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Atorvastatin Restores Lck Expression and Lipid Raft-Associated Signaling in T Cells from Patients with Systemic Lupus Erythematosus

Elizabeth C. Jury,² David A. Isenberg, Claudia Mauri, and Michael R. Ehrenstein²,³

Loss of tolerance to self-Ags in patients with systemic lupus erythematosus (SLE), a prototypic autoimmune disease, is associated with dysregulation of T cell signaling, including the depletion of total levels of lymphocyte-specific protein kinase (Lck) from sphingolipid-cholesterol-enriched membrane microdomains (lipid rafts). Inhibitors of 3-hydroxy-3-methylgluteryl CoA reductase (statins) can modify the composition of lipid rafts, resulting in alteration of T cell signaling. In this study, we show that atorvastatin targets the distribution of signaling molecules in T cells from SLE patients, by disrupting the colocalization of total Lck and CD45 within lipid rafts, leading to a reduction in the active form of Lck. Upon T cell activation using anti-CD3/anti-CD28 in vitro, the rapid recruitment of total Lck to the immunological synapse was inhibited by atorvastatin, whereas ERK phosphorylation, which is decreased in SLE T cells, was reconstituted. Furthermore, atorvastatin reduced the production of IL-10 and IL-6 by T cells, implicated in the pathogenesis of SLE. Thus, atorvastatin reversed many of the signaling defects characteristic of SLE T cells. These findings demonstrate the potential for atorvastatin to target lipid raft-associated signaling abnormalities in autoreactive T cells and provide a rationale for its use in therapy of autoimmune disease.

Inhibitors of 3-hydroxy-3-methylgluteryl CoA (HMG-CoA) reductase, or statins, have been used extensively in clinical practice to reduce cardiovascular-related morbidity and mortality. This effect is partly mediated through beneficial effects on the lipid profile, but it has also been demonstrated that statins have immunomodulatory properties that reduce the inflammatory components of the atherosclerotic plaque (1). The immunological effects of statins have also been investigated in vitro, and in vivo in different models of autoimmunity, including experimental allergic encephalitis, collagen-induced arthritis, and the NZB/W murine lupus model, revealing a marked therapeutic benefit (2–4). A number of studies have addressed the mechanisms through which statins ameliorate autoimmune disease, demonstrating that T cells represent a major target, either directly or indirectly, as shown by the inhibition of IFN-γ-induced expression of MHC class II, down-regulation of pathogenic T cell responses, and the induction of T cell anergy (1, 5).

Lipid rafts, originally defined as regions of the membrane that are resistant to some nonionic detergents (detergent-resistant membranes (DRMs)), are enriched in cholesterol and the ganglioside GM1 (6). The dynamic localization of signaling molecules, including the lymphocyte-specific protein tyrosine kinase (Lck) and linker for activation of T cells (LAT) within lipid rafts, is thought to be essential for the regulation of T cell activation (7, 8). Drugs, including statins, which interfere with raft formation, block signaling and consequently T cell activation (8, 9).

T cells isolated from patients with systemic lupus erythematosus (SLE) display signaling patterns characteristic of abnormal activation, including increased calcium flux and protein tyrosine phosphorylation (10). In addition to other researchers, we have shown that SLE T cells demonstrate an altered distribution of membrane lipid rafts and associated signaling molecules proximal to the Ag receptor, including the reduced expression of Lck (11–13). Along with the pleiotrophic immunomodulatory activity of statins, two specific findings prompted us to investigate the potential effect of statins on the abnormal lipid raft-associated signaling in SLE T cells. Statins not only inhibit cholesterol biosynthesis, but also lead to a reduction in isoprenoids (geranylgeranylpyrophosphate and farnesylpyrophosphate), which participate in effective lymphocyte activation (14).

In this study, we show that in vitro atorvastatin treatment reversed the lipid raft-associated signaling abnormalities in lupus T cells, including the altered expression and distribution of Lck. Furthermore, atorvastatin restored the defective phosphorylation of ERK upon T cell activation. The reversal of signaling defects was paralleled by reduction in the production of pathogenic cytokines IL-10 and IL-6. These results provide insight into the mechanism of action of statins in the context of patients with SLE, and may also have wider implications in the treatment of cardiovascular disease, a serious complication in patients with lupus.

Materials and Methods

Patients and controls

Seventy-one SLE patients fulfilling the American College of Rheumatology revised classification criteria for lupus (15) were assessed for disease activity using the British Isles Lupus Assessment Group index (16). Only patients with active SLE (British Isles Lupus Assessment global score ≥ 6) were included (mean age, 39 years; age range, 19–76 years; 40 women and 4 men). Sixty-one healthy individuals (mean age, 38 years; age range,
24–64 years; 22 women and 4 men) were studied in parallel as controls. The ethics committee of the University College London Hospitals National Health Service Trust approved this study; patients and healthy volunteers were recruited after obtaining informed consent.

Abs and reagents

Purified Abs to human CD3 (OKT3), CD19 (Ref-B9), CD14 (AML), CD56 (B159), and CD16 (3G8) were obtained from Chemicon. Mouse mAb against Lck (3A5), 3A5 conjugated to Sepharose, 3A5 conjugated to PE, CD45 (55-76), and rabbit polyclonal Abs against Lck were obtained from Santa Cruz Biotechnology. Rabbit polyclonal Abs to the active form of Lck (phospho-src pY414) were purchased from New England Biolabs. Abs to phospho-src pY414 are cross-reactive with other src family kinases including Lck, and was used because specific anti-phospho-Lck pY394 was not available. Rabbit polyclonal Ab to LAT was obtained from Upstate Biochemicals. Rabbit polyclonal Abs to ERK1/2 (p44/42), phospho-ERK1/2 and actin were purchased from Cell Signaling Technology. Conjugated Abs to human CD3-FITC, CD4-PE, and CD45-PE and purified mAb to CD28 (CD28.2), and flotilin-1 were obtained from BD Biosciences. HRP- and FITC-conjugated Abs, rabbit anti-mouse and goat anti-rabbit, cholera toxin B (CTB)-FITC, and filipin were purchased from Sigma-Aldrich. Atorvastatin was donated by Pfizer.

Cell culture and isolation of T lymphocytes

PBMC isolated from ~40 mL of heparinized venous blood from SLE patients or controls by centrifugation over Ficoll-Hyphaque (Pharmacia Bio-tech) was cultured at 37°C in 5% CO2 for 72–96 h in RPMI 1640 medium (Invitrogen Life Technologies) with 5–10% (Invitrogen Life Technologies), 100 U/ml penicillin, and 50 mg/ml streptomycin (Sigma-Aldrich). GM1 levels in resting and activated T cells were investigated using CTB-FITC. Cells were surface stained with anti-CD4-PE, fixed with 1% paraformaldehyde in PBS, and permeabilized with PBS staining buffer (PBS, 0.3% saponin, and 1% FCS) before staining with Lck-PE. GM1 levels in resting and activated T cells were investigated using CTB-FITC. Cells were surface stained with anti-CD4-PE, fixed with 1% paraformaldehyde in PBS and stained with CTB-FITC (1/300) for 1 h on ice, washed, and analyzed. To determine the level and distribution of plasma membrane cholesterol ex vivo, resting and activated T cells were labeled with filipin. Briefly, in a method modified from Ref. 17, T cells were fixed with 4% paraformaldehyde, quenched with 50 mM glycine, and incubated with 12.5 μg/mL filipin (Sigma-Aldrich) with or without 10 μM atorvastatin added to the culture medium. To inhibit prenyltransferases, medium was supplemented with 10 μM GGTI-286 and 10 μM FTI-277 (Calbiochem). Cell viability was measured by trypan blue exclusion (>99%). T lymphocyte-enriched populations were obtained by negative selection using a method described previously (11). Where indicated, T cells were activated using magnetic beads coated with anti-CD3 and CD28 (Dynal) for 5–60 min at 37°C.

Flow cytometry

The purity of T lymphocytes in the enriched populations was determined by flow cytometry and was consistently >94%. To measure total Lck, T cells were surface stained with anti-CD3-FITC, fixed with 1% paraformaldehyde in PBS, and permeabilized with PBS staining buffer (PBS, 0.3% saponin, and 1% FCS) before staining with Lck-PE. GM1 levels in resting and activated T cells were investigated using CTB-FITC. Cells were surface stained with anti-CD4-PE, fixed with 1% paraformaldehyde in PBS and stained with CTB-FITC (1/300) for 1 h on ice, washed, and analyzed. To determine the level and distribution of plasma membrane cholesterol ex vivo, resting and activated T cells were labeled with filipin. Briefly, in a method modified from Ref. 17, T cells were fixed with 4% paraformaldehyde, quenched with 50 mM glycine, and incubated with 12.5 μg/mL filipin (Sigma-Aldrich) with or without 10 μM atorvastatin added to the culture medium. To inhibit prenyltransferases, medium was supplemented with 10 μM GGTI-286 and 10 μM FTI-277 (Calbiochem). Cell viability was measured by trypan blue exclusion (>99%). T lymphocyte-enriched populations were obtained by negative selection using a method described previously (11). Where indicated, T cells were activated using magnetic beads coated with anti-CD3 and CD28 (Dynal) for 5–60 min at 37°C.

Preparation of DRMs

T cells were lysed on ice with 500 μL of lysis buffer (1% Triton X-100 in MNE buffer, 23 mM MES (pH 6.5), 2 mM EDTA, and 150 mM NaCl) with phosphatase inhibitors (1 mM sodium orthovanadate and 10 mM sodium fluoride. Sigma-Aldrich), and protease inhibitors (500 μM AEBSF 500 μM, 140 mM aprotinin, 1 μM E-6-41, and 1 μM leupeptin; Calbiochem). For accurate comparison between paired samples (atorvastatin treated/un-treated), DRMs were isolated from equal amounts of total protein by sucrose density ultracentrifugation as described previously (11). Before analysis by Western blotting, lipids were solubilized within the fractions by the addition of 60 mM octyl-glucopyranoside (Sigma-Aldrich) for 20 min at 4°C to facilitate measurement of GM1.

Western blot and immunoprecipitation

RAFT/nonraft fractions or T cell lysates prepared with lysis buffer (Tris-HCl (pH 8), 0.1% Triton X-100, 1 mM MgCl2, and 100 mM NaCl) with freshly added phosphatase and protease inhibitors (Calbiochem) were diluted in Laemmli’s sample buffer, and proteins were separated under reducing conditions on 10 or 8% polyacrylamide gels. Protein concentrations in the whole T cell lysates were determined by bicinchoninic acid protein assay. Lck immunoprecipitation from T cell lysates was performed from 100 μg of protein before separation on 8% SDS-PAGE gels (11). Proteins were transferred to polyvinylidene difluoride membranes (Millipore) and analyzed by immunoblotting as described previously (11).

Statistical analysis

Statistical analyses were made using the Mann-Whitney test, Kruskal-Wallis test, and Wilcoxon-matched pairs test (nonparametric) or Student’s t test (paired and unpaired) (parametric) for comparison between sample groups. Statistical significance of the data was set at p < 0.05.

Results

Atorvastatin treatment restored total Lck expression to normal levels in SLE T cells by reducing Lck activation

We have recently demonstrated that T cells from patients with SLE show a reduced expression, and an abnormal localization of lipid raft-associated signaling molecules proximal to the TCR. In addition to the increased expression of glycosphingolipid, GM1 (11–13), our experiments revealed that cholesterol content was also increased in ex vivo SLE T cells (data not shown). Previous data showing that atorvastatin treatment affects TCR signaling by modulating lipid biosynthesis, resulting in the redistribution of membrane proteins, (Ref. 19 and data not shown) encouraged us to investigate whether this drug could affect the expression and activation of signaling molecules in SLE T cells.

First, the effect of atorvastatin treatment on the expression of total Lck and GM1 was investigated by flow cytometry. PBMC from lupus patients and healthy controls were incubated with or without atorvastatin (10 μM). In untreated lupus T cells, as we have previously shown, GM1 levels, measured by CTB binding, were significantly higher than the levels in healthy controls (Fig. 1A; p = 0.01), whereas Lck expression was significantly reduced (Fig. 1B; p = 0.02) (11). When lupus T cells were cultured with atorvastatin, the expression of GM1 was significantly reduced (Fig. 1A; p = 0.02) and the amount of Lck was restored to levels similar to those observed in T cells from healthy controls (Fig. 1B; p = 0.02).

Our previous work has shown that the reduction in total Lck expression found in lupus T cells was associated with an increase in the activated form of the molecule (pTyr-394) (13). We therefore investigated whether restoration of total Lck levels by atorvastatin in lupus T cells was paralleled by a change in the level of
Atorvastatin normalized raft defects in SLE T cells

In lupus T cells, reduced total Lck expression is associated with translocation of CD45 into lipid raft domains (13). CD45 dephosphorylates the inhibitory tyrosine (Tyr505) of Lck, allowing Lck to adopt an open-active conformation. Therefore, to examine whether normalization of Lck expression was coupled with reduced Lck/CD45 coassociation, we first examined the membrane distribution of CD45 by confocal microscopy. As we have previously shown, CD45 and GM1 (CTB) were colocalized in lupus but not in healthy T cells; however, stimulation with atorvastatin reversed CD45/GM1 colocalization in lupus T cells (Fig. 2A). To further confirm that the change in CD45 membrane distribution was associated with reduced CD45-Lck interaction, we immunoprecipitated Lck from T cell lysates and looked for CD45 coprecipitation. Although the expression of total Lck was increased in the atorvastatin-treated lupus T cells, there was no associated with raft domains to levels similar to those observed in T cells from healthy controls (Fig. 1E).

FIGURE 1. Atorvastatin treatment restored Lck and GM1 expression to normal in lupus T cells. Purified T cells from patients with SLE and healthy controls were isolated by negative selection following a 96-h incubation with or without 10 μM atorvastatin. Membrane GM1 labeled with CTB-FITC (A) or total Lck (B) expression were analyzed by flow cytometry (±SEM; n = 10). Whole T cell lysates were analyzed by Western blotting for Lck, the activated form of Lck (Lck pY414), and actin, as a control for protein loading (C). The semiquantitative density ratio of active Lck:total Lck is shown in D (±SEM, n = 6). Representative Western blots showing DRMs isolated from SLE and healthy T cells by floatation over dense sucrose gradients and analyzed for expression of Lck and LAT (E). Actin expression in the T cell lysates before raft isolation is used as a control for protein loading (n = 9). T cell lipid raft fractions were isolated after culture with 10 and 20 μM atorvastatin or medium alone and analyzed by Western blotting for Lck expression (F). The relative levels of Lck were estimated by comparing the density of the bands to an actin control (±SEM; n = 4).

FIGURE 2. Atorvastatin diminished the coassociation of Lck with CD45 in lipid rafts

Atorvastatin blocked the association of CD45 with Lck leading to reconstitution of Lck in lipid rafts

We have shown previously that total Lck is depleted from lipid raft domains in lupus T cells (11). Since atorvastatin restored total Lck expression and levels of the raft constituent GM1, we next measured its effect on the levels of raft-associated Lck. Culture of lupus T cells with atorvastatin, up-regulated the amount of Lck associated with raft domains to levels similar to those observed in T cells from healthy controls (Fig. 1E). However, another raft-associated signaling molecule, LAT, which has normal expression in lupus T cells (11), remained unaffected by atorvastatin. In contrast, atorvastatin treatment of healthy T cells reduced the levels of raft-associated proteins Lck and LAT (Fig. 1E). Using higher concentrations of atorvastatin, total Lck (and LAT) are depleted from the raft fraction in lupus T cells. Taken together these experiments not only demonstrate that atorvastatin specifically targets Lck in lupus T cells, but also illustrates that lupus T cells have an altered sensitivity to atorvastatin compared with healthy T cells, needing higher concentrations to dissociate raft-associated protein Lck from DRMs (Fig. 1F).
corresponding increase in CD45 coassociation (Fig. 2B), thus resulting in a significant reduction in Lck/CD45 coassociation (p = 0.04) (Fig. 2C). These results suggest that atorvastatin can modulate the aberrant lupus T cell signaling via inhibiting Lck/CD45 association in the lipid raft.

**Inhibition of protein prenylation alone did not restore Lck and GMI expression in SLE cells**

Atorvastatin could modulate lipid raft domains, possibly by inhibiting cholesterol biosynthesis and/or by inhibiting protein prenylation. Protein prenylation (geranylgeranylation or farnesylation) enables membrane targeting of the Ras and Rho family GTPases, both integral to signaling via the T cell Ag receptor (14, 20). Therefore, to test the effect of soluble inhibitors of prenylation on raft expression of Lck, healthy as well as lupus T cells were incubated with and without GGTI-286 and FTI-277 (soluble inhibitors of prenylation). No significant differences in total Lck expression were seen when Lck:flotillin-1 ratios were compared between lupus T cells cultured with or without inhibitors of prenylation (Fig. 3A and B). In addition, specific inhibitors of prenylation did not reduce expression of membrane GM1 (Fig. 3C). Hence, these results suggest that the restoration of a normal Lck expression and distribution within the raft by atorvastatin has an independent mechanism from inhibition of protein isoprenylation.

**Atorvastatin restored the kinetics of TCR activation to normal levels in SLE T cells associated with a reduction in cholesterol**

The restoration of Lck expression within lipid rafts in SLE T cells by atorvastatin suggested that the hyperactivated T cell phenotype

**FIGURE 3.** Inhibition of protein prenylation alone did not restore Lck and GM1 expression in SLE cells. Purified T cells from patients with SLE and healthy controls were isolated by negative selection following incubation (96 h) in the presence of 10 μM GGTI-286 and 10 μM FTI-277 and in RPMI 1640 medium alone. A, DRMs were isolated and analyzed by Western blotting for expression of Lck and the structural protein flotillin-1 as a loading control. Representative blots from three experiments are shown. B, The relative levels of Lck were estimated by comparing the intensity of the bands to the flotillin-1 control (± SEM; n = 4). C, PBMC from patients with SLE and healthy controls were incubated (48 h) in the presence of 10 μM GGTI-286 and 10 μM FTI-277, 1 μM atorvastatin, and in RPMI 1640 medium alone. Membrane GM1 labeled with CTB-FITC was analyzed by flow cytometry (± SEM; n = 10).

**FIGURE 4.** Atorvastatin restored the kinetics of TCR activation to normal levels in SLE T cells which was associated with a reduction in cholesterol. Purified T cells from patients with SLE and healthy controls were isolated following a 96-h culture with or without atorvastatin (10 μM). A, T cells were activated with plate-bound anti-CD3 Abs (5 μg/ml) and soluble anti-CD28 Ab (5 μg/ml) for 60 min and compared with nonactivated cells. Cells were attached to coverslips, stained for CTB binding, and analyzed by microscopy. Representative images from three experiments are shown. B, Purified T cells were activated in 96-well plates precoated with anti-CD3 Abs (5 μg/ml) and soluble anti-CD28 Ab (5 μg/ml) added to the cultures for 48 or 72 h, precoated with anti-CD3 Abs (5 μg/ml) and soluble anti-CD28 Ab (5 μg/ml) for 48 h. Cells were analyzed for membrane GM1 and cholesterol levels by staining with CTB-FITC (B) and filipin, respectively (C), by flow cytometry (n = 8). D, Purified T cells from patients with active SLE and healthy controls (n = 4) were activated using magnetic beads coated with anti-CD3 and anti-CD28 for 5, 15, and 30 min. Cells were analyzed for Lck expression by confocal microscopy. Representative images are shown. E, The numbers of T cell/bead conjugates formed during the T cell activation time course described in D were counted (results are based on counting an average of 50 cells from each sample)(± SEM).
seen in lupus patients may also be reversed by statins. Although in vitro culture of SLE T cells reduces GM1 expression to below basal levels (13), TCR activation is characterized by increases in membrane-associated cholesterol and GM1 (21–23). In SLE T cells, the rise in these membrane-associated lipids was more dramatic than in healthy T cells (Fig. 4, A and B) (cholesterol \( p = 0.03; \) GM1 \( p > 0.05 \)). Atorvastatin abolished this increment, demonstrating that alterations in membrane cholesterol of activated T cells are also sensitive to the actions of atorvastatin. To explore whether the kinetics of SLE T cell activation are influenced by atorvastatin treatment, we evaluated the rate of accumulation of Lck to the immunological synapse following TCR activation. T cells from patients with SLE and healthy controls \( (n = 4) \) with plate-bound anti-CD3 Abs \( (5 \mu g/ml) \) and soluble anti-CD28 Ab \( (5 \mu g/ml) \) with or without \( 1 \mu M \) atorvastatin added to the cultures. Supernatants collected from 3-day cultures were analyzed by cytometric bead array (picograms per milliliter ± SEM).

### Discussion

The key finding from this work is that the HMG-CoA reductase inhibitor, atorvastatin, can reverse the raft-associated proximal signaling abnormalities present in lupus T cells. This was related to the restoration of total Lck expression and associated with reduced levels of activated Lck. Lck activity is regulated by its proximity to positive and negative regulatory molecules, CD45 and C-terminal Src kinase, respectively (27). Although CD45 is largely excluded from raft domains in healthy T cells, several investigators have reported dynamic changes in CD45 localization and raft partitioning during T cell activation (28). CD45 is abnormally localized within lipid rafts in lupus T cells, where it coassociates with, and activates, Lck (Fig. 2), resulting in increased ubiquitin-mediated Lck proteolysis and reduced levels of total Lck (11, 13). Thus, the dissociation of CD45 from Lck- and GM1-enriched membrane with lupus T cells was more rapid than in healthy T cells, again confirming the hyperactivated status of lupus T cells. In contrast, when SLE T cells were cultured with atorvastatin, the visible accumulation of Lck to the cell/bead contact zone was significantly delayed. Thus, a significantly reduced number of lupus T cell/bead conjugates were formed with the atorvastatin-treated cells compared with lupus T cells cultured in medium alone and healthy T cells (Fig. 4E). These results suggest that atorvastatin can modulate the immunological synapse, thereby dampening the hyperactive phenotype of SLE T cells. In summary, these results indicate that atorvastatin can reverse abnormalities in lupus T cells, specifically the proximal events involving T cell-APC interactions, which is likely due to alterations in membrane lipid/protein content and distribution.

### Normalization of intracellular signaling pathways in SLE T cells treated with atorvastatin

During T cell activation, the Ras family GTPases regulate signal transduction via the mitogen-activated protein kinase pathway, leading to ERK phosphorylation (14). In lupus T cells however, TCR-driven phosphorylation of ERK1 and ERK2 is diminished in contrast to an increase in global protein tyrosine phosphorylation (24, 25). SLE T cells failed to display significant ERK1/2 phosphorylation following TCR-mediated activation (Fig. 5, A and B). However, atorvastatin recovered early ERK1/2 phosphorylation to levels similar to those observed in T cells from the healthy controls (Fig. 5, A and B), while abolishing it in the healthy controls. Thus, it appears that atorvastatin can repair abnormal TCR-stimulated signaling events in SLE T cells by recovering Lck expression, reducing global protein tyrosine phosphorylation (data not shown), and restoring signaling via the RAS-ERK pathway.

### Atorvastatin reduced T cell production of IL-10 and IL-6 in SLE

Because statins have been shown to modulate cytokine production in experimental models of arthritis (2, 3) and to link the changes in lipid rafts to the pathophysiology of lupus, we explored the effect of atorvastatin on the production of cytokines relevant to the pathogenesis of SLE. T cells were purified from lupus patients and stimulated for 72 h with anti-CD3/CD28, either with or without atorvastatin. Culture supernatants were recovered and analyzed for the production of IFN-γ, IL10, and IL-6 by cytometric bead array. The results show that atorvastatin did not significantly influence the production of IFN-γ (data not shown). In contrast, the production of IL-10 and IL-6, implicated in the pathogenesis of SLE (26), were significantly reduced by atorvastatin (IL-10 and IL-6, \( p = 0.03; \) Fig. 5C).
microdomains by atorvastatin in unstimulated lupus T cells restored the balanced regulation of Lck activity and subsequent intracellular signal transduction during TCR activation. Despite the low levels of total Lck in lupus T cells, we demonstrate a more rapid accumulation of total Lck to the immunological synapse (a proportionately small area of the cell surface), a phenomenon that has also been described with CD3 and CD45 (12, 29). This more rapid accumulation of signaling molecules to the immune synapse in the T cell membrane (measured by GM1 and cholesterol proportionately small area of the cell surface), a phenomenon that rapid accumulation of total Lck to the immunological synapse (a low levels of total Lck in lupus T cells, we demonstrate a more intracellular signal transduction during TCR activation. Despite the microdomains by atorvastatin in unstimulated lupus T cells reduced formation of bead:cell conjugates (Fig. 4A), a similar reduction was only observed at higher concentrations of atorvastatin (20 μM vs 10 μM) in the lupus T cells (data not shown), again suggesting that these cells have an altered sensitivity to the effects of atorvastatin. Increased membrane cholesterol in resting T cells has been linked to impaired T cell activation and is associated with decreased immune cell function in old age (41). It remains to be determined whether the increase in membrane cholesterol levels observed in T cells from lupus patients contributes to the many abnormalities observed in these cells.

A further paradoxical effect of atorvastatin was the restoration of raft-associated Lck in lupus T cells, whereas in healthy T cells we observed a depletion of Lck from raft fractions. However, at a higher dose (20 μM vs 10 μM), atorvastatin also depleted raft-associated Lck in lupus T cells. This differential sensitivity between healthy and lupus T cells has been observed previously when cellular cholesterol was depleted using methyl-β-cyclodextrin (12) and was confirmed when we examined membrane cholesterol in resting T cells. Although atorvastatin (10 μM) reduced membrane cholesterol in healthy T cells (measured by thin film binding and flow cytometry), a similar reduction was only observed at higher concentrations of atorvastatin (20 μM vs 10 μM) in the lupus T cells (data not shown), again suggesting that these cells have an altered sensitivity to the effects of atorvastatin. Increased membrane cholesterol in resting T cells has been linked to impaired T cell activation and is associated with decreased immune cell function in old age (41). It remains to be determined whether the increase in membrane cholesterol levels observed in T cells from lupus patients contributes to the many abnormalities observed in these cells.

The pleiotropic action of statins contributes to the complexity of the mechanisms underlying their immunological effects (1, 34). We, and others, have proposed that some of these effects could be attributed to a reduction in membrane cholesterol (42, 43). Moreover, a recent report demonstrates that reducing the cholesterol content of lipid rafts using simvastatin, another HMG-CoA reductase inhibitor, alters downstream signaling in prostate cancer cells (44). However, the effect of statins on membrane lipid composition remains controversial, with several reports showing no effect on membrane cholesterol levels and raft architecture (34, 45). Another important action of inhibitors of HMG-CoA reductase is the suppression of protein prenylation which has been shown to underlie the mechanism of action of statins in some animal models, particularly those associated with a marked Th1 response (46). Furthermore, no reduction in cholesterol was seen when statins were used in these Th1-driven models of autoimmunity, whereas when statins were administered to a murine model of lupus, a significant lowering of cholesterol occurred (4). In the work presented in this study, the targeted inhibition of protein prenylation was not sufficient to restore Lck or GM1 levels in lupus T cells (Fig. 3). This result is consistent with Hillyard et al. (47) who have shown that inhibitors of prenylation do not affect raft integrity or associated signaling of human monocytes. Whereas the effects of statins in a Th1-driven disease are likely because of alterations in protein prenylation, modulation of membrane lipids may play a more significant role in non-Th1 autoimmune such as SLE.

Atorvastatin reduced the production of IL-10 and IL-6 by activated T cells, with a more marked effect on lupus T cells than healthy T cells, at least with respect to IL-6. Most studies relating to the immunomodulatory properties of statins in autoimmunity have studied their effects on animal models of disease with a predominant Th1-driven response (2, 3). However, induction of a Th2 cytokine response by statins has not been a universal feature in rodent models of autoimmune disease (48) or in human primary PBMC from patients with multiple sclerosis, where an in vitro study observed an increase in IFN-γ and reduction in IL-10 production (49). Th1 and Th2 cytokines both contribute to the pathogenesis of lupus disease, which is not so easily defined within the classical Th1/Th2 paradigm (50). Moreover, atorvastatin did not alter T cell cytokine production in lupus-prone NZB/W mice (4). Again, these results suggest that the effect of statins depends on the immunopathological environment, but that these agents have a consistent influence on the restoration of T cell tolerance.
Taken together, the results shown here suggest that a normal signaling profile and cellular function can be restored to SLE T cells by in vitro atorvastatin treatment. It is possible that atorvastatin is readjusting autoreactive lupus T cells toward a tolerant phenotype. It is tempting to speculate that part of the pleiotropic effects of statins on the immune system could result from changes in membrane lipids, at least in the context of SLE. These findings may have implications not only in the understanding and potential treatment of SLE and possibly other autoimmune diseases, but could also be applicable in the context of atherosclerosis, itself a late complication seen in patients with SLE.

Disclosures

The authors have no financial conflict of interest.

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