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REVIEW

On the immunoregulatory role of statins in multiple sclerosis: the effects on Th17 cells

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Abstract

Statins, the cholesterol-lowering drugs, also possess immunomodulatory properties, affecting among others T cell activation and differentiation, antigen presentation, and regulatory T cell (Tregs) maintenance and differentiation. Their effects on autoagression have led investigators to assess their clinical significance in autoimmune disease, such as multiple sclerosis (MS), a chronic progressive demyelinating disease of autoimmune nature. The dysregulated immunity noted in MS features a profound shift from Tregs dominance to Th17 cell superiority. In this review, we discuss the immunobiological basis of statins, their role in autoimmunity related to MS, and the data from experimental models and human studies on their effect on Th17 cells.

Keywords Autoimmunity · Demyelination · Inflammation · Regulation · Statin

Abbreviations

APC	Antigen-presenting cell
BBB	Blood–brain barrier
CSF	Cerebrospinal fluid
EDSS	Expanded Disability Status Scale
FACS	Fluorescence-activated cell sorting
FoxP3	Forkhead box P3
IFN	Interferon
IL	Interleukin
mAb	Monoclonal antibodies
MBP	Myelin basic protein
MS	Multiple sclerosis
NMO	Neuromyelitis optica
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cells
RORC	Retinoid-related orphan receptor C
RRMS	Relapsing-remitting MS
SPMS	Secondary progressive MS
T-bet	T-box expressed in T cells
TGF	Transforming growth factor
Th	T-helper
TNF	Tumor necrosis factor
Treg	T regulatory cells
VLA	Very late antigen

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Introduction

Th17 in MS: major aspects

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) and neuromuscular junction, characterized by demyelination, axonal damage, and loss of neuromuscular function [1]. Originally, MS was deemed as an autoreactive Th1 cell disorder; several recent studies however, on both animal models and MS patients, have highlighted the role of CD4+ T-helper 17 (Th17) cells and the cytokine IL-17A in the early stages of MS pathogenesis [2]. Th17 cells express the master

regulator retinoic acid receptor-related orphan receptor-γt (RORγt), encoded by the RORC gene, and can respond to several environmental agents [3]. They exhibit significant plasticity and participate in inflammatory responses as well as in tissue homeostasis and regeneration [4]. In inflammatory states, including experimental autoimmune encephalomyelitis (EAE), the animal model of the disease, Th17 cells, can switch to Th1 cells producing IFN-γ [5]. IL-17A is induced by IL-1β, TGF-β, and IL-6 derived from phagocytes and antigen-presenting cells. Recent studies have shown that evolutionarily conserved prohibitins (PHB1/2) highly expressed on the surface of Th17 cells can activate CRAF/MAPK activation and lead to IL-17A expression [6]. IL-17's physiological function is to combat extracellular bacteria and fungi through induction of defensins and antimicrobial peptides.

Upon recognition of CNS-derived antigens (or cross-recognized microbial antigens), peripheral Th17 cells escape central tolerance surveillance, infiltrate the brain and spinal cord, and mount a robust immune response, leading to maintenance of autoreactive responses [7, 8]. During this scenario, Th17 cells, activated in the periphery, secrete cytokines and chemokines and initiate an inflammatory cascade. Th17-derived pro-inflammatory cytokines including IL-21, IL-22, IL-23, GM-CSF, and IFN-γ have a critical role in the pathogenesis of MS [9, 10]. IL-17A synergizes with these pro-inflammatory cytokines, by inducing other mediators of tissue damage and chemokines that recruit new inflammatory cells [11]. IL-17A adversely affects the functions of microglia, astrocytes, oligodendrocytes, neurons, neural precursor cells, and endothelial cells.

Th17 and Tregs in MS

The dysregulated immune response in MS is characterized by a profound shift in the balance of Th17 cells and suppressive cell subsets, such as regulatory T (Tregs) cells [12]. The increased activity of Th17 cells inversely correlates with percentages and functionality of Tregs and perpetuates autoreactive responses [13, 14].

Tregs defined as CD4 + CD25hiFoxP3+ cells are central in immune tolerance and suppression of autoimmune reactions. The two main Treg subtypes are the natural Tregs (nTreg) which develop in the thymus and the induced Tregs (iTreg), which develop in the periphery, and both of which express the FoxP3 transcription factor [15, 16]. FOXP3 gene is activated by the signal transducer and activator of transcription (STAT) 5. Tregs restrain autoreactive T cell activation either by contact-dependent mechanisms and/or through the production of the anti-inflammatory cytokine IL-10 [17]. Tregs can also maintain peripheral tolerance against self-antigens through additional soluble mediators including IL-35, transforming growth factor (TGF)-β, and cell surface molecules such as CD25, CD39, inducible costimulatory (ICOS), programmed cell death protein 1 (PD-1), and cytotoxic T lymphocyte antigen (CTLA)-4 [18, 19]. CD39, an ectonucleotidase, hydrolyzes extracellular ATP, which is released in inflammatory tissues and inhibits FoxP3+ Tregs thus promoting their transconversion into Th17 cells [20]. IL-35 is associated with upregulation of inhibitory molecules such as PD-1, T cell immunoglobulin and mucin-domain containing-3 (TIM-3), and lymphocyte activation protein 3 (LAG3) on T cells [19]. Recently, a specific Treg subset, namely iTr35, has been identified [21]. Type 1 regulatory T cells (Tr1) are characterized by surface markers CD49b and LAG3 [22]. Tr1 secrete IL-10 but lack FoxP3. B cells can induce Tregs (Treg of B) that express PD-1, ICOS, LAG3, and glucocorticoid-induced tumor necrosis factor receptor (GITR) and produce high levels of IL-10 but low levels of TGF-β [23]. Gut environment induces autoreactive T cells that suppress EAE via LAG3 [24].

TGF-β induces the expression of both RORC and FOXP3 genes of RORγt and FoxP3, respectively, in a CD4 T cell, but FoxP3 binds to and thus inhibits RORγt thus preventing IL-17 expression and Th17 development and promotes iTreg development. TGF-β and IL-2 are essential for iTreg differentiation. TGF-β activates Sma and Mad-regulated protein (SMAD) 2 and SMAD3 which induce the FOXP3 gene, where IL-2 activates STAT5 which also induces FOXP3 expression. However, the presence of inflammatory cytokines IL-6 and IL-21 activates STAT3 which downregulates TGF-β-mediated FoxP3 expression, upregulates RORγt expression, abrogates FoxP3-mediated inhibition of RORγt, and T cells differentiate into Th17. The additional presence of IL-23 upregulates IL-23 receptor and expands Th17 cells [25, 26] .

Tregs employ contact-dependent and cytokine-mediated mechanisms to inhibit effector T cell function. However, some Tregs appear not to be at terminal stage of differentiation and can trans-differentiate into effector cells producing IL-17 [27, 28] or retain their suppressive function while acquiring the ability to secrete pro-inflammatory cytokines such as IL-17 or IFN-γ [29, 30].

In MS, decreases in the frequency of Tregs; defects in FoxP3 and IL-10 expression; variations in CD25, PD-1, and CTLA-4 molecules; and resistance of inflammatory Th17 cells to Treg-mediated suppression are all involved in the maintenance of auto-aggressive responses [15]. Furthermore, recent data have suggested that Tregs have limited capacity of CNS infiltration but are essential to promote oligodendrocyte differentiation and (re)myelination [31]. Tregs contact-dependent production of cytokines can maintain immune homeostasis and ameliorate the progression of MS. In MS, there are increased numbers of Th17 cells and associated cytokines IL-17A, IFN-γ, IL-1, and IL-6 in both peripheral blood and cerebrospinal fluid (CSF) [32]. Latest studies have also revealed that the elevated levels of pro-inflammatory cytokines

in the CNS lead to diminished serotonin (5-HT) synthesis, a neurotransmitter with pleiotropic immune effects [33]. In vitro, 5-HT attenuated Th17 cell proliferation, reduced IFN-γ and IL-17 release, and increased IL-10 production by CD4 + FoxP3+ CD39+ Tregs from MS patients [33]. However, in a humanized TCR transgenic mouse model of MS, removal of FoxP3+ Tregs resulted in exacerbation of the disease without effect on Th17 cells infiltrates in the CNS [34]. Also, CD39-expressing (CD3 + CD4 + CD25hiCD39 + CD127lowFoxp3+) Tregs correlated with Th17 cells in RR MS [35].

Therapeutic targeting of Th17 in MS

Several treatment modalities for MS have been investigated on the principle of restoring homeostasis and function of Treg subsets [15, 36–38]. Most of these also aimed to increase expression of suppressor cytokines such as TGF-β, IDO, IL-10, IL-27, and IL-35. Additional studies have identified CD8+ T cell subsets as potential immunoregulators in MS [39]. For instance, MHC class Ia-restricted neuroantigen-specific "autoregulatory" CD8+ T cells and glatiramer acetate Qa-1/HLA-E-restricted GA-specific CD8+ T cells have been documented [40]. These CD8+ Tregs suppressed proliferation of pathogenic CD4 + CD25 T cells when stimulated by their cognate antigens. Similarly, CD8+ Tregs significantly suppressed experimental autoimmune encephalomyelitis (EAE) when transferred either from pre-disease or during disease peak [41].

Currently, novel therapeutics that directly target Th17 cell effector pathways have been also evaluated in clinical trials and as predictive biomarkers to monitor disease activity [42, 43]. Th17 cells can efficiently cross the blood–brain barrier, promote its disruption, and induce the activation of other inflammatory cells in the CNS. Natalizumab is an effective treatment for relapsing-remitting MS (RRMS), as it makes the blood–brain barrier impenetrable by binding to the alpha4 integrin subunit and therefore prevents central nervous system Th17 infiltration and cytokine expression [44]. Other clinical data have successfully linked the depletion of T and B cell subsets, or the prevention of their migration into the brain with significant reduction in relapses and development of new lesions [45–47]. In vitro studies and preclinical animal model data with the anti-IL-17A antibody Secukinumab indicated that blockade of IL-17A could be beneficial to MS patients not only by inhibiting inflammation and tissue destruction but also by enhancing repair processes [43]. IL-17A blockade is central to MS therapeutics but other agents may also modulate IL-17 expression. One such an example is the statins used to treat hypercholesteremia and prevent atherosclerosis. As statins have remarkable immunoregulatory properties [48], whether statins are able to downregulate autoreactive immune responses via the inhibition of Th17 and the subsequent increase of Tregs in MS remains unclear. Some data from other autoimmune diseases suggest that statins indeed have promising potential but whether this is a likely scenario in MS is not clear. This review is discussing key findings addressing this topic.

Statins: from biochemistry to immunobiology

Statins are oral inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) thus inhibiting L-mevalonic acid synthesis and lowering cholesterol and are widely used in the prevention of cardiovascular disease [49, 50] (Fig. 1).

A number of criteria have been used for the classification of these drugs (Table 1). Statins can also affect downstream steps of the mevalonate pathway thus influencing inflammation, the formation of nitric oxide, the coagulation cascade, and other biochemical processes [51–54] (Fig. 2). Furthermore, certain metabolites of mevalonate can affect the posttranslational modification of key proteins involved in cell proliferation and differentiation.

Statins have been investigated as immunomodulatory and anti-inflammatory drugs [55, 56]. Kobashigawa et al. reported a better outcome of cardiac transplantation in statin-treated patients compared to non-statin-treated patients [57]. Thirteen years later, Pazik J et al. observed that individuals with biopsy-confirmed post-transplant glomerulonephritis who were using statins had a prolonged survival of the transplanted organ, further indicating an immunoregulatory role of statins [58].

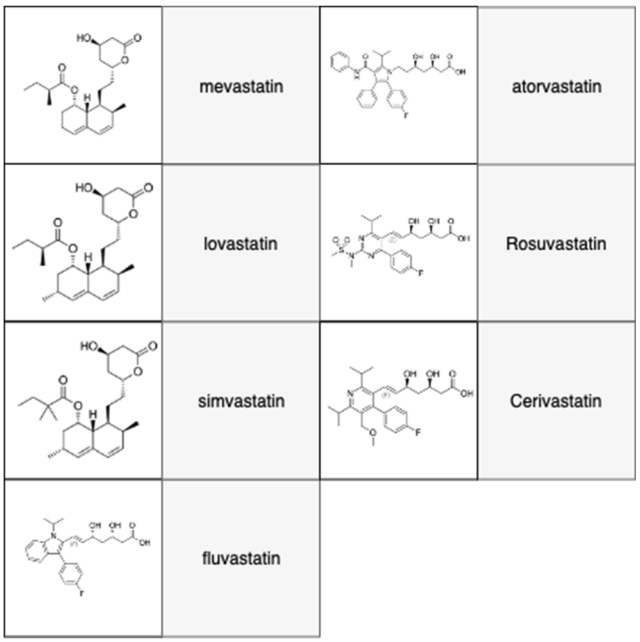

Fig. 1 Statins stereochemical structures

Table 1 Criteria of statins classification

Origin and structural formula	Lovastatin, simvastatin, pravastatin
	- fungi derived
	- similar to the substituted decalin-ring structure
	Fluvastatin, atorvastatin, rosuvastatin, and cerivastatin
	- synthetic drug
	- bigger groups linked to the HMG-like moiety
Physicochemical properties	Both hydrophobic and hydrophilic:
	Fluvastatin
	Hydrophobic
	Lovastatin, simvastatin, atorvastatin, cerivastatin
	Hydrophilic: pravastatin, rosuvastatin
Specific activity	
Liver metabolism	

Statins as immunomodulators

Mevalonate-dependent statin effects

Statins through their ability to interfere with intermediate molecules in the biosynthesis pathway of cholesterol can directly affect membrane localization and functions of several proteins. Statins, through the mevalonate effect, influence molecules, such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) that are involved in isoprenoid synthesis and play a significant role in the activation of GTPases [59]. Members of the Ras and Rho GTPase family are post-translationally modified by prenylation. Statins can inhibit Ras and Rho isoprenylation thus reducing the levels of isoprenoids FPP and GGPP [60]. The inhibition of the above pathways

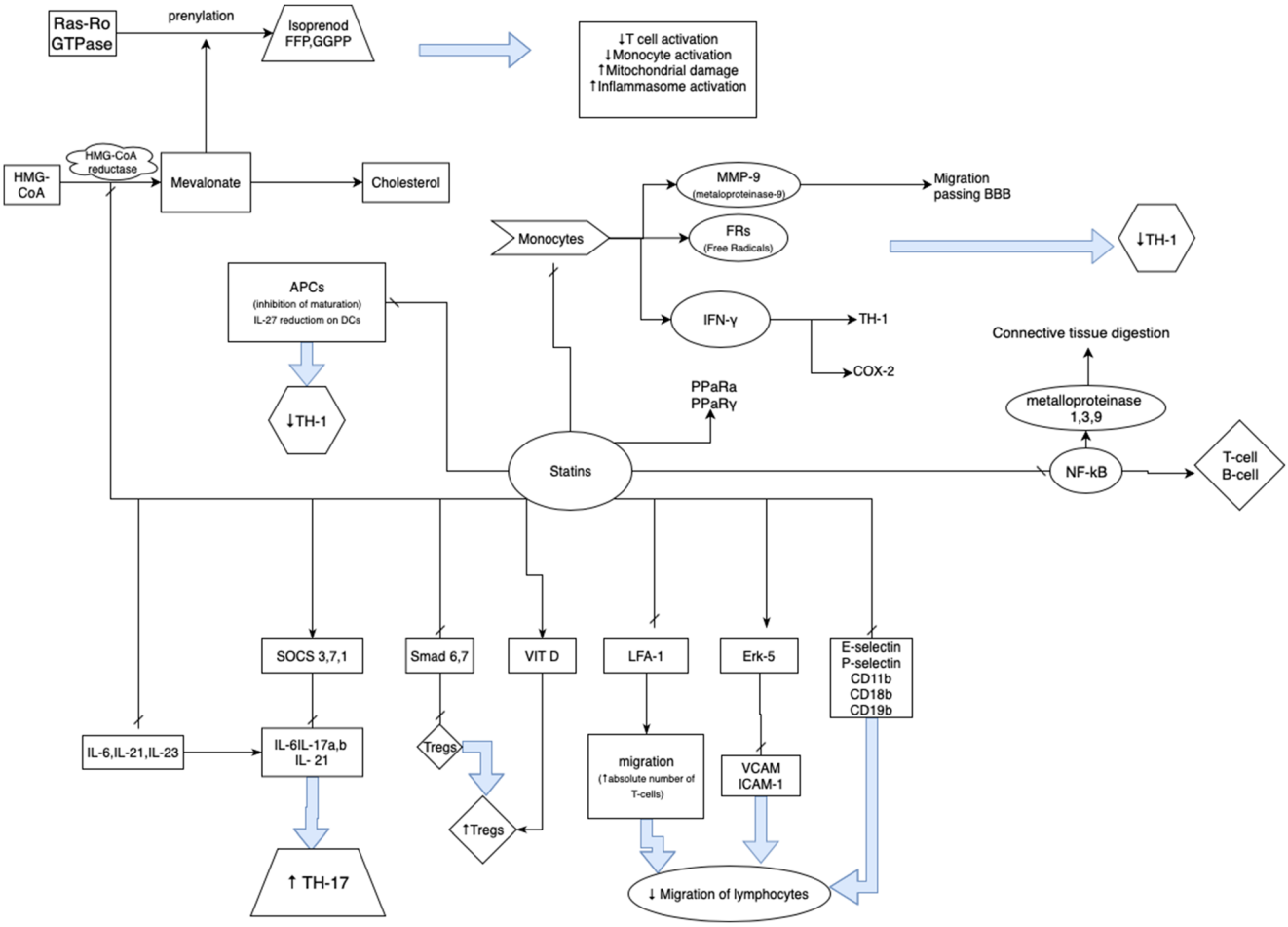

Fig. 2 Immunomodulatory effects of statins. Statins have the ability to regulate the immune system and to suppress autoimmunity through various mechanisms. Their regulatory role is achieved mainly by the decrease of activation of antigen-specific autoreactive immune responses. Also, statins inhibit maturation of antigen-presenting cells, leading to downregulation of Th1 responses. They can also affect/inhibit autoreactive T/B cell responses via a regulation of molecular signaling pathways (i.e., that of Nfκβ, SOCS, etc). Another notable effect of statins related to the upregulation of vitamin D receptor and the induction of regulatory T cells, directly or indirectly. Statins have the ability to inhibit Th17, and the migration of lymphocyte populations and these effects also participate in the amelioration of autoreactive immune responses

leads to defective activation and proliferation of T cells and production of Th1 cytokines [61, 62]. Apart from isoprene compounds, inhibition of mevalonate leads to reduced production of coenzyme Q, an important molecule for the mitochondrial membrane [63, 64]. Mevalonate products are shown to be necessary for TNF-α or angiotensin II-mediated monocyte adhesion to endothelial cells and production of reactive oxygen species (ROS) [65]. Autophagy, a lysosomal inflammatory reaction, is also impaired when mevalonate pathway is inhibited by statins causing over-activation of inflammasome that finally leads to cell death. This is because autophagy-intermediate proteins (autophagy-related [ATG] proteins) need to be prenylated to be functional [64].

Mevalonate-independent statin effects

Statins can also directly regulate gene expression through their mevalonate-independent effects. Thus, besides modulation of the Ras-Rho signaling pathway involved in cell growth and differentiation, statins can also influence cell trafficking, apoptosis, maturation of antigen-presenting cells (APC)s, and T cell activation, differentiation, and interaction with other cell subsets such as Tregs [60, 66–69] .

Cell trafficking Statins can decrease mononuclear cell migration by dampening expression of matrix metalloproteinases (MMP)-9 and MMP-2, which assist cell penetration through the blood–brain barrier (BBB) [70, 71]. They can also suppress MMPs 1 and 3 involved in connective tissue digestion [72]. Leukocyte extravasation could be influenced by inhibition of adhesion molecules and atorvastatin and simvastatin reduced cell surface expression of adhesion molecules L-selectin and VLA4 in a monocyte cell line (THP1 cells) in vitro. Simvastatin along with ezetimibe (a cholesterol absorption inhibiting medication often used in combination with a statin) reduces the gene expression of LFA-1 [73]. Migration of Th1 cells could also be inhibited by atorvastatin and simvastatin through inhibition of CD40-CD40L-dependent B cell activation [74, 75].

Apoptosis Induction of apoptosis by statins is well documented in cancer and stem cell systems [76, 77]. Furthermore, in coronary artery disease, rosuvastatin can cause apoptosis of CD4 + CD28 null cells [78]. Atorvastatin can cause apoptosis of CD4 cells though caspase-independent mechanism [79]. However, the apoptosis-inducing molecules caspase-3, -8, and -9 were found to be elevated as was the Bax/Bcl-2 ratio in apoptotic CD4 cells caused by fluvastatin [80]. Mitochondrial membrane potential impairment along with caspase-3, -8, and -9 increase was found in human lymphoblasts and myeloma cells [81]. These findings indicate that both intrinsic (mitochondrial) and extrinsic (death receptor) pathways are affected.

Antigen-presenting cells Statins suppress antigen presentation and maturation of human dendritic cells (DCs) in a dose-dependent manner [82]. They inhibit major histocompatibility complex (MHC) molecules in monocytes by their suppressive effect on IFN-γ production, as they inhibit transactivator class II. Thus, they block DC antigen presentation as well as antigen-presenting cell (APC)-mediated Th1 differentiation [56, 83, 84]. They suppress B cell ability to present antigen, which implies that T cell response is also impaired [85]. Statins, by affecting both directly and indirectly T-helper cells, regulate Th1/Th2 homeostasis. Therefore, they enhance a Th2 response, as shown by lovastatin's ability to block the expression of T-bet and amplify GATA3 expression, moving the balance towards Th2 response [68]. Simvastatin can limit pro-inflammatory mediators of monocytes, such as the effect of INF-γ on COX-2 expression [86], and the suppression of IL-6 and IL-23, by limiting expression of STAT1 and STAT3 phosphorylation and induction of SOCS3, 7 [87, 88].

T cell activation and differentiation Statins have an inhibitory effect on integrin LFA-1, and thus impair T lymphocyte function [89]. LFA-1, a molecule which is essential for T cell costimulation migration and trafficking of lymphocytes, is inhibited when bound to statins. Statins have effects on Akt/mTOR signaling [90, 91] and inhibit transcription factors such as NF-κB and AP-1 [92] thus limiting pro-inflammatory cytokine synthesis.

Regulatory T cells (Tregs) Statins affect Tregs indirectly through induction of tolerogenic DCs [67, 93]. Statins can also directly influence Tregs [91, 94–96]. In animal models, statins lead to Tregs maintenance and differentiation, by regulating the methylation of FoxP3+ promoter and by inhibiting the induction of Smad 6 and Smad 7. Homing of Tregs to inflamed tissues is positively regulated, as statins decrease pro-inflammatory cytokines IFN-γ IL-12, IL-1α, IL-1β, and TNF-α, while they increase IL-4, IL-10, and TGF-β [67, 84]. The allosteric binding of statin to LFA-1 blocks ICAM-1 function, causing migration of Tregs [67]. Finally, the action of Tregs is favored by the presence of statins and associated vitamin D levels, in a way that is not well-understood yet [60].

Statins in multiple sclerosis

Therapeutic options in MS thus far include drugs or combination of drugs affecting multiple steps in disease progression such as peripheral autoreactive T cell activation, migration into the CNS, and suppression of inflammation [97]. Available disease-modifying therapies (DMTs) for MS include interferon- β (IFN-β) glatiramer acetate (GA), natalizumab, mitoxantrone, and fingolimod [98–100]. First-line therapies, IFN-β and GA, are only partially effective in preventing relapse rate and disability. Mitoxantrone and

natalizumab, second-line therapies, are administered by intravenous infusion and have greater efficacy but have adverse effects. Oral fingolimod is an alternative to regular self-injections.

Statins, having secondary immunomodulatory and anti-inflammatory properties, have been so far only tested as a complementary therapeutic option in MS [101]. Original studies showed beneficial effects of statins on glial cells and EAE and in vitro anti-inflammatory effects on PBMCs from patients with RR MS [71, 102–105]. However, human studies have not consistently confirmed these favorable results and a meta-analysis revealed that the addition of statins to interferon therapy did not significantly influence the relapse risk, disease progression, or expanded disability status scale (EDSS) scores in patients with RRMS [106].

A 2018 systematic review considering the role of statins as immunomodulators in MS, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and graft-versus-host disease (GVHD) indicated that the net outcomes may vary depending on the different types of statins used. Novel aspects of certain statins are the increased angiopoietin-1 production by simvastatin, which thereby impacts on endothelial function, and the direct effects on T cells via calcium influx and IL-2 production by lovastatin [48].

A summary of the beneficial effects that statins may exert in MS are shown in Table 2.

Statins in experimental autoimmune encephalomyelitis (EAE)

A summary of the main studies and reported data on the effect of statins in EAE are presented in Table 3. A seminal study by Bailey et al. have demonstrated that peripheral mDCs can localize in the CNS during relapsing EAE and initiate IL-17 production from CD4+ T cells [113]. The mDCs, by expressing IL-6, IL-23, and TGF-β, skewed T cells towards Th17 and not Th1 differentiation [113]. Zhang et al. have shown that statins (simvastatin) in EAE can prevent expression of RORC [88].

Chen et al. recently evaluated the role of statins as modulators of DCs in EAE, given the knowledge that tolerogenic dendritic cells (TolDCs) negatively regulate autoimmune responses, and that statins might be a promising agent for induction of TolDCs. They used four different groups of mice. (i) the model control (EAE + PBS) group, (ii) the mDCs-administrated EAE model (EAE + mDCs) group, (iii) the TolDCs-administrated EAE model (EAE + TolDCs) group, and (iv) the TolDCs-MOG-administrated EAE model (EAE + TolDCs-MOG). TolDcs. They have found a reduction of Th17 cells and induction of Tregs in the ToLDCs-MOG group [107].

The roles of DCs, Th1, Th2, Tregs, and the associated cytokines are shown in Table 3.

Lovastatin An early study showed that lovastatin and phenylacetate inhibited the induction of nitric oxide synthase and pro-inflammatory cytokines in Lewis rat astrocytes microglia and macrophages [104]. Lovastatin decreased the expression of TNF-α and IFN-γ, decreased MNC infiltration in the brain, and improved the clinical signs of EAE in rats [102, 103]. Lovastatin could decrease the expression of T-bet, a critical transcription factor in the regulation of Th1 responses, and induce the expression of GATA3, a master regulator, which promotes Th2 cytokines in mice with EAE [114]. It inhibited Rho proteins in brain endothelial cells and leukocyte migration across blood–brain barrier and ameliorated both acute and relapsing disease in a mouse model of MS [105]. Lovastatin has also been shown to provide protection in EAE animals by upregulating transcription factors such as peroxisome proliferator-activated receptor (PPAR)-gamma [115] and via inhibition of RhoA-ROCK signaling and enhanced bioavailability of 1,25-dihydroxyvitamin D3 [116]. More specifically, lovastatin increased expression of 1α-hydroxylase in kidneys and the CNS, with corresponding reduction of 24-hydorxylase expression in the CNS of EAE animals via inhibition of RhoA-ROCK signaling [116]. Autoreactive Th1/Th17 cells had higher expression of 24-hydroxylase than Th2/Treg cells, and this was reverted by lovastatin or specific ROCK signaling inhibitors [116, 117].

Atorvastatin: Youssef et al. also described a shift towards a Th2 response using atorvastatin in EAE mice [118]. Atorvastatin administration resulted in activation (phosphorylation) of STAT6 and secretion of Th2 cytokines (IL-4, IL-5, and IL-10) as well as TGF-β. In contrast, STAT4 phosphorylation was markedly inhibited as well as the secretion of Th1 cytokines (IL-2, IL-12, IFN-γ, and TNF-α) [118]. Weber et al. found, however, that atorvastatin failed to increase Th2 cells in vivo [110]. They observed that oral atorvastatin actually prevented EAE in (STAT6−/−) mice, which cannot generate IL-4-producing Th2 cells. On the contrary, atorvastatin in vitro permitted differentiation into Th2 cells and decreased antigen-specific T cell proliferation and

Table 2 Summary of the beneficial effects of statins in MS

A summary of the beneficial effects that statins may exert in MS
Decrease of cell migration and regulation of blood–brain barrier
Shift of Th1 towards TH2 phenotype and inhibition of Th17 cells
Suppression of antigen presentation through MCH II
Inhibition of oligodendrocytes loss [60]

Table 3 A summary of major studies in the EAE model investigating the role of statins in the regulation of Th17 responses

Ref	Year	Country	Type of study	Experimental model	Statin	Doses	Duration of treatment	TH-17	IL-17	TH-1
[107]	2018	China	In vivo	EAE (female C57BL/6 mice, 8–10 weeks old)	Atorvastatin	4 groups: (i) model control (EAE + PBS) group, (ii) mDCs-administrated EAE model (EAE + mDCs) group (iii) TolDCs-administrated EAE model (EAE + TolDCs) group (iv) TolDCs-MOG-administrated EAE model (EAE + TolDCs-MOG)	30 days	↓ (TolDCs-MOG group compared with control)	↓ (TolDCs-MOG group compared with control)	Not tested
[108]	2015	Brazil	In vivo	EAE (C57BL/6 mice female, 8–12 week old)	Simvastatin	Simvastatin orally;5 mg/kg/day; total vol 0.2 ml daily	16 days	↓	↓	↓
[109]	2014	Iran	In vivo	EAE (C57BL/6 female mice,6–8 week old)	Atorvastatin/all-trans retinoic acid	Atorvastatin: 200 mg per mouse/250 mg of all-trans retinoic acid	33 days	Not tested	↓	Not tested
[110]	2014	USA	In vivo/in vitro reactivated T cell atorvastatin-treated group	EAE (C57BL/6 female mice, 5–8 weeks old)	Atorvastatin	Atorvastatin (1 mg/kg/day, 5 mg/kg/day or 10 mg/kg/day) orally in 0.5 ml	10 days	↓	↓	↓
[111]	2012	China	In vivo	EAE (female C57 BL/6 mice)	Atorvastatin/rapamycin	Combination: atorvastatin (1 mg/kg/day) orally + rapamycin (0.3 mg/kg/day) i.p. in 0.1 ml distilled water	30 days	↓	↓	↓
[112]	2008	USA	In vivo	EAE (female Lewis rats)	Lovastatin/rolipram	Separate groups: lovastatin (2 and 5 mg/kg, i.p.) rolipram (2, 5, and 7.5 mg/kg, sc) daily. Combination: lovastatin (1 mg/kg) daily and rolipram (1 mg/k every other day)	5 days/15 days each group	↓	↓	↓

Ref	TH-2	Tregs	TNF-α	IL-4	IL-10	IL-12	TGF-β	IFN-γ	Clinical score	Histological results
[107]	Not tested	↓ (TolDCs-MOG group compared with control)	↓ (TolDCs compared with mDCs)	Not tested	NSD	Not tested	NSD	Not tested	NSD	Reduction of histopathologic presentations of inflammatory cells and decreased demy elination areas in TolDCs-MOG group compared with control
[108]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	↑	Not tested	↓ mean clinical scores (p\0.05)	Not tested
[109]	Not tested	↑	↓ (in vitro)	Not tested	↑	↓ (in vitro)	Not tested	↓ (in vitro)	↓	↓
[110]	↑ Th2 cells in vitro	Not tested	Not tested	Not tested	↑	Not tested	Not tested	↓	↓	↓

Table 3 (continued)

[111]	↑	↑	↑	↑	↑	↑	↓ (0: no paralysis, 1: loss of tone, 2: hindlimb weakness, 3: hindlimb paralysis, 4: hindlimb/forelimb paralysis, 5: moribund/dead)	↓	
[112]	↑	↑	↑	Not tested	↑	↓	↓ (0: no clinical score, 1: piloerection, 2: loss in tail tonicity, 3: hind leg paralysis, 4: paraplegia, 5: moribund/dead)	↓	↓ of infiltration and demyelination

NSD no significant difference

secretion of pro-inflammatory cytokines (IFN-γ, IL-17, TNF, IL-12). The authors concluded that atorvastatin ameliorates CNS autoimmune disease primarily by reducing pro-inflammatory encephalitogenic T cells, and not simply through induction of Th2 cells [110]. Another study showed that atorvastatin administration in EAE on SJL/J genetic background mice resulted in decrease of inflammatory cell infiltration and autoreactive Th1 cell responses in the CNS and reduced disease relapses [119]. Li et al in 2011 showed that atorvastatin ameliorated experimental autoimmune neuritis by decreasing Th1/Th17 cytokines and upregulating T regulatory cells [94].

Simvastatin De Oliveira et al. [108] found that simvastatin at 5 mg/kg/day improved clinical outcome, associated with an increase in TGF-β mRNA expression and inhibition of IL-6 and IL-12 secretion. Furthermore, they showed a significant decrease in central nervous system (CNS) inflammatory mononuclear cell infiltration. Th1 cells were reduced but Th17 cells did not. However, CD4+ IL17+ cells were reduced in the CNS of simvastatin-treated group. The authors concluded that simvastatin modulated Th1 and Th17 cells either by diminishing infiltration of cells in CNS or by inhibiting T cell differentiation. Simvastatin also inhibited the proliferation of T lymphocytes co-cultured with primary microglial cells [108].

Statins in combination with other immunomodulatory agents

Combination of the suboptimal doses of rolipram (a phosphodiesterase-4 inhibitor) and lovastatin significantly reduced transcripts for pro-inflammatory cytokines associated with EAE development, i.e., IL-23, IL-17, IFN-γ, TNF-α, and IL-1β and significantly increased Th2 cytokines, i.e., IL-4 and IL-10. These effects were less evident with the use of either drug alone [112]. Lovastatin was also tested in the EAE model together with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, an immunomodulating agent that activates AMP-activated protein kinase [120]. Suboptimal doses in combination proved efficacious against the induction of EAE with delayed clinical symptoms and reduced severity and duration of disease [120].

Combination therapy using suboptimal doses of atorvastatin and GA also prevented clinical and histologic manifestation in EAE mice [121]. Secretion of Th1 cytokines was decreased whereas Th2 cytokine secretion was elevated, but not in mice treated with each drug alone at the same doses [121]. Treatment with lower doses of atorvastatin and all-trans retinoic acid (ATRA) had important synergistic benefits, causing the regression of clinical and neuropathological features of EAE mice more effectively than treatment with full doses of either drug alone [109]. In addition, combination treatment reduced the secretion of IL-17 and increased the production of IL-10 more prominently than either drug alone. FoxP3 +

Treg cells were increased only in combination treatment group [109]. Furthermore, combination therapy of suboptimal doses of atorvastatin and rapamycin in EAE decreased Th1 and Th17 cytokines and increased Th2 and Treg cell cytokine secretion [111]. Combination therapy promoted induction of Treg cells and reduced the infiltration of IL-17 cells. The combination therapy was as effective as the optimal dose of either drug alone in preventing EAE and was associated with reduced CNS inflammatory lesions and less demyelination [111].

Clinical effects of statins in patients with MS

A systematic review by Pihl-Jensen G. et al. of trials until 2015, in RRMS patients, secondary progressive MS (SPMS), clinically isolated syndrome (CIS), and acute optic neuritis (ON), did not show a clear beneficial effect of statins as monotherapy or in combination with IFN-β [122–124]. However, statins as a combination therapy with IFN-β showed decreased C-reactive protein (CRP) levels [122]. Less but not statistically significant EDSS score progression was reported in patients treated with statins [123, 125]. A summary of the main studies that explored the effects of statins in clinical trials with MS patients and relevant data are shown in (Table 4).

One randomized, placebo-controlled trial of statin monotherapy in SPMS showed decreased brain atrophy and disability in the simvastatin-treated group [127]. IL-17 levels did not change at any time point. The same group performed a secondary analysis on these data from the MS-STAT randomized, placebo-controlled trial in SPMS and found a positive effect of simvastatin on frontal lobe function and physical quality-of-life [130]. Another study concluded that simvastatin ameliorated the magnetic resonance imaging (MRI) contrast-enhancing lesions in patients with RRMS [129]. Atorvastatin alone or in combination with IFN-β in an open-label study of RRMS concluded that the addition of atorvastatin improved MRI brain lesions and increased IL-10 production, but did not suppress T cell responses [131]. However, a follow-up trial failed to find a significant effect of atorvastatin on brain atrophy rate [124]. A study using atorvastatin and methylprednisolone for the treatment of MS-relapsing patients showed that the combined treatment group had significantly higher levels of IL-13, IL-35, and IL-10 in the cerebrospinal fluid than the monotherapy group and significantly lower level of IFN-γ [132]. IL-13 and IL-10 in the combined treatment group positively correlated with EDSS scores [132].

A more recent randomized, double-blind 18-month trial using atorvastatin as add-on therapy to IFN-β treatment or IFN-β monotherapy showed no significant difference in the EDSS scores and imaging lesions between the two groups [126]. Levels of IL-4, IL-10, and TGF-β in the atorvastatin group were significantly higher while levels of TNF-α and lymphocyte proliferation were lower in the atorvastatin-treated patients. Another randomized, placebo-controlled study comparing atorvastatin or placebo add-on therapy to IFN-β for 24 months showed no difference in brain atrophy, EDSS, relapse rate, MS functional composite score, or cognitive changes between groups [124]. In a double-blind study that followed up patients for 2 years, simvastatin elevated the frontal assessment battery score, as well as the mean physical component score [130].

In a randomized trial of RRMS of 18-month duration, atorvastatin in combination with IFN-β significantly decreased IL-17 and TNF-α levels and significantly increased IL-4, IL-10, and TGF-β levels but did not change the clinical outcome of RRMS [126].

Furthermore, the multi-centre Swiss atorvastatin and interferon beta-1b trial in multiple sclerosis (SWABIMS) investigated the effects of atorvastatin and IFN-β in 80 naïve-to-treatment RRMS patients [133] and did not find any beneficial effect of the combined treatment on RRMS compared to IFNB-1b monotherapy over a period of 12 months [134]. Similar results were obtained by another study [135].

In vitro, simvastatin induced IFN-γ, IL-4, and IL-27 production in monocytes and inhibited IL-17 transcription and secretion in CD4+ T cells (Th17 cells). Simvastatin also directly inhibited the expression of RORC, and this effect was reversed by mevalonic acid [88]. Simvastatin inhibited Th17 cell differentiation and IL-17A, IL-17F, IL-21, and IL-22 secretion of in vitro differentiated naive CD4+ T cells from RRMS patients. Less prominent effect was exerted on the cells from healthy controls, as it inhibited only IL-17F secretion. The inhibition of Th17 cell differentiation was mediated via inhibition of IFN regulatory factor 4 (IRF4) expression, which was identified as a key transcription factor for human Th17 cell differentiation using both IRF4 gene knockdown and overexpression experiments [128]. Simvastatin also inhibited IL-1β, IL-23, TGF-β, IL-21, and IL-12p70 and induced IL-27 secretion from DCs of patients with RRMS patients, providing an inhibitory cytokine milieu for Th17 and Th1-cell differentiation [66].

In conclusion, statins have immunoregulatory and anti-inflammatory properties and studies in EAE showed a reduction of IL-17 levels, using statins alone or as a combination treatment. Combination treatments appeared beneficial, giving a greater reduction in IL-17, as well as induction of Th2 cells, than monotherapy treatment. Similarly, studies in MS patients showed a reduction in Th17, but whether combination of statins with standard treatment may be of benefit remains unclear.

Table 4 A summary of major studies investigating the role of statins in the regulation of Th17 responses in patients with MS.

Ref	Year	Country of origin	Type of study	Patients	Statin	Doses	Duration of treatment	TH-17	IL-17	TH-1	TH-2
[126]	2016	Iran	In vivo	RRMS (45 patients control, 50 atorvastatin-treated patients)	Atorvastatin	40 mg/day	18 months	↓	↓	↓	↑
[127]	2014	UK	Double-blind, controlled trial	Progressive multiple sclerosis-140 participants. (70 placebo,70 simvastatin)	Simvastatin	80 mg/day	24 months	NSD	NSD	NSD	NSD
[66]	2013	USA	In vitro	RRMS (31 patients)	Simvastatin	(10 mM)	24 h	↓	↓	↓	Not tested
[128]	2011	USA	In vitro	RRMS (40 patients)	Simvastatin	(10 mM)	2 h, 48 h, or 12 days	↓	↓	Not tested	Not tested
[88]	2008	USA	In vitro	RRMS (34 patients	Simvastatin	(10 mM)	24 h	↓	↓	Not tested	Not tested
[129]	2004	USA	In vivo	RRMS (30 patients)	Simvastatin	80 mg/day	6 months	Not tested	Not tested	Not tested	Not tested

Ref	Tregs	TNF-α	IL-4	IL-10	IL-21	IL-22	TGF-β	IFN-γ	Clinical findings	Histological results
[126]	Not tested	↓	↑	↑	Not tested	Not tested	↑	↓	NSD in EDSS score and the number of gadolinium-enhancing lesions between groups	Not measured
[127]	NSD	Not tested	NSD	NSD	Not tested	Not tested	Not tested	NSD	significant difference between simvastatin and placebo group in EDSS score	↓ mean annualized atrophy rate (↓ 43%) in the simvastatin group
[66]	Not tested	Not tested	NSD	Not tested	↓	Not tested	↓	↓	Not measured	Not measured
[128]	Not tested	Not tested	Not tested	Not tested	↓	↓	Not tested	Not tested	Not measured	Not measured
[88]	Not tested	Not tested	↑ in monocytes	NSD	Not tested	Not tested	Not tested	↑ in monocytes	Not measured	Not measured
[129]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	↓ 44% mean number of gadolinium-enhancing lesions	Not measured

NSD no significant difference

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

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