Simvastatin Inhibits IL-17 Secretion by Targeting Multiple IL-17-Regulatory Cytokines and by Inhibiting the Expression of IL-17 Transcription Factor RORC in CD4+ Lymphocytes

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Simvastatin Inhibits IL-17 Secretion by Targeting Multiple IL-17-Regulatory Cytokines and by Inhibiting the Expression of IL-17 Transcription Factor RORC in CD4+ Lymphocytes

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Statins, extensively used as cholesterol-lowering agents, have recently been identified as immunomodulatory agents. This study investigated the statins’ mechanisms that target the autoimmune response in humans, and evaluated their therapeutic potential in multiple sclerosis. Our results demonstrated statin-mediated increases in suppressor of cytokine secretion (SOCS) 3 and suppressor of cytokine secretion 7, which negatively regulate the STAT/JAK signal transduction pathway and IL-6 and IL-23 gene expression in monocytes. Simvastatin also induced IFN-γ, IL-4, and IL-27 production in monocytes, which together inhibited IL-17 transcription and secretion in CD4+ T cells. IL-17-producing CD4+ cells, referred to as Th17 cells, have recently been found to play a central role in the development of autoimmune diseases. Furthermore, simvastatin directly inhibited the expression of retinoic acid-related orphan nuclear hormone receptor C, a transcription factor that controls IL-17 production in CD4+ T cells. This effect was reversed by mevalonic acid, a downstream metabolite of 3-hydroxy-3-methylglutaryl CoA reductase, confirming that simvastatin’s specific effect is through the inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase. These results provide evidence for the novel immunomodulatory mechanisms of statins, which selectively target the regulation of cytokine transcription involved in the development of the human autoimmune response. Based on the described immunomodulatory mechanisms, good safety profile and oral bioavailability, statins represent a promising therapeutic approach for multiple sclerosis and other chronic inflammatory diseases. The Journal of Immunology, 2008, 180: 6988–6996.

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2 Abbreviations used in this paper: MS, multiple sclerosis; RR, relapsing remitting; GA, glatiramer acetate; IFN, interferon; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor; IL, interleukin; TGF, transforming growth factor; PGE2, prostaglandin E2; TLR, toll-like receptor; CD, dendritic cell; RORC, retinoic acid-related orphan nuclear hormone receptor C; HMG, 3-hydroxy-3-methylglutaryl.

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JAK1 and JAK2, and phosphorylation-dependent activation of STAT3 in response to IL-6. It targets the IL-6 receptor signaling complex gp130 for proteosomal degradation. Similarly, SOCS3 inhibits the STAT3 transduction pathways for IL-23 (9). Therefore, SOCS3 induction by statins may have an inhibitory effect on multiple inflammatory cytokine signal transduction pathways that mediate the autoimmune response.

Th17 is a recently identified subset of CD4+ T cells that is critical for the development of autoimmune diseases. Th17 cells produce IL-17A (also known as IL-17), IL-17F, TNF-α, GM-CSF, and IL-6, in response to TGFβ and IL-6 in mice. The expansion of this T cell subset is mediated by IL-23 and inhibited by IL-27 (10), and its relation to Th1 and Th2 cell lineages is currently investigated. Recent studies have revealed that great differences exist between mice and human Th17 cell lineage differentiation. Several groups have reported that TGFβ does not stimulate, but instead inhibits the human Th17 differentiation (11, 12, 13). As reported by Chen et al. (13), IL-23 induces Th17 differentiation in humans, while it has been established that it does not induce Th17 differentiation in naive animal cells due to the lack of IL23R expression. In humans, IL-23 up-regulates its own receptor on T cells and increases retinoic acid-related orphan nuclear hormone receptor C (RORC) and IL-17 expression (13). IL-21 has recently been identified in animal systems as a cytokine that induces RORγt expression and IL-17 production through the induction of IL23R expression. IL-21 is secreted by Th17 cells and in an autocrine fashion may further enhance IL-17 secretion via induction of STAT3 phosphorylation (14).

IL-17 mRNA is elevated in the active MS brain lesions, as reported by Lock et al. (15) and detected in our unpublished results (Montes et al., submitted for publication). IL-17 gene expression is significantly increased in mononuclear cells derived from the blood and from cerebrospinal fluid of MS patients in comparison to healthy controls (HCs), and numbers of IL-17-expressing blood mononuclear cells are higher during MS exacerbations than in clinically silent periods (16). Because IL-17 has been detected in the target organs of patients with other autoimmune diseases, including rheumatoid arthritis, psoriasis and autoimmune uveitis, it is conceivable that it plays a role in human autoimmune processes (17) and represents a selective target for the treatment of autoimmune diseases.

Materials and Methods

Study subjects

Thirty-four patients with confirmed diagnoses of RR MS and 10 age-, sex-, and race-matched HCs were enrolled in the study upon signing an institutional review board-approved informed consent form. The inclusion criteria for the MS patients consisted of a confirmed diagnosis of RR MS according to McDonald’s diagnostic criteria (18), age 18–55, and an extended disability status score of 1.5–5.5. The exclusion criteria were history of hyperlipidemia treated with statins and immunomodulatory or immunosuppressive medications at the time of blood sample collection. The treatment-free period was at least 6 wk for IV methylprednisolone, 3 mo for IFN-β, 12 mo for IFX, GA, and 6 mo for azathioprine, methotrexate, mitoxantrone, cyclophosphamide, and other immunosuppressive medications.

Gene arrays

PBMCs were separated using Lymphocyte Separation Medium (BioWhittaker) from blood samples of 10 patients with RR MS. Five × 106 cells per condition were stained with plate immobilized antiCD3 (1 μg/ml) and αCD28 (5 μg/ml) mAb (BD Pharmingen) in the absence of or presence of simvastatin (1 μM) (Calbiochem) for 24 h. Total RNA was isolated by Qiagen. cDNA was prepared by reverse transcription and hybridized to a positively charged membrane containing the arrayed DNA. Gene expression of 102 inflammatory genes was measured using GEA Th1−2−3 expression system (SuperArray). Arrays contained cDNA probes including control sequences (blank, Puc-18, β-actin, GAPDH, Cyclophilin-1) in four replicates. Gene expression was measured using Gene Pix Pro 5.0 software (Axon Instruments). The background-subtracted signals were used to calculate the expression level for each gene. The median expression value of all genes on the membrane was used for the normalization of each individual gene on the membrane. Each value was normalized against the median of that gene in all membranes. The genes with <1.2-fold change between untreated and simvastatin-treated cultures and genes with raw expression levels below 600 in 5 out of 20 membranes were filtered out from statistical analysis, which was performed by paired t test comparing each of the normalized values of the simvastatin-treated samples to those of the nontreated samples. Gene tree clustering was conducted using Gene Spring software (Agilent Technologies).

Cell cultures

CD14+ monocytes were isolated from MS patients (n = 10) and age-, sex-, and race-matched HCs (n = 10) by EasySep Negative Selection Monocyte Enrichment Kit (StemCell Technologies) and CD4+ T cells by MACS CD4+ T Cell Isolation Kit (Miltenyi Biotec). Cell purity was confirmed by flow cytometry (~95%). Five × 106 monocytes per condition were pre-treated with simvastatin (10 μM) for 24 h in serum-free media (Opti MEM, Life Technologies), and then stimulated with LPS (1 μg/ml) (Sigma-Alrich) for 2 h before RNA extraction and for 48 h before supernatants collection. Five × 106 CD4+ T cells per condition were pre-treated as above, and then stimulated with plate immobilized αCD3 (1 μg/ml) and αCD28 (5 μg/ml) mAb for 4 h before RNA extraction and for 48 h before supernatant collection. In some experiments, 5 × 106 CD4+ purified T cells per condition from the MS patients were pretreated in the presence or absence of mevalonic acid (Sigma-Alrich) (100 μM) for 24 h before stimulation.

CD45RA cells were separated by MACS CD45RA+ T Cell Isolation Kit (Miltenyi Biotec) and cultured for 24 h in the absence or presence of simvastatin and indicated cytokines (IL-6, IL-23, IFN-γ, IL-4, IL-27 at 50 ng/ml) or cytokine blocking Abs (at concentration 10 μg/ml for 24 h. Cells were then stimulated with plate immobilized αCD3 and αCD28 mAb. After 5 days, IL-2 (20 U/ml) was added and the cultures were expanded for an additional 7 days. At day 12, cells were harvested for quantitative RT-PCR (qRT-PCR), while supernatants were frozen for the measurement of secreted cytokines by ELISA.

qRT-PCR

Total RNA was isolated using a RNeasy kit, and cDNA was synthesized using a High Capacity cDNA Archive Kit (Applied Biosystems). The primers for SOCS1, SOCS3, SOCS7, IL-23A, IL-6, IFN-γ, IL-27, IL-4, IL-17A, IL-21, and RORC were purchased from Applied Biosystems and gene expression was measured by qRT-PCR using Taqman Gene Expression Assays (Applied Biosystems). Results are expressed for each subject as relative gene expression normalized for the 18S mRNA expression.

ELISA

Supernatants from the cell cultures were collected and stored at −80°C until the cytokine measurements by ELISA. IL-17 was measured using a Human IL-17A ELISA Conjugation Kit (Antigenix America). IL-27 using an ELISA kit purchased from R&D Systems. IL-2, IFN-γ, IL-4, IL-6, IL-10, IL-12p40, IL-12p70 (BD Pharmingen), and IL-23 (eBioscience) were measured in duplicate by ELISA following the manufacturer’s recommendation. Results are expressed for each subject as cytokine concentration in pg/ml.

Western blotting

Human negatively selected CD14+ monocytes were plated at 3 × 106 cells per condition in the absence or presence of simvastatin (10 μM) for 24 h in serum-free media, and then stimulated with LPS (1 μg/ml) for 2 h before protein extraction. Proteins from cell lysates were denatured in SDS, resolved by 7.5% SDS-PAGE for STAT detection and by 12.5% SDS-PAGE for STAT3 detection, and transferred on polyvinylidene difluoride membranes prewetted by 100% methanol. Nonspecific binding was blocked for 30 min in the blocking buffer (10 mM Tris (pH 7.5), 150 mM NaCl, 2% FishGelatin, 1% OVA in double distilled water) and incubated overnight with the mouse anti-human Ab for SOCS3, Tubulin (Santa Cruz Biotechnology), and STAT3 (Cell Signaling Technology), and the rabbit anti-phospho-Thr357 of for STAT1, pSTAT1, and pSTAT3 (Cell Signaling Technology). Blots were washed with TBST Tween 20, and then incubated with IRDye 680 conjugated anti-rabbit IgG or anti-mouse IgG secondary Ab (Li-Cor Biosciences) for 1 h. The blots were washed again and protein
bands detected with Odyssey Infrared Imaging System (Li-Cor Biosciences). The intensity of the visualized bands was quantified by Odyssey 2.0 software (Li-Cor Biosciences).

Statistics

A linear regression model in the R package was used to investigate the relationship between SOCS3/SOCS7 and IL-6/IL-23 gene expression. A negative coefficient with \( p \leq 0.05 \) was considered to indicate a change in gene expression.

The statistical analysis for the qRT-PCR and ELISA results was performed by Wilcoxon matched pairs test analysis (GraphPad InStat), followed by a Benjamini and Hochberg correction for multiple comparisons. Adjusted \( p \) values \( <0.05 \) were considered significant.

Results

Simvastatin changes the expression of multiple inflammatory genes in activated PBMCs, most significantly SOCS3 and SOCS7

Our results, and the studies of others, indicate that statins have pleiotropic immunomodulatory effects that include inhibition of Ag presentation, T cell activation and differentiation, and mononuclear cell recruitment into the CNS (19). To simultaneously capture simvastatin-induced changes in the expression of multiple genes involved in the human inflammatory response, and to establish simvastatin’s operative mechanism of action targeting PBMCs in patients with RR MS, we used Th1–2–3 cDNA arrays (SuperArrays). The arrays contained 102 specific cytokines, chemokines, and their receptors; activation-, costimulatory-, and adhesion-molecules; transcription factors; apoptotic molecules; and negative and positive control probes. Because our previous results (19) and a study by Neuhaus et al. (20) reported statins’ anti-proliferative effect only in activated PBMCs, we tested simvastatin’s effect on immune response gene expression in PBMCs stimulated with plate immobilized oCD3 and oCD28 mAbs in the absence or presence of simvastatin (1 \( \mu \)M) in serum-free media for 24 h. Statistical analysis was performed by a paired \( t \) test comparing each of the normalized values of the simvastatin-treated samples to those of the non-treated samples. Asterisk denotes genes with statistically different expression in simvastatin-treated vs untreated samples.

**FIGURE 1.** Simvastatin induces changes in the expression of multiple genes. The gene tree presents an average result for all genes with at least a \( >2 \)-fold change in the simvastatin-treated vs untreated samples. Inflammatory gene expression profiling was performed using a GEA Th1–2–3 expression system. Arrays contained 102 cDNA probes for immune response genes, including control sequences. The PBMCs were derived from 10 patients with RR MS. Gene expression was measured in \( 5 \times 10^6 \) cells stimulated with plate-immobilized oCD3 and oCD28 mAbs in the absence or presence of simvastatin (1 \( \mu \)M) in serum-free media for 24 h. Statistical analysis was performed by a paired \( t \) test comparing each of the normalized values of the simvastatin-treated samples to those of the non-treated samples. Asterisk denotes genes with statistically different expression in simvastatin-treated vs untreated samples.
CD4⁺ and CD8⁺ lymphocytes as measured by flow cytometry (19). The costimulatory molecule CTLA-4 decreased 8.5-fold and CD27 decreased 2.3-fold in the simvastatin-treated PBMCs compared with the untreated samples. IL-4R expression decreased 4.7-fold. These changes likely reflect decreased cell activation.

**FIGURE 2.** Simvastatin induces an increase in SOCS3 and SOCS7 gene expression in monocytes. A, Simvastatin increases SOCS3 and SOCS7 gene expression, and inhibits IL-23A and IL-6 transcription in monocytes. Five × 10⁶ purified monocytes derived from 10 RR MS patients and 10 matched HCs were cultured in the absence or presence of simvastatin (10 μM) for 24 h in serum-free media, and then stimulated with LPS (1 μg/ml) for 2 h. SOCS3, SOCS7, IL-6, and IL-23A gene expression was measured by qRT-PCR. The results are expressed as relative gene expression, normalized for 18S mRNA expression. B, Simvastatin increases SOCS3 protein expression and inhibits STAT1 and STAT3 phosphorylation in monocytes. Three × 10⁶ negatively selected CD14⁺ monocytes were cultured in the absence or presence of simvastatin (10 μM) for 24 h in serum-free media, and then stimulated with LPS for 2 h before protein extraction. SOCS3 expression and STAT1 and STAT3 phosphorylation were measured by Western blotting. The results show a representative blot from three similar experiments.
qRT-PCR experiments confirmed the significant up-regulation of SOCS3 (p = 0.014) and SOCS7 (p = 0.002) in the PBMCs derived from 10 RR MS patients, data not shown. Our qRT-PCR results confirm the gene array results and provide more accurate quantitative measurements; however, they do not reveal which cell subsets contribute to the gene expression changes induced by simvastatin in PBMCs.

**Simvastatin induces an increase in SOCS3 and SOCS7 mRNA expression, which inhibits IL-23 and IL-6 gene expression in monocytes derived from MS patients and HCs**

To identify which cell subsets are selectively targeted by simvastatin, we performed qRT-PCR for multiple members of the SOCS family (SOCS1, 3, 5, 7) and cytokine genes (IFN-γ, IL-27, IL-6, IL-23, and IL-4) in separated CD14+ monocytes and CD3+ (CD4+ and CD8+) lymphocytes. The simvastatin-mediated increase in SOCS3 and SOCS7 gene expression is present in monocytes derived from MS patients (p = 0.006 and p = 0.006, respectively, Fig. 2A), and not in CD3+ T cells (data not shown). LPS-stimulated monocytes derived from age-, sex-, and race-matched HCs also exhibited a significant, but less prominent, increase in SOCS3 expression (p = 0.006 in MS patients; p = 0.0118 in HCs). Up-regulation of SOCS7 in HCs was not statistically significant. Study of simvastatin’s effect on cytokine gene expression in monocytes revealed a significant decrease in IL-23 expression in the MS patients (p = 0.006), while the decrease in the HCs did not reach statistical significance (Fig. 2A). IL-6, another cytokine negatively regulated by SOCS3, exhibited decreased expression in simvastatin-treated cultures in both the MS patients and HCs, but the differences were not significant. Measurement of monocyte-secreted IL-6 in simvastatin-treated cultures revealed a significantly decreased IL-6 secretion in both MS patients (p = 0.003) and the HCs (p = 0.012), data not shown.

A linear regression analysis was used to examine the relationship of statin-induced increase in SOCS3 and SOCS7 gene expression with the decreased gene expression of IL-23 and IL-6 in the same cultures. Up-regulation of SOCS3 had a significant interaction with down-regulation of IL-6 and IL-23 in RR MS patients (SOCS3-IL-6: p = 0.004; SOCS3-IL-23: p = 0.007) while the interaction of SOCS7 gene expression with the IL-6 and IL-23 cytokine gene expression was less prominent (SOCS7-IL-6: p = 0.035; SOCS7-IL-23: p = ns). In contrast, the interaction of SOCS3 and SOCS7 gene expression with down-regulation of cytokine gene expression in HCs was not statistically significant.

To examine the mechanisms by which a statin-induced increase in SOCS3 expression affects cytokine transcription in monocytes, we determined the effect of SOCS3 induction on the phosphorylation of the STAT1 and STAT3 proteins. Simvastatin-induced

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**FIGURE 3.** Simvastatin increases IFN-γ, IL-4, and IL-27 production in monocytes. A, Five × 10⁶ purified monocytes derived from 10 RR MS patients and 10 matched HCs were cultured in the absence or presence of simvastatin (10 μM) for 24 h in serum-free media, and then stimulated with LPS for 2 h before RNA extraction. IFN-γ, IL-4, and IL-27 gene expression was measured by qRT-PCR and the results presented as a relative expression to the 18S mRNA. B, After 48 h of monocyte stimulation with LPS in the absence or presence of simvastatin, IFN-γ, IL-4, and IL-27 were measured in the supernatants by ELISA. The results are expressed as cytokine concentration in pg/ml.
SOCS3 expression in monocytes inhibited phosphorylation of STAT3, a key transcription factor involved in the regulation of IL-6 and IL-23 production, as well as the phosphorylation of STAT1 (Fig. 2B). The statins’ inhibition of STAT3 and STAT1 phosphorylation was reversed by mevalonate, a downstream metabolite of HMG-CoA reductase, confirming the simvastatin’s specific effect through the inhibition of HMG-CoA reductase (Fig. 2B).

Simvastatin induces an increase in IFN-γ, IL-4, and IL-27 production in monocytes derived from MS patients and HCs

Further studies on monocytes have identified a significant simvastatin-induced increase in IFN-γ gene expression in MS patients (4.3-fold) and HCs (10.2-fold), Fig. 3A. Similar changes were detected in IFN-γ protein secretion: simvastatin increased IFN-γ secretion in MS patients (6.5-fold) and 1.7-fold in HCs (Fig. 3B). In MS patients, IL-27, a member of the IL-12 family that is induced by IFN-γ, also displayed significantly increased gene expression (1.6-fold, \( p = 0.025 \)) in monocytes upon treatment with simvastatin, while the difference was not statistically significant in HCs. IL-27 secretion was significantly higher in simvastatin-treated monocytes derived from MS patients (\( p = 0.004 \)), while the difference in HCs was not statistically significant, Fig. 3B. IL-4 gene expression was increased by simvastatin in the monocytes derived from both MS patients (2.3-fold) and HCs (1.6-fold). Similar increase in secreted IL-4 cytokine level was detected in MS patients (2.0-fold, \( p = 0.004 \)) while the increase in HCs was not significant (Fig. 3B). It is of note that the gene expression of SOCS1 and SOCS5, which regulate IFN-γ and IL-4 transcription respectively, was not significantly changed by the treatment of monocytes with simvastatin (data not shown).

The inhibitory effect on IL-17 expression by CD4+ cells is mediated by the cytokine pattern changes induced by simvastatin treatment of monocytes

Simvastatin inhibited IL-6 and IL-23 and induced IFN-γ, IL-4, and IL-27 gene expression. Because the former cytokines were reported to induce IL-17 production in CD4+ cells and the latter to inhibit IL-17 production (10), we predicted that the net effect of statin-induced changes in the cytokine production by monocytes would be the inhibition of IL-17 production in CD4+ cells. We confirmed that culturing of CD45RA+ cells in the presence of IL-6 and IL-23 increased, and the addition of IFN-γ, IL-4, and IL-27 decreased the IL-17 secretion. In contrast, mAb blocking of IL-6 and IL-23 inhibited, while mAb blocking of IFN-γ, IL-4, and IL-27 induced IL-17 secretion in CD45RA+ cells (Fig. 4A). Consistent results were obtained for RORC and IL-17 gene expression in the same samples tested by qRT-PCR (data not shown).

To directly test the prediction that simvastatin-induced changes in monocyte cytokine secretion suppress IL-17 secretion in CD4+ cells, the supernatants from the simvastatin-treated monocytes were transferred to the activated allogenic CD4+ T cells. Supernatants from the simvastatin-treated monocytes inhibited the expression of transcription factor RORC and IL-17 in CD4+ T cells.

**FIGURE 4.** Simvastatin’s effects on cytokine production in monocytes inhibit IL-17 secretion in CD4+ cells. A, IL-17A secretion by αCD3 plus αCD28 mAb-stimulated CD4RA+ cells was induced by IL-6 and IL-23, while the addition of IFN-γ, IL-4, and IL-27 inhibited RORC and IL-17 gene expression. Two × 10^6 negatively selected CD45RA+ naïve cells were cultured in serum-free media with the indicated cytokines or blocking Abs for 24 h, and then stimulated with plate-immobilized αCD3 and αCD28 mAb. After 5 days, IL-2 (20 U/ml) was added and the cultures were expanded for an additional 7 days. At day 12, the supernatants were collected and the secreted IL-17 was measured by ELISA. Representative results from two similar experiments performed in duplicate are shown. B, Supernatants from simvastatin-treated monocytes inhibited RORC and IL-17A gene expression in CD4+ cells. Five × 10^6 purified monocytes derived from three RR MS patients were pretreated with simvastatin (10 μM) for 24 h in serum-free media, and then stimulated with LPS for 24 h. The supernatants from the monocyte cultures were transferred to the negatively separated allogenic CD4+ cells derived from RR MS patients. After 24-h coincubation, the CD4+ cells were stimulated with plate-immobilized αCD3 and αCD28 mAbs for 4 h before RNA extraction. RORC and IL-17A gene expression was measured by qRT-PCR. The results represent one out of three similar experiments, performed in triplicate.
in comparison to the CD4⁺ T cells cultured with the supernatants of untreated monocytes (Fig. 4B).

Simvastatin directly inhibits gene expression and secretion of IL-17 by CD4⁺ lymphocytes in MS patients and HCs

The main finding of our study is that simvastatin significantly inhibited RORC and IL-17A gene expression in the activated CD4⁺ cells derived from MS patients (−2.4 and −5.5-fold, respectively) and HCs (−2.7, and −2.2-fold, respectively). Simvastatin inhibited IL-17A protein secretion in CD4⁺ T cells from MS patients and HCs (−3.3-fold and −2.5-fold, respectively), Fig. 5A. In addition, simvastatin inhibited IL-21 gene expression in the same samples from MS patients (−2.6-fold, p = 0.003) and from HCs (−2.4-fold, p = 0.003), indicating that it may also inhibit the autocrine IL-21-mediated induction of IL-17 transcription in CD4 cells (data not shown).

We also confirmed in long-term cultures that simvastatin inhibits IL-17 secretion in naive CD45RA⁺ cells differentiated in the presence of Th17 polarizing cytokines (IL-6 and IL-23). Simvastatin’s effect was reversed by addition of anti-IFN-γ, IL-4, and IL-27 mAb (Fig. 5B). Similar results were obtained for RORC and IL-17 gene expression in the same samples tested by qRT-PCR (data not shown).
Simvastatin-mediated inhibition of RORC and IL-17A gene expression is reversed by mevalonic acid

Both the simvastatin-mediated decrease in IL-17A and RORC expression were reversed by mevalonic acid, indicating that simvastatin’s effect is mediated by the inhibition of HMG-CoA reductase (Fig. 6).

Discussion

Our data identified that treatment with simvastatin increases SOCS3 and SOCS7 expression in PBMCs and monocytes from RR MS patients and HCs (Figs. 1 and 2). Individual SOCS proteins regulate transcription of multiple cytokines, as well as the expression of MHC class II and costimulatory molecules, and represent an attractive therapeutic target in inflammatory diseases (21). The cell-penetrating SOCS3 protein has been successfully used as a treatment for animal model of inflammatory liver disease. The inhibition of MHC class II expression in CD11b+ splenocytes in cell-penetrating SOCS3-treated mice was mediated by the inhibition of STAT1 phosphorylation that interacts with the type IV CITA promoter for the expression of MHC class II molecules (22). In an animal model of rheumatoid arthritis, intra-articular injection of recombinant SOCS3 reduced joint inflammation and decreased STAT3 phosphorylation and IL-6 production (23).

Therefore, the induction of SOCS3 expression with statins may be therapeutically effective in MS, as supported by studies of the animal model of MS. Li et al. (21) have reported that SOCS3-transduced dendritic cells (DCs) express a decreased level of MHC class II and CD86, have decreased production of IL-12 and IL-23, and were tolerogenic, biasing T cell differentiation toward the Th2 phenotype in myelin oligodendrocyte-specific T cells. Moreover, transfer of the SOCS3-transfected DCs to naive mice prevented the EAE development. Consistent with our results, Huang et al. (24) have demonstrated that statins induced SOCS3 in stimulated mice macrophage cell line, which was reversed by the mevalonic acid.

Our study demonstrated that simvastatin-induced SOCS3 expression in monocytes inhibited phosphorylation of STAT3 and STAT1 (Fig. 2B). Based on these results, we propose that changes in IL-6 and IL-23 are related to the SOCS3-mediated inhibition of STAT3 phosphorylation. Findings of an increased expression of IFN-γ, IL-4, and IL-27 in simvastatin-treated monocytes need to be elucidated, because SOCS1 and SOCS5, which regulate transcription of those cytokines are not significantly inhibited in monocytes upon statin treatment. SOCS1 and SOCS3 have significant sequence homology and both predominantly inhibit STAT1 and STAT3 phosphorylation, however, they are often expressed in a reciprocal fashion (9).

Our data (Fig. 3) and a study by Neuhaus et al. (20) and Kiesier et al. (25) reported an increase in IFN-γ production by statins in the activated PBMCs and monocytes derived from MS patients. IFN-γ has a dual role in the autoimmune inflammatory response: it is a proinflammatory Th1 cytokine, but it also plays a role in controlling autoimmune response by inducing apoptosis of autoreactive T cells in EAE (26). More recently, our understanding of the autoimmune response was significantly altered by reports on the Th17 cell lineage, whose development is suppressed by IFN-γ, IL-12, and IL-27 (27). These studies explored previously contradictory findings that STAT1, IL-12RB, IFN-γ, and IFN-γ receptor-deficient mice develop a severe form of EAE.

We confirmed that inhibition of IL-6 and IL-23 and culturing with IFN-γ, IL-4, and IL-27 inhibited RORC and IL-17 gene expression, as well as IL-17 secretion by CD4 cells. These cytokine changes are consistent with the simvastatin-induced changes in cytokine secretion by monocytes (Fig. 4A). When supernatants from the simvastatin-treated monocytes were used to treat activated allogenic CD4+ cells, they significantly inhibited RORC transcription factor and IL-17A gene expression in CD4+ cells in comparison to the CD4+ cells cultured with the supernatants of untreated monocytes (Fig. 4B). This finding confirmed that statin-induced changes in IL-17-regulatory cytokine production by monocytes indeed suppress IL-17 secretion in CD4 cells.

In addition to this effect of simvastatin on monocytes’ secreted cytokines that regulate IL-17 production by CD4+ cells, we examined whether statins directly affect the IL-17 production by CD4+ T cells. Our results indicated that simvastatin significantly inhibits RORC and IL-17 gene expression, as well as IL-17 secretion in CD4+ cells derived from both RR MS patients and HCs (28) (Fig. 5A). Our previous results indicated that statins also inhibit T-bet and do not significantly change GATA3 expression in the PBMCs derived from MS patients (19). However, at this point it is not clear how RORC, T-bet, and GATA3 cross-regulate each other in the human system, and whether a subset of IL-17-producing CD4+ cells also produce IFN-γ in MS patients, as reported in patients with Crohn’s disease (29) and autoimmune scleritis (17).

In conclusion, our study demonstrated that statin-induced changes in cytokine transcription in monocytes inhibited the production of IL-6 and IL-23, and induced IFN-γ, IL-4, and IL-27, which together suppressed IL-17 secretion in the CD4+ cells derived from both MS patients and HCs. In addition, simvastatin directly suppressed RORC and IL-17 gene expression, as well as IL-17 secretion from the CD4 T cells (30). Our results characterized the novel anti-inflammatory mechanisms of statins, which selectively target the regulation of cytokine transcription and the development of the human autoimmune response. Based on the described immunomodulatory mechanisms, good safety profile, and oral bioavailability, statins represent a promising therapeutic approach for MS and other chronic inflammatory diseases.

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Disclosures

The authors have no financial conflict of interest.

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