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Review

The potential therapeutic role of statins in central nervous system autoimmune disorders

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Abstract. 3-Hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase inhibitors, 'statins' are widely used oral cholesterol-lowering drugs. Statins competitively inhibit HMG-CoA reductase, the enzyme that catalyzes conversion of HMG-CoA to L-mevalonate, a key intermediate in cholesterol synthesis. Certain metabolites of mevalonate are also involved in posttranslational modification of specific proteins involved in cell proliferation and differentiation. Thus, statins have important biologic effects that may be independent of their cholesterol-reducing properties. Recent studies indicate that statins have antiinflammatory and neuroprotective properties which may be beneficial in the treatment of multiple sclerosis as well as other central nervous system (CNS) neurodegenerative diseases. This article will outline current experimental evidence that may suggest potential clinical benefits for patients with CNS autoimmune disorders. Ultimately, clinical trials will have to determine the safety and efficacy of statins in this patient population.

Key words. Multiple sclerosis; experimental autoimmune encephalomyelitis; HMG CoA reductase inhibitors; statins.

Statins

3-Hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase inhibitors, 'statins' are currently the most effective agents available for the treatment of high blood cholesterol levels. Statins can reduce blood tricglyceride levels by 10–30%, total cholesterol levels by 15–40% and low-density lipoprotein (LDL) cholesterol levels by 20–60% [1]. There is overwhelming evidence that statins decrease cardiovascular-related morbidity and mortality in individuals with and without coronary artery disease [1–9]. Statins are administered orally, and they achieve their cholesterol-lowering effects through the inhibition of HMG-CoA reductase (fig. 1). This enzyme catalyzes the conversion of HMG-CoA to L-mevalonate (fig. 1), and through its inhibition, statins prevent several biological activities downstream of L-mevalonate [10].

Since the approval of lovastatin by the Food and Drug Administration (FDA) in 1987, several statins have been demonstrated to be safe and well tolerated [1, 2, 7]. Six different statins are currently available in the United States, including 'natural' statins (or fermentation-derived statins) and 'synthetic' statins. The chemical structure of the natural statins lovastatin, mevastatin, pravastatin and simvastatin is very similar. Alteration of structural characteristics has led to the development of more potent, synthetic statins, including atorvastatin and cerivastatin. The efficacy of several second-generation statins in patients with hypercholesterolemia is currently being evaluated, and has led recently to the approval of rosuvastatin (Crestor) in several countries. The recommended total daily dose of all natural and synthetic statins lies within a similar range (table 1). While oral adminis-

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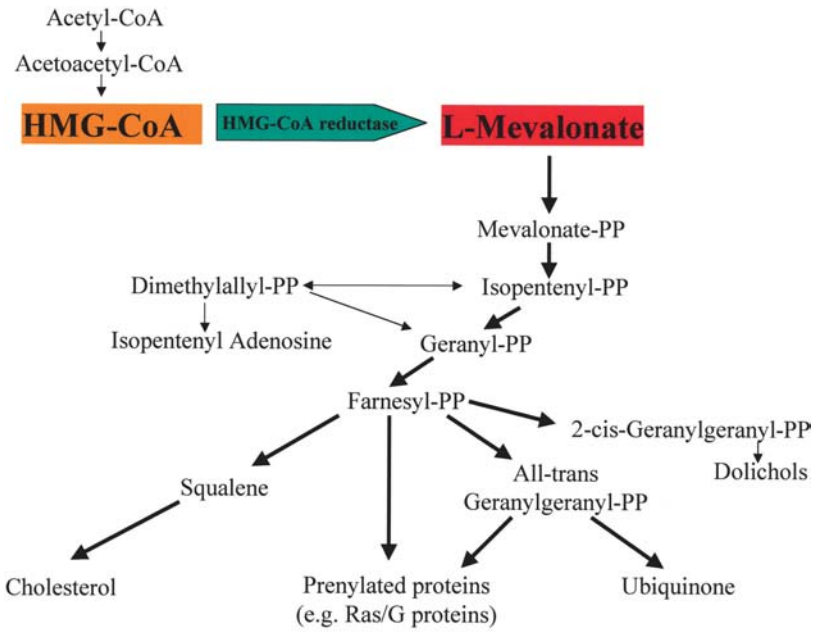

Figure 1. The conversion of HMG-CoA to L-mevalonate is catalyzed by the enzyme HMG-CoA reductase. Through inhibition of HMG-CoA reductase, statins prevent numerous biological activities downstream of L-mevalonate. Isoprenoid intermediates serve as lipid attachments for various intracellular signaling molecules. The inhibition of GTP-binding proteins Rab, Rac, Rap, Ras and Rho may affect cell proliferation, differentiation and migration.

Table 1.

Generic name	Brand name	Daily dose (Range)
Atorvastatin	Lipitor	10–80 mg
Fluvastatin	Lecol	20–80 mg
Lovastatin	Mevacor	10–80 mg
Pravastatin	Pravachol	10–40 mg
Simvastatin	Zocor	10–80 mg

tration results in rapid absorption of all statins, the extent of oral absorption varies between 30 and 98%, depending on the agent [11–13]. Infrequent side effects of statins currently available include a dose-dependent elevation of hepatic transaminases (~2%) [14, 15], and a dose-independent myopathy (~0.1–0.5%) [14]. Cerivastatin was removed from the market after data confirmed that at high doses it caused myopathies and breakdown of muscle tissue ('rhabdomyolysis') significantly more frequently than other statins [16, 17]. A minimal risk of developing a mostly reversible, axonal sensory neuropathy following statin use has been suggested by some studies [18, 19].

The pleiotropic effects of statins can be explained by the metabolic pathway of L-mevalonate: while some of the metabolites are directly involved in cholesterol synthesis, others impact numerous biological systems (fig. 1) [20]. Through inhibition of L-mevalonate catabolism, statins interfere with the synthesis of isoprenoid intermediates of the cholesterol biosynthesis pathway, including farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) (fig. 1). Prenylation by FPP and GGPP is required for the assembly, subcellular localization, intracellular trafficking, and assembly of cell membranes [21–23]. In addition, the modification of small Ras-like proteins, including Rab, Rac, Rap, Ras and Rho, is dependent on FFP and GGPP. Ras-like proteins bind GTP, and they may play an important role in mediating certain effects of statins, including cell proliferation, differentiation and migration [24–26]. Independent of effects downstream of L-mevalonate synthesis, statins may have direct effects on the activity of certain cell surface molecules, including lymphocyte function-associated antigen (LFA)-1, CD40, CD80 and CD86 [24, 26–28].

In 1995, the potential impact of statins as immunomodulators surfaced when it was reported that cardiac transplant patients treated with pravastatin had a decreased incidence of hemodynamically significant rejection episodes, and decreased mortality [29]. Interestingly, these outcomes did not correlate with cholesterol reduction [29]. This landmark observation was followed by a number of studies that have identified immunoregulatory and antiinflammatory properties of statins. As a result of these observations, a number of clinical trials are underway to evaluate the efficacy of statins in several inflammatory central nervous system (CNS) disorders, including multiple sclerosis (MS).

Statins in autoimmune disorders of the CNS

Several immunoregulatory properties of statins have recently been reported, suggesting that these agents may be beneficial in the treatment of MS. MS is a chronic inflammatory demyelinating disease of the CNS that causes relapsing and progressive neurological impairment [30, 31]. MS is considered an autoimmune disease, based on the demonstration of cellular and humoral immune responses [32–34], the genetic association of MS with human leukocyte antigen (HLA) haplotypes [35–38] and the clinical response to immunomodulatory therapies [39, 40].

Several agents are currently approved for the treatment of MS, including three interferon-β (IFN-β) preparations (Avonex, Betaseron and Rebif), glatiramer acetate (Copaxone) and mitoxantrone (Novantrone). All of these agents have significant limitations: they are given parenterally, are only partially effective and may have significant side effects [40].

Experimental autoimmune ('allergic') encephalomyelitis (EAE), the archetypal model for MS [41], has provided much of our knowledge regarding potential mechanisms of statins in CNS autoimmune disorders [25, 27, 42–44]. EAE is induced by immunization with a CNS autoantigen, or through passive transfer of CNS autoantigen-specific, activated $CD4^+$ T_H1 cells [41]. Clinically, acute fulminate, chronic or relapsing-remitting phenotypes can be observed, depending on the strain of the recipient animal [41]. Pathologically, there is CNS demyelination and inflammation.

It was recently demonstrated that orally administered atorvastatin treatment can either prevent or reverse chronic and relapsing EAE [27, 42, 43]. In these studies, atorvastatin doses of 0.1, 1.0 and 10 mg per kilogram of body weight per day significantly suppressed EAE. As a reminder, the maximum dose of atorvastatin approved for patients with hypercholesterolemia is 80 mg per day, translating to approximately 1.1 mg per kilogram of body weight per day. Interestingly, only a minority of experimental animals developed clinically very mild EAE when treatment was discontinued, suggesting a sustained treatment effect of this agent [27]. While these results are very promising, one has to keep in mind that EAE is a model for MS and that the results of such studies in rodents do not always translate to efficient therapies in humans.

There is now overwhelming evidence that statins may affect several steps in the pathogenic cascade of MS: T cell activation, leukocyte entry into the CNS and suppression of several inflammatory mediators (summarized in table 2).

MHC class II and costimulatory signals required for T cell activation are reduced by statins

Activation of $CD4^+$ T cells is required for their efflux from the blood into the CNS parenchyma [45], regardless of their CNS autoantigen specificity [46, 47]. Activation of $CD4^+$ T cells requires recognition of linear peptide antigen (Ag) bound in the Ag-binding groove of major histocompatibility complex (MHC) class II molecules. This interaction is commonly referred two as 'signal 1' (fig. 2). Interferon-γ (IFNγ)-inducible MHC class II expression in 'nonprofessional' antigen-presenting cells (APCs), and constitutive MHC class II expression in 'professional' APCs is regulated by the MHC class II transactivator (CIITA), a transcriptional coactivator [48, 49]. CIITA expression is controlled in a tissue-specific manner at the level of transcription by differential activation of multiple nonhomologous promoters [50]. Originally, it was observed that two CIITA promoter elements, promoter (p) I (pI) and pIII, direct constitutive CIITA expression in dendritic cells and B cells, respectively [50]. Another CIITA promoter, pIV, which contains an IFNγ-activating sequence (GAS) site, E-box and IRF element [50, 51], directs IFNγ-inducible CIITA expression in nonprofessional APCs [50, 52–54]. It was recently shown that statins inhibited IFNγ-inducible MHC class II expression on different nonprofessional APCs [55]. Ator-

Table 2. Evidence for the potential role of statins in multiple sclerosis.

Mechanistic studies	Animal experiments	Clinical trials	Planned trials
MHC class II and costimulatory signals required for T cell activation are reduced [27, 55] Migration of T cells into the CNS is decreased by effects on chemokines and MMPs [28, 66, 77, 80–81] Expression of inflammatory mediators, including T_H1 cytokines by T lymphocytes, is reduced [27] Expression of inflammatory mediators in the CNS, including iNOS, TNF-α, IL-1β, IL-6, is reduced [42, 90]	reduced disease severity in experimental autoimmune encephalomyelitis, an animal model for MS [25, 27, 42–44, 90]	a single-arm (open-label) trial of 80 mg simvastatin (Zocor) in 30 patients with relapsing-remitting MS demonstrated a 43% reduction in number of new gadolinium-enhancing lesions and a 41% reduction in volume of new enhancing lesions [92]	a multicenter placebo-controlled trial to test whether atorvastatin treatment of patients that have experienced their first demyelinating attack (a 'clinically isolared syndrome') will reduce the risk of developing MS activity

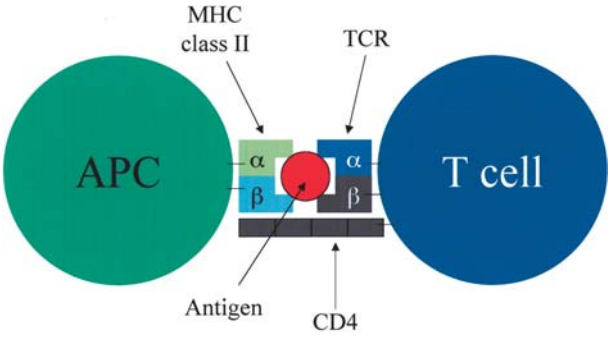

Figure 2. T lymphocytes that express the accessory surface molecule CD4 are capable of recognizing and binding linear peptide antigen (Ag) with their T cell receptor (TCR) in the context of the MHC class II molecules on antigen presenting cells (APC).

vastatin furthermore inhibited IFNγ-inducible MHC class II expression on microglia, an effect that was reversed by L-mevalonate [27]. Atorvastatin almost completely prevented IFNγ-inducible CIITA transcription directed by each CIITA promoter [27]. The effects of statins on MHC class II-restricted Ag presentation were reversed with L-mevalonate [27].

Apart from the binding of the T cell receptor (TCR) to Ag bound in the Ag-binding groove of the MHC class II molecule, a second, costimulatory signal is required for activation of $CD4^+$ T cells [56, 57]. Costimulatory cell-cell interactions are commonly referred to as signal 2. Once activated, T cells express CD40 ligand on their cell surface (CD40L), which engages to the costimulatory molecule CD40 on APCs (fig. 3). Cross-linking of CD40 and CD40L directs expression of other costimulatory molecules on APCs, namely B7-1 (CD80) and B7-2 (CD86), which bind to CD28 on activated T cells (fig. 3). A recent report demonstrated that atorvastatin inhibited IFNγ-inducible expression of several costimulatory molecules, including CD40, CD80 (B7-1) and CD86 (B7-2) on APCs [27].

Migration of T cells into the CNS is decreased by statins

The efflux of T cells from the blood into the CNS is a multistep event involving chemoattraction to the site of inflammation, cell adhesion to the endothelial wall and proteolytic degradation of the basal lamina and extracellular matrix (ECM) [58, 59]. Chemokines (chemoactive cytokines) facilitate the migration of leukocytes into the brain and spinal cord [60, 61]. They are expressed by endothelial cells, microglia, oligodendrocytes and astrocytes, which are cell types relevant in EAE and MS pathogenesis [60, 62–64]. Monocyte chemotactic protein (MCP)-1 is a chemokine that is expressed late in the acute and relapsing phases of EAE, and that is correlated with clinical disease severity [65]. Lovastatin and simvastatin reduced the production of the chemokine MCP-1 in a dose-dependent manner in peripheral blood mononuclear cells and in human endothelial cells [66].

Lymphocyte function-associated antigen (LFA-1) is an integrin on leukocytes which binds to intracellular cell adhesion molecule (ICAM)-1 on endothelial cells. This cell-cell interactions plays a critical role in the adhesion

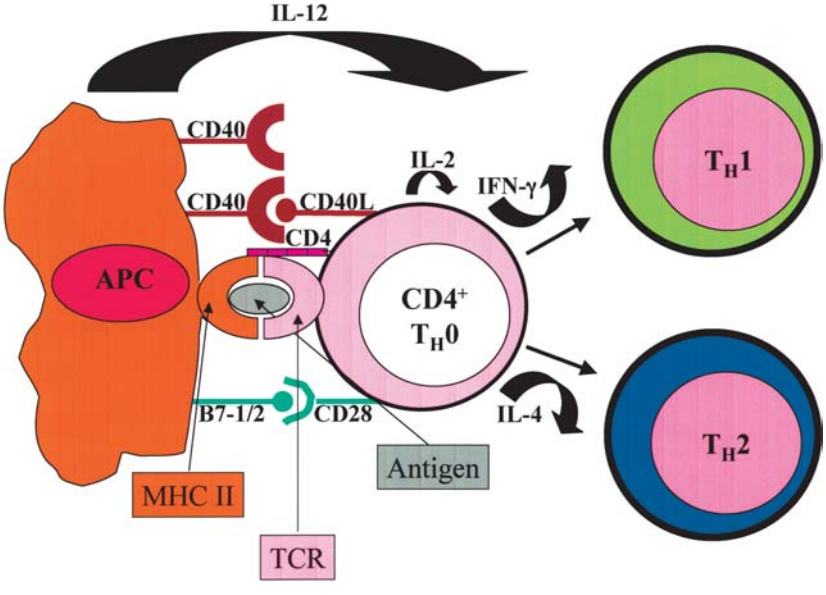

Figure 3. Statins reduce the surface expression of MHC class II molecules on various cell types. Furthermore, statins reduce IFNγ-inducible expression of the costimulatory molecules CD40, CD80 (B7-1) and CD86 (B7-2) on APCs. Statins also promote differentiation of T_H0 cells into T_H2 cells, suppress the secretion of the T_H1 cytokines IL-2 and IFNγ and induce the secretion of the T_H2 cytokines IL-4, IL-5 and IL-10.

of T cells to brain endothelium [67, 68]. Both molecules have been identified on the surface of inflammatory cells and endothelial cells in perivascular MS lesions [69–76]. It was recently reported that independent of its effect on HMG-CoA reductase, lovastatin binds LFA-1 and directly inhibits LFA-1-ICAM-1-mediated cell adhesion [28, 77].

Once T cells cross the endothelial barriers, they still have to break down other physiological barriers to gain access to brain and spinal cord tissue, namely the basement membrane (basal lamina) of blood vessels and ECM within the CNS. Proteolytic enzymes called matrix metalloproteinases (MMPs) are considered the physiologic mediators of T cell migration through biological membranes [78]. It was also shown that MMPs degrade myelin basic protein (MBP), a potential CNS autoantigen in MS, into encephalitogenic peptide fragments [79]. The reduction of leukocyte-MMP-9 secretion by statins was recently demonstrated by two separate groups [80, 81].

Expression of inflammatory mediators by T lymphocytes is reduced by statins

Based on their cytokine profile, $CD4^+$ T cells are currently being categorized into T_H1 and T_H2 subsets [82]. EAE is primarily mediated by $CD4^+$ T_H1 cells, which secrete the proinflammatory cytokines interleukin (IL)-2, IFNγ and TNFα. Specifically, T_H1 T cells are strongly associated with clinical disease severity [83], and it has been demonstrated in several species that T_H1 cytokines are upregulated during acute disease and relapses, but not during clinical remission [84, 85]. Furthermore, IFNγ is considered a key player in the pathogenesis of MS [86]. In contrast, the T_H2 cytokines IL-4, IL-5, IL-10 and IL-13 are thought to have downregulatory properties in EAE. It was recently reported that atorvastatin induces the secretion of the T_H2 cytokines IL-4, IL-5, IL-10, and transforming growth factor (TGF)-β [27]. In addition, atorvastatin promoted differentiation of T_H0 cells into T_H2 cells [27].

Expression of inflammatory mediators in the CNS is reduced by statins

Nitric oxide (NO) is a vascular and neuronal messenger abundantly present in the CNS. NO is the product of NO synthase (NOS), an enzyme that is expressed by astrocytes and abundantly present in MS lesions [87]. The inducible isoform of NOS (iNOS) can be activated by various proinflammatory cytokines, including IFNγ and TNFα [88, 89]. A reduced expression of iNOS and production of NO by rat primary astrocytes after treatment with lovastatin was recently reported [90]. In addition, it was shown that lovastatin administration reduced the expression of iNOS, IFNγ and TNFα in the CNS of EAE animals, while concomitantly improving the clinical signs of EAE [42].

Clinical considerations in human CNS autoimmune disease

Despite these intriguing reports from animal studies, there is only preliminary evidence that statins may be beneficial in MS patients. Retrospective analysis of clinical data to evaluate whether statin treatment of MS patients with hypercholesterolemia had any clinical benefit with regard to their CNS autoimmune disease is challenging for several reasons: (i) the mean age of individuals taking statins for cholesterol reduction or prevention of cardiovascular disease is approximately 57, while the mean age of MS onset is 32 [91]; (ii) several statins with different potency are currently available for treatment of hypercholesterolemia; (iii) as suggested by animal studies, the immunomodulatory effects of statins may be dose dependent [27, 55]. Thus, a lower dose used for cholesterol reduction may not provide immunomodulation.

Ultimately, prospective clinical trials will be necessary to evaluate the clinical benefits of statins during different disease phases of MS. Considering our current knowledge of the mechanisms of action of statins, they may have their most potential benefit in the early inflammatory phase of MS, rather than during the latter neurodegenerative phase.

Prospective, placebo-controlled, randomized trials will ultimately be necessary to validate how efficacious statins may be in MS therapy. However, the results of a small open-label trial single-arm study that tested the efficacy of simvastatin in patients with clinically definite relapsing-remitting MS were recently made public, and suggest a beneficial effect [92]. During a pretreatment period of 3 months, 30 MS patients with relapsing-remitting disease received monthly brain magnetic resonance imaging (MRI) studies of the brain, followed by 6 months of treatment with 80 mg of simvastatin daily, the highest FDA-approved dose. The patients also received brain MRIs at months 4, 5 and 6 of simvastatin treatment. Analysis of pre- and post-treatment MRI data indicated a decrease of ~45% in the mean number and volume of gadolinium (Gd)-enhancing lesions in simvastatin-treated subjects. Due to the design of this study, the results have to be interpreted with caution, but suggest that treatment with a high dose of simvastatin is safe and partially effective for the treatment of relapsing remitting MS. A multicenter placebo-controlled trial that anticipates enrolling 152 patients is planned to start in late 2003, and will examine whether treatment with 80 mg of atorvastatin in patients that have experienced their first demyelinating attack (a 'clinically isolated syndrome') will reduce the risk of developing MS activity.

Statins have different mechanisms of action than immunomodulatory agents currently approved for the treatment of MS. Thus, statins may not only be useful as monotherapy, but also in combination with established therapies. For example, the principal mechanism of action of glatiramer acetate (Copaxone) seems to be the induction of T_H2 cells that downregulate T cell responses to CNS autoantigens through 'bystander suppression' [93]. As statins also induce a T_H2 bias and can promote naive T_H0 cells to differentiate into T_H2 cells, statin therapy may facilitate induction of T_H2 glatiramer acetate-reactive T cells or enhance their activity. A combination therapy of statins with IFN-β may provide an additive effect on MMP-9 suppression, one of the principal effects of IFN-β in MS therapy [94–96].

An important aspect of combination therapies is the occurrence of adverse effects. Given the side-effect profile of individual agents, there is a potential danger of their addition, synergism and potentiation when used in combination. Therefore, the overlapping adverse effects, such as potential hepatotoxicity of statins and IFN-β, will require careful evaluation of this particular combination in clinical trials.

Conclusions

In recent years there have been major advances in understanding how cellular and humoral immune responses contribute to the pathogenesis of CNS autoimmune diseases, including MS. These discoveries have led to new therapies, all of which are administered parenterally and have significant side effects. There is now emerging evidence that statins have beneficial clinical effects in MS, at least some of which may be independent of their cholesterol-lowering properties. Recent observations from animal studies and in vitro experiments have demonstrated that statins have numerous immunomodulatory properties, including the suppression of cell adhesion, migration and activation. Larger-scale, randomized, double-blind trials are needed to evaluate the role of statins as a treatment of autoimmune CNS diseases.

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