BMC Cardiovascular Disorders

BioMed Central

Research article

Open Access

HMG-CoA reductase inhibition aborts functional differentiation and triggers apoptosis in cultured primary human monocytes: a potential mechanism of statin-mediated vasculoprotection

Joannis E Vamvakopoulos*[1,3] and Colin Green[2]

Address: [1]Department of Surgery, University of Cambridge, Cambridge, UK, [2]Northwick Park Institute for Medical Research, Harrow, UK and [3]Current address: Rational Drug Design Program, Biomedicum Helsinki, Helsinki, Finland

Email: Joannis E Vamvakopoulos* - joannis@scientist.com; Colin Green - s.jenks@ic.ac.uk

* Corresponding author

Published: 19 July 2003

BMC Cardiovascular Disorders 2003, **3**:6

Received: 26 February 2003
Accepted: 19 July 2003

This article is available from: http://www.biomedcentral.com/1471-2261/3/6

Abstract

Background: Statins effectively lower blood cholesterol and the risk of cardiovascular death. Immunomodulatory actions, independent of their lipid-lowering effect, have also been ascribed to these compounds. Since macrophages participate in several vascular pathologies, we examined the effect of statin treatment on the survival and differentiation of primary human monocytes.

Methods: Peripheral blood mononuclear cells (PBMCs) from healthy individuals were cultured in the presence or absence of mevastatin. Apoptosis was monitored by annexin V / PI staining and flow cytometry. In parallel experiments, cultures were stimulated with LPS in the presence or absence of mevastatin and the release of IL-1β and IL-1Ra was measured by ELISA.

Results: Among PBMCs, mevastatin-treated monocytes were particularly susceptible to apoptosis, which occurred at doses >1 microM and was already maximal at 5 microM. However, even at the highest mevastatin dose used (10 microM), apoptosis occurred only after 24 h of culture, possibly reflecting a requirement for cell commitment to differentiation. After 72 h of treatment the vast majority (>50%) of monocytes were undergoing apoptosis. Stimulation with LPS revealed that mevastatin-treated monocytes retained the high IL-1β output characteristic of undifferentiated cells; conversely, IL-1Ra release was inhibited. Concurrent treatment with mevalonolactone prevented the induction of apoptosis and suppressed both IL-1β and IL-1Ra release in response to LPS, suggesting a rate-limiting role for HMG-CoA reductase in monocyte differentiation.

Conclusions: Our findings indicate that statins arrest the functional differentiation of monocytes into macrophages and steer these cells into apoptosis, suggesting a novel mechanism for the vasculoprotective properties of HMG-CoA reductase inhibitors.

Background

Advanced coronary artery disease (CAD) is currently a leading cause of morbidity and mortality in the western world and the most common indication for heart transplantation. Even after successful transplantation, allograft vasculopathy affects as many as 60% of cardiac grafts

within one year [1] and is the principal cause of late graft loss. While their natural histories differ, CAD and allograft vasculopathy share certain features of their pathogenesis and histopathology. Prominent among these features is the recruitment and retention of peripheral blood monocytes in the vascular wall, an event that is thought to trigger the formation of vascular lesions.

Monocyte-derived macrophages play a central role in the pathogenesis of both native atherosclerosis and allograft vasculopathy [2,3]. Macrophages are an integral cellular component of the atherosclerotic plaque where they function to sequester lipids, giving rise to "foam cells". The release of extracellular matrix-degrading proteases by these cells, combined with their pro-apoptotic effect on adjacent vascular smooth muscle cells [4], are thought to destabilise the plaque, progressively leading to rupture. Furthermore, atherosclerotic plaque macrophages promote local coagulation by releasing prothrombotic mediators such as tissue factor. Similarly, though their precise role remains poorly defined, macrophages abound in the neointimal lesions associated with allograft vasculopathy and formation of these lesions is defective in macrophage-deficient mice [3]. Moreover, treatments that have proven effective in reducing neointimal lesion formation also reduced the macrophage burden of the lesion [5,6]. It seems likely, therefore, that macrophage turnover in the vascular wall may influence the rate of progression of both native atherosclerosis and allograft vasculopathy.

Statins are highly efficacious in controlling hyperlipidaemia and reducing the risk of acute coronary events and cardiovascular death [7]. The biological activity of statins stems from their chemical structure, which resembles that of mevalonic acid. Statins suppress *de novo* cholesterol biosynthesis by inhibiting HMG-CoA reductase, the rate-limiting enzyme of the mevalonate pathway [8]. Amazingly, statin therapy is well tolerated with few major adverse effects, usually attributable to metabolic interactions with other drugs [9].

Early animal studies suggested that, in addition to its anti-atherosclerotic effect, statin treatment might also attenuate the development of allograft vasculopathy [10,11]. The first evidence for an association of post-transplant statin treatment with reduced incidence and progression of allograft vasculopathy in human cardiac allograft recipients came from a prospective study by Kobashigawa *et al* [12]. This finding was subsequently corroborated by others [13] and spurred interest in characterising the vasculoprotective effects of statins [14,15].

Statins are now known to have multiple effects on native cellular components of the vascular wall as well as on monocytes / macrophages [16]. Given the involvement of macrophages in CAD and allograft vasculopathy, one plausible mechanism through which statins exert their vasculoprotective actions could be the induction of macrophage apoptosis. When grown *in vitro* monocytes differentiate into macrophages, a phenotypic transition heralded by down-regulation of the IL-1β response to lipopolysaccharide (LPS) [17,18]. Using this simple model, we explored the hypothesis that mevastatin treatment arrests monocyte-to-macrophage differentiation and, instead, steers these cells into apoptosis.

Methods

All aqueous solutions were prepared using endotoxin-free water from a MilliQ Biocel purification unit (Millipore, Bedford MA) and filter-sterilised. Reagents were obtained from Sigma (St Louis MO) unless otherwise indicated.

Subjects and materials

Peripheral blood mononuclear cells (PBMCs) obtained by venipuncture from six individuals were used in this study. All subjects were healthy volunteers recruited using procedures and documentation approved by the Cambridge Local Ethics Committee.

Mevastatin was reconstituted to 4 mM in dimethyl sulfoxide (DMSO), stored at 4°C in the dark and added to cell cultures at a final concentration of 10 μM. Mevalonolactone (ICN Biochemicals, Cleveland OH), a membrane-permeable internal ester of mevalonate that is hydrolysed by cytoplasmic esterases, was reconstituted to 40 mM in DMSO, stored at 4°C in the dark and used at a final concentration of 100 μM. Lipopolysaccharide (LPS; from E coli strain 055:B5) was dissolved in phosphate-buffered saline (PBS) and used at 5 μg/mL final concentration. Recombinant human gamma-interferon (γIFN; Peprotech, London UK) was reconstituted in PBS and used at 50 ng/mL (1000 U/mL) final concentration.

PBMC purification and handling

Blood was processed immediately after phlebotomy. PBMCs were purified by centrifugation over Percoll (AP Biotech, Piscataway NJ), spin-washed once with cold modified Hanks' Balanced Salt Solution (containing 5 mM Na_4EDTA, 1% BSA) and once with cold PBS at 250 × g for 5 min, resuspended in PBS and counted. The monocytic ($CD33^+hi$) content of the PBMC population and the extent of neutrophil contamination were quantified by immunofluorescent staining of 5×10^5 cells with an anti-human CD33-FITC MAb (clone WM53; Serotec, Oxford UK) and analysis by flow cytometry. All PBMC preparations used in this study were essentially neutrophil-free (neutrophil content <0.5%).

The remaining PBMCs were resuspended in RPMI 1640 medium and recounted. Aliquots of this suspension,

calculated to contain 2.5 × 10^5 $CD33^+$hi cells each, were seeded in individual wells of a 24-well tissue culture plate. Each well contained 1 mL of pre-warmed complete growth medium (RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin). Cell culture was performed at 37°C / 5% CO_2 in a humidified incubator.

Analysis of apoptotic monocytes

Apoptotic cells were identified by double supravital staining with recombinant FITC-conjugated annexin V and propidium iodide (PI) [19], using the TACS Apoptosis Detection kit (R&D Systems, Minneapolis MN) according to the manufacturer's instructions. All cultures were performed and assayed in triplicate. Flow cytometric analysis was performed immediately after supravital staining. For each sample, 2,500 events were obtained in the monocyte region and gated onto a fluorescence dot plot, where the fraction of total annexin V-positive cells was determined. Common settings for forward / side scatter, fluorescence gains, colour compensation and fluorescence threshold were used throughout the study. These were determined by assaying unstained and single-stained cells (with PI or annexin V alone).

Measurement of cytokine release

The concentrations of IL-1β and IL-1Ra in cell culture supernatants were determined by ELISA using human Cytoset™ kits (BioSource, Camarillo CA) according to the manufacturer's instructions. Cell culture supernatants were depleted of residual cells and cell debris by centrifugation, collected in microtubes and stored at -20°C prior to the assay. Each supernatant was thawed once and both cytokines were assayed in parallel. All standards, samples and controls were assayed in duplicate.

Statistical analysis

Data were compiled and analysed using MicroSoft Excel™ (MicroSoft, Redmont WA). Descriptive statistics (mean; standard deviation; standard error of mean) were calculated for each treatment group. For both the apoptosis and cytokine release studies, group means were compared using the appropriate version of Student's unpaired t-test, as determined by the F-test for equality of variances. Test results are reported as two-tailed p values, where $p < 0.05$ was considered statistically significant. Summary data are reported as mean +/- SEM.

Results

Mevastatin-treated differentiating human monocytes undergo delayed apoptosis

Prolonged mevastatin treatment was associated with massive apoptosis of cultured human peripheral blood monocytes, but not lymphocytes (figure 1). Incubation with mevastatin for 24 h did not compromise monocyte viability when compared to untreated or vehicle-treated cell cultures. However, a 48-h treatment with mevastatin resulted in the appearance of a large apoptotic monocyte population, as defined by light scatter criteria (granular cells of diminishing size) and annexin V-positive staining. At this time-point, 27.5 +/-1.8% of mevastatin-treated monocytes were undergoing apoptosis compared with 8.8 +/-1.6% of vehicle-treated monocytes ($p = 0.001$; figure 2).

Monocyte viability was severely compromised after 72 h of mevastatin treatment, when 53.0 +/- 3.9% of monocytes were undergoing apoptosis compared to 12.9 +/-1.3% of vehicle-treated monocytes ($p = 0.0006$; figure 2). Dose-response experiments showed that mevastatin triggered apoptosis at concentrations >1 μM ($EC_{50} \sim 2.5$ μM at 72 h; data not shown) and that this effect was already maximal at 5 μM. Consistent with 48-h time-point observations, flow cytometric analysis revealed relatively few annexin V single-positive monocytes (<18% of apoptotic population), indicating rapid progression to apoptosis. In contrast to the lymphocyte population, the size of which did not vary significantly over time, viable monocyte numbers had plummeted after 72 h of treatment with mevastatin (figure 1C).

Supplementation of mevastatin-treated cultures with mevalonolactone attenuated monocyte apoptosis by 83.1% (range 72.6–99.3%; $p = 0.01$) and 77.4% (range 56.4–90.9%; $p = 0.01$) at 48 h and 72 h, respectively (figure 2).

Mevastatin treatment arrests monocyte differentiation in vitro

Interleukin-1β release after LPS stimulation is suppressed early during monocyte-to-macrophage differentiation *in vitro* and *in vivo*. Continuous mevastatin treatment of non-stimulated human PBMCs for 48 h did not affect constitutive IL-1β output (47.7 +/- 13.6 *vs* 45.1 +/- 9.0 pg/mL in vehicle-treated cultures). However, when LPS was added at 24 h mevastatin-treated cells released approximately 40-fold more IL-1β compared to vehicle-treated cells (15,559.1 +/- 5,518 *vs* 389.2 +/- 163 pg/mL; $p = 0.05$), a level comparable to that obtained with freshly harvested, undifferentiated monocytes (figure 3A).

Gamma-interferon is known to potentiate induction of IL-1β by LPS [20]. In this study, incubation of vehicle-treated monocytes with γIFN for 6 h prior to addition of LPS increased IL-1β output approximately 4-fold. Under the same conditions, mevastatin-treated monocytes released approximately 11-fold more IL-1β than vehicle-treated cells (18,423.4 +/- 4,152 *vs* 1,649.7 +/- 338.4 pg/mL; $p = 0.03$), although the enhancing effect of γIFN was no longer apparent.

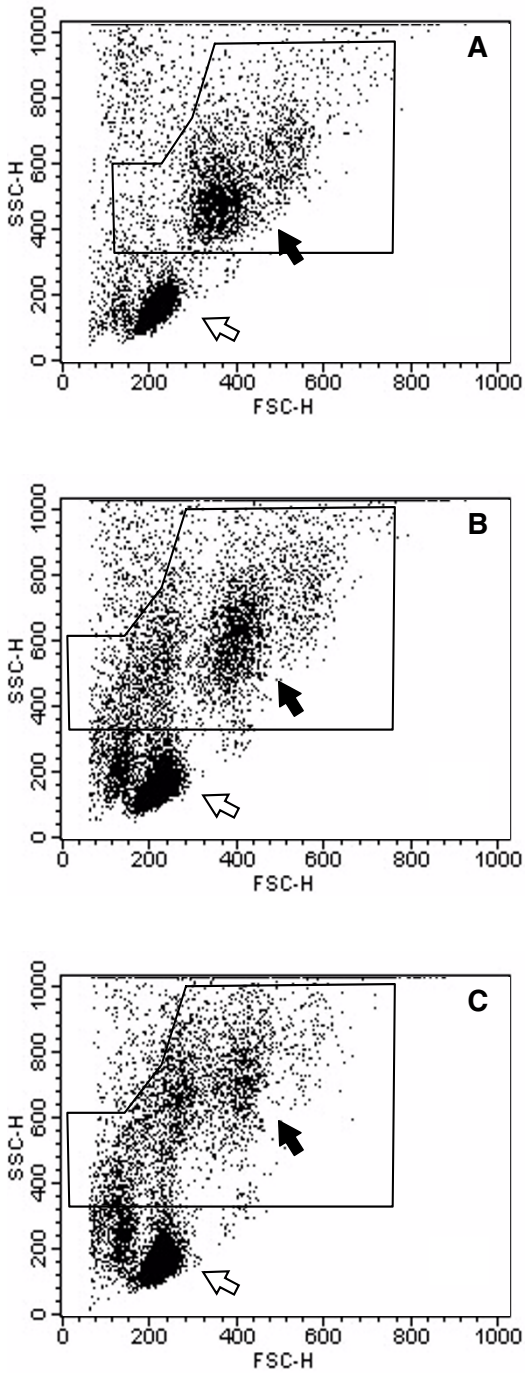

Figure 1
Mevastatin-treated differentiating monocytes are particularly susceptible to apoptosis. After 24 h of statin treatment monocytes still constituted a single, viable cell population (panel **A**, black arrow). By 48 h this viable monocytic population had decreased in size and a second population of smaller, annexin V-positive cells had appeared **(B)**. By 72 h the majority of monocytes were undergoing apoptosis **(C)**. Conversely, peripheral blood lymphocytes (white arrow) remained largely viable throughout the observation period. Results shown here are representative of 18 experiments (three per individual).

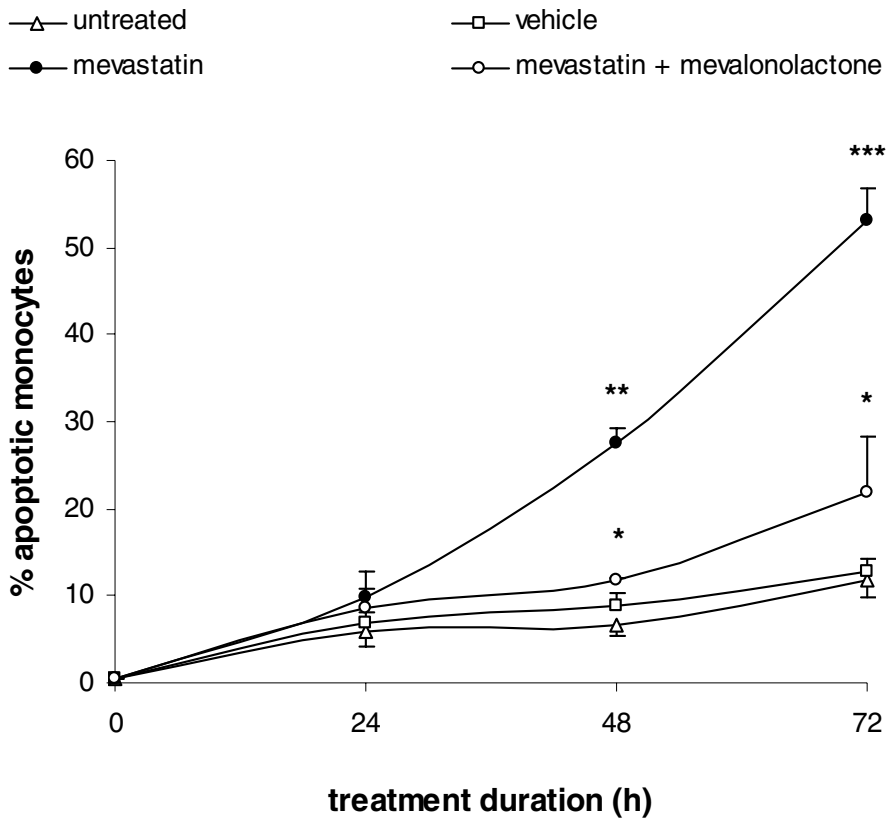

Figure 2
Kinetics of apoptosis in mevastatin-treated human peripheral blood monocytes. Cultures were treated with vehicle (0.25% DMSO); 10 μM mevastatin; or mevastatin plus 100 μM mevalonolactone. Error bars represent SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Inclusion of mevalonolactone in the growth medium completely prevented the mevastatin-induced blockade of monocyte differentiation, both in the presence and in the absence of γIFN (p = 0.02 and 0.05, respectively; figure 3A). In fact, mevalonolactone supplementation further suppressed IL-1β release below that of vehicle-treated monocytes. When LPS was used alone, mevalonolactone-treated monocytes released 62% less IL-1β than vehicle-treated cells (147.7 +/- 38.3 *vs* 389.2 +/- 163.0 pg/mL; p = 0.25). When LPS was used in combination with γIFN, mevalonolactone suppressed IL-1β release by 81.5%, compared to vehicle treatment (305.2 +/- 65.3 *vs* 1,649.7 +/- 338.4 pg/mL; p = 0.03).

Mevastatin suppresses LPS-induced IL-1Ra release from differentiating monocytes

The interleukin-1 receptor antagonist is an important endogenous regulator of IL-1. IL-1Ra inducibility by LPS is also progressively down-regulated during monocyte-to-macrophage differentiation [21]. In contrast to our observations with IL-1β, mevastatin treatment of non-stimulated PBMCs down-regulated constitutive IL-1Ra output at 48 h by approximately 40% (p = 0.24; figure 3B); interestingly, the magnitude of this reduction is similar to the rate of monocyte apoptosis seen at that same time-point. Following LPS stimulation, mevastatin-treated monocytes again released less IL-1Ra than vehicle-treated cells (p = 0.29). This consistent observation reached statistical significance after combined γIFN / LPS stimulation, when mevastatin-treated monocytes released approximately

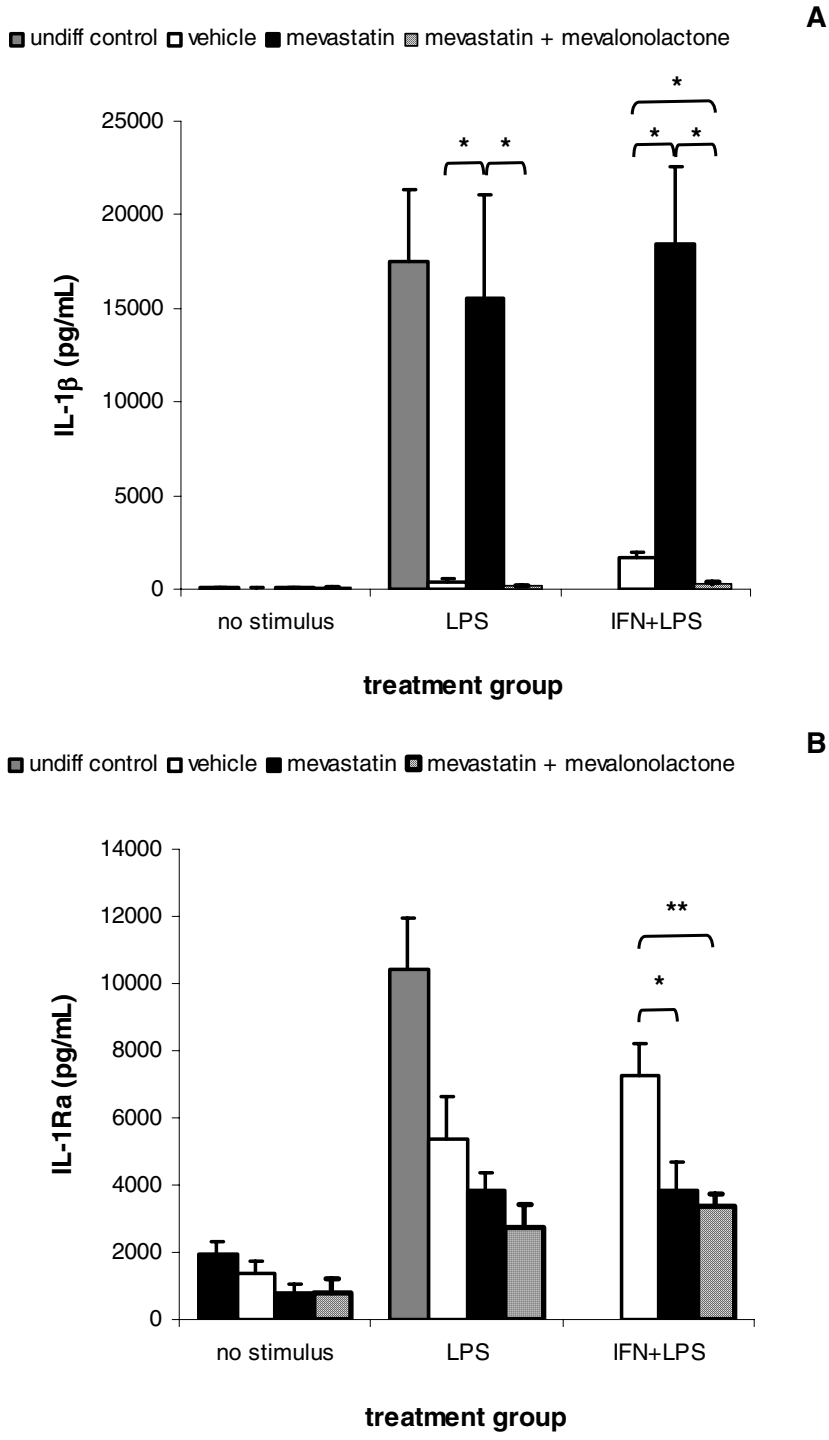

Figure 3
Mevastatin arrests the functional maturation of cultured human peripheral blood monocytes. LPS was added to PBMC cultures either at the onset (undifferentiated control), or after 24 h, of cell culture and supernatants were harvested 24 h later in each case. Pre-treatment with γIFN was only applicable to differentiating cells. Mevastatin treatment preserved monocyte responsiveness to LPS in terms of IL-1β release **(A)** but suppressed IL-1Ra release **(B)**. *, $p < 0.05$; **, $p < 0.01$ (brackets indicate groups being compared).

50% less IL-1Ra than vehicle-treated cells (3,836.2 +/- 841.6 *vs* 7,286.9 +/- 928.8 pg/mL; p = 0.03). Crucially, mevalonolactone apparently failed to prevent the suppression of IL-1Ra release by mevastatin (figure 3B).

Discussion

Fibroproliferative vascular disease, thought to arise as a result of endothelial stress or trauma, is characterised by a complex pathology that culminates in a spectrum of manifestations, depending on the nature of the triggering event. Thus, native atherosclerosis is thought to occur mainly as a result of chronic oxidative stress, while mechanical endothelial trauma initiates restenosis after angioplasty or stenting. Lastly, allograft vasculopathy is thought to arise chiefly as a result of endothelial dysfunction precipitated by both immune and non-immune injury. Monocyte recruitment and retention in the vascular wall is a unifying feature of these diverse outcomes and correlates strongly with the timing and severity of vascular lesion formation [22–25].

Peripheral blood monocytes represent a heterogeneous population of immature cells that differentiate upon exiting the circulation. The tissue microenvironment plays a crucial role in initiating and guiding monocyte differentiation: arrest on a solid matrix is generally sufficient to initiate this process, while the presence of particular cytokines and growth factors functions to steer it towards a variety of phenotypes [26–28]. This process can be faithfully reproduced *in vitro* where, in the absence of exogenous growth factors, approximately 80% of peripheral blood monocytes spontaneously develop into macrophages [29]. One of the earliest functional indices of monocyte-to-macrophage differentiation, both *in vitro* and *in vivo*, is a markedly attenuated IL-1β release in response to LPS [17,18].

Here we have demonstrated that mevastatin, an inhibitor of HMG-CoA reductase, abolishes the profound down-regulation of IL-1β release associated with monocyte differentiation; and that this effect coincides temporally with the induction of monocyte-specific apoptosis in human PBMC cultures. While monocyte differentiation proceeds spontaneously *in vitro*, lymphocytes differentiate only in response to signals via the T/B cell receptor complexes, which are lacking in pure PBMC cultures from single individuals; notably, these cells remained viable throughout the observation period. Therefore, unlike previous observations with murine J744 myeloid cells [30], the susceptibility of primary monocytes to statin-induced apoptosis appears to be tightly linked to the process of cell differentiation.

The precise molecular mechanisms underlying these findings are currently under investigation; depletion of downstream products of the mevalonate pathway such as the phosphorylated geranylgeranyl and farnesyl isoprenoids, which would compromise G-protein function, is likely to be involved. Shortage of such intermediates may be exacerbated in the face of increased utilization of the mevalonate pathway that occurs during monocyte differentiation [31], explaining the failure of mevalonolactone supplementation to completely prevent apoptosis in our experimental set-up. Conversely, mevalonolactone appeared to accelerate the suppression of LPS-induced IL-1β release in differentiating monocytes, hinting that the mevalonate pathway regulates this process both dynamically and bi-directionally. Hence this metabolic pathway may represent a promising therapeutic target for diseases involving dysregulated macrophage function.

Monocyte turnover in solid tissue is determined by the balance between recruitment and clearance. Overproduction of monocyte chemoattractant protein (MCP-1) by native vascular cells and macrophages is a major mechanism promoting macrophage accumulation in atherosclerotic [32,33] and neointimal [34,35] lesions. Statin treatment was recently shown to down-regulate MCP-1 production *in vitro* and *in vivo* [36]; and to suppress the release of various other atherothrombotic mediators by plaque macrophages [15,36–40]. However, in most cases, the potential contribution of cell death to these findings was not concurrently investigated. Here we show that HMG-CoA reductase inhibition suppressed the release of IL-1Ra from LPS-stimulated monocytes; but that this suppression was largely accounted for by apoptotic cell death. Unlike IL-1β, the release of which occurs *via* stimulation of the P_2X_7 receptor at the level of the cell surface [41] and is thus refractory to apoptosis, secretion of IL-1Ra follows the classical pathway, which is effectively shut down during apoptosis [42]. While suppression of endothelial MCP-1 synthesis might genuinely contribute to the benefits of statin therapy, we suggest that induction of monocyte apoptosis may underlie many downstream statin actions, including the reduction in plaque macrophage counts and inhibition of *in situ* MMP and TF production [37–40] (figure 4). It is noteworthy that, since osteoclasts are also developmentally derived from peripheral blood monocytes, this hypothesis fits well with the recently proposed role of statins as modulators of bone formation [43].

Interestingly, *in vivo* studies so far do not lend support to our hypothesis. Libby and colleagues have postulated that foam cell apoptosis might perpetuate vascular lipid accumulation, contributing to atherosclerotic plaque growth [2]. Furthermore, in a recent, very elegant study these authors concluded that apoptosis is not involved in the reduction of plaque macrophage counts by cerivastatin and, instead, attributed these effects to suppression of cell

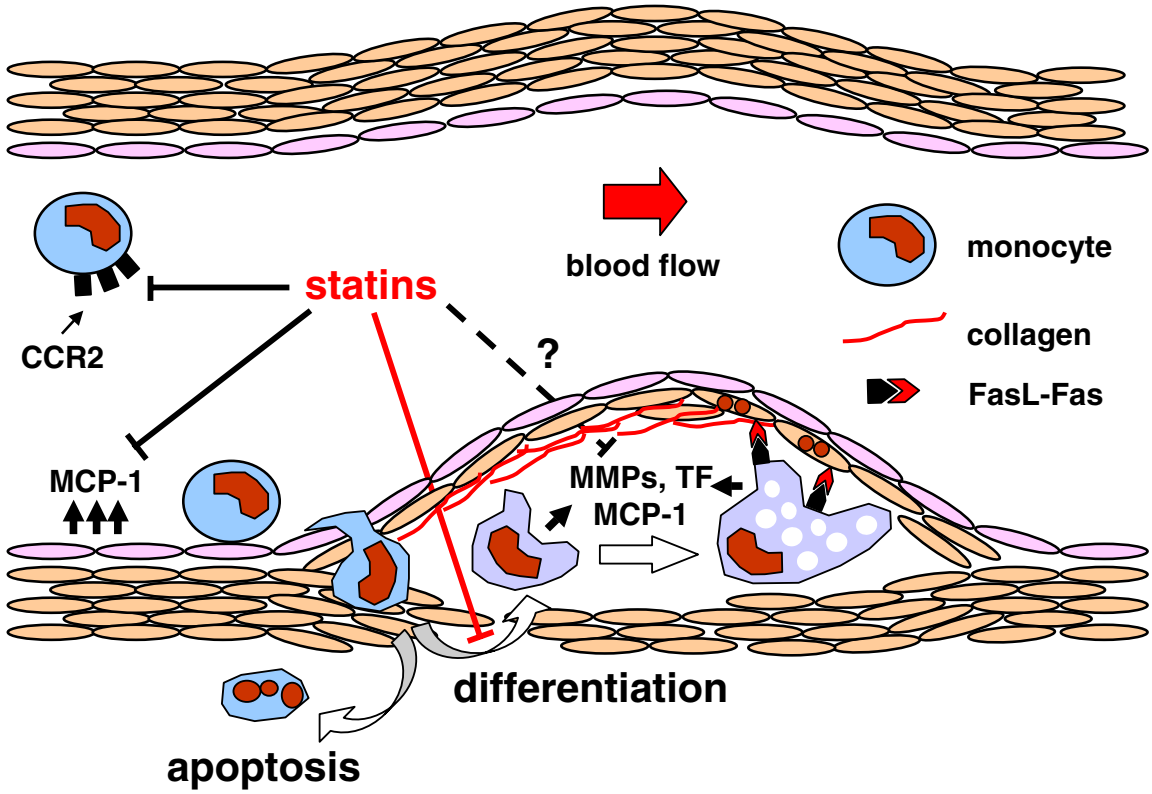

Figure 4
Statin-mediated monocyte apoptosis in the context of atherosclerosis. During formation of the atherosclerotic plaque monocytes are recruited and retained in the vascular wall, where they differentiate into macrophages. Macrophages incorporate lipids giving rise to foam cells, which exhibit a dysregulated phenotype possibly due to oxidative stress. Mature macrophages and foam cells are thought to promote plaque rupture by eroding the fibromuscular cap. This is likely achieved through release of matrix-degrading proteases and also *via* FasL-mediated cytotoxic effects on adjacent vascular smooth muscle cells. Statins modulate both primary vascular release of MCP-1 and monocyte chemotaxis. *In situ* monocyte apoptosis might also contribute to their downstream effects on plaque stability.

growth [38]. The data presented here clearly challenge this conclusion and we feel that rejecting apoptosis as a possible mechanism of statin vasculoprotection may be premature on several counts. Firstly, it is well known that apoptotic cells are rapidly cleared *in vivo* and, in their paper, Aikawa and colleagues acknowledged that very few apoptotic cells were actually observed in both the control and statin-treated groups [38]. In view of this, it is unfortunate that these authors did not assess the role of apoptosis in their *in vitro* studies of monocyte differentiation. Moreover, several reports have cautioned that the TUNEL assay, used by Aikawa *et al*, may not allow accurate quantification of apoptotic cells [44,45]. It might be argued that cerivastatin doses investigated in the aforementioned study (0.01–0.05 μM) reflect therapeutic plasma levels of that drug, which may not be pro-apoptotic. However, in support of our own findings, a recent study by Kaneider *et al* [46] showed significant monocyte pro-apoptotic activity of low-dose (0.01 μM) cerivastatin, in terms of caspase-3 activation. It should be noted that cerivastatin is relatively hydrophilic and a more potent inhibitor of HMG-CoA reductase than mevastatin; thus our findings with mevastatin at relatively high doses may still be therapeutically relevant. Further *in vitro* studies with clinically used statins are currently in progress to address this issue.

Conclusions

HMG-CoA reductase inhibition in human peripheral blood monocytes appears to maintain them in a functionally immature state and to render them susceptible to apoptosis. We suggest that this may represent one mechanism whereby statins decrease macrophage load and the release of prothrombotic mediators in vascular lesions, potentially leading to their prevention or regression.

Competing interests

None declared.

Authors' contributions

JEV conceived and led the design of this study; carried out all experimental work and statistical analysis; and drafted the manuscript. CG participated in the design of this study, supervised its conduct and secured funding. Both authors read and approved the final manuscript.

Acknowledgements

This work was funded by the Northwick Park Institute for Medical Research (NPIMR) and the Papworth Hospital NHS Trust Fund. JV is a CIMO scholar-in-residence (Finnish Ministry of Education) whose work is also supported by grants from the Institute of Biomedical Science (UK).

References

1. Julius BK, Attenhofer Jost CH, Sutsch G, Brunner HP, Kuenzli A, Vogt PR, Turina M, Hess OM and Kiowski W: **Incidence, progression and functional significance of cardiac allograft vasculopathy after heart transplantation** *Transplantation* 2000, **69:**847-853.
2. Libby P, Geng YJ, Aikawa M, Schoenbeck U, Mach F, Clinton SK, Sukhova GK and Lee RT: **Macrophages and atherosclerotic plaque stability** *Curr Opin Lipidol* 1996, **7:**330-335.
3. Shi C, Lee W-S, He Q, Zhang D, Fletcher DL Jr, Newell JB and Haber E: **Immunologic basis of transplant-associated arteriosclerosis** *Proc Natl Acad Sci USA* 1996, **93:**4051-4056.
4. Boyle JJ, Bowyer DE, Weissberg PL and Bennett MR: **Human blood-derived macrophages induce apoptosis in human plaque-derived vascular smooth muscle cells by Fas-ligand / Fas interactions** *Arterioscler Thromb Vasc Biol* 2001, **21:**1402-1407.
5. Azuma H, Nadeau KC, Ishibashi M and Tilney NL: **Prevention of functional, structural and molecular changes of chronic rejection of rat renal allografts by a specific macrophage inhibitor** *Transplantation* 1995, **60:**1577-1582.
6. Niebauer J, Schwarzacher SP, Hayase M, Wang B, Kernoff RS, Cooke JP and Yeung AC: **Local L-arginine delivery after baloon angioplasty reduces monocyte binding and induces apoptosis** *Circulation* 1999, **100:**1830-1835.
7. Rosenson RS and Tangney CC: **Antiatherothrombotic properties of statins: implications for cardiovascular event reduction** *J Am Med Assoc* 1998, **279:**1643-1650.
8. Endo A, Kuroda M and Tanzawa K: **Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity** *FEBS Lett* 1976, **72:**323-326.
9. Christians U, Jacobsen W and Floren LC: **Metabolism and drug interactions of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in transplant patients: are the statins mechanistically similar?** *Pharmacol Ther* 1998, **80:**1-34.
10. Meiser BM, Wenke K, Thiery J, Wolf S, Devens C, Seidel D, Hammer C, Billingham ME and Reichart B: **Simvastatin decreases accelerated graft vessel disease after heart transplantation in an animal model** *Transplant Proc* 1993, **25:**2077-2079.
11. Maggard MA, Ke B, Wang T, Kaldas F, Seu P, Busuttil RW and Imagawa DK: **Effects of pravastatin on chronic rejection of rat cardiac allografts** *Transplantation* 1998, **65:**149-155.
12. Kobashigawa JA, Katznelson S, Laks H, Johnson JA, Yeatman L, Wang XM, Chia D, Terasaki PI, Sabad A, Cogert GA, Trosian K, Hamilton MA, Hage A, Drinkwater D and Stevenson LW: **Effect of pravastatin on outcomes after cardiac transplantation** *N Engl J Med* 1995, **333:**621-627.
13. Wenke K, Meiser B, Thiery J, Nagel D, von Scheidt W, Steinbeck G, Seidel D and Reichart B: **Simvastatin reduces graft vessel disease and mortality after heart transplantation: a four-year randomized trial** *Circulation* 1997, **96:**1398-1402.
14. Katznelson S and Kobashigawa JA: **Dual roles of HMG-CoA reductase inhibitors in solid organ transplantation: lipid lowering and immunosuppression** *Kidney Intl* 1995, **48:**S112-S115.
15. Diomede L, Albani D, Sottocorno M, Donati MB, Bianchi M, Fruscella P and Salmona M: ***In vivo* anti-inflammatory effect of statins is mediated by nonsterol mevalonate products** *Arterioscler Thromb Vasc Biol* 2001, **21:**1327-1332.
16. Koh KK: **Effects of statins on vascular wall: vasomotor function, inflammation, and plaque stability** *Cardiovasc Res* 2000, **47:**648-657.
17. Elias JA, Schreiber AD, Gustilo K, Chien P, Rossman MD, Lammie PJ and Daniele RP: **Differential interleukin-1 elaboration by unfractionated and density fractionated human alveolar macrophages and blood monocytes: relationship to cell maturity** *J Immunol* 1985, **135:**3198-3204.
18. Burchett SK, Weaver WM, Westall JA, Larsen A, Kronheim S and Wilson CB: **Regulation of tumor necrosis factor / cachectin and IL-1 secretion in human mononuclear phagocytes** *J Immunol* 1988, **140:**3473-3481.
19. Vermes I, Haanen C, Steffens-Nakken H and Reutelingsperger C: **A novel assay for apoptosis: flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein-labelled annexin V** *J Immunol Meth* 1995, **184:**39-51.
20. Newton RC: **Effect of interferon on the induction of human monocyte secretion of interleukin-1 activity** *Immunology* 1985, **56:**441-446.
21. Vamvakopoulos JE, Green C and Metcalfe S: **Genetic control of IL-1β bioactivity through differential regulation of the IL-1 receptor antagonist** *Eur J Immunol* 2002, **32:**2988-2996.
22. Moreno PR, Bernardi VH, Lopez-Cuellar J, Newell JB, McMellon C, Gold HK, Palacios IF, Fuster V and Fallon JT: **Macrophage infiltration predicts restenosis after coronary intervention in patients with unstable angina** *Circulation* 1996, **94:**3098-3102.
23. Kim C-J, Khoo JC, Gillotte-Taylor K, Li A, Palinski W, Glass CK and Steinberg D: **Polymerase chain reaction-based method for quantifying recruitment of monocytes to mouse atherosclerotic lesions *in vivo* : enhancement by tumor necrosis factor-alpha and interleukin-1beta** *Atheroscler Thromb Vasc Biol* 2000, **20:**1976-1982.
24. Kitagawa-Sakakida S, Tori M, Li Z, Horiguchi K, Izutani H, Matsuda H and Shirakura R: **Active cell migration in retransplanted rat cardiac allografts during the course of chronic rejection** *J Heart Lung Transplant* 2000, **19:**584-590.
25. Lehr H-A, Sagban TA, Ihling C, Zähringer U, Hungerer KD, Blumrich M, Reifenberg K and Bhakdi S: **Immunopathogenesis of atherosclerosis: endotoxin accelerates atherosclerosis in rabbits on hypercholesterolemic diet** *Circulation* 2001, **104:**914-920.
26. Chomarat P, Banchereau J, Davoust J and Palucka AK: **IL-6 switches the differentiation of monocytes from dendritic cells to macrophages** *Nat Immunol* 2000, **1:**510-514.
27. Abe R, Donnelly SC, Peng T, Bucala R and Metz CN: **Peripheral blood fibrocytes: differentiation pathway and migration to wound sites** *J Immunol* 2001, **166:**7556-7562.
28. Mohamadzadeh M, Berard F, Essert G, Chalouni C, Pulendran B, Davoust J, Bridges G, Palucka AK and Banchereau J: **Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells** *J Exp Med* 2001, **194:**1013-1020.
29. Grage-Griebenow E, Flad H-D and Ernst M: **Heterogeneity of human peripheral blood monocyte subsets** *J Leukoc Biol* 2001, **69:**11-20.
30. Coxon FP, Benford HL, Russell GG and Rogers MJ: **Protein synthesis is required for caspase activation and induction of apoptosis by bisphosphonate drugs** *Mol Pharmacol* 1998, **54:**631-638.
31. Knight BL, Patel DD and Soutar AK: **The regulation of 3-hydroxy-3-methylglutaryl-CoA reductase activity, cholesterol esterification and the expression of low-density lipoprotein recep-**

tors in cultured monocyte-derived macrophages *Biochem J* 1983, **210:**523-532.
32. Nelken NA, Coughlin SR, Gordon D and Wilcox JN: **Monocyte chemoattractant protein-1 in human atheromatous plaques** *J Clin Invest* 1991, **88:**1121-1127.
33. Boring L, Gosling J, Cleary M and Charo IF: **Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis** *Nature* 1998, **394:**894-897.
34. Furukawa Y, Matsumori A, Ohashi N, Shioi T, Ono K, Harada A, Matsushima K and Sasayama S: **Anti-monocyte chemoattractant protein-1 / monocyte chemotactic and activating factor antibody inhibits neointimal hyperplasia in injured rat carotid arteries** *Circulation Res* 1999, **84:**306-314.
35. Gao W, Topham PS, King JA, Smiley ST, Csizmadia V, Lu B, Gerard CJ and Hancock WW: **Targeting of the chemokine receptor CCR1 suppresses development of acute and chronic cardiac allograft rejection** *J Clin Invest* 2000, **105:**35-44.
36. Romano M, Diomede L, Sironi M, Massimiliano M, Sottocorno M, Polentarutti N, Guglielmotti A, Albani D, Bruno A, Fruscella P, Salmona M, Vecchi A, Pinza M and Mantovani A: **Inhibition of monocyte chemotactic protein-1 synthesis by statins** *Lab Invest* 2000, **80:**1095-1100.
37. Bellosta S, Via D, Canavesi M, Pfister P, Fumagalli R, Paoletti R and Bernini F: **HMG-CoA reductase inhibitors reduce MMP-9 secretion by macrophages** *Arterioscler Thromb Vasc Biol* 1998, **18:**1671-1678.
38. Aikawa M, Rabkin E, Sugiyama S, Voglic SJ, Fukumoto Y, Furukawa Y, Shiomi M, Schoen FJ and Libby P: **An HMG-CoA reductase inhibitor, cerivastatin, suppresses growth of macrophages expressing matrix metalloproteinases and tissue factor *in vivo* and *in vitro*** *Circulation* 2001, **103:**276-283.
39. Fukumoto Y, Libby P, Rabkin E, Hill CC, Enomoto M, Hirouchi Y, Shiomi M and Aikawa M: **Statins alter smooth muscle cell accumulation and collagen content in established atheroma of watanabe heritable hyperlipidemic rabbits** *Circulation* 2001, **103:**993-999.
40. Baetta R, Camera M, Comparato C, Altana C, Ezekowitz MD and Tremoli E: **Fluvastatin reduces tissue factor expression and macrophage accumulation in carotid lesions of cholesterol-fed rabbits in the absence of lipid lowering** *Arterioscler Thromb Vasc Biol* 2002, **22:**692-698.
41. MacKenzie A, Wilson HL, Kiss-Toth E, Dower SK, North RA and Surprenant A: **Rapid secretion of interleukin-1beta by microvesicle shedding** *Immunity* 2001, **15:**825-835.
42. Nozawa K, Casiano C, Hamel J, Molinaro C, Fritzler M and Chan E: **Fragmentation of Golgi complex and Golgi autoantigens during apoptosis and necrosis** *Arthritis Res* 2002, **4:**R3.
43. Edwards CJ and Spector TD: **Statins as modulators of bone formation** *Arthritis Res* 2002, **4:**151-153.
44. Del Bino G, Darzynkiewicz Z, Degraef C, Mosselmans R, Fokan D and Galand P: **Comparison of methods based on annexin-V binding, DNA content or TUNEL for evaluating cell death in HL-60 and adherent MCF-7 cells** *Cell Prolif* 1999, **32:**25-37.
45. Leite M, Quinta-Costa M, Leite PS and Guimaraes JE: **Critical evaluation of techniques to detect and measure cell death – study in a model of UV radiation of the leukaemic cell line HL60** *Anal Cell Pathol* 1999, **19:**139-151.
46. Kaneider NC, Reinisch CM, Dunzendorfer S, Meierhofer C, Djanani A and Wiedermann CJ: **Induction of apoptosis and inhibition of migration of inflammatory and vascular wall cells by cerivastatin** *Atherosclerosis* 2001, **158:**23-33.

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2261/3/6/prepub