

ORIGINAL ARTICLE

Fluvastatin and lovastatin inhibit granulocyte macrophage–colony stimulating factor–stimulated human eosinophil adhesion to inter-cellular adhesion molecule–1 under flow conditions

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Clinical & Experimental Allergy

Summary

Background Eosinophil accumulation in the lung is an important feature of airway inflammation in asthma. There is therefore much interest in developing novel therapies to prevent this process. Accumulating evidence suggests that statins have anti-inflammatory properties, including inhibition of leucocyte accumulation. We therefore assessed the ability of five statins to inhibit human eosinophil adhesion to recombinant human inter-cellular adhesion molecule (rhICAM)-1 under physiologically relevant flow conditions.

Methods Purified eosinophils were pre-treated with a panel of statins before elucidation of the adhesion profiles of resting and granulocyte macrophage–colony stimulating factor (GM-CSF)-stimulated cells to rhICAM-1-coated microchannels at a flow rate of 0.5 dynes/cm². Images were recorded in real-time at 1 min intervals and analysed using Ducocell software.

Results Fluvastatin and lovastatin (both 10 nM) significantly inhibited GM-CSF-stimulated eosinophil adhesion to rhICAM-1 after 2 min ($34.4 \pm 3.0\%$ inhibition and $37.8 \pm 12.6\%$ inhibition, respectively, $n = 4$, $P < 0.05$) but had no significant inhibitory effect on unstimulated eosinophil adhesion. Mevastatin, simvastatin, and pravastatin (all 10 nM) had no significant effect on GM-CSF-stimulated eosinophil adhesion to rhICAM-1. A concentration range of fluvastatin and lovastatin inhibited GM-CSF stimulated eosinophil adhesion with significant ($P < 0.05$) inhibition observed at low concentrations of 1 nM for both drugs. Mevalonate (100 nM) reversed fluvastatin-mediated but not lovastatin-mediated inhibition of eosinophil adhesion.

Conclusions Inhibition of eosinophil adhesion to ICAM-1 by fluvastatin and lovastatin under physiological shear stress represent novel actions by these drugs that may inform the development of anti-inflammatory therapy for allergic disease.

Keywords adhesion, asthma, eosinophils, fluvastatin, ICAM-1, lovastatin, statins

Submitted 23 February 2009; revised 19 May 2009; accepted 30 June 2009

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Introduction

Despite remarkable advances in diagnosis and long-term management, asthma remains a serious public health problem world-wide. Eosinophil accumulation in the lungs is a well-defined feature of the inflammatory processes underlying allergic asthma even in mild forms of the disease [1]. Eosinophils contain potent histotoxic cationic granule proteins, membrane derived lipid mediators, pro-inflammatory cytokines and chemokines leading to the paradigm that they are major effector cells in the inflammatory processed

underlying much of the pathogenesis of asthma and other allergic diseases [2]. Eosinophils are also thought to contribute to the pathophysiology of airway remodelling primarily through the release of substances, particularly TGF β , involved in extra cellular matrix deposition leading to sub-epithelial membrane thickening. Eosinophil deficient mouse models have provided strong support for eosinophil involvement in asthma [3, 4] while more recent evidence from mouse models of asthma suggests that eosinophils may be critical in the regulation of Th2 immune responses in the lung following allergen provocation [5].

The preferential accumulation of eosinophils in the asthmatic lung suggests the existence of pathways for their selective accumulation. This involves enhanced eosinophil production and release from the bone marrow followed by a combination of selective adhesion to the post-capillary vascular endothelium and trans-endothelial migration (TEM) into the surrounding tissues [6]. There is general agreement that the introduction of flow conditions to *in vitro* models of leucocyte adhesion is crucial to the generation of data relevant to the *in vivo* situation [7]. Our previous studies utilized a novel state of the art microflow system [8] and real-time microscopic imaging to demonstrate inhibitory effects by the histamine H₁-receptor antagonist, levocetirizine [9] and the cysteinyl leukotriene cysLT₁ receptor (cysLT₁R) antagonist montelukast [10], on resting and granulocyte macrophage-colony stimulating factor (GM-CSF)-stimulated human eosinophil adhesion to recombinant human vascular cell adhesion molecule (rhVCAM)-1 under flow conditions.

Statins reduce cholesterol levels by inhibiting the first enzyme in the cholesterol pathway, namely 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and have established effectiveness in the treatment of atherosclerotic disease. They also exhibit anti-inflammatory properties, most likely by inhibiting the prenylation of signalling molecules such as Rho GTPases [11]. A number of clinical studies have provided evidence that statins may have beneficial effects in respiratory diseases [12]. Moreover, animal models of asthma have demonstrated significant inhibitory effects by statins on pro-inflammatory cell accumulation, including that of eosinophils [13, 14]. We therefore investigated the effect of a panel of statins on resting and GM-CSF-stimulated adhesion to intercellular adhesion molecule (ICAM)-1 under flow conditions. Of these only fluvastatin and lovastatin exhibited significant inhibitory effects on GM-CSF-stimulated eosinophil adhesion. These were therefore subjected to more intensive study.

Materials and methods

Materials

CD-16 immunomagnetic beads and magnetically activated separation columns were from Miltenyi Biotec (Surrey, UK). Allophycocyanin (APC)-labelled CR3 monoclonal antibody (mAb) and Annexin V-fluorescent isothiocyanate (FITC) apoptosis detection kit I were both from Becton Dickinson (Oxford, UK). The isotype-matched control mAb was from Serotec Ltd (Kidlington, Oxon, UK). Human GM-CSF was purchased from Peprotech (London, UK). Human anti-CR3 (CD11b) mAb was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Recombinant human ICAM-1 were purchased from R&D Systems (Abingdon, UK). Hank's buffered salt solution,

with or without Mg²⁺ (0.09767 g/mL) and Ca²⁺ (0.14 g/mL), were from PAA Laboratories (Yeovil, UK). Fluvastatin, lovastatin, pravastatin, mevastatin, simvastatin, and bovine serum albumin (BSA) were purchased from Sigma (Poole, Dorset, UK). Foetal cloned serum was obtained from Fisher Scientific (Loughborough, UK). All other chemicals were of analytical grade.

Eosinophil isolation

Human eosinophils were obtained from blood donated by normal volunteers, or individuals with mild allergic disease who were not taking any medication and who gave informed consent (eosinophilia $\leq 0.5 \times 10^6$ eosinophils/mL). Eosinophils were purified using our standard technique using dextran sedimentation and centrifugation on Percoll gradients followed by CD16-dependent negative immunomagnetic selection as described [15]. Using this method, eosinophils with a purity of at least 99% were obtained, with >98% viability as assessed by trypan blue exclusion. Depending on the donor, between 5 and 10 million eosinophils were isolated from 80 ml of human blood.

Microflow system

This study used a novel microflow system consisting of a novel syringe pump with microfluidic biochip and flow sensor controlled by a PC using dedicated software (Cellix Ltd., Trinity College, Dublin, Ireland). The system is described in full elsewhere [8]. Briefly, the microfluidic syringe pump allows very accurate and reproducible flow rates to be achieved. Importantly, flow rates are very low (5 pL/min to 10 μ L/min) and those used in this study (1–2 dyn/cm²) mimic those found in the post-capillary venules during inflammatory processes [16]. The biochip is comprised of eight channels, each with small diameter channels that mimic the dimensions of the post-capillary venules and these are coated with the recombinant human (rh) adhesion protein of interest.

Eosinophil adhesion under flow conditions

Each microchannel was coated for three hours in humid conditions at ambient temperature with either recombinant human ICAM-1 (rhICAM-1) or BSA [both 10 μ g/mL in phosphate-buffered saline (PBS) containing Ca²⁺ and Mg²⁺]. All channels were then coated with BSA (as before) to occupy non-specific binding sites. Resting or GM-CSF-treated eosinophils were preincubated at 37 °C in a water bath for 10 min before incubation for a further 20 min with/without statin (0.1–100 nM). Anti-CR3, or appropriate isotype-matched Ab controls were added under the same conditions at a final concentration of 10 μ g/mL. Eosinophils were then infused into the channels at a shear

stress level of 0.5 dynes/cm². Images were taken at 1 min intervals in real time and were captured using the accompanying PixeLINK microscopy software. For experiments with GM-CSF (10 ng/mL)-stimulated eosinophils these were added to the warmed cells at the same time as statins or other treatments and incubated at 37 °C for 20 min before commencing the flow assay.

Determination of eosinophil apoptosis and CR3 expression by flow cytometry

Following isolation from human blood, eosinophils (2.5×10^5) were cultured in RPMI 1640 culture medium supplemented with 10% fetal calf for 24 h. The degree of apoptosis in these cells was measured using our established technique [15]. Briefly, eosinophils were washed in cold PBS and resuspended in binding buffer [4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES)-buffered PBS supplemented with 2.5 mM CaCl₂] before the addition of FITC-conjugated annexin V for 10 min at room temperature. Cells were then washed, resuspended in binding buffer and propidium (PI) iodide added to give a final concentration of 1 mg/mL. They were then analysed immediately by flow cytometry performed on the basis of eosinophils gated for their forward and side scatter profiles, with any cell debris excluded from the analysis.

Analysis of data

Three images per shear stress level were captured, and adhered eosinophil numbers were recorded using Ducocell application software (Cellix Ltd.). Data were exported into Excel for interpretation. Statistical significance was determined by Students unpaired *t*-test, and $P < 0.05$ was considered statistically significant. All data is presented as mean $\pm$ SEM. The *n* in the text refers to the number of separate experiments.

Results

Effect of five different statins on granulocyte macrophage-colony stimulating factor-stimulated eosinophil adhesion to inter-cellular adhesion molecule-1 under flow conditions

In total, approximately 50 000 eosinophils were passed through each channel for the duration of each assay. Initially, a total of 70 000 cells were aspirated into the flow system, so that the cell flow rate would remain constant throughout the assay. The eosinophils were then pumped into the Vena8 biochip channel coated with rhICAM-1 at a shear stress level of 0.5 dynes/cm². Unstimulated eosinophils that passed through the biochip channel showed minimal binding to rhICAM-1 (< 10%), and those that did bind showed no morphological changes

after adherence. Similar levels of adherence were observed when unstimulated cells were pumped through biochips channels coated with BSA.

Cells were then pre-incubated with 10 ng/mL GM-CSF, the optimal concentration as established by previous studies [9, 10], and their adhesion to rhICAM-1 was observed. After application of 0.5 dynes/cm² shear stress, GM-CSF-stimulated eosinophils adhered to rhICAM-1 (approximately 50% maximal adherence was observed after 3 min), and the cells demonstrated 'rolling' and 'tethering' characteristics.

Figure 1 is a representative experiment that illustrates unstimulated eosinophil adhesion or GM-CSF-stimulated adhesion at 0.5 dynes/cm² to ICAM-1, together with the inhibitory effect of pre-incubation with fluvastatin or lovastatin (both 10 nM), or anti-CR3 integrin mAb. Images were taken after 0.5 dynes/cm² shear stress was applied for 3 min.

Both fluvastatin and lovastatin (10 nM) significantly inhibited GM-CSF-stimulated eosinophil adhesion to rhICAM-1 under 0.5 dynes/cm² shear stress ($n = 6$, $P < 0.05$). Simvastatin, mevastatin, and pravastatin (all 10 nM) had no significant affect on GM-CSF-stimulated eosinophil adhesion to rhICAM-1 (Fig. 2).

Effect of fluvastatin on granulocyte macrophage-colony stimulating factor-stimulated eosinophil adhesion to inter-cellular adhesion molecule-1 under flow conditions

We next examined the effect of a concentration range of fluvastatin (10 pM–100 nM) on GM-CSF-stimulated eosinophil adhesion to rhICAM-1 under constant flow of 0.5 dynes/cm². Isolated eosinophils were exposed to same conditions described above, with the relevant concentration of fluvastatin added at the same point as GM-CSF, anti-CR3 integrin, or control mAb. As before, the non-GM-CSF-stimulated eosinophils demonstrated a very low adhesion profile to rhICAM-1. GM-CSF-stimulated eosinophils, in the absence of fluvastatin, again demonstrated rolling and 'tethering' characteristics after application of flow, which resulted in a high level of adherence.

Figure 3 demonstrates that at low concentrations of fluvastatin (10 and 100 pM), there was no significant effect on GM-CSF-stimulated eosinophil adhesion to rhICAM-1 after 3 min. However, higher but physiologically relevant concentrations (1 and 10 nM) demonstrated a significant decrease in eosinophil 'tethering' to rhICAM-1 ($P < 0.05$, $n = 5-10$). Interestingly, 100 nM fluvastatin also produced a decrease in eosinophil adhesion to rh-ICAM-1, but this decrease was not as pronounced as that observed with 1 nM and 10 nM and was not significantly different compared with GM-CSF only. The anti-CR3 integrin mAb again reduced eosinophil adhesion levels to rhICAM-1 to those found in BSA-coated channels with no significant inhibitory effect given by the isotype control mAb.

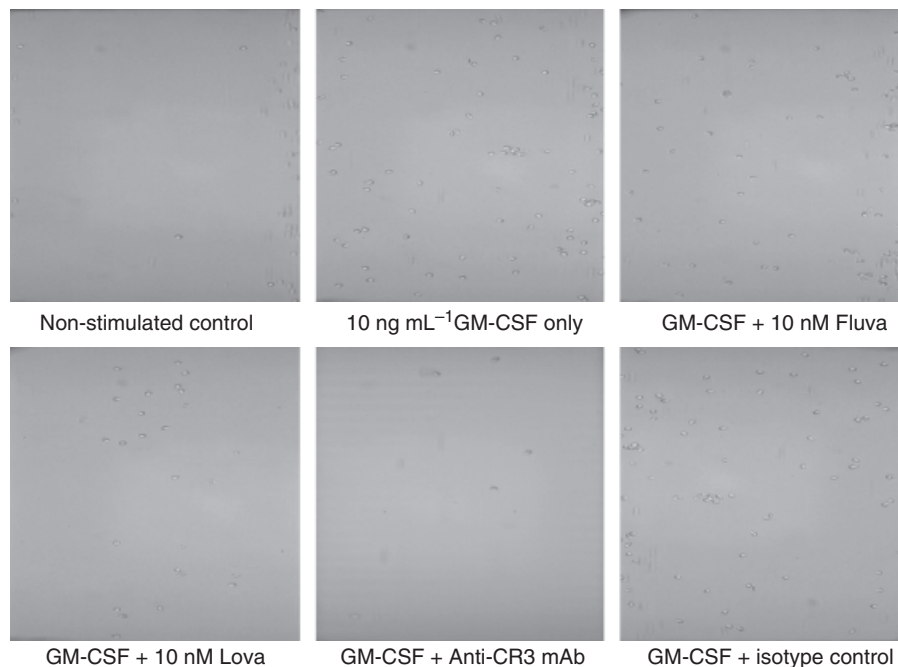


Fig. 1. Representative microscope images ($\times 200$ magnification) showing the effect of a range of statins (10 nM) on granulocyte macrophage-colony stimulating factor (GM-CSF)-stimulated eosinophil adhesion to recombinant human inter-cellular adhesion molecule (10 $\mu\text{g/mL}$) at a flow rate of 0.5 dynes/ cm^2 . Cells were pre-incubated in $\text{Ca}^{2+}/\text{Mg}^{2+}$ -containing Hank's buffered salt solution for 30 min at 37 $^{\circ}\text{C}$, including 20 min pre-incubation with 10 ng/mL GM-CSF and the designated statin, anti-CR3 monoclonal antibody (mAb) or isotype control where appropriate, before being passed through the biochip channel (400 μm wide, 100 μm deep). Isotype control mAb had no effect on either unstimulated or GM-CSF-stimulated eosinophils (not shown).

A change in eosinophil morphology was also observed in GM-CSF-stimulated cells, i.e. adhesion to rhICAM-1 led to cell flattening, which was a precursor to diapedesis. However, fluvastatin had no visible effect on eosinophil cell flattening on rhICAM-1.

Effect of lovastatin on granulocyte macrophage-colony stimulating factor-stimulated eosinophil adhesion to inter-cellular adhesion molecule-1 under flow conditions

Figure 4 illustrates the effect of a concentration range of lovastatin (10 pM–100 nM) on eosinophil adhesion to rhICAM-1 under flow conditions. Similar to fluvastatin, no significant effect on GM-CSF-stimulated eosinophil adhesion to rhICAM-1 was observed with the lower concentrations of lovastatin (10 and 100 pM, $n = 5-10$, $P < 0.05$) after 3 min. However, at the higher concentration range (1, 10, and 100 nM), lovastatin significantly inhibited GM-CSF-stimulated eosinophil adhesion to rhICAM-1 ($n = 5-9$, $P < 0.05$). Lovastatin had no effect on cell flattening following adhesion to rhICAM-1. Lovastatin inhibition was not reversed by pre-incubation with mevalonate (100 nM), this was in contrast to fluvastatin whose inhibition of eosinophil adhesion was significantly reversed by pre-incubation with mevalonate (Fig. 5).

Effect of fluvastatin and lovastatin on CR3 expression

CR3 (CD11b/CD18), is a granulocyte-bound surface integrin that has an essential role in leucocyte diapedesis [17]. Statin-induced downregulation of eosinophil CR3 expression or avidity is a potential inhibitory mechanism of action with respect to cell adhesion to ICAM-1. Determination of CR3 expression was assessed using flow cytometry and APC-labelled anti-CR3 mAbs. GM-CSF (10 ng/mL) induced significant ($P > 0.05$) increases in eosinophil CR3 expression that was not significantly modulated by pre-incubation with a concentration range (10 nM, 100 nM, and 1 μM) of either fluvastatin or lovastatin ($n = 4$, data not shown).

Effect of fluvastatin and lovastatin on eosinophil viability and apoptosis

Viability of untreated eosinophils and GM-CSF-stimulated eosinophils treated with a concentration range of lovastatin or fluvastatin (10 pM–100 nM) was determined by trypan blue exclusion and averaged 98% and 97%, respectively. Furthermore, no concentration of fluvastatin or lovastatin tested accelerated eosinophil apoptosis or inhibited GM-CSF prolonged survival or necrosis as judged by annexin V binding or propidium iodide exclusion, respectively (data not shown), quantified as described [15].

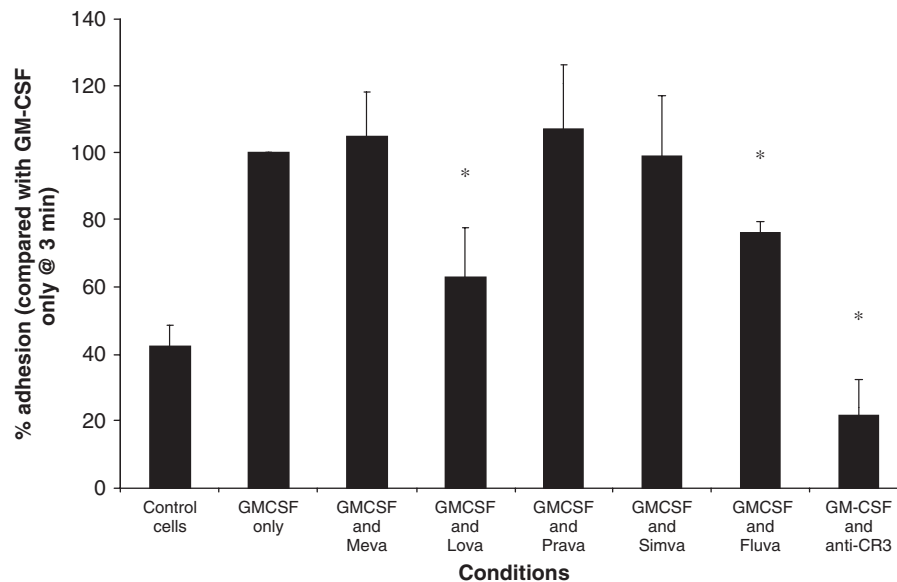


Fig. 2. Effect of a range of statins (all 10 nM) on granulocyte macrophage-colony stimulating factor (GM-CSF)-stimulated eosinophil adhesion to recombinant human inter-cellular adhesion molecule (rhICAM-1) under flow conditions of 0.5 dynes/cm². Data expressed as a percentage of GM-CSF-stimulated eosinophil adhesion to rhICAM-1 at 0.5 dynes/cm². Each bar represents the mean $\pm$ SEM of at least 4 independent experiments. *Significantly different ($P < 0.05$) from GM-CSF value at 0.5 dynes/cm². Isotype control monoclonal antibody had no effect on eosinophil adhesion (not shown).

Eosinophil adhesion to vascular cell adhesion molecule-1

As previously reported, GM-CSF enhanced eosinophil adhesion to rhVCAM-1 under flow [10]. However, none of the statins tested had any inhibitory on resting or GM-CSF stimulated eosinophil adhesion to rhVCAM-1 (data not shown).

Discussion

Adhesion of eosinophils to post-capillary venules is an essential part of TEM. This process is, in part, dependent on β_2 -integrin/ICAM-1 interaction on the surface of the endothelium. Our previous studies demonstrated that both unstimulated and GM-CSF-stimulated eosinophil adhesion to VCAM-1 were significantly inhibited under flow conditions by physiologically relevant concentrations of the H₁-receptor antagonist levocetirizine [9] or the cysteinyl leukotriene receptor antagonist montelukast [10]. In the present study, we analysed the effects of a panel of statins on GM-CSF-stimulated eosinophil adhesion to ICAM-1 under constant flow conditions, and have shown for the first time that fluvastatin and lovastatin inhibit eosinophil adhesion to rhICAM-1. However, the other three statins analysed (mevastatin, pravastatin, and simvastatin) had no significant effect on eosinophil/ICAM-1 adhesion. The fluvastatin- and lovastatin-mediated inhibition occurred at shear stress levels equivalent to those found *in vivo*, in post-capillary venules (i.e. 0.5 dynes/cm²), [16] and at physiologically relevant concentrations. These findings are highly significant, as they give evi-

dence of a potential anti-inflammatory role for fluvastatin and lovastatin independent of their cholesterol-reducing mechanism of action.

Fluvastatin or lovastatin had no effect on eosinophil flattening after adhesion to ICAM-1. Therefore, the inhibitory effect of both statins appears to be confined to the initial interaction between ICAM-1 and β_2 -integrins contained within certain eosinophil cell surface proteins [e.g. lymphocyte function-associated antigen-1 (LFA-1) and CR3]. This hypothesis is supported by *in vivo* rodent studies that show fluvastatin-mediated inhibition of the initial rolling and tethering of leucocytes to the endothelium [18].

GM-CSF-mediated eosinophil accumulation in inflamed tissue is thought to originate in the bone marrow, where the cytokine enhanced eosinophil proliferation and differentiation. It also increases eosinophil adhesion to the endothelium, enhances TEM, and inhibits apoptosis of the pro-inflammatory cells, after being expressed in and released from inflamed endothelial tissue [19, 20]. The increase in eosinophil adhesion to the endothelium mediated by GM-CSF is thought to occur via upregulation and/or increasing the affinity of cell surface proteins such as very late antigen-4 (VLA-4), LFA-1 and CR3, which then increases binding to adhesion proteins on the endothelium, such as VCAM-1 and ICAM-1. Our findings demonstrate that both fluvastatin and lovastatin have no significant effect on CR3 expression on eosinophils, and also do not influence the rate of eosinophil apoptosis. Therefore, these two statins most likely act through inhibition of the increase in affinity of eosinophil CR3

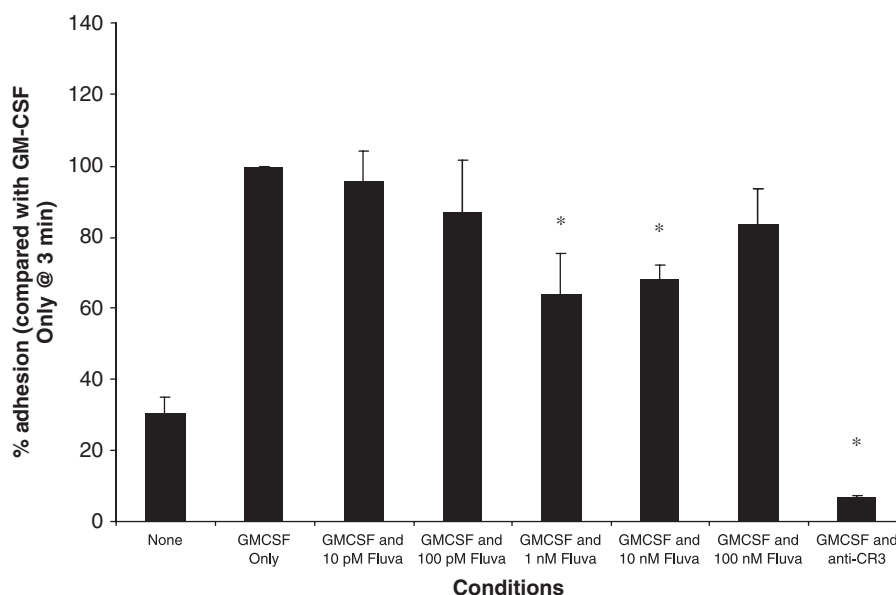


Fig. 3. Effect of a concentration range of fluvastatin (10 pM–100 nM) on granulocyte macrophage–colony stimulating factor (GM-CSF)-stimulated eosinophil adhesion to recombinant human inter-cellular adhesion molecule (rhICAM-1) under flow conditions of 0.5 dynes/cm². Data expressed as a percentage of GM-CSF-stimulated eosinophil adhesion to rhICAM-1 at 0.5 dynes/cm². Each bar represents the mean±SEM of at least 5 independent experiments. *Significantly different ($P < 0.05$) from GM-CSF value at 0.5 dynes/cm². Isotype control monoclonal antibody had no effect on eosinophil adhesion (not shown).

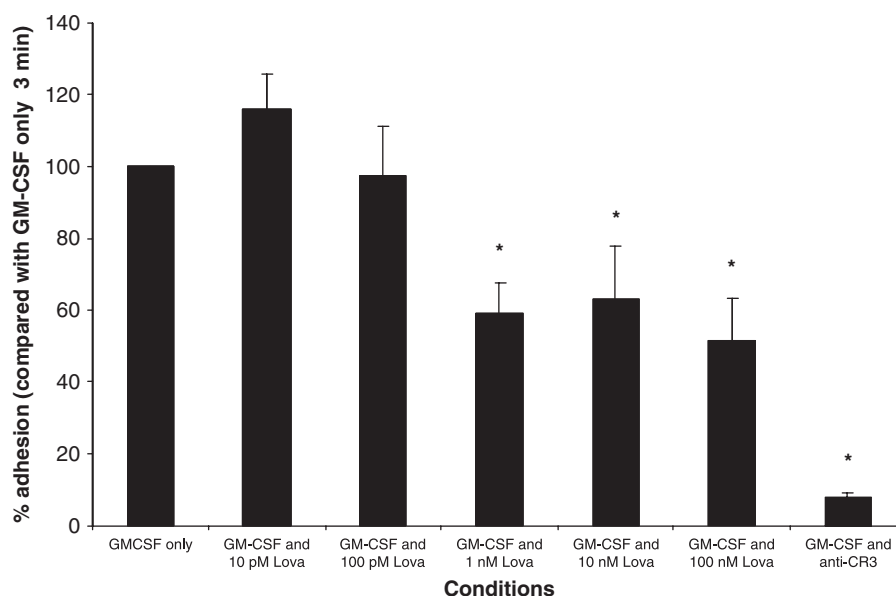


Fig. 4. Effect of a concentration range of lovastatin (10 pM–100 nM) on granulocyte macrophage–colony stimulating factor (GM-CSF)-stimulated eosinophil adhesion to recombinant human inter-cellular adhesion molecule (rhICAM-1) under flow conditions of 0.5 dynes/cm². Data expressed as a percentage of GM-CSF-stimulated eosinophil adhesion to rhICAM-1 at 0.5 dynes/cm². Each bar represents the mean±SEM of at least 5 independent experiments. *Significantly different ($P < 0.05$) from GM-CSF value at 0.5 dynes/cm². Isotype control monoclonal antibody had no effect on eosinophil adhesion (not shown).

following GM-CSF stimulation or through inhibitory action via another adhesion pathway, for example LFA-1/ICAM-1. In this respect statins, and in particular lovastatin, can bind to an allosteric site located within the LFA-1

molecule in T lymphocytes [21], thereby inhibiting LFA-1/ICAM-1 interactions, resulting in decreased adhesion to the endothelium [22]. Furthermore, statins are known to post-translationally modify an extensive array of

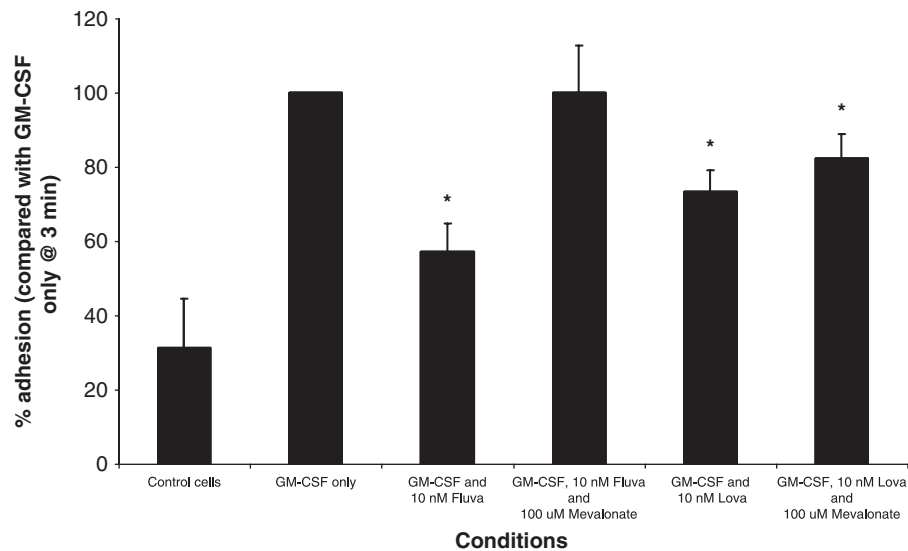


Fig. 5. Effect of mevalonate (100 nM) on fluvastatin- and lovastatin-induced inhibition of granulocyte macrophage-colony stimulating factor (GM-CSF)-stimulated eosinophil adhesion to recombinant human inter-cellular adhesion molecule-1. Each bar represents the mean $\pm$ SEM of at least 4 independent experiments. *Significantly different ($P < 0.05$) from GM-CSF value at 0.5 dynes/cm².

intracellular signalling molecules, including the Rho family of GTPases, and as a result, statins impact on many cellular functions essential for normal cell homeostasis including proliferation, differentiation, and cytoskeletal organization, including cell surface protein expression. Thus, statins may interfere with adherence to ICAM-1 by modulating LFA-1 expression and/or affinity through the Rho signalling pathway, possibly via inhibition of prenyl group synthesis [23]. It is of interest that inhibition of early adhesive events (initial tethering/rolling) by neutrophils to HUVEC *in vitro* by statins required a pre-incubation period of 6 h [24]; presumably to ensure total depletion of cellular mevalonate pools. In contrast, we observed significant effects by both fluvastatin and lovastatin following 30 min of pre-incubation. Whether this observation is indicative of eosinophils having smaller pools of mevalonate than neutrophils is unclear and would require further investigation.

Previous studies with lovastatin have demonstrated anti-inflammatory actions in endothelial cells, such as protein kinase B inhibition, and downregulation of ICAM-1 expression via C-reactive protein disruption [25, 26]. Other studies have shown lovastatin-induced upregulation of CCL-1 in T-helper 2 (Th2) cells, leading to increased Th2 recruitment to the site of inflammation [27]. It has also been shown that the effect of fluvastatin on endothelial cells appears mixed, with evidence for pro-inflammatory upregulation of ICAM-1, monocyte chemoattractant protein-1 (MCP-1), and E-selectin [28] and anti-inflammatory downregulation of ICAM-1 and platelet endothelial cell adhesion molecule-1 (PECAM-1) [29]. Leucocyte-specific actions of fluvastatin also appear to be contradictory, with evidence of a pro-inflammatory in-

crease of TNF- α , IFN- γ and IL-18 release in human peripheral blood mononuclear cells [30], and anti-inflammatory suppression of IFN- γ and IL-12 production in human monocytes [31], in addition to reducing LFA-1 expression in human monocyte cell lines [32].

Interestingly, fluvastatin-mediated inhibition of eosinophil adhesion to rhICAM-1 was reversed with the addition of mevalonate, but the latter did not reverse lovastatin-mediated inhibition. This suggests that the anti-adhesive action of fluvastatin in eosinophils is dependent on HMG-CoA reductase inhibition, whereas lovastatin-mediated inhibitory effects may be independent of its inhibitory action on HMG-CoA reductase. Evidence to support a statin-mediated inhibitory mechanism of action independent of HMG-CoA reductase inhibition includes rosuvastatin appearing to downregulate expression of the genes encoding the inflammatory response proteins orosomucoid (ORM1) and IL18 receptor accessory protein (IL18RAP) in peripheral blood leucocytes [33].

It should be noted, however, that while mevastatin, pravastatin, and simvastatin had no effect on eosinophil adhesion to ICAM-1, this does not preclude them having other anti-inflammatory effects. For example, simvastatin has a number of immunosuppressive actions, including induction of apoptosis in eosinophils [34], and inhibition of the protein kinase C pathway in leucocytes via activation of the peroxisome proliferator-activated receptor [35]. We have also demonstrated that pravastatin inhibited T cell adhesion to ICAM-1, but not VCAM-1, under flow conditions [unpublished observations], while it also suppresses IFN- γ and IL-12 production by human monocytes [31]. Mevastatin also has a number of anti-inflammatory actions [36, 37].

Although our findings are suggestive that fluvastatin and lovastatin may have inhibitory effects on eosinophil accumulation; a key question is whether their use, or of other drugs in this class, might be of benefit to patients with asthma. To date only two randomized placebo-controlled clinical trials have addressed this issue: the first examined the anti-inflammatory and clinical effect of simvastatin in patients with asthma [38]. With the exception of a modest (14%) reduction in exhaled nitric oxide, a marker of lung inflammation, this study did not demonstrate significant differences in other inflammatory outcomes, lung volumes, or airway resistance between simvastatin and placebo. In the second study, addition of atorvastatin to inhaled corticosteroid therapy in patients with mild to moderate atopic asthma did not improve short-term asthma control [39]. These studies suggest that statins are not effective in patients with mild-to-moderate asthma who are responsive to treatment with inhaled steroids. However, further randomized clinical trials are needed to establish whether statins might be effective co-therapies in asthma or whether they would benefit patients with more chronic severe disease that may be resistant to treatment with corticosteroids.

In conclusion, we have analysed stimulated eosinophil adhesive behavior under flow conditions, and found that physiologically relevant concentrations of fluvastatin and lovastatin inhibit GM-CSF-stimulated eosinophil adhesion to ICAM-1 in an *in vitro* model of post-capillary venules. This demonstrates potential anti-inflammatory effects by both statins in addition to their cholesterol-lowering abilities. This may provide important evidence for developing alternative treatments for eosinophil-induced inflammation in allergic-based disease.

Acknowledgements

This study was supported by Tenovus Scotland (AR, GMW) and a FP6-2004-Mobility-3 Marie Curie grant, 'ASTHMA,' contract number MTKI-CT-2005-029541 (VW and GMW).

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