Original Article

Atorvastatin Fails to Prevent the Development of Autoimmune Diabetes Despite Inhibition of Pathogenic β-Cell–Specific CD8 T-Cells

Biliana Lozanoska-Ochser,[1] Francesca Barone,[2] Costantino Pitzalis,[2] Mark Peakman[1]

Statins, the widely used inhibitors of cholesterol biosynthesis, also have immunomodulatory properties. Statins have recently been shown to have beneficial prophylactic and therapeutic effects in actively induced, short-term animal models of the autoimmune diseases multiple sclerosis and rheumatoid arthritis, leading to clinical trials. We therefore investigated whether statins' protective effects could be reproduced in the nonobese diabetic (NOD) mouse, a spontaneous, chronic model of autoimmune diabetes. Mice were treated with 0, 1, 10, or 50 mg · kg^{-1} · day^{-1} oral atorvastatin from 6 or 12 weeks of age, without effect on the rate or prevalence of diabetes development, islet infiltration, or islet major histocompatibility complex class II expression. However, there was clear evidence of a disease-relevant immunological effect of statins in vivo, since short-term (12-day) treatment significantly reduced the number of proinflammatory (γ-interferon–producing) CD8 cells recognizing a dominant pathogenic epitope. This effect was absent in mice treated for longer periods, suggesting that atorvastatin loses efficiency in inhibiting autoantigen-specific T-cells over time. This observation may explain the discrepancy between the reported success of statins in acutely induced models and the lack of it in a chronic, spontaneous model of autoimmune disease and has implications for the adoption of such therapy in humans. *Diabetes* 55:1004–1010, 2006

In recent years there has been a notable surge of interest in the effects of the commonly used cholesterol-lowering drugs called statins or 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Independent of their lipid-lowering properties, statins have been shown to have wide-ranging immunomodulatory and anti-inflammatory properties including inhibition of γ-interferon (IFN-γ)–inducible major histocompatibility complex (MHC) class II expression, proinflammatory cytokine and chemokine production, and expression of adhesion molecules (1–3). Statins were also found to reduce the expression of the costimulators CD40, CD40-ligand, CD80, and CD86 on various cell types and block lymphocyte function–associated antigen-1–mediated T-cell adhesion and costimulation (4,5). The discovery of these novel immunomodulatory properties provided researchers with the rationale for using statins to treat immune-mediated inflammatory diseases. In key preclinical studies, atorvastatin treatment was found to inhibit the development of actively induced experimental encephalomyelitis (EAE) in SJL/J mice (4,6) while simvastatin treatment inhibited the development of arthritis induced by immunization with collagen (collagen-induced arthritis [CIA]) in mice (7), prompting open-label and small-scale intervention studies of the equivalent diseases in humans: multiple sclerosis and rheumatoid arthritis, respectively (8,9).

Against this background, statin therapy is worthy of consideration in the prevention or treatment of autoimmune diabetes. Two features of these drugs are particularly attractive. First is the potent suppression of T-helper 1 (Th1) proinflammatory cytokine production by autoreactive T-cells (4). The islet destruction that is characteristic of type 1 diabetes is considered to be the result of a Th1-mediated inflammatory process, and circulating Th1 autoreactive T-cells recognizing islet autoantigens are a feature of the disease at onset (10). A second potentially beneficial feature of statins is their downregulatory effect on inducible MHC class II expression (3). Post mortem studies show that islet vessel endothelial cells express induced MHC class II molecules at diagnosis of disease (11), and we previously demonstrated in vitro that presentation of islet autoantigens by MHC class II–expressing endothelium markedly enhances the transmigration of islet autoreactive CD4 T-cells (12), suggesting that this feature of insulitis could promote islet damage and inflammation.

To obtain preclinical evidence of efficacy, we elected to examine the effect of statins in the nonobese diabetic (NOD) mouse, a spontaneous and chronic model of type 1 diabetes. Mice were treated at different disease stages, using both preventive and curative protocols, and at three different doses. Measurable outcomes included progression to diabetes as well as changes in immunological parameters. We report that, contrary to expectations, statin treatment fails to prevent autoimmune diabetes, despite powerful acute effects on T-cell autoreactivity.

From the [1]Department of Immunobiology, King's College London, School of Medicine, London, U.K.; and the [2]Department of Rheumatology, King's College London, School of Medicine, London, U.K.

Address correspondence and reprint requests to Professor Mark Peakman, Department of Immunobiology, King's College London, School of Medicine, 2nd Floor, New Guy's House, Guy's Hospital, London, SE1 9RT, U.K. E-mail: mark.peakman@kcl.ac.uk.

Received for publication 27 September 2005 and accepted in revised form 4 January 2006.

CIA, collagen-induced arthritis; CTL, cytotoxic T-lymphocyte; EAE, experimental encephalomyelitis; FITC, fluorescein isothiocyanate; IEC, islet endothelial cell; IFN-γ, γ-interferon; IGRP, islet-specific glucose 6-phosphatase catalytic subunit–related protein; IL, interleukin; MHC, major histocompatibility complex; Th1, T-helper 1.

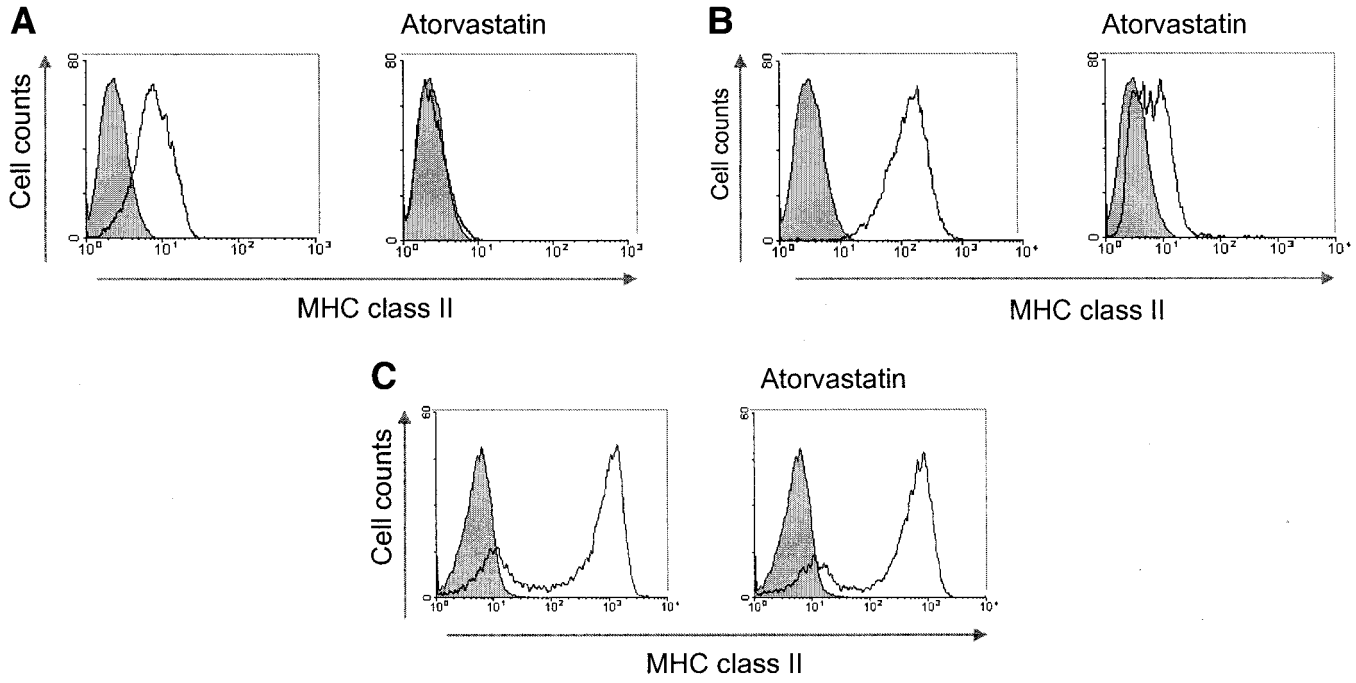

FIG. 1. Effect in vitro of atorvastatin on IFN-γ–inducible MHC class II expression. Flow cytometric analysis of MHC class II expression on resting (shaded histogram) or IFN-γ–stimulated (unshaded histogram) human IECs (*A*) and NOD mouse peritoneal macrophages (*B*). Preincubation with atorvastatin (10 μmol/l) inhibited the IFN-γ–inducible MHC class II expression on IECs and macrophages. In contrast, *C* shows NOD mouse splenocytes cultured in the absence (shaded histogram) or presence (unshaded histogram) of atorvastatin (10 μmol/l) for 48 h. Atorvastatin does not alter constitutive MHC class II expression, which is present on B220+ and CD11c+ splenocytes (data not shown).

RESEARCH DESIGN AND METHODS

Female NOD mice were obtained from our breeding colony (13–15), and studies were performed with approval from the institute's ethical review committee.

Atorvastatin effects on NOD mouse macrophages and human islet endothelial cells. NOD mouse peritoneal macrophages (>95% CD14+) and splenocytes were cultured in RPMI-1640 media supplemented with 100 μg/ml penicillin/streptomycin and 10% FCS (Invitrogen, Paisley, U.K.). Human islet

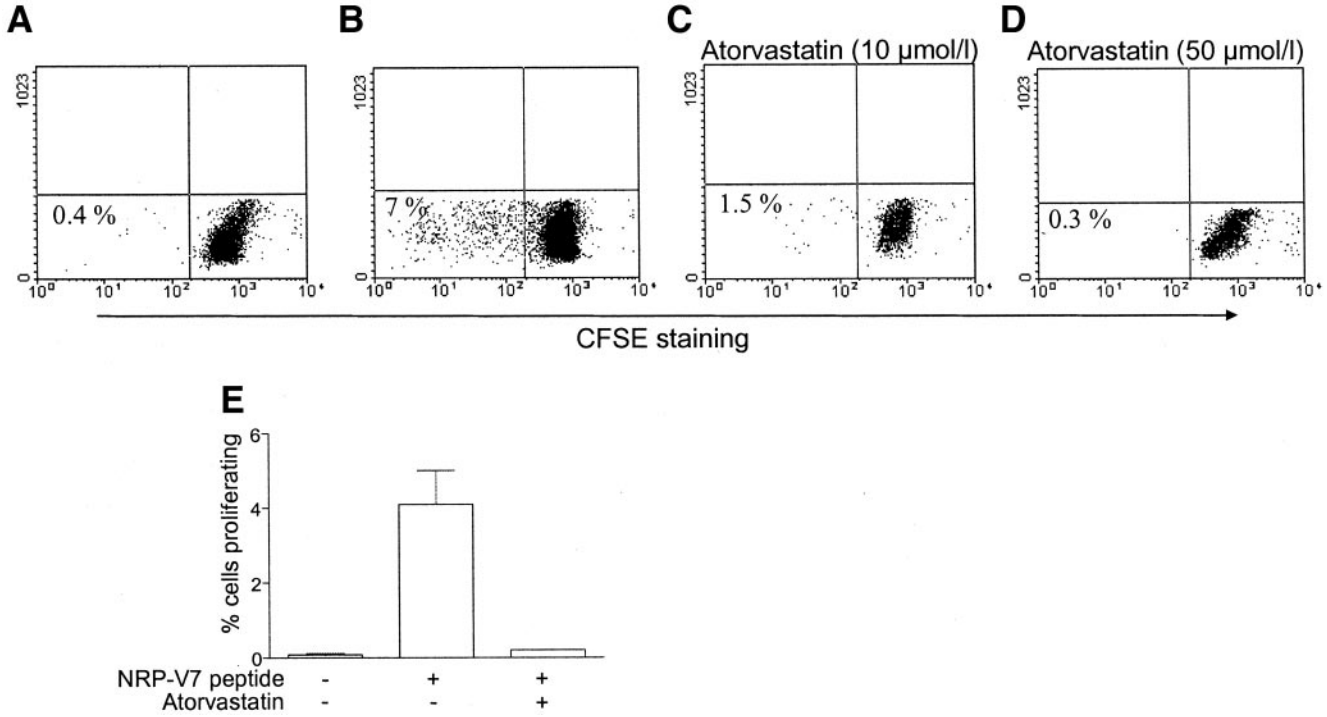

FIG. 2. Effect in vitro of atorvastatin on islet autoreactive T-cell proliferation. Graph shows proliferation, as measured by reduction in carboxyfluorescein diacetate succinimidly ester (CFSE) staining intensity, of CD8 T-cells among splenocytes in the absence of stimulus (*A*) and in the presence of NRP-V7 peptide (10 μmol/l) (*B*). Considerable CD8 T-cell proliferation is observed in response to the autoantigen. In the presence of atorvastatin at 10μmol/l (*C*) and 50 μmol/l (*D*) there is a dose-dependent and complete inhibition of proliferation in response to the NRP-V7 peptide. *E*: Means ± SE percentage of proliferating splenocytes under similar conditions (atorvastatin at 10 μmol/l) from three separate experiments.

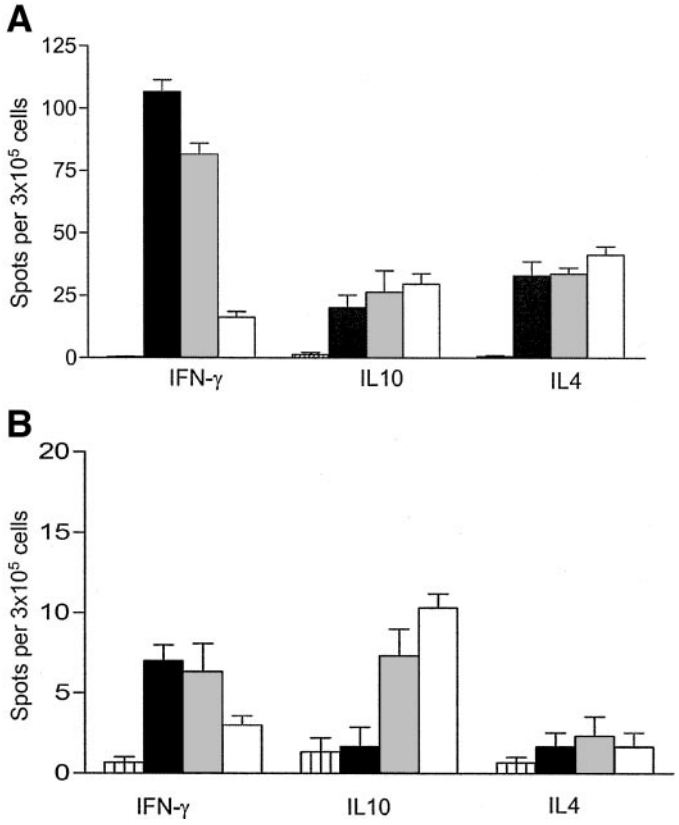

FIG. 3. Effect in vitro of atorvastatin on proinflammatory cytokine production. *A*: Cytokine ELISPOT analysis for production of IFN-γ, IL-10, and IL-4 following incubation of NOD splenocytes alone (□), with the polyclonal stimulus ConA (10 μg/ml) (■) or with ConA and atorvastatin at 10 μmol/l (▩) or 50 μmol/l (□). Atorvastatin inhibited polyclonal IFN-γ production but moderately enhanced IL-10 and IL-4 production in a dose-dependent fashion. *B*: Cytokine ELISPOT analysis following incubation of NOD mouse splenocytes alone (□) or with the diabetogenic peptide NRP-V7 alone (■) or together with atorvastatin at 10 μmol/l (▩) or 50 μmol/l (□). Atorvastatin treatment inhibited Ag-specific IFN-γ production and increased the production of IL-10 but not IL-4. Data are representative of at least three separate experiments.

endothelial cells (IECs) (16) (>95% CD105+, CD31+) were cultured in microvascular endothelial cell growth medium complete with endothelial cell growth factors (TCS CellWorks, Buckinghamshire, U.K.) supplemented with 100 μg/ml penicillin/streptomycin and 20% FCS. Macrophages and IECs were then preincubated with different statins including atorvastatin (Pfizer, Eastleigh, U.K.), pravastatin (Bristol-Myers Squibb, Hounslow, U.K.), and simvastatin (Merck Sharp & Dohme, Hoddesdon, U.K.) at 1, 5, and 10 μmol/l for 24 h at 37°C and 5% CO_2 and activated with IFN-γ (R&D Systems Europe, Oxon, U.K.) at 500 IU/ml for a further 48 h. To examine the effects of statins on constitutive MHC class II expression, NOD mouse splenocytes were incubated in the present of statins for 48 h. Adherent cells were detached (Accutase; TCS CellWorks), washed in PBS/2% FCS, and stained for 30 min at 4°C with rat anti–I-A-RPE (MRC OX-6; Serotec, Oxford, U.K.) and rat anti-mouse CD14-fluorescein isothiocyanate (FITC) (BD Pharmingen, Oxford, U.K.) for peritoneal macrophages; rat anti-I-A-RPE and rat anti-mouse B220 (BD Pharmingen) for splenocytes; and mouse anti-human CD105-RPE, CD31-RPE (Serotec, Oxford, U.K.), and HLA-DR-FITC (BD Pharmingen) for IECs, as well as appropriate isotype controls. Cells were then washed and analyzed using a FACSCalibur with CellQuest software (Becton Dickinson).

Autoreactive T-cell proliferation. To measure T-cell proliferation, 2×10^6 splenocytes were labeled with 2 μmol/l carboxyfluorescein diacetate succinimidyl ester (Molecular Probes, Leiden, the Netherlands) and cultured with either the mimotope of the dominant cytotoxic T-lymphocyte (CTL) epitope of islet-specific glucose-6-phosphatase-related protein (islet-specific glucose 6-phosphatase catalytic subunit–related protein $[IGRP]_{206–214}$, KYNKANVFL; epitope is termed NRP-V7 throughout) at 10 μmol/l (Thermo Electron, Ulm, Germany) in the presence or absence of 1, 5, or 10 μmol/l atorvastatin for 5 days in Dulbecco's modified Eagle's media supplemented with 2 nmol/l L-glutamine, 100 μg/ml penicillin/streptomycin, and 10% FCS and proliferation analyzed by flow cytometry.

Atorvastatin therapy. Female NOD mice at 4–6 (prevention study) and 12 (treatment study) weeks of age received atorvastatin daily as a suspension in 0.5 ml PBS by oral gavage using 20-mm feeding needles at 1, 10, and 50 mg/kg with an equal number of mice contemporaneously receiving vehicle (PBS) alone. Mice were monitored daily for glycosuria and maintained until 30 weeks of age or the development of diabetes, indicated by urinary glucose >8.3 mmol/l on two occasions 72 h apart and confirmed by blood glucose measurement >16.7 mmol/l as described (14).

Effector T-cell responses. These were detected ex vivo by an indirect cytokine ELISPOT as described (10) using 2×10^6 splenocytes isolated from treated and control mice, cultured in Dulbecco's modified Eagle's media supplemented with 2 nmol/l L-glutamine, 100 μg/ml penicillin/streptomycin, and 10% FCS in 48-well plates in the presence of autoantigenic peptides NRP-V7, $GAD65_{217–236}$, insulin B9-23, or concanavalin A (ConA, 10 μg/ml; Sigma-Aldrich, Poole, U.K.), all at 10-μmol/l final concentration for 48 h. Irrelevant control peptides comprised the coxsackievirus B4 $P2C_{11371–145}$ CTL epitope (17) and promiscuous tetanus p731 and p734 epitopes (10) for CD8 and CD4 responses, respectively. Nonadherent cells were harvested and washed and 10^6 cells divided and aliquoted in triplicate into 96-well plates precoated with cytokine capture antibodies for IFN-γ, interleukin (IL)-4 or IL-10 using U-Cytech kits (Utrecht, the Netherlands). The 96-well detection plates were dried, spots of >100 μm diameter counted in a BioReader 3000 (BioSys, Karben, Germany), and results expressed as mean spots/300,000 cells, representing the approximate number of cells in each triplicate well.

Islet histopathology and immunohistochemistry. Pancreas tissue was embedded in Tissue-Tec OCT Compound and snap-frozen in cold isopentane (VWR Scientific Products, Poole, U.K.) and liquid N_2 and stored at −80°C until use. Cryostat sections (6 μm) were fixed in acetone and stained with hematoxylin and eosin. Islets were scored for the extent of infiltration independently by two observers unaware of animal treatments. At least 30 islets per three nonoverlapping pancreatic sections/animal were analyzed. For the evaluation of islet and vessel MHC class II expression, acetone fixed sections were stained with biotinylated anti-mouse I-A (MRC OX-6, Serotec), rat anti-mouse CD105, anti-rat IgG-FITC (BD Pharmingen), and staining revealed using avidin-biotin Vector Red reagents (Vector Laboratories, Burlingame, CA).

Statistical analysis. Student's *t* test was used for intergroup comparisons of T-cell responses. The incidence of diabetes in female mice in our colony at the age of 30 weeks is ~90%. At minimum ($n = 9$ per group), NOD prevention studies were powered to detect an approximate reduction in diabetes incidence among females at 30 weeks, from 90% in untreated to 30% in treated mice, with 80% power and $P < 0.05$. Differences in diabetes-free survival between treated and control mice were examined using life table analysis and the log-rank test. In NOD intervention studies initiated at diagnosis, $n = 6$ mice were used, powered to detect an approximate cure rate of >50% with 80% power. For these statistical analyses, $P < 0.05$ was considered significant.

RESULTS

Effect of atorvastatin in vitro on IFN-γ–inducible MHC class II expression on islet endothelium, peritoneal macrophages, and splenocytes. The anti-inflammatory effects of statins have been attributed to pleiotropic properties that include downregulation of inducible (but not constitutive) MHC class II molecules and inhibition of type 1 cytokine effector function. Since both of these properties are relevant to the prevention of autoimmune diabetes, which is associated with islet hyperexpression of MHC class II molecules on islet endothelium and Th1-like autoreactivity (10,11), we carried out assays in vitro, designed to assess these parameters.

Human islet endothelial cells (Fig. 1*A*) and NOD mouse peritoneal macrophages (Fig. 1*B*) were stimulated with IFN-γ in the presence or absence of atorvastatin. In agreement with previous observations, we found that atorvastatin inhibits IFN-γ–dependent MHC class II upregulation on these cell types, which represent nonprofessional and professional antigen-presenting cells, respectively (2–4). Prior studies have shown that this immunomodulatory effect of atorvastatin is mediated by the inhibition of the inducible promoter IV of the class II

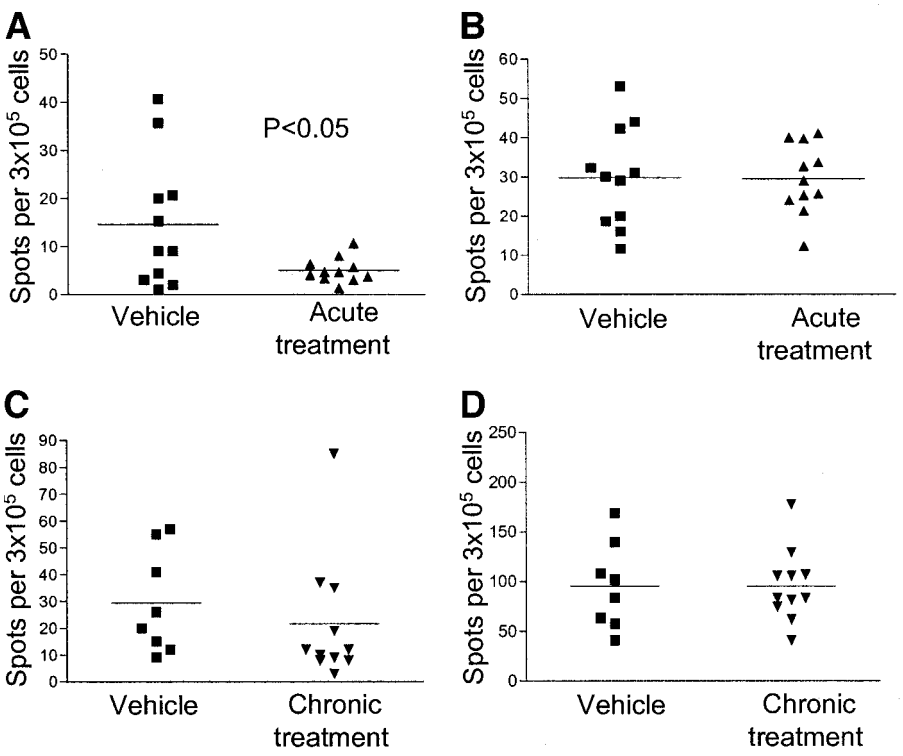

FIG. 4. Effect in vivo of atorvastatin treatment on pathogenic CD8 T-cell function. ELISPOT measurement of IFN-γ–producing cells in response to NRP-V7 peptide or polyclonal stimulus (10 μg/ml ConA) by splenocytes isolated from control, vehicle-treated (n = 11), and atorvastatin-treated (50 mg · kg^{-1} · day^{-1}) (n = 11) mice. *A*: Acute statin treatment for the duration of 12 days resulted in a significant inhibition of IFN-γ production following NRP-V7 peptide stimulation in the treated group; $P < 0.05$. *B*: This reduction in IFN-γ production was antigen specific since there was no difference between Con A-mediated IFN-γ production in the control and treated mice. *C*: In contrast, chronic atorvastatin treatment (n = 12, mean treatment duration of 9 weeks) administered until mice became diabetic has no effect on IFN-γ production in response to NRP-V7 peptide by splenocytes when compared with vehicle-treated control mice (n = 8; P = 0.36). No response was seen in the control peptides CVB4 P2C$_{11371–145}$ and tetanus p731, p734. *D*: IFN-γ production in response to the polyclonal stimulus ConA was similar in the chronic statin-treated and control groups.

transactivator (3). Atorvastatin had a negligible effect on MHC class II expression by splenocytes (Fig. 1*C*). This is an expected finding since the majority of MHC class II–positive cells in the murine spleen are B-cells, which express constitutive MHC class II that is not susceptible to modulation by statins.

In vitro effect of atorvastatin on islet autoreactive T-cell proliferation and cytokine production. Although both CD4 and CD8 T-cells are required for the development of autoimmune diabetes in the NOD mouse, CD8 T-cells appear responsible for the initial β-cell insult and are essential for the destruction of insulin-producing β-cells and the progression of insulitis to overt diabetes (18). On encountering antigen, effector CD8 T-cells produce cytokines such as IFN-γ, observed within 48 h, and enter the cell cycle, which can be detected within 3–5 days. We therefore examined the effect of atorvastatin on both of these parameters, examining autoreactive CD8 T-cell proliferation and cytokine profile using the NRP-V7 mimotope of the IGRP autoantigen recognized by pathogenic CTLs as stimulus. The CTL response against NRP-V7 is an early and progressive event in NOD pre-diabetes, mirroring progression to overt disease, and CD8 T-cells reactive against this epitope are highly potent in disease transfer (19,20). As shown (Fig. 2), atorvastatin treatment in vitro completely blocked the proliferation of CD8 T-cells specific for NRP-V7 peptide. Considering that atorvastatin treatment has no effect on MHC class I expression, the likely explanation for this finding is a direct effect of atorvastatin on cell cycle regulation via inhibition of the synthesis of isoprenoid intermediates, as reported previously (6,21).

Atorvastatin also inhibited polyclonal and IFN-γ production in response to NRP-V7 peptide stimulation in a dose-dependent fashion (Fig. 3*A* and *B*). Interestingly, in line with previous observations (4,6) atorvastatin treatment increased antigen-specific IL-10 production.

In vivo effect of atorvastatin treatment on pathogenic CD8 T-cell function. To establish whether these anti-inflammatory activities of statins are generated and maintained in vivo, we next treated 12-week-old NOD mice for 12 days with 50 mg/kg oral atorvastatin or vehicle and examined NRP-V7 reactivity. As shown in Fig. 4*A*, 12 days of oral atorvastatin significantly reduced the frequency of splenic CD8 T-cells making IFN-γ in response to NRP-V7. This reduction was specific for the autoreactive cells, since the frequency of IFN-γ–producing cells in response to ConA stimulation was identical in treated and control animals (Fig. 4*B*). There was no evidence of expansion of effector cells making IL-4 or IL-10 and no effect of this acute statin therapy on islet infiltration or islet MHC class II molecule expression (data not shown).

CD4 cells producing IFN-γ in response to GAD65$_{217–236}$ and insulin B9-23 were detected infrequently in control mice (2 of 11) and at low levels and were not detected in any atorvastatin-treated mice (data not shown).

Effect of atorvastatin treatment on autoimmune diabetes development. These data, collected both in vitro and in vivo, were resonant with previous reports of the anti-inflammatory effects of statins and provided strong experimental support for the antidiabetic potential of this therapy. We therefore initiated both prevention (therapy starting at 6 weeks of age when islet infiltration is minimal) and intervention (12 weeks of age when the majority of NOD mice have extensive islet infiltration and overt diabetes is incipient) studies using a range of atorvastatin doses. None of these doses were effective at any disease stage (Fig. 5*A–F*). Analysis of splenocyte CD8 T-cell responses in these mice receiving statins for a median of 9 weeks indicated that this failure of therapy was associated with a recovery of NRP-V7 reactivity, such that the frequency of CD8 T-cells making IFN-γ in response to this epitope was not statistically different between vehicle- and drug-treated mice (Fig. 4*C*). Likewise, there was no differ-

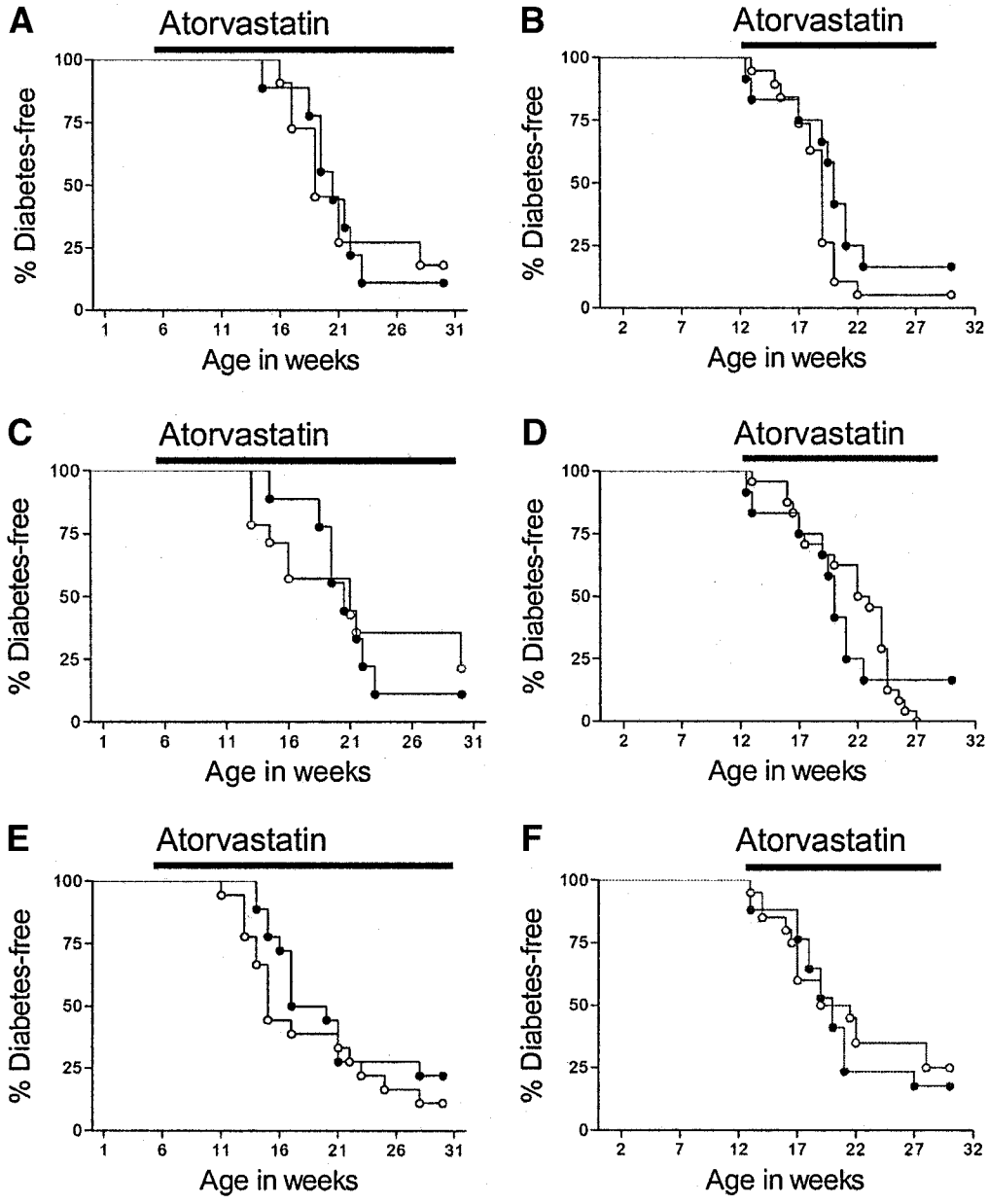

FIG. 5. Effect of atorvastatin treatment on autoimmune diabetes development. *A*: Low dose of atorvastatin (1 mg/kg) (○, $n = 11$) or PBS control (●, $n = 9$) has no effect on diabetes incidence when given orally as a preventive treatment from 6 weeks of age or as a curative treatment from 12 weeks of age (treated $n = 19$ and PBS control $n = 12$) (*B*). A moderate atorvastatin dose of 10 mg/kg was also ineffective at reducing the incidence of diabetes whether given from 6 weeks of age (treated $n = 14$ and PBS control $n = 9$) (*C*) or from 12 weeks of age (treated $n = 24$ and PBS control $n = 11$) (*D*). Finally, high-dose therapy at 50 mg/kg had no preventive or treatment effect when given from 6 weeks of age (treated $n = 17$ and PBS control $n = 18$) (*E*) or 12 weeks of age (treated $n = 19$ and PBS control $n = 17$) (*F*). Duration of atorvastatin therapy is represented by horizontal line.

ence in the frequency of IFN-γ–producing cells in response to ConA stimulation (Fig. 4*D*).

Effect of atorvastatin on established disease. In light of our observation that statin-mediated effects on autoreactive CD8 T-cells were acute and short-lived, we reasoned that a beneficial effect of these drugs might be seen if they were given at disease onset. However, oral atorvastatin given at the stage of overt diabetes had no effect in controlling rising blood glucose concentration (Fig. 6).

DISCUSSION

This is the first study to examine the effect of statin treatment using a spontaneous and chronic model of autoimmunity, as well as the first to do so in a model of autoimmune diabetes. We selected a statin with a powerful profile of effects in vitro on several of the key immunopathological features of autoimmune diabetes, which include upregulated MHC class II expression on islet vessels (11) and activation of islet-specific CD8 effector T-cells (18), and one that has previous efficicacy in Th1-mediated autoimmune disease models (4). Indications of therapeutic potential that were obtained in our studies in vitro were bolstered by an equally powerful effect in vivo on effector T-cells, demonstrated by a 66% diminution in the frequency of pathogenic NRP-V7 reactive CD8 T-cells, the circulating number of which is tightly linked to diabetes development (19). Remarkably, cytokine production by T-cells receiving a polyclonal stimulus was unaffected,

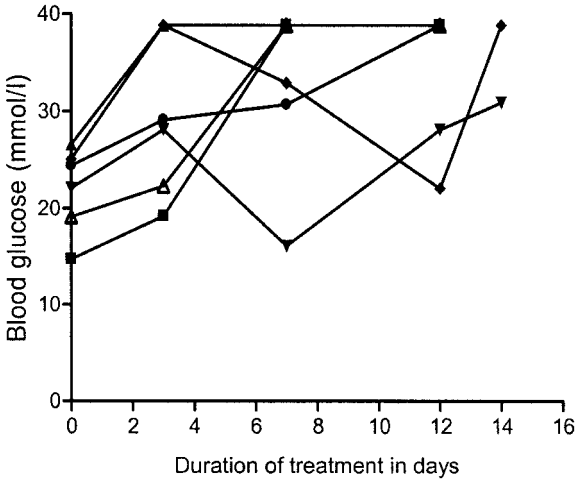

FIG. 6. Effect of atorvastatin on established disease treatment of mice ($n = 6$) at the stage of diabetes diagnosis with 50 mg/kg of atorvastatin did not control or reduce blood glucose concentration. Each symbol represents a single diabetic mouse.

presumably reflecting the greater effect of statins on concurrently activated cells. However, this beneficial effect on autoantigen-specific T-cells was short-lived. As the number of NRP-V7 reactive CD8 T-cells recovered over subsequent weeks, progression to overt diabetes development was undiminished. Therapy administered at 12 weeks of age, when diabetes onset is imminent, was also ineffective. Moreover, the acute beneficial effects of statins were insufficiently powerful to rescue NOD mice at the stage of overt diabetes.

In keeping with guidelines suggested recently for ensuring appropriate study of potential therapies in NOD mice (22), we assessed low, medium, and high doses of statins and used protocols that would evaluate early and late prevention as well as late intervention. Nonetheless, the outcome of this comprehensive analysis is that there is no suggestion from the survival tables of an effect of atorvastatin on diabetes progression. These results contrast with the clear success of statins in other models of human autoimmune disease, such as EAE and CIA (4,6,7). However, key features of these models are the accurate control of their induction, their short natural history, and their tendency, especially in the case of EAE, toward remission in the medium term. Human autoimmune diseases, on the other hand, typically have an unknown initiation, and varying but long preclinical prodromes. In this respect, the NOD mouse is a more faithful model of human autoimmune disease, being spontaneous and chronic. It is not immediately apparent why atorvastatin, which demonstrates such powerful effects in vitro, should have failed to abrogate progression to diabetes in NOD mice. One possibility is that the anti-inflammatory effects of statins lack sufficient power to impact upon established, chronic disease processes, and consistent with this is the observation that prolonged clinical use of statins has not been associated with any adverse events that could be attributed to chronic immune suppression. The observed effect on pathogenic CD8 T-cells was transient, and it is known from studies in which the high-avidity clonotypes of IGRP-reactive CD8 T-cells are deleted that such a maneuver may still fail to affect the rate of disease progression, as new clonotypes emerge (23). A second possibility is that our result reflects the known interstrain variations in sensitivity to the effects of statins (24). Finally, our findings might also be explained by metabolic compensation for the enzyme-blocking effects, since rodents are known to be capable of rapid upregulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (25) and the inhibition of pathogenic CD8 effector cells by atorvastatin that we demonstrate was very transient in nature. This explanation would account for the apparent powerful therapeutic effects of statins in induced acute models of autoimmunity such as EAE and CIA but not in the chronic model of inflammation represented by the NOD mouse.

The results from the initial small-scale clinical trials in the chronic human homologues of EAE and CIA illustrate the aforementioned difficulties associated with translating and relating results obtained from mouse studies to human disease. Vollmer et al. (9) recently reported the results of the first human clinical trial of the efficacy of statin treatment in multiple sclerosis patients, in which 30 patients with relapsing/remitting disease were given 80 mg/day of oral simvastatin for 6 months. In contrast to the results obtained from mouse studies, there were no changes in immunological parameters and cytokine profiles after statin treatment. Nevertheless, using baseline versus treatment comparison, they observed a reduction in disease activity. Considering the study design (lack of placebo control, small size, short follow-up), these results should be considered preliminary in nature. Similarly, McCarey et al. (8) reported a modest effect on clinical disease and inflammatory markers following a 6-month controlled trial in patients with rheumatoid arthritis. The effect of statin treatment on established disease was marginal compared with existing antirheumatic therapy, and again, this was a study of limited size and short term. Given the improvement in symptoms and the excellent safety profile of statins, it is possible that in rheumatoid arthritis, it will represent a useful adjunctive therapy rather than a disease-modifying one.

The results of our study reflect some of the vicissitudes of attempting to explore potential therapies for human disease using animal models, an area of intense recent debate (22,26). The question remains as to whether statins should be discarded as potential agents in the prevention or treatment of type 1 diabetes in humans because of the failed efficacy in NOD mice or whether the many caveats that apply to lessons from animal models should be heeded. High doses were used in our study to induce changes in pathogenic CD8 T-cells, and perhaps higher doses than are currently used will be required in humans to realize similar effects. It is noteworthy that the cholesterol-lowering properties of statins in mice are minimal and transient, whereas they are effective and sustained in humans. Thus maintained, high doses in humans may be worthy of consideration. Small-scale clinical studies may be required in the future to focus on evaluating the immunological efficacy of statin treatment using surrogate markers, as well as the dose required. However, it is possible that statins, like other immunomodulatory agents, may be beneficial in certain inflammatory diseases, have no effect in some (27), and cause deterioration of others (22).

ACKNOWLEDGMENTS

This work was supported by Diabetes UK.

REFERENCES

1. Sadeghi MM, Collinge M, Pardi R, Bender JR: Simvastatin modulates cytokine-mediated endothelial cell adhesion molecule induction: involvement of an inhibitory G protein. *J Immunol* 165:2712–2718, 2000
2. Sadeghi MM, Tiglio A, Sadigh K, O'Donnell L, Collinge M, Pardi R, Bender JR: Inhibition of interferon-gamma-mediated microvascular endothelial cell major histocompatibility complex class II gene activation by HMG-CoA reductase inhibitors. *Transplantation* 71:1262–1268, 2001
3. Kwak B, Mulhaupt F, Myit S, Mach F: Statins as a newly recognized type of immunomodulator. *Nat Med* 6:1399–1402, 2000
4. Youssef S, Stuve O, Patarroyo JC, Ruiz PJ, Radosevich JL, Hur EM, Bravo M, Mitchell DJ, Sobel RA, Steinman L, Zamvil SS: The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature* 420:78–84, 2002
5. Weitz-Schmidt G, Welzenbach K, Brinkmann V, Kamata T, Kallen J, Bruns C, Cottens S, Takada Y, Hommel U: Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat Med* 7:687–692, 2001
6. Aktas O, Waiczies S, Smorodchenko A, Dorr J, Seeger B, Prozorovski T, Sallach S, Endres M, Brocke S, Nitsch R, Zipp F: Treatment of relapsing paralysis in experimental encephalomyelitis by targeting Th1 cells through atorvastatin. *J Exp Med* 197:725–733, 2003
7. Leung BP, Sattar N, Crilly A, Prach M, McCarey DW, Payne H, Madhok R, Campbell C, Gracie JA, Liew FY, McInnes IB: A novel anti-inflammatory role for simvastatin in inflammatory arthritis. *J Immunol* 170:1524–1530, 2003
8. McCarey DW, McInnes IB, Madhok R, Hampson R, Scherbakov O, Ford I, Capell HA, Sattar N: Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. *Lancet* 363:2015–2021, 2004
9. Vollmer T, Key L, Durkalski V, Tyor W, Corboy J, Markovic-Plese S, Preiningerova J, Rizzo M, Singh I: Oral simvastatin treatment in relapsing-remitting multiple sclerosis. *Lancet* 363:1607–1608, 2004
10. Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, Roep BO, Peakman M: Autoreactive T-cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J Clin Invest* 113:451–463, 2004
11. Somoza N, Vargas F, Roura-Mir C, Vives-Pi M, Fernandez-Figueras MT, Ariza A, Gomis R, Bragado R, Marti M, Jaraquemada D, et al.: Pancreas in recent onset insulin-dependent diabetes mellitus: changes in HLA, adhesion molecules and autoantigens, restricted T-cell receptor V beta usage, and cytokine profile. *J Immunol* 153:1360–1377, 1994
12. Greening JE, Tree TI, Kotowicz KT, van Halteren AG, Roep BO, Klein NJ, Peakman M: Processing and presentation of the islet autoantigen GAD by vascular endothelial cells promotes transmigration of autoreactive T-cells. *Diabetes* 52:717–725, 2003
13. Smerdon RA, Peakman M, Hussain MJ, Vergani D: Lymphocyte vaccination prevents spontaneous diabetes in the non-obese diabetic mouse. *Immunology* 80:498–501, 1993
14. Xu XJ, Gearon C, Stevens E, Vergani D, Baum H, Peakman M: Spontaneous T-cell proliferation in the non-obese diabetic mouse to a peptide from the unique class II MHC molecule, I-Ag7, which is also protective against the development of autoimmune diabetes. *Diabetologia* 42:560–565, 1999
15. Gearon CL, Hussain MJ, Vergani D, Peakman M: Lymphocyte vaccination protects prediabetic non-obese diabetic mice from developing diabetes mellitus. *Diabetologia* 40:1388–1395, 1997
16. Zanone MM, Favaro E, Doublier S, Lozanoska-Ochser B, Deregibus MC, Greening J, Huang GC, Klein N, Cavallo Perin P, Peakman M, Camussi G: Expression of nephrin by human pancreatic islet endothelial cells. *Diabetologia*, 2005
17. Varela-Calvino R, Skowera A, Arif S, Peakman M: Identification of a naturally processed cytotoxic CD8 T-cell epitope of coxsackievirus B4, presented by HLA-A2.1 and located in the PEVKEK region of the P2C nonstructural protein. *J Virol* 78:13399–13408, 2004
18. Liblau RS, Wong FS, Mars LT, Santamaria P: Autoreactive CD8 T-cells in organ-specific autoimmunity: emerging targets for therapeutic intervention. *Immunity* 17:1–6, 2002
19. Trudeau JD, Kelly-Smith C, Verchere CB, Elliott JF, Dutz JP, Finegood DT, Santamaria P, Tan R: Prediction of spontaneous autoimmune diabetes in NOD mice by quantification of autoreactive T-cells in peripheral blood. *J Clin Invest* 111:217–223, 2003
20. Amrani A, Verdaguer J, Serra P, Tafuro S, Tan R, Santamaria P: Progression of autoimmune diabetes driven by avidity maturation of a T-cell population. *Nature* 406:739–742, 2000
21. Liao JK: Isoprenoids as mediators of the biological effects of statins. *J Clin Invest* 110:285–288, 2002
22. Shoda LK, Young DL, Ramanujan S, Whiting CC, Atkinson MA, Bluestone JA, Eisenbarth GS, Mathis D, Rossini AA, Campbell SE, Kahn R, Kreuwel HT: A comprehensive review of interventions in the NOD mouse and implications for translation. *Immunity* 23:115–126, 2005
23. Han B, Serra P, Amrani A, Yamanouchi J, Maree AF, Edelstein-Keshet L, Santamaria P: Prevention of diabetes by manipulation of anti-IGRP autoimmunity: high efficiency of a low-affinity peptide. *Nat Med* 11:645–652, 2005
24. Gegg ME, Harry R, Hankey D, Zambarakji H, Pryce G, Baker D, Adamson P, Calder V, Greenwood J: Suppression of autoimmune retinal disease by lovastatin does not require Th2 cytokine induction. *J Immunol* 174:2327–2335, 2005
25. Kita T, Brown MS, Goldstein JL: Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in livers of mice treated with mevinolin, a competitive inhibitor of the reductase. *J Clin Invest* 66:1094–1100, 1980
26. Roep BO, Atkinson M, von Herrath M: Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes. *Nat Rev Immunol* 4:989–997, 2004
27. Raki M, Molberg O, Tollefsen S, Lundin KE, Sollid LM: The effects of atorvastatin on gluten-induced intestinal T cell responses in coeliac disease. *Clin Exp Immunol* 142:333–340, 2005