLT1177 (60 mg/kg). In line with the OLT1177-enriched food data reported above,

we found that the low dose of OLT1177 exerted greater efficacy in enhancing functional outcomes in EAE mice. Moreover, the low OLT1177 dose also reduced myelin loss in the spinal cord. Finally, we also uncovered that the twice-daily administration of 60 mg/kg of OLT1177 by oral gavage protected against neurological decline and demyelination when treatment was initiated after disease onset. Therefore, our data suggest that OLT1177 might be promising treatment for MS. Since this drug is safe in humans (Marchetti et al., 2018a), this drug may progress rapidly to the clinic.

In chapter two, we focused our attention on IL-37. Similar to IL-1β and IL-18, IL-37 belongs to the IL-1 family (Garlanda et al., 2013; Keyel, 2014; Palomo et al., 2015). IL-37 is the only member of this cytokine family that has anti-inflammatory properties (Nold et al., 2010). Similar to all the members of IL-1 family, *IL-37* is located in the human chromosome 2. There is, however, no mouse gene coding for *il-37*. There is also no IL-37 gene in chimpanzees, our closest genetic relative, although IL-37 is present in the gorilla and other primates (Newman et al., 2005). It is not possible to know whether there was an ancient IL-37 in the mouse or chimpanzee that was lost through evolution. The first exon of IL-37 is present in the mouse but there is no open reading frame. One explanation is that an insertion by endogenous retroviruses disrupted an ancient IL-37 in the mouse, as well as, in the chimpanzee. Nevertheless, the development of a transgenic mouse expressing the human *IL-37b* (hIL-37tg) has been crucial for revealing its broad anti-inflammatory actions. These mice showed resistance against a myriad of pathological conditions, such as colitis (McNamee et al., 2011), cancer (Henry et al., 2015) or arthritis (Ye et al., 2015), among others. Indeed, three years ago we uncovered for the first time that IL-37 is also protective against CNS trauma (Coll-Miró et al., 2016). However, there is not studies exploring the actions of this cytokine in other neurological conditions. This milestone encouraged us to explore the role of IL-37 in a neuroinflammatory disease, such as MS.

For this purpose, we induced EAE in hIL-37tg mice and their WT littermate mice. Although the expression of *IL-37* in hIL-37tg mice is regulated by the constitute CMV promoter, it has been described that under physiological conditions the mRNA is barely detected (Coll-Miró et al., 2016) due to an instability sequence present in the

exon 5 (Bufler et al., 2004). Nevertheless, the inflammatory stimuli stabilize *IL-37* transcripts and the protein can be then produced (Bufler et al., 2004; Coll-Miró et al., 2016). We found that the *IL-37* transcripts were barely detected in the spinal cord of hIL-37tg in physiological conditions, but they were up-regulated at the peak and chronic phase of EAE disease. The cells that secrete IL-37 in the CNS remains elusive, but we hypothesize that infiltrating immune cell and/or activated microglia are the source, since its expression correlates with the infiltration of immune cells and microglia expansion in the CNS (Caravagna et al., 2018). Importantly, transgenic expression of *IL-37* resulted in beneficial effects in EAE diseased severity, since it protected against neurological impairments and demyelination.

We also uncovered some of the mechanisms underlying the protective actions of IL-37 in EAE. As reported before, cytokines play a key role in the activation of resident glial cells and in the recruitment of immune cells from the circulation (Reviewed at Becher et al. 2017). We demonstrated that IL-37 reduced dramatically the levels of some of the key cytokines that contribute to MS and EAE: IL-1α and IL-1β (Symons et al., 1987; Baker et al., 1991; Chung et al., 2009; Rossi et al., 2014; Lévesque et al., 2016), IL-6 (Eugster et al., 1998; Linker et al., 2008; Erta et al., 2016), TNFα and IFNγ (Kallaur et al., 2013; Valentin-Torres et al., 2016). Previous studies revealed that IL-37 inhibits the NLPR3 inflammasome (Moretti et al., 2014), which may explain the decrease in some of the cytokines reduced by OLT1177. The reduction of cytokine levels was accompanied by attenuation in the infiltration of immune cells within the spinal cord at the peak of the disease in hIL-37tg mice. This is likely to be the more related event underlying the protective effects conferred by IL-37 in our model, since leukocytes appear accumulated in the lesioned areas in the CNS, which correlates with tissue damage and functional outcomes (Ajami et al., 2011; Duffy et al., 2014; Caravagna et al., 2018). Nonetheless, not only the counts of immune cells are related to the physiopathology of EAE, but also their phenotype. For instance, T regulatory cells are the cells with the strongest suppressive actions on inflammation are found in the recovery phase of EAE (O'Connor et al., 2007; Koutrolos et al., 2014). In contrast, Th1 cells, which express the transcription factor tBet, secrete IFNγ and TNFα and are presumed to be the principal mediators of MS development (Reviewed at O'Brien et al. 2010), since a previous work showed that tBet KO mice were resistant to the development of EAE (Nath et al., 2006). Importantly, IL-37 was

able to increase the percent of T regulatory cells and decrease Th1 cells, which may explain its protective actions in the CNS. We also uncovered that IL-37 reduced the numbers of immune cells in the lymph nodes, especially, the counts of dendritic cells. These cells are considered the main initiator of innate and adaptive immunity since they are important, not only for the generation of T cell-mediated immune responses, but also for the induction and maintenance of central and peripheral tolerance (Sellés-Moreno et al., 2012; Mansilla et al., 2015; Zhou et al., 2017; Chen et al., 2018; Zubizarreta et al., 2019). Hence, the reduction in dendritic cell numbers in the lymph nodes by IL-37 may lead to immune tolerance and explain, in part, the lower infiltration and activation of immune cells within the CNS parenchyma of hIL-37tg mice. In this line, a recent a phase Ib clinical trial with tolerogenic dendritic cells in patients with MS or neuromyelitis optica has resulted in promising results (Zubizarreta et al., 2019).

IL-37 is as a dual-function cytokine since it can function as a extracellular cytokine by signalling via the receptor complex IL-18Rα-SIGIRR, but this can also translocate to the nucleus by binding SMAD3. In this thesis, we elucidated that the extracellular function of IL-37 is critical for mediating its therapeutic effects against EAE. We uncovered that genetic deletion of *sigirr*, one of the main components of the IL-37 surface receptor, abolished the beneficial actions of IL-37 in this MS mouse model. Indeed, previous works also revealed that IL-37 fails to reduce several pro-inflammatory cytokines when the extracellular complex is blocked (Nold-Petry et al., 2015). A recent study demonstrated that nuclear function of IL-37 also plays a key role in supressing cytokine expression, especially, at early stages upon the inflammatory challenge (Li et al., 2019). Thus, further experiments are needed to elucidate to what degree the nuclear function of IL-37 mediates anti-inflammatory actions in EAE.

Similar to hIL-37tg, mice, we found that *IL-37* transcripts were barely detected in human brain and PBMC samples of healthy individuals, although the components of the receptor complex, *IL-18Rα* and *SIGIRR* were expressed. Importantly, *IL-37* mRNA levels were not increased in PBMC and brain active lesions of MS patients despite the presence of the surface receptor. These data suggest that MS is associated to a deficiency of IL-37, as previously observed in other inflammatory

conditions, such as, systemic lupus erythematosus (Wu et al., 2016), psoriasis (Sehat et al., 2000), hepatitis (Li et al., 2013), asthma (Elfeky et al., 2018) and periodontal disease (Sağlam et al., 2015). This is interesting since MS patients may mimic a similar scenario than that observed in EAE mice, since they lack *il-37* despite expressing the IL-37 receptor complex. Importantly, we also demonstrated that daily delivery of recombinant human IL-37 protein ameliorated functional and histological outcomes in EAE mice albeit IL-37 treatment was initiated when mice showed the first signs of the disease.

IL-37 may organize as dimeric structures with nanomolar affinity, which reduce the bioactivity of the molecule (Ellisdon et al., 2017). A recent report showed that the most active conformation of IL-37 is the monomeric form (Eisenmesser et al., 2019). To address whether the effects obtained using the recombinant human IL-37 protein could be enhanced in EAE, we tested the effects treatment with native IL-37, and two other recombinant forms, IL-37^{D73K} and IL-37^{Y85A}, which carry mutations that specifically disrupt the dimer formation. Nonetheless, we found that the native and mutated forms of IL-37 exerted similar therapeutic effects in EAE mice, although the monomeric form, IL-37^{D73K}, showed a slight greater efficacy.

Finally, in the third chapter, we laid aside the anti-inflammatory molecules to attenuate inflammation and addressed a different novel strategy to reach this aim: to stimulate the resolution of inflammation by using resolution agonists. Although the resolution of inflammation was thought to be passive process mediated by the dilution of the inflammatory mediators in the inflammatory milieu, it is now known that this is an active and tightly regulated event (Serhan et al., 2010; Serhan, 2014). The resolution of inflammation is triggered by a family of bioactive lipids known as SPMs, which are naturally produced from PUFAs (omega-3 or omega-6) (Serhan et al., 2015; Chiang and Serhan, 2017). This family of lipids includes LX, RvD, RvE, PD and MaR (Schwab et al., 2007; Buckley et al., 2014; Serhan, 2014, 2017). Failure in the synthesis of SPMs have been associated to chronic or unwanted inflammation in Alzheimer's diseases (Wang et al., 2015b), atherosclerosis (Fredman et al., 2016) or spinal cord injury (Francos-Quijorna et al., 2017). There are currently two studies addressing the resolution of inflammation in MS. In the first study, the authors uncovered that the lipid mediator pathways are dysregulated in the CSF of MS

individuals, suggesting aberrant production of SPMs in the CNS of these patients (Prüss et al., 2013). The second study reported that the metabolites derived from PUFAs (omega-3 and omega-6) were decreased in EAE mice and MS patients, and demonstrated that oral administration of RvD1 ameliorated the clinical signs of EAE (Poisson et al., 2015). In the present thesis, we revealed that the key enzymes involved in the synthesis of SPMs, *LOX-12* and *LOX-15*, are aberrantly induced in the PBMCs and in brain active lesions of MS patients, but also in the spinal cord of mice undergoing EAE. The failure of MS patients to induce these enzymes may explain, in part, the lack of positive results observed by omega-3 supplementation in this disease (Torkildsen et al., 2012; Riccio et al., 2016), because despite the bioavailability of PUFA, SPMs are unlikely to be produced due to the aberrant production of LOX enzymes.

We therefore investigated whether exogenous delivery of different SPMs derived from omega-3 PUFAs attenuated neurological impairments in EAE mice. We found that RvE1 failed to ameliorate the clinicals signs of the disease. However, MaR1 and RvD1 conferred protection against functional deficits when treatment was initiated at disease. This supports the beneficial actions of RvD1 in EAE described previously (Poisson et al., 2015). In its noteworthy to mention that MaR1 showed greater therapeutic effects than RvD1, and therefore, we focused our studies on this SPM.

MaR1 is one of the less characterised SPMs. However, as we reported here, several studies have demonstrated that this SPM is able to enhance the resolution of inflammation in animal models of disease, such as, spinal cord injury (Francos-Quijorna et al., 2017), brain ischemia (Xian et al., 2016), sepsis (Li et al., 2016), or spinal muscular atrophy (Ohuchi et al., 2018). We found that MaR1 reduced the protein levels of many pro-inflammatory cytokines in the spinal cord, such as IL-1α, IL-1β, TNFα, IFNγ and IL-6, which play a critical role in development of MS (Kallaur et al., 2013; Rossi et al., 2014) and EAE (Eugster et al., 1998; Schrijver et al., 2003; Serada et al., 2008; Lévesque et al., 2016; Valentin-Torres et al., 2016). Similar to OLT1177 and IL-37, the modulation of the pro-inflammatory milieu by MaR1 caused the reduction in the infiltration of most of the leukocyte populations into the spinal cord, which may explain the protective effects conferred by this SPM in EAE mice. MaR1 also hampered the efflux of leukocytes into the blood stream from lymphatic

organs or bone marrow since their numbers were reduced in the circulation of hIL-37tg mice but not in the lymph nodes. The only immune population that was reduced by MaR1 in lymph nodes was the dendritic cells. This may by crucial to explain immunomodulatory effects of this SPM in EAE, and consequently, on disease progression (Sellés-Moreno et al., 2012; Mansilla et al., 2015; Zhou et al., 2017; Chen et al., 2018; Zubizarreta et al., 2019).

As reported before, lymphocytes and macrophages are heterogenous populations that can be divided into different categories with differential involvement in the pathology. Interestingly, we found that MaR1 not only reduced the counts of T cells in the spinal cord of EAE mice, but it also switched their phenotype towards a more regulatory and anti-inflammatory state. A previous work reveals that MaR1 is able to alter the phenotype of lymphocytes to a more anti-inflammatory suppressive (Chiurchiù et al., 2016). Here, we found that MaR1 boosted the percentage of non-classic CD49b+ T regulatory cells in the spinal cord and blood. This might be important for the protective action of this SPM since these cells play a critical role in the maintenance of peripheral immune tolerance (Gagliani et al., 2013) and MS patients have impaired infiltration of T regulatory cells cells within the lesioned CNS (Fritzsching et al., 2011). MaR1 also reduced the percent of the pro-inflammatory Th1 cells (tBet) in the spinal cord and blood, as well, as the number of T cells that release IFNγ. This effect might be due to the increased counts in T regulatory cells observed upon MaR1 treatment. Importantly, MaR1 also modulated the phenotype of macrophages in the spinal cord but not in the lymph node or circulation, as revealed increased the proportion of anti-inflammatory macrophages. The effect of MaR1 on macrophage polarization was also described in mice after spinal cord injury (Francos-Quijorna et al., 2017) and colitis (Marcon et al., 2013). Since pro-inflammatory macrophages have a key role in demyelination in MS and EAE (reviewed at Chu *et al.,* 2018), the effect of MaR1 on this myeloid cell population is likely to contribute to the therapeutic action of this SMP in EAE.

As a corollary, the present thesis reported that the modulation of the inflammatory response by administrating anti-inflammatory approaches such as OLT1177 and IL-37, or by delivering resolution agonist ameliorates neurological decline and demyelination in EAE. Although these approaches have different mechanisms of

action, we revealed that they shared some immunomodulatory effects, such as the ability to decrease the levels of pro-inflammatory cytokines and the counts of immune cells in the CNS. Besides, IL-37 and MaR1 also alter the polarization of CD4 T cells and/or macrophages driving these immune cells towards a more anti-inflammatory phenotype. These three novel approaches that have not been previously tested in EAE. We demonstrate that they mediate potent therapeutic actions in EAE even if the administration is initiated after the clinical signs of the disease. Therefore, these drugs may lead to the development of novel avenues for the treatment of MS.

CONCLUSIONS

Chapter 1. OLT1177 (Dapansutrile), an NLRP3 inflammasome inhibitor, ameliorates experimental autoimmune encephalomyelitis.

- Prophylactic treatment with OLT1177-enriched food (3.75 mg/kg of food) reduces neurological decline and demyelination in experimental autoimmune encephalomyelitis.
- OLT1177-enriched food decreases the levels of pro-inflammatory cytokines and the infiltration of immune cells in the spinal cord of mice with experimental autoimmune encephalomyelitis when given prophylactically.
- Twice-daily intraperitoneal administration of OLT1177 (60mg/kg) protects from functional impairments and demyelination in mice with experimental autoimmune encephalomyelitis when given prophylactically.
- Therapeutic treatment with twice-daily oral administration of OLT1177 (60mg/kg) mediates therapeutic effects in experimental autoimmune encephalomyelitis

Chapter 2. IL-37 exerts therapeutics effects in experimental autoimmune encephalomyelitis.

- *IL-37* is expressed at low levels in the spinal cord of hIL-37tg mice in physiological conditions and it is upregulated at the peak and chronic phases of experimental autoimmune encephalomyelitis.
- hIL-37tg mice are protected against functional decline and demyelination in experimental autoimmune encephalomyelitis.
- The transgenic expression of human *IL-37* reduces the levels of pro-inflammatory cytokines in the spinal cord of mice with experimental autoimmune encephalomyelitis at the peak of the disease.
- The transgenic expression of human *IL-37* in mice reduces the accumulation of immune cells in the lymph nodes and spinal cord and drives immune cells

towards a more anti-inflammatory phenotype in experimental autoimmune encephalomyelitis.

- The components of the IL-37 surface receptor, *il-18rα* and *sigirr*, are expressed in the spinal cord of mice at physiological conditions and after induction of experimental autoimmune encephalomyelitis.
- The extracellular function of IL-37 is critical for its beneficial actions in experimental autoimmune encephalomyelitis
- The components of the IL-37 extracellular receptor, *IL-18Rα* and *SIGIRR*, are expressed in the peripheral blood mononuclear cells and brain of healthy and multiple sclerosis individuals, but IL-37 is dysregulated in multiple sclerosis patients.
- Therapeutic treatment with native IL-$37^{46\text{-}218}$, IL-37^{D73K} or IL-37^{Y85A} protects against functional deficits and demyelination in mice with experimental autoimmune encephalomyelitis.

Chapter 3. Administration of Maresin-1 ameliorates the physiopathology of experimental autoimmune encephalomyelitis.

- There is defective induction of the enzymes that produce the specialized pro-resolving lipid mediators in peripheral blood mononuclear cells and brain active lesion of multiple sclerosis patients, as well as, in the spinal cord of mice with experimental autoimmune encephalomyelitis.
- Therapeutic treatment with Maresin-1 and Resolvin-D1, but not Resolvin-E1, reduces experimental autoimmune encephalomyelitis symptomatology in mice.
- Administration of Maresin-1 silences cytokine protein levels in the spinal cord of mice with experimental autoimmune encephalomyelitis
- Maresin-1 reduces the trafficking of immune cells from lymphatic organs and/or bone morrow to the blood stream, decreases the infiltration of immune cells into the spinal cord, and drives CD4 T cells and macrophages towards a more anti-inflammatory phenotype in mice with experimental autoimmune encephalomyelitis.

REFERENCES

Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM V (2011) Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nat Neurosci 14:1142–1150 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21804537 [Accessed April 13, 2019].

Allen I V, McQuaid S, Mirakhur M, Nevin G (2001) Pathological abnormalities in the normal-appearing white matter in multiple sclerosis. Neurol Sci 22:141–144 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11603615 [Accessed July 24, 2018].

Arend WP, Malyak M, Guthridge CJ, Gabay C (1998) INTERLEUKIN-1 RECEPTOR ANTAGONIST: Role in Biology. Annu Rev Immunol 16:27–55 Available at: http://www.ncbi.nlm.nih.gov/pubmed/9597123 [Accessed April 17, 2019].

Ariel A, Li P-L, Wang W, Tang W-X, Fredman G, Hong S, Gotlinger KH, Serhan CN (2005) The docosatriene protectin D1 is produced by TH2 skewing and promotes human T cell apoptosis via lipid raft clustering. J Biol Chem 280:43079–43086 Available at: http://www.jbc.org/lookup/doi/10.1074/jbc.M509796200 [Accessed July 20, 2018].

Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN (2005) Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J Exp Med 201:713–722 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15753205 [Accessed June 21, 2018].

Baecher-Allan C, Kaskow BJ, Weiner HL (2018) Multiple Sclerosis: Mechanisms and Immunotherapy. Neuron 97:742–768 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29470968 [Accessed July 25, 2018].

Baker D, O'Neill JK, Turk JL (1991) Cytokines in the central nervous system of mice during chronic relapsing experimental allergic encephalomyelitis. Cell Immunol 134:505–510 Available at: http://www.ncbi.nlm.nih.gov/pubmed/1902401 [Accessed February 21, 2019].

Baranzini SE, Oksenberg JR (2017) The Genetics of Multiple Sclerosis: From 0 to 200 in 50 Years. Trends Genet 33:960–970 Available at: https://www.sciencedirect.com/science/article/pii/S0168952517301634?via%3Dihub [Accessed January 23, 2018].

Bazan NG (2006) Neuroprotectin D1 (NPD1): A DHA-Derived Mediator that Protects Brain and Retina Against Cell Injury-Induced Oxidative Stress. Brain Pathol 15:159–166 Available at: http://doi.wiley.com/10.1111/j.1750-3639.2005.tb00513.x [Accessed July 20, 2018].

Becher B, Spath S, Goverman J (2017) Cytokine networks in neuroinflammation. Nat Rev Immunol 17:49–59 Available at: http://www.nature.com/articles/nri.2016.123 [Accessed February 21, 2019].

Bennett M, Gilroy DW (2016) Lipid Mediators in Inflammation. Microbiol Spectr 4:1–21.

Bhise V, Dhib-Jalbut S (2016) Further understanding of the immunopathology of multiple sclerosis: impact on future treatments. Expert Rev Clin Immunol 12:1069–1089 Available at: https://www.tandfonline.com/doi/full/10.1080/1744666X.2016.1191351 [Accessed May 14, 2019].

Bieber AJ, Kerr S, Rodriguez M (2003) Efficient central nervous system remyelination requires T cells. Ann Neurol 53:680–684 Available at: http://doi.wiley.com/10.1002/ana.10578 [Accessed August 1, 2018].

Bittner S, Afzali AM, Wiendl H, Meuth SG (2014) Myelin oligodendrocyte glycoprotein (MOG35-55) induced experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice. J Vis Exp:e51275 Available at: http://www.jove.com/video/51275/myelin-oligodendrocyte-glycoprotein-mog35-55-induced-experimental.

Bjelobaba I, Begovic-Kupresanin V, Pekovic S, Lavrnja I (2018) Animal models of multiple sclerosis: Focus on experimental autoimmune encephalomyelitis. J Neurosci Res 96:1021–1042 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29446144 [Accessed July 31, 2018].

Bjelobaba I, Savic D, Lavrnja I (2017) Multiple Sclerosis and Neuroinflammation: The Overview of Current and Prospective Therapies. Curr Pharm Des 23:693–730 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27981909 [Accessed May 13, 2019].

Blakemore WF (1972) Observations on oligodendrocyte degeneration, the resolution of status spongiosus and remyelination in cuprizone intoxication in mice. J Neurocytol 1:413–426 Available at: http://www.ncbi.nlm.nih.gov/pubmed/8530973 [Accessed August 1, 2018].

Blakemore WF (2005) The case for a central nervous system (CNS) origin for the Schwann cells that remyelinate CNS axons following concurrent loss of oligodendrocytes and astrocytes. Neuropathol Appl Neurobiol 31:1–10 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15634226 [Accessed July 31, 2018].

Bo L (2009) The histopathology of grey matter demyelination in multiple sclerosis. Acta Neurol Scand 120:51–57 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19566500 [Accessed July 24, 2018].

Born TL, Thomassen E, Bird TA, Sims JE (1998) Cloning of a Novel Receptor Subunit, AcPL, Required for Interleukin-18 Signaling. J Biol Chem 273:29445–29450 Available at: http://www.jbc.org/content/273/45/29445?ijkey=84aff9ebdc3239e2a2c24b9cff6c8f2d52a7822c&keytype2=tf_ipsecsha [Accessed April 17, 2019].

Buckley CD, Gilroy DW, Serhan CN (2014) Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 40:315–327 Available at: http://linkinghub.elsevier.com/retrieve/pii/S1074761314000776 [Accessed July 23, 2018].

Buckley CD, Gilroy DW, Serhan CN, Stockinger B, Tak PP (2013) The resolution of inflammation. Nat Rev Immunol 13:59–66 Available at: http://www.nature.com/articles/nri3362 [Accessed May 10, 2018].

Bufler P, Gamboni-Robertson F, Azam T, Kim S-H, Dinarello C a (2004a) Interleukin-1 homologues IL-1F7b and IL-18 contain functional mRNA instability elements within the coding region responsive to lipopolysaccharide. Biochem J 381:503–510 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15046617 [Accessed August 9, 2018].

Bufler P, Gamboni-Robertson F, Azam T, Kim S-H, Dinarello CA (2004b) Interleukin-1 homologues IL-1F7b and IL-18 contain functional mRNA instability elements within the coding region responsive to lipopolysaccharide. Biochem J 381:503–510 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15046617 [Accessed March 20, 2019].

Bulau A-M, Nold MF, Li S, Nold-Petry CA, Fink M, Mansell A, Schwerd T, Hong J, Rubartelli

A, Dinarello CA, Bufler P (2014) Role of caspase-1 in nuclear translocation of IL-37, release of the cytokine, and IL-37 inhibition of innate immune responses. Proc Natl Acad Sci 111:2650–2655 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24481253 [Accessed August 9, 2018].

Busfield SJ, Comrack CA, Yu G, Chickering TW, Smutko JS, Zhou H, Leiby KR, Holmgren LM, Gearing DP, Pan Y (2000) Identification and gene organization of three novel members of the IL-1 family on human chromosome 2. Genomics 66:213–216 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0888754300961846 [Accessed August 9, 2018].

Caravagna C, Jaouën A, Desplat-Jégo S, Fenrich KK, Bergot E, Luche H, Grenot P, Rougon G, Malissen M, Debarbieux F (2018) Diversity of innate immune cell subsets across spatial and temporal scales in an EAE mouse model. Sci Rep 8:5146 Available at: http://www.nature.com/articles/s41598-018-22872-y [Accessed April 12, 2019].

Carlton WW (1967) Studies on the induction of hydrocephalus and spongy degeneration by cuprizone feeding and attempts to antidote the toxicity. Life Sci 6:11–19 Available at: http://www.ncbi.nlm.nih.gov/pubmed/6030552 [Accessed August 1, 2018].

Cavalli G, Dinarello CA (2018) Suppression of inflammation and acquired immunity by IL-37. Immunol Rev 281:179–190 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29247987 [Accessed August 9, 2018].

Cavalli G, Justice JN, Boyle KE, D'Alessandro A, Eisenmesser EZ, Herrera JJ, Hansen KC, Nemkov T, Stienstra R, Garlanda C, Mantovani A, Seals DR, Dagna L, Joosten LAB, Ballak DB, Dinarello CA (2017) Interleukin 37 reverses the metabolic cost of inflammation, increases oxidative respiration, and improves exercise tolerance. Proc Natl Acad Sci 114:2313–2318 Available at: http://www.pnas.org/lookup/doi/10.1073/pnas.1619011114 [Accessed August 10, 2018].

Cavalli G, Koenders M, Kalabokis V, Kim J, Tan AC, Garlanda C, Mantovani A, Dagna L, Joosten LAB, Dinarello CA (2016) Treating experimental arthritis with the innate immune inhibitor interleukin-37 reduces joint and systemic inflammation. Rheumatology 55:2220–2229 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27567100 [Accessed August 10, 2018].

Charcot JM (1880) Leçons sur les maladies du système nerveux : faites à la Salpêtrière : Charcot, J. M. (Jean Martin), 1825-1893 : Free Download, Borrow, and Streaming : Internet Archive. Available at: https://gallica.bnf.fr/ark:/12148/bpt6k98763k.image [Accessed July 24, 2018].

Chen Z, Yang D, Peng X, Lin J, Su Z, Li J, Zhang X, Weng Y (2018) Beneficial effect of atorvastatin-modified dendritic cells pulsed with myelin oligodendrocyte glycoprotein autoantigen on experimental autoimmune encephalomyelitis. Neuroreport 29:317–327 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29394220 [Accessed May 14, 2019].

Chiang N, Serhan CN (2017a) Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Aspects Med 58:114–129 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0098299717300195 [Accessed July 23, 2018].

Chiang N, Serhan CN (2017b) Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Aspects Med 58:114–129 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28336292 [Accessed June 21, 2018].

Chiurchiù V, Leuti A, Dalli J, Jacobsson A, Battistini L, Maccarrone M, Serhan CN (2016) Proresolving lipid mediators resolvin D1, resolvin D2, and maresin 1 are critical in modulating T cell responses. Sci Transl Med 8:353ra111-353ra111 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27559094 [Accessed February 25, 2019].

Chu F, Shi M, Zheng C, Shen D, Zhu J, Zheng X, Cui L (2018a) The roles of macrophages and microglia in multiple sclerosis and experimental autoimmune encephalomyelitis. J Neuroimmunol 318:1–7 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0165572817305660 [Accessed July 30, 2018].

Chu F, Shi M, Zheng C, Shen D, Zhu J, Zheng X, Cui L (2018b) The roles of macrophages and microglia in multiple sclerosis and experimental autoimmune encephalomyelitis. J Neuroimmunol 318:1–7 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29606295 [Accessed February 25, 2019].

Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, Ma L, Watowich SS, Jetten AM, Tian Q, Dong C (2009) Critical Regulation of Early Th17 Cell Differentiation by Interleukin-1 Signaling. Immunity 30:576–587 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19362022 [Accessed April 12, 2019].

Clària J, Serhan CN (1995) Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proc Natl Acad Sci U S A 92:9475–9479 Available at: http://www.ncbi.nlm.nih.gov/pubmed/7568157 [Accessed June 21, 2018].

Colas RA, Dalli J, Chiang N, Vlasakov I, Sanger JM, Riley IR, Serhan CN (2016) Identification and Actions of the Maresin 1 Metabolome in Infectious Inflammation. J Immunol 197:4444–4452 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27799313 [Accessed May 16, 2018].

Coll-Miró M, Francos-Quijorna I, Santos-Nogueira E, Torres-Espin A, Bufler P, Dinarello CA, López-Vales R (2016) Beneficial effects of IL-37 after spinal cord injury in mice. Proc Natl Acad Sci 113:1411–1416 Available at: http://www.pnas.org/lookup/doi/10.1073/pnas.1523212113.

Constantinescu CS, Farooqi N, O'Brien K, Gran B (2011) Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol 164:1079–1106 Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3229753&tool=pmcentrez&rendertype=abstract.

Correale J, Balbuena Aguirre ME, Farez MF (2013) Sex-specific environmental influences affecting MS development. Clin Immunol 149:176–181 Available at: https://ac.els-cdn.com/S1521661613000417/1-s2.0-S1521661613000417-main.pdf?_tid=b6ef5efc-118a-11e8-8021-00000aacb362&acdnat=1518614991_747796c7b50a4a7c63b55cdd4774691f [Accessed February 14, 2018].

Correale J, Farez MF (2015) The role of astrocytes in multiple sclerosis progression. Front Neurol 6:1–12.

Dalli J, Serhan CN (2018) Identification and structure elucidation of the pro-resolving mediators provides novel leads for resolution pharmacology. Br J Pharmacol Available at: http://doi.wiley.com/10.1111/bph.14336 [Accessed July 19, 2018].

Dalli J, Vlasakov I, Riley IR, Rodriguez AR, Spur BW, Petasis NA, Chiang N, Serhan CN (2016) Maresin conjugates in tissue regeneration biosynthesis enzymes in human

macrophages. Proc Natl Acad Sci 113:12232–12237 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27791009 [Accessed July 20, 2018].

Dalli J, Zhu M, Vlasenko NA, Deng B, Haeggström JZ, Petasis NA, Serhan CN (2013) The novel 13 *S* ,14 *S* -epoxy-maresin is converted by human macrophages to maresin 1 (MaR1), inhibits leukotriene A $_4$ hydrolase (LTA $_4$ H), and shifts macrophage phenotype. FASEB J 27:2573–2583 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23504711 [Accessed July 20, 2018].

David S (2015) Neuroinflammation: New Insights into Beneficial and Detrimental Functions.

Dendrou CA, Fugger L, Friese MA (2015) Immunopathology of multiple sclerosis. Nat Rev Immunol 15:545–558 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26250739 [Accessed May 1, 2019].

Didonna A, Oksenberg JR (2017) The Genetics of Multiple Sclerosis. Codon Publications. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29261259 [Accessed January 23, 2018].

Dinarello CA (1997) Interleukin-1. Cytokine Growth Factor Rev 8:253–265 Available at: http://www.ncbi.nlm.nih.gov/pubmed/9620641 [Accessed April 17, 2019].

Dinarello CA (1999) IL-18: A TH1 -inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol 103:11–24 Available at: https://www.jacionline.org/article/S0091-6749(99)70518-X/fulltext [Accessed April 17, 2019].

Dinarello CA (2007) Interleukin-18 and the Pathogenesis of Inflammatory Diseases. Semin Nephrol 27:98–114 Available at: http://www.ncbi.nlm.nih.gov/pubmed/17336692 [Accessed April 17, 2019].

Duffy SS, Lees JG, Moalem-Taylor G (2014) The contribution of immune and glial cell types in experimental autoimmune encephalomyelitis and multiple sclerosis. Mult Scler Int 2014:285245 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25374694 [Accessed February 21, 2019].

Dunn E, Sims JE, Nicklin MJ, O'Neill LA (2001) Annotating genes with potential roles in the immune system: six new members of the IL-1 family. Trends Immunol 22:533–536 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11574261 [Accessed August 9, 2018].

Dyment DA, Ebers GC, Sadovnick AD (2004) Genetics of multiple sclerosis. Lancet Neurol 3:104–110 Available at: http://www.ncbi.nlm.nih.gov/pubmed/14747002 [Accessed July 24, 2018].

Edenius C, Kumlin M, Björk T, Änggård A, Lindgren JÅ (1990) Lipoxin formation in human nasal polyps and bronchial tissue. FEBS Lett 272:25–28 Available at: http://doi.wiley.com/10.1016/0014-5793%2890%2980440-T [Accessed July 23, 2018].

Eisenmesser EZ, Gottschlich A, Redzic JS, Paukovich N, Nix JC, Azam T, Zhang L, Zhao R, Kieft JS, The E, Meng X, Dinarello CA (2019) Interleukin-37 monomer is the active form for reducing innate immunity. Proc Natl Acad Sci U S A 116:5514–5522 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30819901 [Accessed April 11, 2019].

Elfeky OA, Abed NT, Emam SM, Elsayed HA (2018) Assessment of Serum Level of Interleukin-37 in Asthmatic Children at Benha University Hospital. Egypt J Immunol 25:53–60 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30600948 [Accessed

April 10, 2019].

Ellisdon AM, Nold-Petry CA, D'Andrea L, Cho SX, Lao JC, Rudloff I, Ngo D, Lo CY, Soares da Costa TP, Perugini MA, Conroy PJ, Whisstock JC, Nold MF (2017) Homodimerization attenuates the anti-inflammatory activity of interleukin-37. Sci Immunol 2:eaaj1548 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28783685 [Accessed March 28, 2019].

Erta M, Giralt M, Jiménez S, Molinero A, Comes G, Hidalgo J (2016) Astrocytic IL-6 Influences the Clinical Symptoms of EAE in Mice. Brain Sci 6:15 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27196935 [Accessed May 4, 2019].

Eugster H-P, Frei K, Kopf M, Lassmann H, Fontana A (1998) IL-6-deficient mice resist myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. Eur J Immunol 28:2178–2187 Available at: http://www.ncbi.nlm.nih.gov/pubmed/9692887 [Accessed April 12, 2019].

Fan X, Moltedo B, Mendoza A, Davydov AN, Faire MB, Mazutis L, Sharma R, Pe'er D, Chudakov DM, Rudensky AY (2018) CD49b defines functionally mature Treg cells that survey skin and vascular tissues. J Exp Med 215:2796–2814 Available at: http://jem.rupress.org/content/215/11/2796 [Accessed February 21, 2019].

Fernandes De Abreu DA, Landel V, Féron F (2011) Seasonal, gestational and postnatal influences on multiple sclerosis: The beneficial role of a vitamin D supplementation during early life. J Neurol Sci 311:64–68 Available at: http://dx.doi.org/10.1016/j.jns.2011.08.044.

Filippini G (2017) Ocrelizumab appears to reduce relapse and disability in multiple sclerosis but quality of evidence is moderate. Evid Based Med 22:215–216 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29117998 [Accessed July 31, 2018].

Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G (2009) The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol 10:241–247 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19221555 [Accessed May 10, 2019].

Francos-Quijorna I, Santos-Nogueira E, Gronert K, Sullivan AB, Kopp MA, Brommer B, David S, Schwab JM, López-Vales R (2017) Maresin 1 Promotes Inflammatory Resolution, Neuroprotection, and Functional Neurological Recovery After Spinal Cord Injury. J Neurosci 37:11731–11743 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29109234 [Accessed July 23, 2018].

Fredman G, Hellmann J, Proto JD, Kuriakose G, Colas RA, Dorweiler B, Connolly ES, Solomon R, Jones DM, Heyer EJ, Spite M, Tabas I (2016) An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat Commun 7:12859 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27659679 [Accessed May 8, 2019].

Fritzsching B, Haas J, König F, Kunz P, Fritzsching E, Pöschl J, Krammer PH, Brück W, Suri-Payer E, Wildemann B (2011) Intracerebral Human Regulatory T Cells: Analysis of CD4+CD25+FOXP3+ T Cells in Brain Lesions and Cerebrospinal Fluid of Multiple Sclerosis Patients Dieli F, ed. PLoS One 6:e17988 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21437244 [Accessed February 25, 2019].

Frohman EM, Racke MK, Raine CS (2006) Multiple Sclerosis — The Plaque and Its Pathogenesis. N Engl J Med 354:942–955 Available at: http://www.nejm.org/doi/abs/10.1056/NEJMra052130 [Accessed February 22, 2018].

Fukunaga K, Kohli P, Bonnans C, Fredenburgh LE, Levy BD (2005) Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. J Immunol 174:5033–5039 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15814734 [Accessed July 23, 2018].

Gabay C, Lamacchia C, Palmer G (2010) IL-1 pathways in inflammation and human diseases. Nat Rev Rheumatol 6:232–241 Available at: http://www.ncbi.nlm.nih.gov/pubmed/20177398 [Accessed April 17, 2019].

Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P, Guo B, Herbert DR, Bulfone A, Trentini F, Di Serio C, Bacchetta R, Andreani M, Brockmann L, Gregori S, Flavell RA, Roncarolo M-G (2013) Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nat Med 19:739–746 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23624599 [Accessed February 21, 2019].

Garlanda C, Dinarello CA, Mantovani A (2013) The interleukin-1 family: back to the future. Immunity 39:1003–1018 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24332029 [Accessed August 9, 2018].

Genain CP, Cannella B, Hauser SL, Raine CS (1999) Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med 5:170–175 Available at: http://www.nature.com/articles/nm0299_170 [Accessed February 22, 2018].

Ghasemi N, Razavi S, Nikzad E (2017) Multiple Sclerosis: Pathogenesis, Symptoms, Diagnoses and Cell-Based Therapy. Cell J 19:1–10 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28367411 [Accessed May 30, 2019].

Grigoriadis N, van Pesch V (2015) A basic overview of multiple sclerosis immunopathology. Eur J Neurol 22:3–13.

Hall SM (1972) The effect of injections of lysophosphatidyl choline into white matter of the adult mouse spinal cord. J Cell Sci 10:535–546 Available at: http://www.ncbi.nlm.nih.gov/pubmed/5018033 [Accessed August 1, 2018].

Harbo HF, Gold R, Tintoré M (2013) Sex and gender issues in multiple sclerosis. Ther Adv Neurol Disord 6:237–248 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23858327 [Accessed January 23, 2018].

Hauser SL et al. (2017) Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N Engl J Med 376:221–234 Available at: http://www.nejm.org/doi/10.1056/NEJMoa1601277 [Accessed July 31, 2018].

He Y, Hara H, Núñez G (2016) Mechanism and Regulation of NLRP3 Inflammasome Activation. Available at: http://dx.doi.org/10.1016/j.tibs.2016.09.002 [Accessed April 17, 2019].

Heidary M, Rakhshi N, Pahlevan Kakhki M, Behmanesh M, Sanati MH, Sanadgol N, Kamaladini H, Nikravesh A (2014) The analysis of correlation between IL-1B gene expression and genotyping in multiple sclerosis patients. J Neurol Sci 343:41–45 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24867167 [Accessed May 10, 2019].

Henry CJ, Casás-Selves M, Kim J, Zaberezhnyy V, Aghili L, Daniel AE, Jimenez L, Azam T, McNamee EN, Clambey ET, Klawitter J, Serkova NJ, Tan AC, Dinarello CA, DeGregori J (2015) Aging-associated inflammation promotes selection for adaptive oncogenic events in B cell progenitors. J Clin Invest 125:4666–4680 Available at: https://www.jci.org/articles/view/83024 [Accessed August 10, 2018].

Hong S, Gronert K, Devchand PR, Moussignac R-L, Serhan CN (2003) Novel

Docosatrienes and 17 *S* -Resolvins Generated from Docosahexaenoic Acid in Murine Brain, Human Blood, and Glial Cells. J Biol Chem 278:14677–14687 Available at: http://www.ncbi.nlm.nih.gov/pubmed/12590139 [Accessed July 20, 2018].

Huppert J, Closhen D, Croxford A, White R, Kulig P, Pietrowski E, Bechmann I, Becher B, Luhmann HJ, Waisman A, Kuhlmann CRW (2010) Cellular mechanisms of IL-17-induced blood-brain barrier disruption. FASEB J 24:1023–1034 Available at: http://www.fasebj.org/doi/10.1096/fj.09-141978 [Accessed July 25, 2018].

Inoue M, Shinohara ML (2013) NLRP3 Inflammasome and MS/EAE. Autoimmune Dis 2013:859145 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23365725 [Accessed April 17, 2019].

Jansen T, Klück V, Janssen M, Comarniceanu A, Efdé M, Scribner C, Barrow R, Skouras D, Dinarello C, Joosten L (2019) P160 The first phase 2A proof-of-concept study of a selective NLRP3 inflammasome inhibitor, dapansutrile™ (OLT1177™), in acute gout. Ann Rheum Dis 78:A70–A71 Available at: https://ard.bmj.com/content/78/Suppl_1/A70 [Accessed May 13, 2019].

Jeffery ND, Blakemore WF (1995) Remyelination of mouse spinal cord axons demyelinated by local injection of lysolecithin. J Neurocytol 24:775–781 Available at: http://link.springer.com/10.1007/BF01191213 [Accessed August 1, 2018].

Ji RR, Xu ZZ, Strichartz G, Serhan CN (2011) Emerging roles of resolvins in the resolution of inflammation and pain. Trends Neurosci 34:599–609 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21963090 [Accessed June 1, 2019].

Jia H, Liu J, Han B (2018) Reviews of Interleukin-37: Functions, Receptors, and Roles in Diseases. Biomed Res Int 2018:3058640 Available at: https://www.hindawi.com/journals/bmri/2018/3058640/ [Accessed August 9, 2018].

Jörg S, Grohme DA, Erzler M, Binsfeld M, Haghikia A, Müller DN, Linker RA, Kleinewietfeld M (2016) Environmental factors in autoimmune diseases and their role in multiple sclerosis. Cell Mol Life Sci 73:4611–4622 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5097114/pdf/18_2016_Article_2311.pdf [Accessed February 13, 2018].

Kallaur AP, Oliveira SR, Simao ANC, De Almeida ERD, Morimoto HK, Lopes J, De Carvalho Jennings Pereira WL, Andrade RM, Pelegrino LM, Borelli SD, Kaimen-Maciel DR, Reiche EMV (2013) Cytokine profile in relapsing-remitting Multiple sclerosis patients and the association between progression and activity of the disease. Mol Med Rep 7:1010–1020 Available at: https://www.spandidos-publications.com/10.3892/mmr.2013.1256 [Accessed February 21, 2019].

Kamińska J, Koper OM, Piechal K, Kemona H (2017) Multiple sclerosis - etiology and diagnostic potential. Postepy Hig Med Dosw (Online) 71:551–563 Available at: http://phmd.pl/gicid/01.3001.0010.3836 [Accessed May 30, 2019].

Kapsogeorgou EK, Tzioufas AG (2016) Autoantibodies in Autoimmune Diseases: Clinical and Critical Evaluation. Isr Med Assoc J 18:519–524 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28471596 [Accessed May 13, 2019].

Keyel PA (2014) How is inflammation initiated? Individual influences of IL-1, IL-18 and HMGB1. Cytokine 69:136–145 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24746243 [Accessed May 13, 2019].

Khan N, Kuo A, Brockman DA, Cooper MA, Smith MT (2018) Pharmacological inhibition

of the NLRP3 inflammasome as a potential target for multiple sclerosis induced central neuropathic pain. Inflammopharmacology 26:77–86 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28965161 [Accessed May 10, 2019].

Kirby TO, Ochoa-Repáraz J (2018) The Gut Microbiome in Multiple Sclerosis: A Potential Therapeutic Avenue. Med Sci (Basel, Switzerland) 6 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30149548 [Accessed May 30, 2019].

Koutrolos M, Berer K, Kawakami N, Wekerle H, Krishnamoorthy G (2014) Treg cells mediate recovery from EAE by controlling effector T cell proliferation and motility in the CNS. Acta Neuropathol Commun 2:163 Available at: http://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-014-0163-1 [Accessed May 6, 2019].

Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN (2012) Resolvin D1 receptor stereoselectivity and regulation of inflammation and proresolving microRNAs. Am J Pathol 180:2018–2027 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22449948 [Accessed July 19, 2018].

Krumm B, Xiang Y, Deng J (2014) Structural biology of the IL-1 superfamily: key cytokines in the regulation of immune and inflammatory responses. Protein Sci 23:526–538 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24677376 [Accessed April 17, 2019].

Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Brück W (2002) Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain 125:2202–2212 Available at: http://www.ncbi.nlm.nih.gov/pubmed/12244078 [Accessed July 24, 2018].

Kumar S, McDonnell PC, Lehr R, Tierney L, Tzimas MN, Griswold DE, Capper EA, Tal-Singer R, Wells GI, Doyle ML, Young PR (2000) Identification and initial characterization of four novel members of the interleukin-1 family. J Biol Chem 275:10308–10314 Available at: http://www.ncbi.nlm.nih.gov/pubmed/10744718 [Accessed August 9, 2018].

Lafage M, Maroc N, Dubreuil P, de Waal Malefijt R, Pébusque MJ, Carcassonne Y, Mannoni P (1989) The human interleukin-1 alpha gene is located on the long arm of chromosome 2 at band q13. Blood 73:104–107 Available at: http://www.ncbi.nlm.nih.gov/pubmed/2783369 [Accessed April 17, 2019].

Lassmann H, Brück W, Lucchinetti CF (2007) The immunopathology of multiple sclerosis: An overview. Brain Pathol 17:210–218.

Lattanzi S, Cagnetti C, Danni M, Provinciali L, Silvestrini M (2017) Oral and intravenous steroids for multiple sclerosis relapse: a systematic review and meta-analysis. J Neurol 264:1697–1704 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28492970 [Accessed May 31, 2019].

Lee J-K, Kim S-H, Lewis EC, Azam T, Reznikov LL, Dinarello CA (2004) Differences in signaling pathways by IL-1 and IL-18. Proc Natl Acad Sci 101:8815–8820 Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.0402800101 [Accessed April 17, 2019].

Lévesque SA, Paré A, Mailhot B, Bellver-Landete V, Kébir H, Lécuyer M-A, Alvarez JI, Prat A, Vaccari JP de R, Keane RW, Lacroix S (2016) Myeloid cell transmigration across the CNS vasculature triggers IL-1β–driven neuroinflammation during autoimmune encephalomyelitis in mice. J Exp Med 213:929–949 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27139491 [Accessed February 21, 2019].

Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN (2001) Lipid mediator class switching during acute inflammation: Signals in resolution. Nat Immunol 2:612–619 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11429545 [Accessed May 10, 2018].

Levy BO, Romano M, Chapman HA, Reilly JJ, Drazen J, Serhan CN (1993) Human alveolar macrophages have 15-lipoxygenase and generate 15(S)-hydroxy-5,8,11-cis-13-trans-eicosatetraenoic acid and lipoxins. J Clin Invest 92:1572–1579 Available at: http://www.ncbi.nlm.nih.gov/pubmed/8376607 [Accessed July 23, 2018].

Li C, Ji H, Cai Y, Ayana DA, Lv P, Liu M, Jiang Y (2013) Serum interleukin-37 concentrations and HBeAg seroconversion in chronic HBV patients during telbivudine treatment. J Interferon Cytokine Res 33:612–618 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23697556 [Accessed April 10, 2019].

Li R, Wang Y, Ma Z, Ma M, Wang D, Xie G, Yin Y, Zhang P, Tao K (2016) Maresin 1 Mitigates Inflammatory Response and Protects Mice from Sepsis. Mediators Inflamm 2016:1–9 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28042205 [Accessed May 8, 2019].

Li S, Amo-Aparicio J, Neff CP, Tengesdal IW, Azam T, Palmer BE, López-Vales R, Bufler P, Dinarello CA (2019) Role for nuclear interleukin-37 in the suppression of innate immunity [Immunology and Inflammation]. Proc Natl Acad Sci U S A 116:4456–4461 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30792349 [Accessed March 28, 2019].

Linker RA, Lühder F, Kallen K-J, Lee D-H, Engelhardt B, Rose-John S, Gold R (2008) IL-6 transsignalling modulates the early effector phase of EAE and targets the blood-brain barrier. J Neuroimmunol 205:64–72 Available at: http://www.ncbi.nlm.nih.gov/pubmed/18950871 [Accessed May 4, 2019].

Losy J, Niezgoda A (2001) IL-18 in patients with multiple sclerosis. Acta Neurol Scand 104:171–173 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11551238 [Accessed May 10, 2019].

Lu L-F, Rudensky A (2009) Molecular orchestration of differentiation and function of regulatory T cells. Genes Dev 23:1270–1282 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19487568 [Accessed July 25, 2018].

Lublin FD, Reingold SC (1996) Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology 46:907–911 Available at: http://www.ncbi.nlm.nih.gov/pubmed/8780061 [Accessed May 30, 2019].

Ludwin SK (1978) Central nervous system demyelination and remyelination in the mouse: an ultrastructural study of cuprizone toxicity. Lab Invest 39:597–612 Available at: http://www.ncbi.nlm.nih.gov/pubmed/739762 [Accessed August 1, 2018].

Luo Y, Cai X, Liu S, Wang S, Nold-Petry CA, Nold MF, Bufler P, Norris D, Dinarello CA, Fujita M (2014) Suppression of antigen-specific adaptive immunity by IL-37 via induction of tolerogenic dendritic cells. Proc Natl Acad Sci 111:15178–15183 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25294929 [Accessed August 10, 2018].

Maker-Clark G, Patel S (2013) Integrative therapies for multiple sclerosis. Disease-a-Month 59:290–301 Available at: http://dx.doi.org/10.1016/j.disamonth.2013.03.017.

Malpass K (2012) Multiple sclerosis: “Outside-in” demyelination in MS. Nat Rev Neurol

8:61 Available at: http://www.nature.com/articles/nrneurol.2011.217 [Accessed July 25, 2018].

Mansilla MJ, Sellès-Moreno C, Fàbregas-Puig S, Amoedo J, Navarro-Barriuso J, Teniente-Serra A, Grau-López L, Ramo-Tello C, Martínez-Cáceres EM (2015) Beneficial Effect of Tolerogenic Dendritic Cells Pulsed with MOG Autoantigen in Experimental Autoimmune Encephalomyelitis. CNS Neurosci Ther 21:222–230 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25403984 [Accessed May 14, 2019].

Marchetti C et al. (2018a) OLT1177, a β-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation. Proc Natl Acad Sci 115:E1530–E1539 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29378952 [Accessed May 13, 2019].

Marchetti C, Swartzwelter B, Koenders MI, Azam T, Tengesdal IW, Powers N, de Graaf DM, Dinarello CA, Joosten LAB (2018b) NLRP3 inflammasome inhibitor OLT1177 suppresses joint inflammation in murine models of acute arthritis. Arthritis Res Ther 20:169 Available at: http://www.ncbi.nlm.nih.gov/pubmed/30075804 [Accessed May 13, 2019].

Marcon R, Bento AF, Dutra RC, Bicca MA, Leite DFP, Calixto JB (2013) Maresin 1, a Proresolving Lipid Mediator Derived from Omega-3 Polyunsaturated Fatty Acids, Exerts Protective Actions in Murine Models of Colitis. J Immunol 191:4288–4298 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24038091 [Accessed February 25, 2019].

McNamee EN, Masterson JC, Jedlicka P, McManus M, Grenz A, Collins CB, Nold MF, Nold-Petry C, Bufler P, Dinarello CA, Rivera-Nieves J (2011a) Interleukin 37 expression protects mice from colitis. Proc Natl Acad Sci 108:16711–16716 Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.1111982108 [Accessed August 10, 2018].

McNamee EN, Masterson JC, Jedlicka P, McManus M, Grenz A, Collins CB, Nold MF, Nold-Petry C, Bufler P, Dinarello CA, Rivera-Nieves J (2011b) Interleukin 37 expression protects mice from colitis. Proc Natl Acad Sci 108:16711–16716 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21873195 [Accessed August 10, 2018].

Merrill JE (2009) In Vitro and In Vivo Pharmacological Models to Assess Demyelination and Remyelination. Neuropsychopharmacology 34:55–73 Available at: http://www.ncbi.nlm.nih.gov/pubmed/18800062 [Accessed July 31, 2018].

Milo R, Kahana E (2010) Multiple sclerosis: Geoepidemiology, genetics and the environment. Autoimmun Rev 9 Available at: https://ac.els-cdn.com/S1568997209001888/1-s2.0-S1568997209001888-main.pdf?_tid=a45ce662-10ed-11e8-b7aa-00000aacb361&acdnat=1518547529_0b4215fff31a9f2825a7988e3fd9d51f [Accessed February 13, 2018].

Montalban X et al. (2017) Ocrelizumab versus Placebo in Primary Progressive Multiple Sclerosis. N Engl J Med 376:209–220 Available at: http://www.nejm.org/doi/10.1056/NEJMoa1606468 [Accessed July 31, 2018].

Moretti S, Bozza S, Oikonomou V, Renga G, Casagrande A, Iannitti RG, Puccetti M, Garlanda C, Kim S, Li S, van de Veerdonk FL, Dinarello CA, Romani L (2014) IL-37 Inhibits Inflammasome Activation and Disease Severity in Murine Aspergillosis Klein BS, ed. PLoS Pathog 10:e1004462 Available at: http://dx.plos.org/10.1371/journal.ppat.1004462 [Accessed August 10, 2018].

Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG (2004) From The Cover:

Neuroprotectin D1: A docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc Natl Acad Sci 101:8491–8496 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15152078 [Accessed July 20, 2018].

Murphy AC, Lalor SJ, Lynch MA, Mills KHG (2010) Infiltration of Th1 and Th17 cells and activation of microglia in the CNS during the course of experimental autoimmune encephalomyelitis. Brain Behav Immun 24:641–651 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0889159110000413 [Accessed July 25, 2018].

Nair A, Frederick TJ, Miller SD (2008) Astrocytes in multiple sclerosis: A product of their environment. Cell Mol Life Sci 65:2702–2720.

Nakamura K, Okamura H, Wada M, Nagata K, Tamura T (1989) Endotoxin-induced serum factor that stimulates gamma interferon production. Infect Immun 57:590 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC313137/ [Accessed April 17, 2019].

Nath N, Prasad R, Giri S, Singh AK, Singh I (2006) T-bet is essential for the progression of experimental autoimmune encephalomyelitis. Immunology 118:384–391 Available at: http://www.ncbi.nlm.nih.gov/pubmed/16827899 [Accessed May 6, 2019].

Newman TL, Tuzun E, Morrison VA, Hayden KE, Ventura M, McGrath SD, Rocchi M, Eichler EE (2005) A genome-wide survey of structural variation between human and chimpanzee. Genome Res 15:1344–1356 Available at: http://www.ncbi.nlm.nih.gov/pubmed/16169929 [Accessed June 3, 2019].

Nicoletti F, Di Marco R, Mangano K, Patti F, Reggio E, Nicoletti A, Bendtzen K, Reggio A (2001) Increased serum levels of interleukin-18 in patients with multiple sclerosis. Neurology 57:342–344 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11468327 [Accessed May 10, 2019].

Nold-Petry C a et al. (2015a) IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. Nat Immunol 16:354–365 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25729923.

Nold-Petry CA et al. (2015b) IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. Nat Immunol 16:354–365 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25729923 [Accessed August 9, 2018].

Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA (2010) IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol 11:1014–1022 Available at: http://www.ncbi.nlm.nih.gov/pubmed/20935647 [Accessed August 9, 2018].

O'Brien K, Gran B, Rostami A (2010) T-cell based immunotherapy in experimental autoimmune encephalomyelitis and multiple sclerosis. Immunotherapy 2:99–115 Available at: http://www.ncbi.nlm.nih.gov/pubmed/20231863 [Accessed May 6, 2019].

O'Connor RA, Malpass KH, Anderton SM (2007) The inflamed central nervous system drives the activation and rapid proliferation of Foxp3+ regulatory T cells. J Immunol 179:958–966 Available at: http://www.ncbi.nlm.nih.gov/pubmed/17617587 [Accessed February 25, 2019].

Ohuchi K, Ono Y, Joho M, Tsuruma K, Ogami S, Yamane S, Funato M, Kaneko H, Nakamura

S, Hara H, Shimazawa M (2018) A Docosahexaenoic Acid-Derived Pro-resolving Agent, Maresin 1, Protects Motor Neuron Cells Death. Neurochem Res 43:1413–1423 Available at: http://link.springer.com/10.1007/s11064-018-2556-1 [Accessed February 20, 2019].

Palomo J, Dietrich D, Martin P, Palmer G, Gabay C (2015) The interleukin (IL)-1 cytokine family – Balance between agonists and antagonists in inflammatory diseases. Cytokine 76:25–37 Available at: https://www.sciencedirect.com/science/article/pii/S1043466615300090?via%3Dihub [Accessed May 13, 2019].

Pan G, Risser P, Mao W, Baldwin DT, Zhong AW, Filvaroff E, Yansura D, Lewis L, Eigenbrot C, Henzel WJ, Vandlen R (2001) IL-1H, AN INTERLEUKIN 1-RELATED PROTEIN THAT BINDS IL-18 RECEPTOR/IL-1Rrp. Cytokine 13:1–7 Available at: http://www.ncbi.nlm.nih.gov/pubmed/11145836 [Accessed August 9, 2018].

Pérez-Cerdá F, Sánchez-Gómez MV, Matute C (2016) The link of inflammation and neurodegeneration in progressive multiple sclerosis. Mult Scler Demyelinating Disord 1:9 Available at: http://msddjournal.biomedcentral.com/articles/10.1186/s40893-016-0012-0 [Accessed May 2, 2019].

Poisson LM, Suhail H, Singh J, Datta I, Denic A, Labuzek K, Hoda MN, Shankar A, Kumar A, Cerghet M, Elias S, Mohney RP, Rodriguez M, Rattan R, Mangalam AK, Giri S (2015) Untargeted Plasma Metabolomics Identifies Endogenous Metabolite with Drug-like Properties in Chronic Animal Model of Multiple Sclerosis. J Biol Chem 290:30697–30712 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26546682 [Accessed May 8, 2019].

Ponath G, Park C, Pitt D (2018) The Role of Astrocytes in Multiple Sclerosis. Front Immunol 9:217 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29515568 [Accessed July 30, 2018].

Prayson RA (2012) Neuropathology. Elsevier/Saunders.

Procaccini C, De Rosa V, Pucino V, Formisano L, Matarese G (2015) Animal models of Multiple Sclerosis. Eur J Pharmacol 759:182–191 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25823807 [Accessed August 1, 2018].

Prüss H, Rosche B, Sullivan AB, Brommer B, Wengert O, Gronert K, Schwab JM (2013) Proresolution Lipid Mediators in Multiple Sclerosis — Differential, Disease Severity-Dependent Synthesis — A Clinical Pilot Trial Bozza PT, ed. PLoS One 8:e55859 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23409068 [Accessed July 23, 2018].

Recchiuti A (2013) Resolvin D1 and its GPCRs in resolution circuits of inflammation. Prostaglandins Other Lipid Mediat 107:64–76 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23500003 [Accessed June 1, 2019].

Riccio P, Rossano R, Larocca M, Trotta V, Mennella I, Vitaglione P, Ettorre M, Graverini A, De Santis A, Di Monte E, Coniglio MG (2016) Anti-inflammatory nutritional intervention in patients with relapsing-remitting and primary-progressive multiple sclerosis: A pilot study. Exp Biol Med 241:620–635 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26785711 [Accessed May 8, 2019].

Rivers TM, Sprunt DH, Berry GP (1933) OBSERVATIONS ON ATTEMPTS TO PRODUCE ACUTE DISSEMINATED ENCEPHALOMYELITIS IN MONKEYS. J Exp Med 58:39–53 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19870180 [Accessed August 1, 2018].

Robbins SL (Stanley L, Cotran RS (1979) Pathologic basis of disease. W.B. Saunders Co.

Rossi S, Motta C, Studer V, Macchiarulo G, Volpe E, Barbieri F, Ruocco G, Buttari F, Finardi A, Mancino R, Weiss S, Battistini L, Martino G, Furlan R, Drulovic J, Centonze D (2014) Interleukin-1β causes excitotoxic neurodegeneration and multiple sclerosis disease progression by activating the apoptotic protein p53. Mol Neurodegener 9:56 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25495224 [Accessed April 13, 2019].

Sağlam M, Köseoğlu S, Savran L, Pekbağriyanik T, Sağlam G, Sütçü R (2015) Levels of interleukin-37 in gingival crevicular fluid, saliva, or plasma in periodontal disease. J Periodontal Res 50:614–621 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25399716 [Accessed April 10, 2019].

Schett G, Neurath MF (2018) Resolution of chronic inflammatory disease: universal and tissue-specific concepts. Nat Commun 9:3261 Available at: http://www.nature.com/articles/s41467-018-05800-6 [Accessed May 1, 2019].

Schrijver HM, Van As J, Crusius JBA, Dijkstra CD, Uitdehaag BMJ (2003) Interleukin (IL)-1 gene polymorphisms: Relevance of disease severity associated alleles with IL-1β and IL-1ra production in multiple sclerosis. Mediators Inflamm 12:89–94 Available at: http://www.ncbi.nlm.nih.gov/pubmed/12775358 [Accessed February 21, 2019].

Schwab JM, Chiang N, Arita M, Serhan CN (2007) Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature 447:869–874 Available at: http://www.nature.com/articles/nature05877 [Accessed June 21, 2018].

Sehat M, Talaei R, Dadgostar E, Nikoueinejad H, Akbari H (2000) Iranian journal of allergy, asthma and immunology. TUMS Publications. Available at: http://ijaai.tums.ac.ir/index.php/ijaai/article/view/1434 [Accessed April 10, 2019].

Sellés-Moreno C, Ramo-Tello C, Grau-López L, Pujol-Borrel R, Martínez-Cáceres EM (2012) Tolerogenic dendritic cells in experimental autoimmune encephalomyelitis, specific tolerance induction? J Transl Med 10:P27 Available at: https://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-10-S3-P27 [Accessed May 14, 2019].

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. Proc Natl Acad Sci 105:9041–9046 Available at: http://www.ncbi.nlm.nih.gov/pubmed/18577591 [Accessed May 8, 2019].

Serhan C, Ward P, Gilroy D (2010) Fundamentals of Inflammation. Available at: www.cambridge.org [Accessed February 23, 2018].

Serhan CN (1997) Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? Prostaglandins 53:107–137 Available at: http://www.ncbi.nlm.nih.gov/pubmed/9112289 [Accessed June 21, 2018].

Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510:92–101 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24899309 [Accessed July 20, 2018].

Serhan CN (2017) Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. Mol Aspects Med 58:1–11 Available at: https://doi.org/10.1016/j.mam.2017.03.001.

Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LAJ, Perretti M, Rossi AG, Wallace JL (2007) Resolution of inflammation: state of the art, definitions and terms. FASEB J 21:325–332 Available at: http://www.ncbi.nlm.nih.gov/pubmed/17267386 [Accessed May 16, 2018].

Serhan CN, Chiang N, Dalli J, Levy BD (2015a) Lipid mediators in the resolution of inflammation. Cold Spring Harb Perspect Biol 7:a016311 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25359497 [Accessed July 20, 2018].

Serhan CN, Dalli J, Colas RA, Winkler JW, Chiang N (2015b) Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim Biophys Acta - Mol Cell Biol Lipids 1851:397–413 Available at: http://www.ncbi.nlm.nih.gov/pubmed/25139562 [Accessed July 20, 2018].

Serhan CN, Dalli J, Karamnov S, Choi A, Park C-K, Xu Z-Z, Ji R-R, Zhu M, Petasis NA (2012) Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. FASEB J 26:1755–1765 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22253477 [Accessed July 20, 2018].

Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA (2006) Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. J Immunol 176:3843–3843 Available at: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.176.6.3843-a [Accessed July 20, 2018].

Serhan CN, Sheppard KA (1990) Lipoxin formation during human neutrophil-platelet interactions: Evidence for the transformation of leukotriene A4 by platelet 12-lipoxygenase in vitro. J Clin Invest 85:772–780 Available at: http://www.ncbi.nlm.nih.gov/pubmed/2155925 [Accessed July 23, 2018].

Shao B-Z, Xu Z-Q, Han B-Z, Su D-F, Liu C (2015) NLRP3 inflammasome and its inhibitors: a review. Front Pharmacol 6:262 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26594174 [Accessed April 17, 2019].

Sharaf N, Nicklin MJ, di Giovine FS (2014) Long-range DNA interactions at the IL-1/IL-36/IL-37 gene cluster (2q13) are induced by activation of monocytes. Cytokine 68:16–22 Available at: http://linkinghub.elsevier.com/retrieve/pii/S1043466614000702 [Accessed August 9, 2018].

Shi F-D, Takeda K, Akira S, Sarvetnick N, Ljunggren H-G (2000) The Journal of Immunology. J Immunol 157:3223–3227 Available at: http://www.jimmunol.org/content/165/6/3099 [Accessed May 10, 2019].

Simpson S, Blizzard L, Otahal P, Van der Mei I, Taylor B (2011) Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. J Neurol Neurosurg Psychiatry 82:1132–1141 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21478203 [Accessed May 30, 2019].

Sintzel MB, Rametta M, Reder AT (2017) Vitamin D and Multiple Sclerosis: A Comprehensive Review. Neurol Ther:1–27 Available at: http://link.springer.com/10.1007/s40120-017-0086-4 [Accessed February 14, 2018].

Sloka JS, Stefanelli M (2005) The mechanism of action of methylprednisolone in the treatment of multiple sclerosis. Mult Scler J 11:425–432 Available at: http://www.ncbi.nlm.nih.gov/pubmed/16042225 [Accessed July 31, 2018].

Steinman L (2014) Immunology of Relapse and Remission in Multiple Sclerosis. Annu

Rev Immunol 32:257–281 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24438352 [Accessed May 2, 2019].

Sun Q, Wu Y, Zhao F, Wang J (2017) Maresin 1 Ameliorates Lung Ischemia/Reperfusion Injury by Suppressing Oxidative Stress via Activation of the Nrf-2-Mediated HO-1 Signaling Pathway. Oxid Med Cell Longev 2017:1–12 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28751936 [Accessed July 20, 2018].

Symons JA, Bundick R V, Suckling AJ, Rumsby MG (1987) Cerebrospinal fluid interleukin 1 like activity during chronic relapsing experimental allergic encephalomyelitis. Clin Exp Immunol 68:648–654 Available at: http://www.ncbi.nlm.nih.gov/pubmed/3498582 [Accessed February 21, 2019].

Thompson AJ (2017) Challenge of progressive multiple sclerosis therapy. Curr Opin Neurol 30:237–240 Available at: http://insights.ovid.com/crossref?an=00019052-201706000-00007 [Accessed May 13, 2019].

Torkildsen Ø, Wergeland S, Bakke S, Beiske AG, Bjerve KS, Hovdal H, Midgard R, Lilleås F, Pedersen T, Bjørnarå B, Dalene F, Kleveland G, Schepel J, Olsen IC, Myhr K-M (2012) ω-3 Fatty Acid Treatment in Multiple Sclerosis (OFAMS Study). Arch Neurol 69:1044–1051 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22507886 [Accessed May 8, 2019].

Tramacere I, Del Giovane C, Salanti G, D'Amico R, Filippini G (2015) Immunomodulators and immunosuppressants for relapsing-remitting multiple sclerosis: a network meta-analysis. Cochrane database Syst Rev 9:CD011381 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26384035.

Trapp BD, Nave K-A (2008) Multiple Sclerosis: An Immune or Neurodegenerative Disorder? Annu Rev Neurosci 31:247–269 Available at: http://www.ncbi.nlm.nih.gov/pubmed/18558855 [Accessed July 25, 2018].

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L (1998) Axonal Transection in the Lesions of Multiple Sclerosis. N Engl J Med 338:278–285 Available at: http://www.nejm.org/doi/abs/10.1056/NEJM199801293380502 [Accessed July 24, 2018].

Traugott U, Reinherz EL, Raine CS (1983) Multiple sclerosis. Distribution of T cells, T cell subsets and Ia-positive macrophages in lesions of different ages. J Neuroimmunol 4:201–221 Available at: http://www.ncbi.nlm.nih.gov/pubmed/6222066 [Accessed July 25, 2018].

Tselis A (2012) Epstein–Barr virus cause of multiple sclerosis. Curr Opin Rheumatol 24:424–428 Available at: http://www.ncbi.nlm.nih.gov/pubmed/22617821 [Accessed April 16, 2019].

Tsunoda I, Sato F, Omura S, Fujita M, Sakiyama N, Park A-M (2016) Three immune-mediated disease models induced by Theiler's virus: Multiple sclerosis, seizures and myocarditis. Clin Exp Neuroimmunol 7:330–345 Available at: http://doi.wiley.com/10.1111/cen3.12341 [Accessed August 1, 2018].

Tur C, Montalban X (2017) Progressive MS trials: Lessons learned. Mult Scler J 23:1583–1592 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29041872 [Accessed May 13, 2019].

Valentin-Torres A, Savarin C, Hinton DR, Phares TW, Bergmann CC, Stohlman SA (2016) Sustained TNF production by central nervous system infiltrating macrophages promotes progressive autoimmune encephalomyelitis. J Neuroinflammation 13:46

Available at: http://www.ncbi.nlm.nih.gov/pubmed/26906225 [Accessed May 14, 2019].

Vandewalle J, Luypaert A, De Bosscher K, Libert C (2018) Therapeutic Mechanisms of Glucocorticoids. Trends Endocrinol Metab 29:42–54 Available at: http://www.ncbi.nlm.nih.gov/pubmed/29162310 [Accessed June 2, 2019].

Velnar T, Bailey T, Smrkolj V (2009) The Wound Healing Process: An Overview of the Cellular and Molecular Mechanisms. J Int Med Res 37:1528–1542 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19930861 [Accessed May 1, 2019].

Wagner CA, Goverman JM (2015) Novel Insights and Therapeutics in Multiple Sclerosis. F1000Research 4:517 Available at: /pmc/articles/PMC4544373/?report=abstract.

Wang L, Wang F-S, Gershwin ME (2015a) Human autoimmune diseases: a comprehensive update. J Intern Med 278:369–395 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26212387 [Accessed May 13, 2019].

Wang X, Zhu M, Hjorth E, Cortés-Toro V, Eyjolfsdottir H, Graff C, Nennesmo I, Palmblad J, Eriksdotter M, Sambamurti K, Fitzgerald JM, Serhan CN, Granholm AC, Schultzberg M (2015b) Resolution of inflammation is altered in Alzheimer's disease. Alzheimer's Dement 11:40–50.e2 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24530025 [Accessed February 20, 2019].

Weber A, Wasiliew P, Kracht M (2010) Interleukin-1 (IL-1) Pathway. Sci Signal 3:cm1-cm1 Available at: http://www.ncbi.nlm.nih.gov/pubmed/20086235 [Accessed April 17, 2019].

Wekerle H (2008) Lessons from multiple sclerosis: Models, concepts, observations. In: Annals of the Rheumatic Diseases, pp iii56-iii60 Available at: http://www.ncbi.nlm.nih.gov/pubmed/19022815 [Accessed January 23, 2018].

Wu C, Sakorafas P, Miller R, McCarthy D, Scesney S, Dixon R, Ghayur T (2003) The Journal of Immunology. J Immunol 161:3400–3407 Available at: http://www.jimmunol.org/content/170/11/5571 [Accessed April 17, 2019].

Wu G-C, Li H-M, Wang J-B, Leng R-X, Wang D-G, Ye D-Q (2016) Elevated plasma interleukin-37 levels in systemic lupus erythematosus patients. Lupus 25:1377–1380 Available at: http://www.ncbi.nlm.nih.gov/pubmed/27125292 [Accessed April 10, 2019].

Wu GF, Alvarez E (2011) The immunopathophysiology of multiple sclerosis. Neurol Clin 29:257–278 Available at: http://www.ncbi.nlm.nih.gov/pubmed/21439440 [Accessed February 22, 2018].

Xian W, Wu Y, Xiong W, Li L, Li T, Pan S, Song L, Hu L, Pei L, Yao S, Shang Y (2016) The pro-resolving lipid mediator Maresin 1 protects against cerebral ischemia/reperfusion injury by attenuating the pro-inflammatory response. Biochem Biophys Res Commun 472:175–181 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26915798 [Accessed May 8, 2019].

Yang Y, Wang H, Kouadir M, Song H, Shi F (2019) Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis 10:128 Available at: http://www.nature.com/articles/s41419-019-1413-8 [Accessed May 10, 2019].

Ye L, Jiang B, Deng J, Du J, Xiong W, Guan Y, Wen Z, Huang K, Huang Z (2015a) IL-37 Alleviates Rheumatoid Arthritis by Suppressing IL-17 and IL-17–Triggering Cytokine Production and Limiting Th17 Cell Proliferation. J Immunol 194:5110–5119 Available at: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1401810 [Accessed

August 10, 2018].

Ye L, Jiang B, Deng J, Du J, Xiong W, Guan Y, Wen Z, Huang K, Huang Z (2015b) IL-37 Alleviates Rheumatoid Arthritis by Suppressing IL-17 and IL-17–Triggering Cytokine Production and Limiting Th17 Cell Proliferation. J Immunol 194:5110–5119 Available at: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1401810 [Accessed August 10, 2018].

Zang YCQ, Li S, Rivera VM, Hong J, Robinson RR, Breitbach WT, Killian J, Zhang JZ (2004) Increased CD8+ cytotoxic T cell responses to myelin basic protein in multiple sclerosis. J Immunol 172:5120–5127 Available at: http://www.ncbi.nlm.nih.gov/pubmed/15067096 [Accessed July 25, 2018].

Zeng Q, Song R, Fullerton DA, Ao L, Zhai Y, Li S, Ballak DB, Cleveland JC, Reece TB, McKinsey TA, Xu D, Dinarello CA, Meng X (2017) Interleukin-37 suppresses the osteogenic responses of human aortic valve interstitial cells in vitro and alleviates valve lesions in mice. Proc Natl Acad Sci 114:1631–1636 Available at: http://www.pnas.org/lookup/doi/10.1073/pnas.1619667114 [Accessed August 10, 2018].

Zhou Y, Leng X, Li H, Yang S, Yang T, Li L, Xiong Y, Zou Q, Liu Y, Wang Y (2017) Tolerogenic dendritic cells induced by BD750 ameliorate proinflammatory T cell responses and experimental autoimmune encephalitis in mice. Mol Med 23:204–214 Available at: http://www.ncbi.nlm.nih.gov/pubmed/28960227 [Accessed May 14, 2019].

Zubizarreta I et al. (2019) Immune tolerance in multiple sclerosis and neuromyelitis optica with peptide-loaded tolerogenic dendritic cells in a phase 1b trial. Proc Natl Acad Sci 116:8463–8470 Available at: https://www.pnas.org/content/116/17/8463 [Accessed May 14, 2019].

ABBREVIATIONS

AA: arachidonic acid

ASC: protein apoptosis-associated speck-like protein

ATLs: aspirin-triggered LXs

BBB: blood-brain barrier

CFA: complete Freund's adjuvant

CIS: clinical isolated syndrome

CNS: central nervous system

COX: cyclooxygenase

CSF: cerebrospinal fluid

DHA: docosahexaenoic acid

DMDs: disease-modifying drugs

EAE: experimental autoimmune encephalomyelitis

EBV: Epstein-Barr virus

EMA: European Medicines Agency

EPA: eicosapentanoic acid

FDA: Food and Drug administration

FPRL1: formyl peptide receptor-like-1

GAWS: Genome-wide association studies

hIL-37tg: human IL-37 transgenic mouse

HLA: human leukocyte antigen

IFA: incomplete Freund's adjuvant

IL: interleukin

IL-1F: interleukin 1 family

IL-1R1: IL-1 receptor type 1

LM: lipid mediator

LOX: lipoxygenase

LT: leukotriene

LX: lipoxin

MaR: maresin

MHC: major histocompatibility complex

MS: multiple sclerosis

NAWM: normal apparent white matter

NLRP3: Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing type 3

NPD: neuroprotectin

OMS: Overcoming Multiple Sclerosis organization

PD: protectins

PG: prostaglandins

PMN: polymorphonuclear leukocytes

PPMS: primary progressive multiple sclerosis

PRMS: progressive relapsing multiple sclerosis

RRMS: Relapsing-remitting multiple sclerosis

RvD: resolvin D

RvE: resolvin E

SIGIRR (IL-1R8): single immunoglobulin IL-1 receptor related protein

SPM: specialized pro-resolving mediator

SPMS: secondary progressive multiple sclerosis

TMEV: Theiler's murine encephalomyelitis