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A Novel Anti-Inflammatory Role for Simvastatin in Inflammatory Arthritis[1]

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3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) exert favorable effects on lipoprotein metabolism, but may also possess anti-inflammatory properties. Therefore, we explored the activities of simvastatin, a lipophilic statin, in a Th1-driven model of murine inflammatory arthritis. We report in this study that simvastatin markedly inhibited not only developing but also clinically evident collagen-induced arthritis in doses that were unable to significantly alter cholesterol concentrations in vivo. Ex vivo analysis demonstrated significant suppression of collagen-specific Th1 humoral and cellular immune responses. Moreover, simvastatin reduced anti-CD3/anti-CD28 proliferation and IFN-γ release from mononuclear cells derived from peripheral blood and synovial fluid. Proinflammatory cytokine production in vitro by T cell contact-activated macrophages was suppressed by simvastatin, suggesting that such observations have direct clinical relevance. These data clearly illustrate the therapeutic potential of statin-sensitive pathways in inflammatory arthritis. *The Journal of Immunology,* **2003, 170: 1524–1530.**

The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)[3] reductase inhibitors (statins) have been unequivocally shown to reduce cardiovascular morbidity and mortality (1, 2). Although such clinical benefits are mediated in part through lipid modulation, recent studies demonstrate broader properties for statins, particularly in modifying inflammatory pathways ongoing within the atherosclerotic lesion (3). The molecular mechanisms subserving such immunomodulatory activities remain unclear. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonic acid (MVA) during cholesterol synthesis. Downstream metabolites, including geranylgeranyl pyrophosphate and farnesyl pyrophosphate regulate prenylation within several critical signaling pathways (4). Statins inhibit IFN-γ-inducible macrophage MHC class II expression via class II transactivator suppression (5) and activate peroxisome proliferator-activated receptor-α via inhibition of Rho-dependent pathways (6). Moreover, some statins (e.g., lovastatin, simvastatin) may modulate T cell costimulation through direct effects on LFA-1/ICAM-1 interactions, dependent upon recognition of a novel statin binding site on β_2 integrins (7). These properties indicate that statins might modulate functional maturation of T lymphocytes.

Following Ag exposure, T lymphocytes mature to different functional phenotypes distinguished on the pattern of cytokine production. Th1 responses, characterized by IFN-γ release, are critical to the development and course of several important autoimmune diseases, including rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease (8), and have a postulated role in vascular wall inflammation (9, 10). However, definitive proof that statins can modify Th1/Th2 responses with immunopathologic consequence in vivo has until now been lacking.

We have used inflammatory arthritis as a model in which to test the above hypothesis. Elevated levels of proinflammatory cytokine production characterize RA synovial inflammation (11). Moreover, successful therapeutic targeting of cytokines in RA, particularly TNF-α, has demonstrated their critical pathogenetic importance. Th1 responses predominate within RA synovial T cell subsets and contribute significantly to dysregulated cytokine production (12–14). Synovial Th1 cells may drive macrophage cytokine release through secretion of IFN-γ or IL-17 (15), or may act through direct cognate cell-cell membrane interactions, involving several ligand pairs including LFA-1/ICAM-1 (16, 17). In this study, we document for the first time a novel anti-arthritic effect of statins. We show that simvastatin can effectively suppress murine collagen-induced arthritis (CIA), either prophylactically, or if administered after clinically evident onset of disease, via specific suppression of the pathologic Th1 and proinflammatory responses. The clinical relevance of these observations is illustrated by parallel studies in RA-derived human cells in which simvastatin suppressed cytokine release by PBMCs and by macrophages following cell contact-dependent interaction with activated T lymphocytes.

Materials and Methods

Preparation of simvastatin

Simvastatin (Merck Sharp & Dohme, Middlesex, U.K.) was prepared as a 4 mg/ml stock. Briefly, 4 mg was dissolved in 100 μl of ethanol and 150 μl of 0.1 N NaOH, incubated at 50°C for 2 h, and then pH adjusted to 7 and volume corrected to 1 ml.

Induction and assessment of CIA

Male DBA/1 mice (8–10 wk old; Harlan Olac, Bicester, U.K.) received 200 μg of bovine type II collagen (CII; Sigma-Aldrich, Poole, U.K.) in CFA (Difco, Detroit, MI) by intradermal injection (day 0). Collagen (200

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[3] Abbreviations used in this paper: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MVA, mevalonic acid; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; CII, type II collagen; PB, peripheral blood.

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μg in PBS) was given again on day 21 by i.p. injection. Mice were monitored daily for signs of arthritis for which severity scores were derived as follows: 0 = normal, 1 = erythema, 2 = erythema plus swelling, 3 = extension/loss function, and total score = sum of four limbs. Disease onset characterized by erythema and/or paw swelling was seen between days 25 and 35. No difference was seen in the degree of response between early and late responders. Paw thickness was measured with a dial-caliper (Kroeplin, Munich, Germany). For histological assessment, mice were sacrificed and the hind limbs removed and fixed in 10% neutral-buffered formalin, then decalcified in 5% formic acid and embedded in paraffin. Sections (5 μm) were stained with H&E or toluidine blue (Sigma-Aldrich). Quantification of arthritis was performed by two treatment-blinded observers as described (18).

Treatment protocols

For the prophylactic protocol, DBA/1 mice were administered daily i.p. with increasing doses of simvastatin (10, 20, or 40 mg/kg) from days 21 to 40. For the therapeutic approach, DBA/1 mice were treated with simvastatin (40 mg/kg) i.p. for a total of 14 days 1 day after CIA became clinically detectable. Control mice received PBS alone in both experiments. The doses of statin chosen are consistent with the primary bioactive dose used in vivo in rat/murine studies (typically 10–100 mg/kg/day) (19–21). These doses are higher than used in man but are necessary in rat/murine studies due to rapid up-regulation (3- to 8-fold) of HMG-CoA reductase with statin drugs (22).

Cell culture

Murine studies. Draining lymph nodes (popliteal and inguinal; four per mouse) were aseptically removed from arthritic mice and passed through Nytex to prepare a single-cell suspension. Cells were cultured at 2×10^6/ml for up to 96 h in RPMI medium, supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES buffer, and 10% heat-inactivated FCS (all Life Technologies, Paisley, U.K.). Cells were stimulated with graded concentrations of CII. Proliferation assays were performed in triplicate in U-bottom 96-well plates (Nunc, Roskilde, Denmark). Supernatants from parallel triplicate cultures were stored at −70°C until estimation of cytokine content by ELISA.

Human studies. Samples were derived following approval from Glasgow Royal Infirmary Ethical Committee (Glasgow, U.K.). RA patients met ACR diagnostic criteria (23). PBMCs and synovial fluid mononuclear cells were prepared using histopaque (Sigma-Aldrich) following the manufacturer's instructions. Cells derived from normal controls or RA patients were stimulated with plate-bound anti-CD3 Ab (0.2 μg/ml; Skybio, Wyboston, U.K.) and anti-CD28 Ab (0.5 μg/ml; BD PharMingen, Oxford, U.K.) in the presence or absence of simvastatin. Cultures were maintained in RPMI, with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (all Sigma-Aldrich). After 48 h, [^{3}H]thymidine (Amersham Pharmacia Biotech, Little Chalfont, U.K.) was added (1 μCi/ml) and the cultures harvested following a further 18-h incubation. For IFN-γ release, culture supernatants were collected at 48 h. Cell contact experiments were performed as previously described (17). Briefly, peripheral blood (PB) T cells were activated with PHA (5 μg/ml)/PMA (25 ng/ml) then fixed in 4% paraformaldehyde. Control nonactivated T cells were also fixed for comparative purposes. Fixed PB T cells were cocultured with autologous monocytes that were pretreated with simvastatin ± MVA (100 μM) for 24 h. Supernatants were harvested at 48 h for TNF-α estimation.

Serum collection

Using cardiac puncture, serum was collected at day 41 (prophylactic protocol) or day 15 (therapeutic protocol). Blood was allowed to clot, then centrifuged and aliquots of serum stored at −70°C before ELISA.

ELISA

Murine TNF-α (BioSource International, Nivelles, Belgium), IL-4, IL-5, IL-6, IL-10, IL-12 (p40 + p70), and IFN-γ (BD PharMingen) were assayed by ELISA using paired Abs according to the manufacturer's instructions. Lower limits of detection were as follows: IL-4, IL-5, IL-6, IL-12, and TNF-α all at 10 pg/ml; IL-10 and IFN-γ at 40 pg/ml. Similarly, human TNF-α and IFN-γ (Biosource) were assayed by ELISA. Lower limits of detection were 30 pg/ml. Serum anti-collagen II Ab titers were measured by ELISA as described (24).

Statistical analysis

Clinical and histological scores were analyzed with the nonparametric Mann-Whitney *U* test. Cytokine and collagen-specific IgG levels were compared using the Student's *t* test.

Results

Simvastatin suppresses CIA

To determine whether statins might suppress immune-mediated pathology in vivo, we investigated the effect of simvastatin on the development of CIA in DBA/1 mice (prophylactic protocol). Simvastatin dose-dependently suppressed the incidence and severity of developing CIA when administered daily from days 21 to 40, following i.p. CII challenge (Fig. 1, *A* and *B*). A reduction in serum IL-6 levels (Fig. 2*A*) was observed, a surrogate for suppression of the acute phase response, suggesting that systemic inflammatory responses were modified.

It was important to determine whether similar effects could be obtained in mice after onset of CIA. Therefore, simvastatin was administered to DBA/1 mice after CIA became clinically detectable (therapeutic protocol). Significant reduction of arthritis progression was apparent within 3 days of treatment compared with vehicle-treated controls. This was evident in total articular index

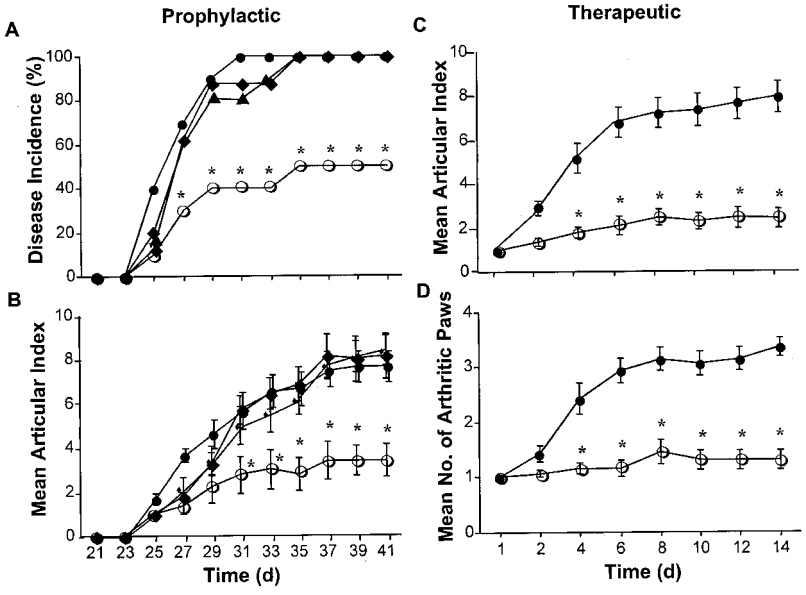

FIGURE 1. Effects of simvastatin on developing (prophylactic) and clinically evident (therapeutic) CIA. DBA/1 mice were immunized with CII and an i.p. challenge injection was given at day 21. *A* and *B*, Prophylactic protocol: mice were treated daily from days 21 to 40 with increasing doses of simvastatin starting from 10 mg/kg (♦; $n = 10$), 20 mg/kg (▲; $n = 8$), 40 mg/kg (○; $n = 10$), or PBS (●; $n = 10$). The appearance of arthritis was monitored daily and presented as disease incidence or mean articular index (calculated only from arthritic mice). Simvastatin administered at 40 mg/kg significantly lowered incidence and severity of arthritis (*, $p < 0.05$ by Mann-Whitney *U* test). There was no statistically significant difference between the 10 mg/kg, 20 mg/kg, and PBS groups. *C* and *D*, Therapeutic protocol: mice were treated 1 day post onset of clinically evident articular disease for a total of 14 days either with simvastatin 40 mg/kg (○; $n = 13$) or PBS (●; $n = 14$). Significant suppression of disease activity (*, $p < 0.05$) was observed in simvastatin-treated mice as indicated by mean articular index and mean number of arthritic paws. Data are expressed as mean ± SEM.

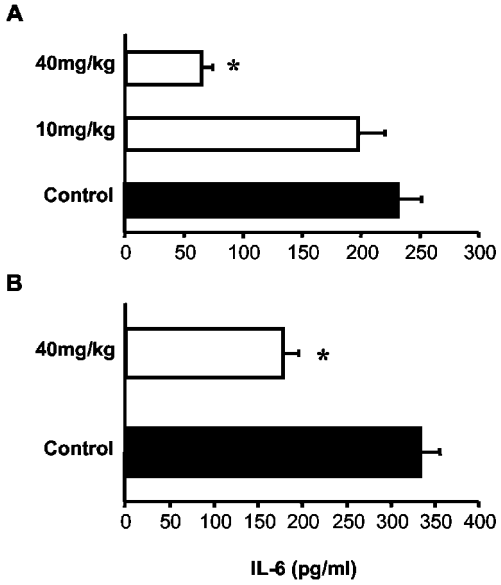

FIGURE 2. Serum IL-6 levels and Ab titers in simvastatin-treated mice. Serum levels of IL-6 were determined by ELISA in both prophylactic (day 41) and therapeutic (day 15) studies. Data are expressed as mean ± SEM of individual serum concentrations.

and in the number of subsequently recruited arthritic joints (Fig. 1, *C* and *D*), but also in reduced progression of articular swelling in the initially inflamed joint (Table I). Disease was suppressed until at least 14 days after cessation of treatment. Commensurate with clinical measurements, serum IL-6 levels were also significantly suppressed (Fig. 2*B*). To determine whether simvastatin administration prevented articular destruction, we evaluated cartilage and bone integrity histologically. Progression of synovial hyperplasia, and of cartilage and bone erosion was significantly suppressed by simvastatin (Fig. 3). Total cholesterol plasma levels did not change significantly compared with placebo, suggesting that the dose of simvastatin used is of likely physiological relevance, since it was not sufficient to induce cholesterol reduction. (e.g., 40 mg/kg of simvastatin: control 2.87 ± 0.09 mmol/L vs simvastatin 2.35 ± 0.21 mmol/L; mean ± SEM $p > 0.05$, Student's *t* test). Together these data clearly indicate that simvastatin potently suppressed both developing and after onset phases of inflammatory CIA, largely independent of effects on total cholesterol modulation. Moreover, such activity can prevent progression of articular damage.

Simvastatin suppresses collagen-specific Th1 immune response in vivo

We next investigated mechanisms whereby such effects were achieved. CIA is associated with a Th1-polarized immune response, rendering it an excellent model to explore the effect of statins upon functional T cell maturation in vivo. Therefore, CII-specific immune responses were examined in vitro in draining lymph nodes obtained at day 41 in the prophylactic protocol. Spontaneous and CII-induced IFN-γ release was significantly suppressed in mice receiving either 10 or 40 mg/kg simvastatin (Fig. 4*A*) associated with significant reduction in CII-induced proliferation (Fig. 4*B*). CII-induced production of TNF-α, IL-12, and IL-6 was also significantly reduced in the 40 mg/kg-treated group, whereas IL-10 production was unaltered (Fig. 4, *C* and *D* and data not shown). In contrast, production of IL-4 or IL-5 was not detected. Immune modulation by simvastatin in vivo was Ag-specific since Con A-induced production of IFN-γ, TNF-α, and IL-5 in parallel cultures was not affected. Similarly, draining lymph node class II transactivator mRNA levels measured by TaqMan PCR at day 40 were unaltered, suggesting that general suppression of inducible class II MHC expression was unlikely to explain our observations. Finally, serum collagen-specific IgG1 and IgG2a levels were reduced in the simvastatin recipients (Fig. 5). Together, these data indicate that simvastatin exerts anti-inflammatory activity through suppression of the Th1 response, without necessarily enhancing a "compensatory" Th2 response.

We next determined whether simvastatin could modify an ongoing collagen-specific Th1 response when the drug was administered after the onset of clinical disease. Draining lymph nodes were obtained 14 days after daily simvastatin administration (therapeutic protocol). Simvastatin significantly reduced spontaneous and CII-induced IL-12 and IFN-γ production and cellular proliferation (Fig. 4, *A*, *B*, and *D*). Neither IL-4 nor IL-5 production was detected. Significant suppression of CII-induced TNF-α and IL-6 release was also evident (Fig. 4*C* and data not shown), as was production of IL-10 (control 272 ± 48 pg/ml vs simvastatin 169 ± 2 pg/ml; mean ± SD, $p < 0.05$). Con A-induced IFN-γ, TNF-α, and IL-5 productions were similar in simvastatin and control-treated groups (data not shown), indicating that immune modulation was Ag-specific. Finally, serum levels of CII-specific IgG2a, and to a lesser extent IgG1, were reduced by simvastatin administration (Fig. 5). These data indicate that simvastatin can effectively and specifically suppress ongoing Th1 cytokine responses and anti-collagen B cell responses, and strongly support the notion that such immune modulation ameliorated the progression of articular inflammation in vivo.

Effect of simvastatin on anti-CD3/anti-CD28 stimulation of human cells in vitro

To further investigate the therapeutic potential of statins, we performed parallel studies in which the ability of simvastatin to modify T cell regulatory and proinflammatory cytokine production by cells from RA patients in vitro was evaluated. We examined the effects of simvastatin on PB-derived mononuclear cells stimulated with anti-CD3/anti-CD28 Abs. Simvastatin reduced proliferation in a dose-dependent manner in cultures derived from normal ($n = 7$) and RA blood ($n = 4$), (Fig. 6*A*, $p < 0.05$ at 1, 5, and 10 μM simvasatin). IFN-γ release was similarly reduced in the presence of simvastatin (Fig. 6*B*, $p < 0.05$ at 5 and 10 μM simvastatin). In RA synovial fluid cells ($n = 3$), proliferation was inhibited significantly by simvastatin at 5 and 10 μM, (Fig. 6*C*, $p < 0.05$), as was IFN-γ release at 10 μM, (Fig. 6*D*, $p < 0.05$). There was no significant change in IL-10 levels in the culture supernatants while levels of IL-4 and IL-5 were undetectable. We observed no reduction in T cell viability at these concentrations of simvastatin. These data are consistent with our prior observations ex vivo in the murine CIA model.

Table I. *Reduced progression of initial arthritic joint*[a]

Δ Difference	PBS ($n = 14$)	Simvastatin ($n = 13$)
Articular index	1.64 ± 0.17	0.15 ± 0.30*
Paw thickness (mm)	0.53 ± 0.09	0.05 ± 0.08*

[a] DBA/1 mice immunized with CII were treated with simvastatin according to the therapeutic protocol as described in *Materials and Methods.* Articular swelling in the initially diseased joint was scored for severity (0–3), and paw thickness (millimeters) was measured with a dial-calliper. Δ Difference was calculated by subtracting the value of day 1 (beginning of treatment) from day 14 (end of therapy) of individual mice. Results are mean ± SEM of this difference per group.

* $p < 0.01$, compared with PBS control by Mann-Whitney U test.

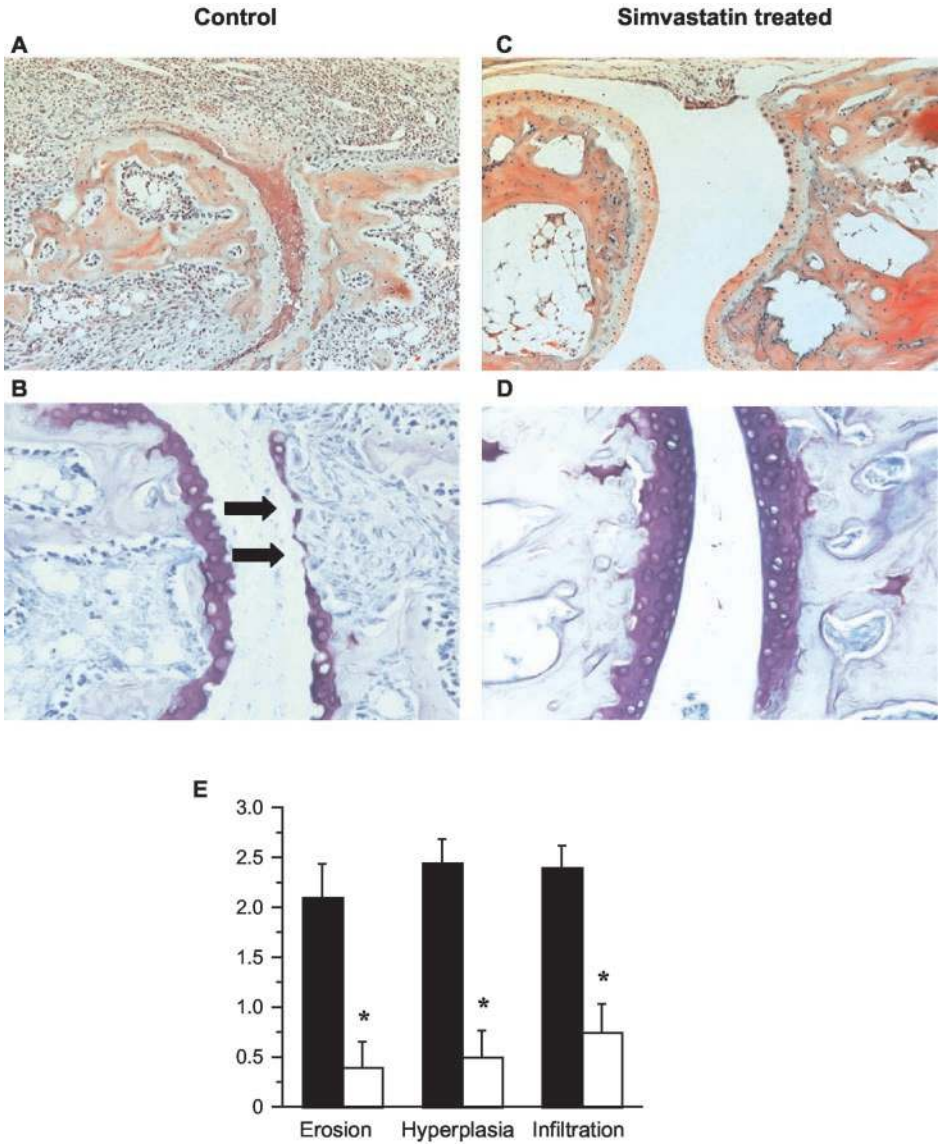

FIGURE 3. Simvastatin administration to mice with after onset CIA resulted in significantly reduced joint pathology. *A–D*, Mice were treated with PBS or simvastatin (40 mg/kg) following detection of arthritis, as described in the therapeutic protocol. After 14 days of simvastatin administration, arthritic paws were removed and stained with H&E or toluidine blue. Profound cartilage surface erosion and loss of proteoglycan was observed in PBS controls (arrows), whereas simvastatin recipients exhibited significantly reduced histologic evidence of destruction. *E*, Histological appearances were scored for the presence of synovial bone erosion, hyperplasia, and cellular infiltration following administration of PBS (■; $n = 10$) or simvastatin (□; $n = 10$). ∗, $p < 0.01$ vs PBS, by Mann-Whitney U test, data are mean ± SEM.

Effects of simvastatin on RA derived T cells and macrophages in vitro

It is increasingly accepted that T cells drive RA synovial proinflammatory cytokine production primarily through cell contact with macrophages (16, 17). Because statins can block T cell costimulation by binding to the β_2 integrin L-site (7), we reasoned that similar effects might suppress synovial T cell-macrophage interaction. PHA/PMA-activated PB T cells from normal human

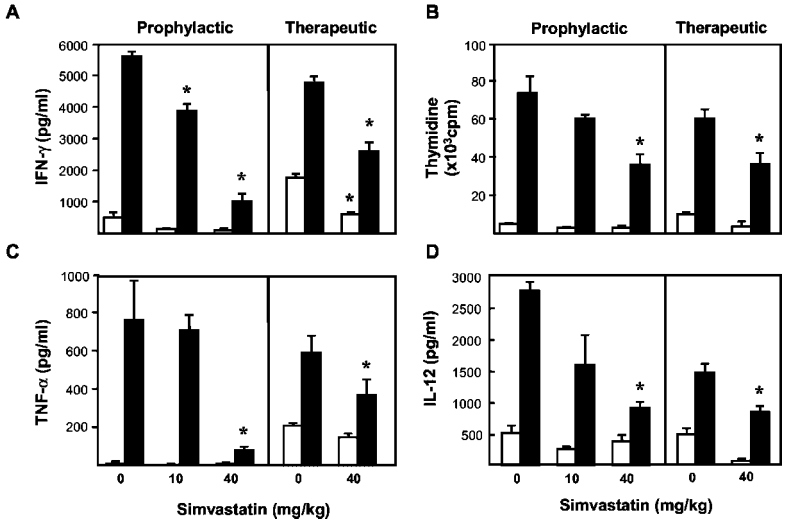

FIGURE 4. Simvastatin reduces in vitro Ag-specific and in vivo anti-CII responses. Draining lymph node cells ($n = 5$ mice/group) were harvested from arthritic mice on day 41 (prophylactic protocol) or day 15 (therapeutic protocol) and cultured either with medium alone (□) or CII (■; 50 μg/ml) for up to 96 h. *A*, *C*, and *D*, Cytokine concentrations (IFN-γ 96 h; TNF-α, IL-12 72 h) in the culture supernatant were determined by ELISA. *B*, T cell proliferation was assayed by uptake of [^{3}H]thymidine after 96 h. Significant suppression of induced and in some cases spontaneous cytokine production and proliferation was evident in lymph node cultures removed from simvastatin-treated mice compared with PBS-treated controls. ∗, $p < 0.05$, whereby simvastatin groups are compared with respective PBS controls by Student's t test. Data are expressed as mean ± SEM of triplicate cultures of pooled lymph node cell suspensions.

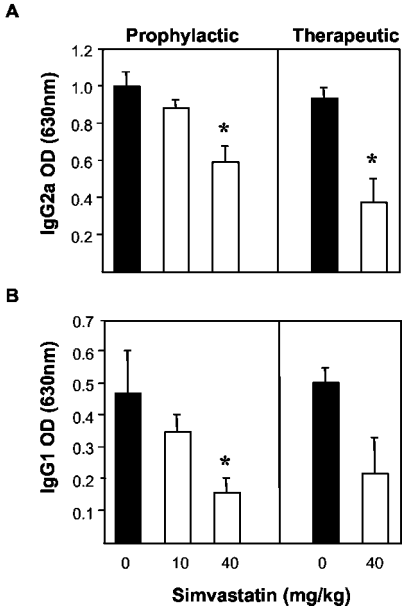

FIGURE 5. Serum anti-collagen IgG2a and IgG1 titers in simvastatin-treated mice. Anti-collagen IgG2a and IgG1 Ab levels were measured from PBS (■; $n = 5$) or simvastatin-treated mice (□; $n = 5$) in both prophylactic (day 41) and therapeutic (day 15) studies. Data are expressed as mean absorbance (OD_{630}) ± SEM of individual serum measurements; *, $p < 0.05$, 40 mg/kg vs PBS.

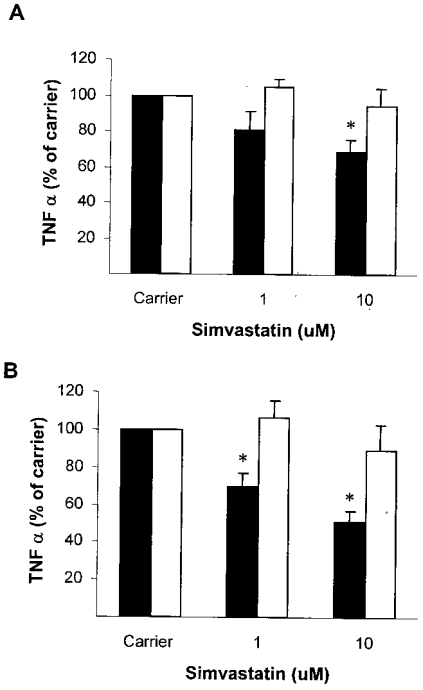

FIGURE 7. Simvastatin suppresses cell-mediated macrophage TNF-α release induced via cognate interactions. TNF-α production was measured in a cell contact assay in which normal (*A*) or RA patient (*B*) derived PB T cells were stimulated for 48 h with PMA/PHA (per *Materials and Methods*), then fixed in paraformaldehyde before coincubation with autologous monocytes for 48 h. Monocytes were incubated with simvastatin alone (■) or in the additional presence of 100 μM MVA (□) for 24 h before and during coculture. Significant suppression of TNF-α production in coculture was observed by simvastatin that was reversed by MVA in all cases. Values are mean ± SEM from nine separate experiments, each performed in triplicate (except RA PB T cell/monocyte coculture in the presence of MVA where $n = 6$ patients).

subjects were paraformaldehyde-fixed then cocultured with autologous blood-derived monocytes in the presence of increasing concentrations of simvastatin. Such coculture-induced macrophage TNF-α release was significantly suppressed by simvastatin by ~30% (Fig. 7*A*). Importantly, similar or greater (~50%) suppression was observed in experiments using PB T cells and monocytes derived from RA patients (Fig. 7*B*). The suppression was reversed by coincident addition of MVA. These data show that the HMG-CoA reductase pathway is operative in blocking macrophage activation via T cell contact. No evidence of macrophage apoptosis was observed (using annexin V/propidium iodide staining by flow cytometric analysis) at the concentrations of simvastatin used (data not shown), indicating that reduced cell survival is unlikely to explain the observed inhibition.

We finally investigated whether prior exposure to simvastatin modified the capacity of T cells to activate macrophages upon subsequent cell contact. Addition of simvastatin (up to 10 μM) during PB T cell activation by either PHA/PMA or cytokine (IL-15 100 ng/ml) had no effect on the subsequent ability of those T cells to activate macrophages via cell contact ($n = 10$ experiments; data

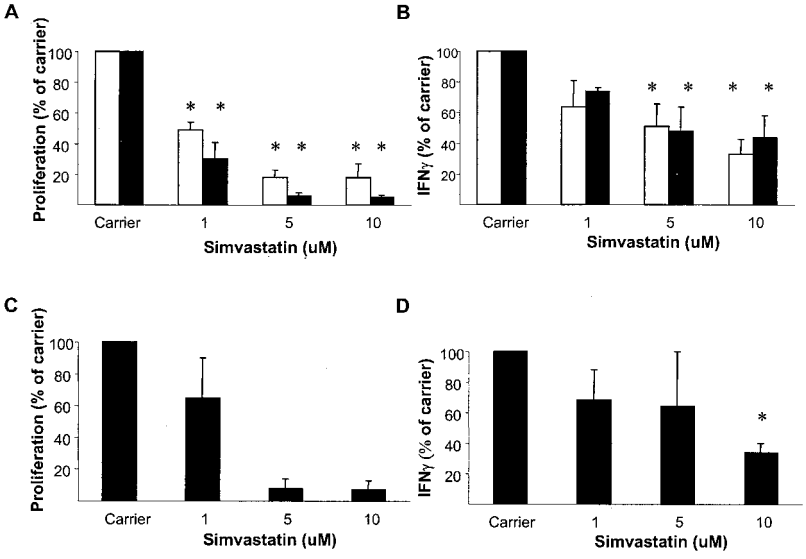

FIGURE 6. Dose-dependent effects of simvastatin on human leukocytes in vitro. Addition of increasing concentrations of simvastatin inhibited anti-CD3/anti-CD28-induced proliferation (72 h) of normal PBMC ($n = 7$ donors) as well as RA PBMC ($n = 4$ donors) (*A*), and synovial fluid mononuclear cells ($n = 3$ donors) (*C*). *B* and *D*, IFN-γ release (48 h) was also reduced in a dose-dependent manner. Experiments (from each donor) were done in triplicate and graphs show mean ± SEM from all donors, *, $p < 0.05$. □, Normal donors; ■, RA patients.

not shown). Commensurate with this, using FACS analysis we observed no change in expression of either LFA-1 on $CD3^+$ PB T cells (median MFI: 135 carrier vs 140 simvastatin, $n = 3$) or of ICAM-1 (median MFI: 116 carrier vs 113 simvastatin, $n = 3$) on $CD14^+$ monocytes, following addition of simvastatin (1–50 μM) in vitro for up to 72 h. These data indicate that simvastatin must be present during the coculture of T cells with macrophages to exert the suppressive effects.

Discussion

Elucidating the potential effects of statins on inflammatory responses in vitro and in vivo is currently of major importance. Our data demonstrate that simvastatin provides a highly effective therapy in treating murine CIA, a surrogate model for human RA. Previous studies of thioglycolate peritonitis or carrageenan-induced paw pad inflammation suggested that statins modify acute inflammation (7, 25). We now show that simvastatin can suppress the progression of acute to chronic inflammation in vivo. Importantly, simvastatin administration was effective even after the onset of arthritis. These data indicate that statins, or "statin-sensitive" pathways, may find therapeutic utility in a variety of autoimmune conditions. They further suggest that exploration of statin-targeted pathways may elicit novel T cell regulatory events.

There is currently considerable interest in the potential of immune modulatory therapies in the treatment of RA, particularly those targeting cytokine expression (26, 27). Pathways that drive cytokine release in RA synovium are unclear but include a significant contribution by T cells, either through the direct release of IFN-γ or via cell contact (28). Our data indicate that simvastatin might suppress synovitis through effects on both such pathways. We have clearly shown simvastatin suppression of Th1 immune responses in vivo and subsequently on disease relevant cells ex vivo derived from RA patients. Effects upon T cell proliferation and IFN-γ release were manifest in both RA and healthy donors. This study was not powered or designed for formal comparison of statin sensitivity in RA compared with healthy donor PBMC, and our data should suggest only that statin tractible pathways are maintained in the disease state. These data are compatible with prior studies in vitro in which MHC class II expression was altered (5) and T cell costimulation was also suppressed through direct allosteric inhibition via an integrin L-site (7). Our in vivo observations that simvastatin inhibits Th1 cell development and effector functions have broader significance for other disease states including vascular disease. In particular, there is increasing evidence implicating Th1-mediated mechanisms in the pathogenesis of atherogenesis (9, 10).

Simvastatin was also effective in blocking T cell-macrophage interactions. Statins have previously been shown to exert multiple effects on macrophages including suppression of monocyte chemotaxis, inducible NO synthase activation, and reduction in LPS-induced TNF-α release (4, 29, 30). We now demonstrate using patient-derived cells that simvastatin targets a physiologically relevant pathway in which T cells drive macrophages through cognate interactions. Several ligand pairs have been implicated in these interactions including CD40/CD154 and LFA-1/ICAM-1. Because the latter may be directly targeted by statins, this pathway may partially explain our observations. However, it is of interest that statins were most effective in suppressing macrophage activation when incubated with macrophages before as well as during T cell addition. This strongly suggests modulation of macrophage activation status independent of membrane ligand interactions. Our data in vitro do not readily assimilate the effects that statins may also moderate via effects on lipid metabolism. For example, in vivo in humans, statins elevate HDL and apoA1 levels that in turn have been shown to specifically suppress T cell-macrophage cognate interactions mediated by apoA1 binding to T cells (31). Thus, several mechanisms, direct and indirectly via lipid modulation, likely subserve the anti-inflammatory effects of simvastatin on T cell-macrophage interactions.

Our studies in mice were performed at doses higher than used in man. Higher statin doses in rat/murine studies are used (19–21) due to rapid up-regulation of HMG-CoA reductase in these species (22). It is notable that no significant reduction in plasma cholesterol was observed in our studies using up to 40 mg/kg. Moreover, liver function analysis (aspartate transaminase, alanine transaminase) was similar between placebo and simvastatin-treated mice. In comparison, in humans typical cholesterol reductions of ~45% are achieved using ~1.5 mg/kg. As such, our immune modulatory effects have been achieved at doses of simvastatin that were well tolerated and lie below the optimal cholesterol-lowering dose required in mice. Clearly, our studies represent a proof of concept approach that illustrates not only the potential of statins to directly modulate inflammatory arthritis, but indicate a future development potential for statin-like drugs selected on their anti-inflammatory rather than lipid-lowering activities.

In conclusion, we demonstrate in this study a novel therapeutic role for simvastatin that may be applicable to RA. Statins have an after onset role in the reduction of cardiovascular risk and there is some evidence to suggest that they reduce acute rejection episodes after transplantation (32). We now show in this study that simvastatin markedly reduces Ag-specific inflammatory responses in vitro and in vivo. Simvastatin significantly attenuated the development and expansion of Th1 cells, which, at least in part, drive the production of proinflammatory cytokines by macrophages in a cell contact-dependent manner. These mechanisms may also partially explain reported benefits of statin in other inflammatory diseases such as dementia and diabetes where inflammation is increasingly a recognized component of pathogenesis (33, 34). Appropriate clinical studies are now required to test this hypothesis in vivo in inflammatory arthritis. Similarly, the intracellular signaling consequences of statins operating during T cell maturation deserve explanation.

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