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Effects of Oxidized Low Density Lipoprotein, Lipid Mediators and Statins on Vascular Cell Interactions[1)]

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The integrin heterodimer CD11b/CD18 ($\alpha M\beta 2$, Mac-1, CR3) expressed on monocytes or polymorphonuclear leukocytes (PMN) is a receptor for iC3b, fibrinogen, heparin, and for intercellular adhesion molecule (ICAM)-1 on endothelium, crucially contributing to vascular cell interactions in inflammation and atherosclerosis. In this report, we summarize our findings on the effects of lipid mediators and lipid-lowering drugs. Exposure of endothelial cells to oxidized low density lipoprotein (oxLDL) induces upregulation of ICAM-1 and increases adhesion of monocytic cells expressing Mac-1. Inhibition experiments show that monocytes use distinct ligands, i.e. ICAM-1 and heparan sulfate proteoglycans for adhesion to oxLDL-treated endothelium. An albumin-transferable oxLDL activity is inhibited by the antioxidant pyrrolidine dithiocarbamate (PDTC), while 8-epi-prostaglandin F2α (8-epi-PGF2α) or lysophosphatidylcholine had no effect, implicating yet unidentified radicals. Sequential adhesive and signaling events lead to the firm adhesion of rolling PMN on activated and adherent platelets, which may occupy areas of endothelial denudation. Shear-resistant arrest of PMN on thrombin-stimulated platelets in flow conditions requires distinct regions of Mac-1, involving its interactions with fibrinogen bound to platelet αIIbβ3, and with other platelet ligands. Both arrest and adhesion strengthening under flow are stimulated by platelet-activating factor and leukotriene B4, but not by the chemokine receptor CXCR2. We tested whether Mac-1-dependent monocyte adhesiveness is affected by inhibitors of hydroxy-methylglutaryl-Coenzyme A reductase (statins) which improve morbidity and survival of patients with coronary heart disease. As compared to controls, adhesion of isolated monocytes to endothelium *ex vivo* was increased in patients with hypercholesterolemia. Treatment with statins decreased total and low density lipoprotein (LDL) cholesterol plasma levels, surface expression of Mac-1, and resulted in a dramatic reduction of Mac-1-mediated monocyte adhesion to endothelium. The inhibition of monocyte adhesion was reversed by mevalonate but not LDL *in vitro*, indicating that isoprenoid precursors are crucial for adhesiveness of Mac-1. Such effects may crucially contribute to the clinical benefit of statins, independent of cholesterol-lowering, and may represent a paradigm for novel, anti-inflammatory mechanisms of action by this class of drugs.

Key words: Adhesion; Endothelium; Lipids; Monocytes; Platelets.

Monocyte Adhesion to Oxidized Low Density Lipoprotein-Stimulated Endothelium Mediated by Distinct Ligands

The process of atherogenesis involves the actions of oxidized low density lipoprotein (oxLDL). An extensive oxidative modification of LDL may occur in an antioxidant-depleted subendothelial microenvironment (1). OxLDL can induce the initial adhesiveness and transmigration of human blood monocytes or monocytic cell lines on vascular endothelium, possibly enhancing monocyte recruitment and retention (2–5). In addition, stimulation of endothelial cells with oxLDL has been reported to increase adhesion of human blood monocytes (6). However, the mechanisms involved in this effect remain to be completely elucidated. Studies in human umbilical vein endothelial cells (HUVEC) have demonstrated that intercellular adhesion molecule (ICAM)-1 expression is upregulated by oxLDL or by long-term incubation with native LDL (6, 7). Two novel endothelial adhesion proteins for monocytic cells have recently been described to be induced by minimally modified LDL (mmLDL) (8, 9). We have shown that the human monocytic cell line Mono Mac 6 (10) is an appropriate *in vitro* tool to study monocyte interactions with cytokine-stimulated endothelium (11). In the following section, we have summarized findings on the adhesion of Mono Mac 6 cells to oxLDL-stimulated HUVEC, in an attempt to characterize the endothelial ligands involved in this interaction and to identify responsible active components in the oxLDL preparations used.

Treatment of HUVEC with oxLDL for 24 hours dose-dependently increases the adhesion of human monocytic Mono Mac 6 cells but not of U937 cells. Mac-1 (CD11b/CD18), a monocytic counter-receptor for ICAM-1 that also binds to heparin (12), is present on Mono Mac 6 but not U937 cells, and may thus explain these differences in adhesion (11). Consistently, oxLDL induces a two-fold upregulation of ICAM-1 but not vas-

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cular cell adhesion molecule (VCAM)-1 or E-selectin surface expression on HUVEC. Maltose-1-phosphate (M1P) or heparin but not monoclonal antibodies (mAbs) to ICAM-1 reduce Mono Mac 6 cell adhesion to untreated HUVEC. Induction of unidentified endothelial molecules by mmLDL has been suggested to mediate monocyte adhesion, as inferred by inhibition with M1P (13), and this may explain the lack of inhibition with ICAM-1 mAbs. Notably, combinations of mAbs to ICAM-1 with either M1P or heparin, a recently identified Mac-1 ligand (12), inhibited Mono Mac 6 adhesion to oxLDL-stimulated HUVEC by more than 50 %, while either alone had no effect. This suggests that two distinct endothelial ligands for Mac-1, inducible ICAM-1 and heparan sulfate proteoglycans, mediate monocyte interaction with oxLDL-treated HUVEC. The composition of oxLDL particles is subjected to considerable variation (1). Our preparations are thoroughly washed in EDTA-containing phosphate buffer saline (PBS) after oxidation, comparable to dialyzing. Consistently, toxic effects (e.g. lactate dehydrogenase (LDH) elevation or detachment) are detectable after 24 hours incubation of HUVEC with unwashed but not washed oxLDL. The extraction of oxLDL components by treatment with bovine serum albumin (BSA) and subsequent separation results in diminished activity of oxLDL and a transfer of active components to BSA, as indicated by the induction of ICAM-1 expression and monocyte adhesion. However, candidate lipid peroxidation products, lysophosphatidylcholine (LPC) or 8-epi $PGF_2\alpha$ which have been reported as active components in oxLDL (14, 15), produce no stimulatory effects. Thus, formation of other oxidation products is responsible for the activity of oxLDL, and the identification of such compounds is currently underway. Multiple radicals and peroxides are generated during the oxidation processes, and presence of the antioxidant and radical scavenger pyrrolidine dithiocarbamate (PDTC) prevents induction of Mono Mac 6 cell adhesion in oxLDL-stimulated HUVEC, likely due to co-antioxidative, radical scavenging properties. In conclusion, oxidatively modified LDL induces adhesion of monocytic cells, which utilize at least two distinct ligands on endothelium, identified as ICAM-1 when sufficiently upregulated and heparan proteoglycans (16). This may provide a possible mechanism for atherogenic effects of oxLDL.

Similarly, an upregulation of ICAM-1 by oxLDL has been described in HUVEC or porcine coronary artery endothelium (6, 17). In contrast, mmLDL appears to decrease endothelial ICAM-1 expression (13). This may be due to differences in cell culture conditions and LDL preparation as evident from the distinct electrophoretic mobility of mmLDL and oxLDL. The late time point of ICAM-1 induction by oxLDL has not been previously noted, in part due to toxic effects on prolonged incubation (6). Using washed oxLDL may be comparable to long-term incubation with native LDL protected against extensive cellular oxidation, which also enhances ICAM-1 expression (7). Both effects may be due to induction of secondary endothelial mediators, resulting in a delayed ICAM-1 upregulation. Consistently, recent studies in a rat model have shown that the injection of LDL is associated with the appearance of vascular reactivity for oxLDL epitopes and endothelial ICAM-1 expression on endothelium after 24 hours (18). In addition, expression of ICAM-1 on endothelium subsequently serves to support dynamically regulated and lymphocyte function associated-1 (LFA-1)-mediated transmigration of monocytes (Figure 1), e.g. in response to monocyte chemotactic protein-1 (MCP-1) (19).

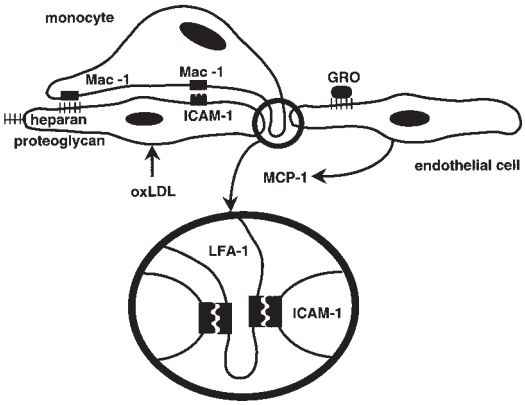

Fig. 1 Distinct ligands induced by oxLDL on endothelium involved in monocyte adhesion and transmigration. GRO, growth-stimulatory activity; ICAM-1, intercellular cell adhesion molecule-1; LFA-1 lymphocyte function associated-1, CD11a/CD18 integrin; Mac-1, CD11b/CD18 integrin; MCP-1, monocyte chemotactic protein-1; oxLDL, oxidized low-density lipoprotein.

A clear inhibition of monocyte adhesion to oxLDL-stimulated HUVEC with ICAM-1 mAbs requires their combination with M1P or heparin, indicating an essential involvement of at least two distinct endothelial ligands in increased monocyte adhesion, i.e. ICAM-1 following initial upregulation by oxLDL and heparan sulfate proteoglycans (16). Consistently, ICAM-1 mAb only slightly inhibits monocyte adhesion to oxLDL-treated vascular smooth muscle cells despite induction of ICAM-1, and blocking one endothelial ligand marginally affects monocyte binding to cytokine-stimulated HUVEC, whereas combinations of mAbs cause strong inhibition (20, 21), indicating that alternative ligands can compensate for blocking one interaction. An inhibition of adhesion with M1P in combination with ICAM-1 mAbs suggests a contribution of ligands for monocytes, which are also induced by mmLDL and can be blocked with M1P (14). Distinct endothelial ligands induced by mmLDL (8, 9) do not appear to be identical with such ligands (16), since they mediate U937 adhesion, which is not increased by oxLDL. An inhibition of monocyte adhesion with the soluble Mac-1 ligand heparin (12) indicates that cellular heparan sulfate proteoglycans are involved in monocyte adhesion to untreated HUVEC. While heparin alone has no effect, combining heparin with ICAM-1 mAbs significantly inhibits monocyte adhesion to oxLDL-stimulated HUVEC. This indicates that, in addition to ICAM-1, the presence of such heparan sulfate proteoglycans is required to mediate monocytic cell adhesion to oxLDL-stimulated HUVEC, emphasizing the involvement of at least two distinct li-

gands. Heparin can release and neutralize heparan-bound chemokines such as growth-stimulating activity (GRO), but not MCP-1, both of which are induced by mmLDL and are involved in mediating monocyte adhesion (22, 23). GRO may participate in the heparin-inhibitable adhesion, since heparin inhibits adhesion to untreated endothelium where little GRO is expressed (22). The endothelial ligands for monocytes induced by oxLDL are schematically depicted in Figure 1.

The properties of the oxLDL preparations used appear to resemble those of LDL modified by coincubation with cultured cells (17). In search for active components in oxLDL, we found that physiologically relevant concentrations of 8-epi $PGF_2\alpha$ (14) do not enhance monocytic cell adhesion to HUVEC. In contrast to previous studies (15, 24), LPC does not contribute to the effects of oxLDL. This is consistent with results showing that effects of oxLDL are not necessarily mediated by LPC (25). Optimal treatment with LPC requires binding to BSA (24), causing differences in effectiveness. The transfer of active components in oxLDL to BSA indicates the presence of lipophilic oxidation products other than LPC, such as lipid peroxides or radicals, that are responsible for induction of ICAM-1 and monocyte adhesion. This is also supported by the prevention of oxLDL-induced effects with PDTC, a potent radical scavenger (20). Recently, it has been demonstrated that oxLDL activates peroxisome proliferator-activated receptor γ (PPARγ)-dependent transcription through a novel signaling pathway involving scavenger receptor-mediated particle uptake, and two of the major oxidized lipid components of oxLDL, 9-hydroxy octadecadienoic acid (9-HODE) and 13-HODE, have been identified as endogenous activators and ligands of PPARγ (26). Such ligand activation can induce changes characteristic of monocytic differentiation and promote uptake of oxLDL through transcriptional induction of the scavenger receptor CD36 (27). This may be relevant to the signal transduction following activation by oxLDL in endothelial cells, which may be mediated by endothelial CD36 as a receptor binding oxLDL (28). Taken together, our data suggest that washed oxLDL without cytotoxicity upregulates ICAM-1 on endothelium and induces monocyte adhesion that can be mediated by ICAM-1 and additional endothelial ligands (16). The induction of ICAM-1 and the involvement of heparan sulfate proteoglycans in adhesion could further explain the atherogenic potential of oxLDL and may lead to the development of potential therapeutic strategies in the prevention of atherogenesis.

Involvement of Platelet-Activating Factor (PAF) and Leukotriene B4 (LTB4) in Leukocyte Arrest on Adherent Platelets

In attempt to define the process of transendothelial leukocyte extravasation, a multistep model has proposed a sequential involvement of various traffic signal molecules. Selectins initiate tethering and rolling of leukocytes as a prerequisite for their stable arrest due to stimulation of β_2 integrins in flow (29, 30). Platelets express P-selectin, which is mobilized from α-granules to the plasma membrane upon activation, and the β_2 integrin ligand ICAM-2 (31–33). Thus, a similar multistep model may apply to the highly efficient accumulation of PMN on surface-adherent platelets under flow. Indeed, rolling and arrest of PMN on activated platelets under flow requires the action of P-selectin and β_2 integrins, respectively (34–37). Fibrinogen, a Mac-1 ligand (38, 39) which can bind to activated platelets via αIIbβ3 (40), has been implicated in platelet-PMN interactions in cell suspension or whole blood (41). However, the role of Mac-1 in adherence of PMN in flow and the identity of the β_2 integrin ligands mediating PMN arrest on activated platelets in flow remain to be elucidated. Chemoattractants and CXC chemokines have been shown to stimulate adhesiveness of Mac-1 in PMN (42, 43) and convert selectin-mediated rolling of PMN into firm arrest (29). Interactions of PMN and platelets are required for the production of certain chemokines, and for transcellular leukotriene and arachidonic acid metabolism by lipoxygenases (44). Activated platelets produce chemoattractants, e.g. the lipid mediator leukotriene B4 (LTB4) or the hydrolytic phospholipid product, platelet-activating factor (PAF), which may stimulate PMN integrins. Platelets express the CXC chemokines ENA-78, GRO-α and β-thromboglobulin, a precursor for neutrophil activating peptide-2 (NAP-2) released from α-granules and processed by cathepsin G (45-47), which act via the receptor CXCR2 on PMN (48). The receptor for PAF and the CXCR2 differ in signal transduction and coupling to Gα proteins (49). Moreover, lipid mediators but not CXC chemokines are soluble in the plasma membrane, while chemokines but not lipid mediators bind to heparin or proteoglycans. This might lead to a differential role for these chemoattractants in activation of PMN under flow.

We studied the pathways that lead to arrest and firm adhesion of rolling PMN on activated, surface-adherent platelets (50). Stable arrest and adhesion strengthening of PMN on thrombin-stimulated, surface-adherent platelets in flow requires distinct Ca^{2+}- and Mg^{2+}-dependent regions of Mac-1 (αMβ2) and involves interactions of Mac-1 with fibrinogen bound to platelets via αIIbβ3. Mac-1 also binds to unidentified ligands on platelets other than ICAM-2, heparin or heparan sulfate proteoglycans, as seen by inhibition with mAbs or peptides, treatment of platelets with heparitinase and by using platelets with defective αIIbβ3 from a patient with Glanzmann thrombasthenia (50). Tethering of PMN on platelet ICAM-2 via LFA-1 may facilitate the transition between rolling on selectins and Mac-1-dependent arrest. Chemoattractants and chemokines stimulate the avidity of Mac-1 in PMN (43,44) and may convert selectin-mediated rolling into firm arrest (29). Hence, chemoattractive lipid mediators may play a role in accumulation and adhesion strengthening of PMN on activated platelets in flow. Pretreatment of PMN with the PAF receptor antagonist UK-74,505 (51) results in a dose-dependent inhibition of PMN accumulation (50). The LTB4 receptor antagonist SC-53228 is less po-

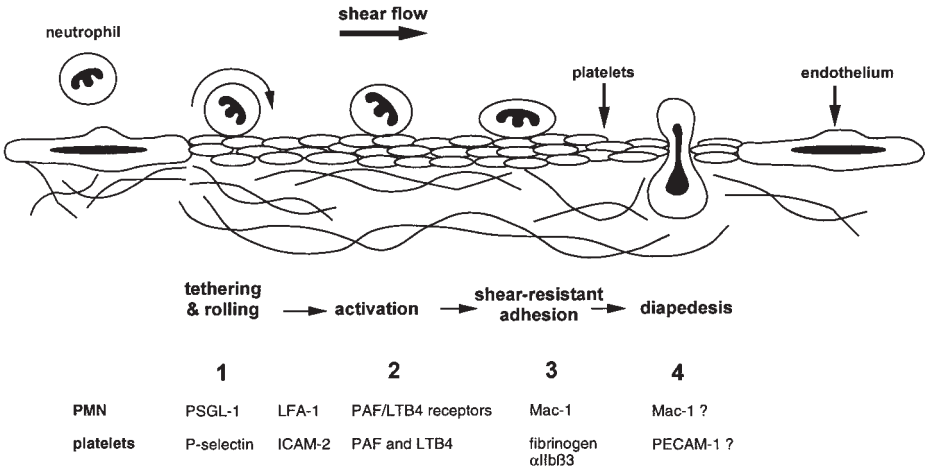

Fig. 2 Sequential involvement of signal molecules in accumulation of neutrophils on adherent platelets under shear flow. ICAM-2, intercellular cell adhesion molecule-2; LFA-1 lymphocyte function associated-1, CD11a/CD18 integrin; LTB4, leukotriene B4; Mac-1, CD11b/CD18 integrin; PAF, platelet-activating factor; PSGL-1, P-selectin glycoprotein-ligand 1; PECAM-1, platelet-endothelial cell adhesion molecule-1.

tent, while no additive effects are seen with both antagonists over UK-74,505 alone. Likewise, pertussis toxin (PTX) inhibits accumulation and shows little additive effect with UK-74,505, whereas pretreatment with the CXCR2 mAb 10H2 did not inhibit arrest. Similar effects were seen for adhesion strengthening (50). UK-74,505, SC-53228 and pertussis toxin PTX but not CXCR2 mAb reduced the shear resistance of PMN adhesion and increased rolling. Contrasting effects were obtained for spreading of PMN on activated platelets in stasis. Thus, PMN arrest and adhesion strengthening on activated platelets under flow conditions appeared to be stimulated by PAF and, to a lesser extent, LTB4 via PTX-sensitive signaling pathways but not by activation of CXCR2 (50).

It has been intriguing to apply a multistep model to the highly efficient accumulation of PMN on activated, surface-adherent platelets in flow. Rolling mediated by P-selectin is important as a prerequisite for firm attachment, and rolling on activated platelets was rapidly followed by Mac-1 mediated arrest on fibrinogen (50). We suggest that distinct functional regions of Mac-1 contribute to PMN arrest and adhesion strengthening on activated platelets in flow. Seven N-terminal repeats of integrin α subunits have been predicted to fold into a β-propeller structure containing putative Ca^{2+}-binding motifs located on the lower face, and the I or inserted domain with a Mg^{2+} at its binding site to be tethered to the top of this structure (52). The arrest of PMN may involve Ca^{2+}-dependent regions of Mac-1, in particular a putative β-propeller domain, while the Mg^{2+}-dependent regions in the I domain may contribute to PMN arrest on activated platelets. Taken together, Mac-1 may be functionally subdivided into regions which contribute to initial arrest and regions which are required for subsequent shear resistance in flow (50).

Arrest and adhesion strengthening of PMN on platelets in flow appears to be stimulated by the lipid mediators PAF and, to a lesser extent, LTB4, via PTX-sensitive signaling pathways, but not by activation of CXCR2 (50). Several reasons may account for differential effectiveness of platelet chemoattractants during PMN activation in shear flow. Firstly, lipid mediators but not CXC chemokines are soluble in the lipid membrane, whereas chemokines but not lipid mediators can be bound to proteoglycans. The differential binding of chemokines to proteoglycan subpopulations may determine the presence and distribution of chemokines in the cellular microenvironment (53). Thus, PAF or LTB4 may be retained in the platelet plasma membrane by partition, whereas relevant platelet CXC chemokines may not be bound to platelet proteoglycans, and may be washed away under flow. Moreover, PMN enzymes required for processing platelet precursors into active CXC chemokines (47) may not be immobilized and may be removed by flow. This would be consistent with inhibition of adhesion strengthening and spreading by the CXCR2 mAb in stasis but not in flow. Alternatively, the PAF receptor may differ from CXCR2 in its PTX-sensitive signal transduction pathways and coupling to Gα proteins (48), which may enable a more rapid activation of Mac-1 adhesiveness under flow conditions. In parallel, endothelial PAF has been implicated in juxtacrine stimulation of integrin function in PMN tethered through P-selectin on thrombin-stimulated endothelium (54). Although P-selectin itself does not trigger arrest of PMN under flow, it may cooperate with chemoattractants to stimulate leukocytes (55). It has recently been confirmed that PMN adhesion on immobilized platelets under shear flow requires both a juxtacrine activation mechanism involving PAF and an enhancing signal from P-selectin (56). Finally, Mac-1 and homologous interactions of platelet-endothelial cell adhesion molecule-1 (PECAM-1) may contribute to transmigration of PMN through platelet layers, e.g. towards IL-8, thus completing extravasation (37). Figure 2 illustrates the multistep model with a sequential involvement of signal molecules including PAF in the efficient accumulation of PMN on adherent platelets under shear flow conditions.

The proposed mechanisms of PMN arrest on activated, adherent platelets contribute to a more elaborate

definition of the multistep model by demonstrating a differential involvement of integrins, their ligands and of chemoattractive lipid mediators in sequential steps of this process. Interactions between platelets and leukocytes occur at sites of vascular damage, as in hemostasis. Platelets bind to prothrombotic endothelium and the underlying basement membrane in vascular injury, occupying a position analogous to the endothelium. Binding of PMN to platelets immobilized in a thrombus may facilitate immigration into thrombosed areas, wound healing, tissue repair, or protection from infection, and may contribute to the maintenance of vascular integrity, as well as to its impairment in pathological states. Platelets and PMN indeed colocalize at sites of hemorrhage, vascular grafts, atherosclerotic lesions and myocardial infarction (57–60). Activation of PMN and platelet adhesion occurs after coronary angioplasty and is associated with late clinical events (61). Understanding the mechanisms of PMN accumulation on platelets in flow may thus lead to clinical applications and interventions to influence the consequences of leukocyte-platelet interactions in vascular disease.

Statins Inhibit Monocyte Adhesiveness in Patients with Hypercholesterolemia

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (statins) are used in the treatment of hypercholesterolemia. They also improve the survival of patients with coronary heart disease (CHD) and prevent CHD in hypercholesterolemic men (62, 63). However, the clinical benefit of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, such as simvastatin and pravastatin, is not strictly correlated to their lipid lowering effects (62, 64). The action of statins on cellular cholesterol biosynthesis in the liver, blood cells, and vasculature may affect membrane integrity and regulation of membrane-related processes (65) which are associated with changes in multiple cellular functions. Cholesterol precursors, i.e. farnesyl or geranyl pyrophosphates, have been implicated in interactions of proteins and protein anchoring to lipid membranes (66, 67), essentially contributing to receptor-stimulated Ca^{2+}-increase, superoxide anion formation, LDL oxidation, eicosanoid synthesis and differentiation in monocytic cells (68-71). Functional consequences of such effects may be relevant for CHD and atherosclerosis. Monocyte recruitment into the vascular wall is crucial for initiation and progression of atherosclerotic lesions (58). Interaction of the monocytic surface receptor Mac-1 with ICAM-1 has been involved in the adhesion to and migration across endothelium of monocytes, particularly when stimulated (5, 30). Hence, we studied effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on monocyte CD11b surface expression and adhesion to endothelium *in vitro,* as well as on increased adhesiveness of monocytes (72) isolated from hypercholesterolemic patients before and after cholesterol-lowering treatment, using a recently established *ex vivo* assay with fixed human endothelial cells (73).

In hypercholesterolemic patients, Mac-1-dependent adhesion of isolated monocytes to fixed endothelium expressing ICAM-1 *ex vivo* is dramatically increased, as compared to healthy controls. Treatment of these patients with the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors lovastatin or simvastatin for 6 weeks decreases total and LDL cholesterol plasma levels, as well as monocyte CD11b surface expression, and results in a substantial reduction of monocyte adhesion to endothelium (74). The regulation of CD11b surface expression is dissociated from adhesive function, which requires its activation (75, 76). Thus, the pronounced decrease in adhesion after treatment with statins appears to be independent of the slight reduction in CD11b expression. Adhesiveness of Mac-1 may rather be mediated by a small subpopulation of molecules undergoing conformational changes in response to stimulation (77). Moreover, treatment of monocytes with lovastatin *in vitro* dose-dependently reduces surface expression of CD11b on monocytes, and inhibits CD11b-dependent adhesiveness to fixed endothelium of monocytes stimulated with MCP-1. Coincubation with mevalonate, but not LDL, reverses these effects (74), suggesting that early cholesterol precursors but not cholesterol are crucial for adhesiveness of CD11b. Hence, lovastatin may interfere with activation of Mac-1 adhesiveness by chemokines in monocytes, due to decreased availability of isoprenoids.

Monocyte accumulation following adhesion to endothelium and extravasation plays a crucial role in atheroma formation due to a perpetuation of chronic inflammatory reaction and lipid deposition into monocyte-derived foam cells (58). These cells further contribute to the instability and disruption of atherosclerotic plaques by secreting metalloproteases that degrade extracellular matrix constituents, and may thus trigger clinical manifestations of atherosclerosis (78). Isoprenoids posttranslationally modify various proteins and mediate protein-membrane anchoring and interactions of proteins. Some of the isoprenylated proteins identified are small guanosine triphosphate (GTP)-binding proteins of the *ras* family, such as *rac* and *rho,* as well as the γ-subunits of heterotrimeric G-proteins (67). Chemoattractant-receptors are coupled to G-protein heterotrimers, and integrin-dependent binding of leukocytes to endothelium can involve a G-protein regulated activation event (30). Lovastatin has been shown to inhibit Ca^{2+}-influx stimulated by G-protein-coupled receptors and localization of *ras*-like proteins into the membrane of monocytic cells (68). Expression of a constitutively active form of the GTPase R-*ras* results in activation of β_1 and β_3 integrin avidity, and inactivation of the GTPase *rho* suggests its participation in signaling via chemoattractant receptors to trigger activation of Mac-1 (79, 80). Hence, statins may impair activation of Mac-1 involving small GTPases, or stimulation of G-protein-heterotrimer coupled receptors by MCP-1 or other autocrine chemokines. Alternatively, these drugs may act by interfering with the transport, dimerization or anchoring of Mac-1 in the plasma membrane to maintain its cell surface expression. Statins may affect GTP-bind-

Tab. 1 Examples of cholesterol-independent effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins).

Effect	Reference
Decrease in receptor-stimulated Ca2+-increase	68
Reduction in NADPH-dependent superoxide anion formation	69
Inhibition of cellular LDL oxidation	69
Increase in arachidonic acid metabolism	70
Monocytic cell differentiation	71
Interference with monocyte chemotaxis	83
Normalization of platelet function	84
Reduction in smooth muscle cell proliferation migration	85
Upregulation of endothelial nitric oxide synthetase	86
Inhibition of metalloprotease secretion	87
Inhibition of monocyte and leukocyte adhesion	74, 82

ing protein-membrane or protein interactions by preventing their prenylation or farnesylation, thus impairing activation and expression of Mac-1.

Increased adhesion of monocytes to endothelium *ex vivo* occurs in hypercholesteremic patients, however inhibitory effects of statins have not been demonstrated (72). The use of fixed endothelium from one preparation allows to minimize interassay variations, particularly in longitudinal adhesion studies (73), and may have thus enabled us to detect inhibitory effects of statins on monocyte adhesion. The increased adhesion of monocytes from hypercholesterolemic patients has been attributed to abnormal monocyte function with respect to eicosanoid metabolism and superoxide anion generation (72, 81). Hence, statins may act by inhibiting superoxide anion formation and subsequent LDL oxidation by an NAPDH oxidase in activated monocytes which requires assembly of various cytosolic components including small GTP-binding proteins (67, 69). Consistently, intake of the antioxidant vitamin C has been demonstrated to prevent the increased CD11b-dependent adhesiveness of monocytes from smokers (73), which may involve similar oxidative pathways.

The finding that reductions in relative risk of CHD after simvastatin reported in the 4S trial do not significantly differ between quartiles for any lipid variable (62) implies effects on mechanisms involved in atherogenesis other than cholesterol lowering. Moreover, the efficacy of pravastatin in the REGRESS study (64) is reduced by smoking, which increases oxidative stress and monocyte adhesiveness (73). In conclusion, the reduction of monocyte CD11b expression and CD11b-dependent adhesiveness to endothelium by statins provides a novel mechanism of action of these compounds and may explain beneficial effects in the prevention and treatment of CHD (62, 63). Inhibition of leukocyte adhesion and chemotaxis (73, 82, 83) highlights actions of statins which are not related to their cholesterol lowering effects but may be due to interference with cellular isoprenoid metabolism or prenylation-dependent processes. As summarized in Table 1, other direct vascular effects of statins have emerged, i.e. reduction of platelet aggregation (84), inhibition of smooth muscle cell proliferation and migration (85), upregulation of endothelial NO synthetase (86) and inhibition of metalloprotease secretion (87). Our findings may be crucial for clinical benefits of statins, and may represent a novel anti-inflammatory paradigm for the mechanisms of action by this class of drugs (88), which warrants further detailed investigation with respect to leukocyte biology.

References

1. Parthasarathy S, Rankin SM. Role of oxidized low density lipoprotein in atherogenesis. Prog Lipid Res 1992; 31:127–43.
2. Lehr H-A, Becker M, Marklund SL, Hübner C, Arfors KE, Kohlschütter A, *et al.* Superoxide-dependent stimulation of leukocyte adhesion by oxidatively modified LDL in vivo. Arterioscler Thromb 1992; 12:824–9.
3. Quinn MT, Parthasarathy S, Fong GL, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sci USA 1987; 84:2995–8.
4. Frostegård J, Nilsson J, Haegerstrand A, Hamsten A, Wigzell H, Gidlund M. Oxidized low density lipoprotein induces differentiation and adhesion of human monocytes and the monocytic cell line U937. Proc Natl Acad Sci USA 1990; 87:904–8.
5. Weber C, Erl W, Weber PC. Enhancement of monocyte adhesion to endothelial cells by oxidatively modified low-density lipoprotein is mediated by activation of CD11b. Biochem Biophys Res Commun 1995; 206:621–8.
6. Jeng JR, Chang CH, Shieh SM, Chiu HC. Oxidized low-density lipoprotein enhances monocyte-endothelial cell binding against shear-stress-induced detachment. Biochem Biophys Acta 1993; 1178:221–7.
7. Smalley DM, Lin JHC, Curtis ML, Kobari Y, Stemerman MB, Pritchard KA Jr. Native LDL increases endothelial cell adhesiveness by inducing intercellular adhesion molecule-1. Arterioscler Thromb Vasc Biol 1996; 16:585–90.
8. Calderon TM, Factor SM, Hatcher VB, Berliner JA, Berman JW. An endothelial cell adhesion protein for monocytes recognized by monoclonal antibody IG9. Lab Invest 1994; 70:836–49.
9. McEvoy LM, Sun H, Tsao PS, Cooke JP, Berliner JA, Butcher EC. Novel vascular molecule involved in monocyte adhesion to aortic endothelium in models of atherogenesis. J Exp Med 1997; 185:2069–77.
10. Weber C, Aepfelbacher M, Haag H, Ziegler-Heitbrock HWL, Weber PC. Tumor necrosis factor induces enhanced responses to platelet activating factor and differentiation in human monocytic Mono Mac 6 cells. Eur J Immunol 1993; 23:852–7.
11. Erl W, Weber C, Wardemann C, Weber PC. Adhesion properties of Mono Mac 6, a monocytic cell line with characteristics of mature human monocytes. Atherosclerosis 1995; 113:99-107.

12. Diamond MS, Alon R, Parkos CA, Quinn MT, Springer TA. Heparin is an adhesive ligand for leukocyte integrin Mac-1 (CD11b/CD18). J Cell Biol 1995; 130:1473–82.
13. Kim JA, Territo MC, Wayner E, Carlos TM, Parhami F, Smith CW, *et al*. Partial characterization of leukocyte binding molecules on endothelial cells induced by minimally oxidized LDL. Arterioscler Thromb 1994; 14:427–33.
14. Lynch SM, Morrow JD, Roberts LJ, Frei B. Formation of non-cyclooxygenase-derived prostanoids (F2-isoprostanes) in plasma and low density lipoprotein exposed to oxidative stress in vitro. J Clin Invest 1994; 93:998–1004.
15. Kume N, Cybulsky MI, Gimbrone MA Jr. Lyso-phosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. J Clin Invest 1992; 90:1138–44.
16. Erl W, Weber PC, Weber C. Monocytic cell adhesion to endothelial cells stimulated by oxidized low density lipoprotein is mediated by distinct endothelial ligands. Atherosclerosis. 1998; 136: 297–303.
17. Frostegård J, Haegerstrand A, Diglund M, Nilsson J. Biologically modified LDL increases the adhesive properties of endothelial cells. Atherosclerosis 1991; 90:119–26.
18. Calara F, Dimayuga P, Niemann A, Thyberg J, Diczfalusy U, Witztum JL, *et al*. An animal model to study local oxidation of LDL and its biological effects in the arterial wall. Arterioscler Thromb Vasc. Biol 1998; 18:884–93.
19. Weber C, Lu CF, Casasnovas JM, Springer TA. Role of αLβ2 integrin avidity in transendothelial chemotaxis of mononuclear cells. J Immunol 1997; 159: 3968–75.
20. Yue TL, Wang XK, Ruffolo RR, Feuerstein GZ. Carvedilol, a new vasodilating β-adreno-receptor blocker, inhibits oxidation of low-density lipoproteins by vascular smooth muscle cells and prevents leukocyte adhesion to smooth muscle cells. J Pharmacol Exp Ther 1995; 273:1442-9.
21. Weber C, Erl W, Pietsch A, Weber PC. Aspirin inhibits nuclear factor-κB mobilization and monocyte adhesion in stimulated human endothelial cells. Circulation 1995; 91:1914–8.
22. Schwartz D, Andalibi A, Chaverri-Almada L, Berliner JA, Kirchgessner T, Fang ZT, *et al*. Role of the GRO family of chemokines in monocyte adhesion to mm-LDL-stimulated endothelium. J Clin Invest 1994; 94:1968–73.
23. Weber C, Alon R, Moser B, Springer TA. Sequential regulation of α4β1 and α5β1 integrin avidity by CC chemokines in monocytes: implications for transendothelial chemotaxis. J Cell Biol 1996; 134:1063–74.
24. Sugiyama S, Kugiyama K, Ohgushi M, Fujimoto K, Yasue H. Lysophosphatidylcholine in oxidized low-density lipoprotein increases endothelial susceptibility to polymorphonuclear leukocyte-induced endothelial dysfunction in porcine coronary arteries. Circ Res 1994; 74:565–75.
25. Khan BV, Parthasarathy SS, Alexander W, Medford RM. Modified low density lipoprotein and its constituents augment cytokine-activated vascular cell adhesion molecule-1 gene expression in human vascular endothelial cells. J Clin Invest 1995; 95:1262–70.
26. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. Cell 1998; 93:229–40.
27. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARγ promotes monocyte/ macrophage differentiation and uptake of oxidized LDL. Cell 1998; 93:241–52.
28. Dawson DW, Pearce SF, Zhong R, Silverstein RL, Frazier WA, Bouck NP. CD36 mediates the in vitro inhibitory effects of thrombospondin-1 on endothelial cells. J Cell Biol 1997; 138:707–17.
29. Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell 1991; 65:859–73.
30. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multi-step paradigm. Cell 1994; 76:301–14.
31. Stenberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF. A platelet alpha granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. J Cell Biol 1985; 101:880–6.
32. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, *et al*. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 1989; 59:305–12.
33. Diacovo TG, de Fougerolles AR, Bainton DF, Springer TA. A functional integrin ligand on the surface of platelets: intercellular adhesion molecule-2. J Clin Invest 1994; 94:1243–1.
34. Palabrica T, Lobb R, Furie BC, Aronivitz M, Benjamin C, Hsu Y-M, *et al*. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. Nature 1992; 359:848–51.
35. Buttrum SM, Hatton R, Nash GB. Selectin-mediated rolling of neutrophils on immobilized platelets. Blood 1993; 82:1165–74.
36. Yeo EL, Sheppard J-AI, Feuerstein IA. Role of P-selectin and leukocyte activation in polymorphonuclear cell adhesion to surface adherent activated platelets under physiologic shear conditions (an injury vessel wall model). Blood 1994; 83:2498–507.
37. Diacovo TG, Roth SJ, Buccola JM, Bainton DF, Springer TA. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the β_2-integrin CD11b/CD18. Blood 1996; 88:146–57.
38. Altieri DC, Bader R, Mannucci PM, Edgington TS. Oligospecificity of the cellular adhesion receptor Mac-1 encompasses an inducible recognition specificity for fibrinogen. J Cell Biol 1988; 107:1893–900.
39. Wright SD, Weitz JI, Huang AD, Levin SM, Silverstein SC, Loike JD. Complement receptor type three (CD11b/CD18) of human polymorphonuclear leukocytes recognizes fibrinogen. Proc Natl Acad Sci USA 1988; 85:7734–8.
40. Pytela R, Pierschbacher MD, Ginsberg MH, Plow EF, Ruoslahti E. Platelet membrane glycoprotein IIb/IIIa: member of a family of Arg-Gly-Asp-specific adhesion receptors. Science 1986; 231:1559–62.
41. Ruf A, Schlenk RF, Maras A, Morgenstern E, Patscheke H. Contact-induced neutrophil activation by platelets in human cell suspensions and whole blood. Blood 1992; 80:1238–46.
42. Lo SK, Detmers PA, Levin SM, Wright SD. Transient adhesion of neutrophils to endothelium. J Exp Med 1989; 169:1779–93.
43. Detmers PA, Lo SK, Olsen-Egbert E, Walz A, Baggiolini M, Cohn ZA. Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. J Exp Med 1990; 171:1155–62.
44. Marcus AJ, Safier LB, Ullman HL, Islam N, Broekman MJ, Falck JR, *et al*. Platelet-neutrophil interactions: (12*S*)-hydroxyeicosatetraen-1 20-dioic acid: a new eicosanoid synthesized by unstimulated neutrophils from (12-*S*)-20-dihydroxyeicosatetraenoic acid. J Biol Chem 1988; 263:2223–9.
45. Power CA, Furness RB, Brawand C, Wells TNC. Cloning of a full-length cDNA encoding the neutrophil-activating peptide ENA-78 from human platelets. Gene 1994; 151:333–4.

46. Walz A, Baggiolini M. A novel cleavage product of beta-thromboglobulin formed in cultures of stimulated mononuclear cells activates human neutrophils. Biochem Biophys Res Commun 1989; 159:969–75.
47. Brandt E, Van Damme J, Flad H-D. Neutrophils can generate their activator neutrophil-activating peptide 2 by proteolytic cleavage of platelet-derived connective tissue-activating peptide III. Cytokine 1991; 3:311–21.
48. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines-CXC and CC chemokines. Adv Immunol 1994; 55:97–179.
49. Amatruda TT, Gerard NP, Gerard C, Simon MI. Specific interactions of chemoattractant factor receptors with G-proteins. J Biol Chem 1993; 268:10139–44.
50. Weber C, Springer TA. Neutrophil accumulation on activated, surface-adherent platelets in flow is mediated by interaction of Mac-1 with fibrinogen bound to αIIbβ3 and stimulated by platelet activating factor. J Clin Invest 1997; 100: 2085–93.
51. Pons F, Rossi AG, Norman KE, Williams TJ, Nourshargh S. Role of platelet-activating factor (PAF) in platelet accumulation in rabbit skin: effect of the novel long-acting PAF antagonist, UK-74,505. Br J Pharmacol 1993; 109:234–42.
52. Springer TA. Folding of the N-terminal, ligand-binding region of integrin α-subunits into a β-propeller domain. Proc Natl Acad Sci USA 1997; 94:65–72.
53. Witt DP, Lander AD. Differential binding of chemokines to glycosaminoglycan subpopulations. Curr Biol 1994; 4:394–400.
54. Lorant DE, Patel KD, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. J Cell Biol 1991; 115:223–34.
55. Weyrich AS, Elstad MR, McEver RP, McIntyre TM, Moore KL, Morrissey JH, *et al.* Activated platelets signal chemokine synthesis by human monocytes. J Clin Invest 1996; 97:1525–34.
56. Olstrowsky L, King AJ, Bond S, Mitchell D, Lorant DE, Zimmerman GA, *et al.* A juxtracrine mechanism for neutrophil adhesion on platelets involves platelet-activating factor and a selectin-dependent activation process. Blood 1998; 91:3028–36.
57. Laws KH, Clanton JA, Starnes VA. Kinetics and imaging of indium-111-labeled autologous platelets in experimental myocardial infarction. Circulation 1983; 67:110–6.
58. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362:801–9.
59. Bednar MM, Raymond S, McAuliffe T, Lodge PA, Gross CE. The role of neutrophils and platelets in a rabbit model of thromboembolic stroke. Stroke 1991; 22:44–50.
60. Del Maschio A, Dejana E, Bazzoni G. Bidirectional modulation of platelet and polymorphonuclear leukocyte activities. Ann Hematol 1993; 67:23–31.
61. Mickelson JK, Lakkis NM, Villarreal-Levy G, Hughes BJ, Smith CW. Leukocyte activation with platelet adhesion after coronary angioplasty: a mechanism for recurrent disease? J Am Coll Cardiol 1996; 28:345–53.
62. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994; 344:1383–9.
63. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, *et al.* Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995; 333:1301–7.
64. Jukema JW, Bruschke AVG, van Boven AJ, Reiber JH, Bal ET, Zwinderman AH, *et al.* Effects of lipid lowering by pravastatin on progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels. The regression growth evaluation statin study (REGRESS). Circulation 1995; 91:2528–40.
65. Yeagle PL. Lipid regulation of cell membrane structure and function. FASEB J 1989; 3:1833–42.
66. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature 1990; 343:425–30.
67. Glomset JA, Gelb MH, Farnsworth CC. Prenyl proteins in eukaryotic cells: a new type of membrane anchor. Trends Biochem Sci 1990; 15:139–42.
68. Haag H, Grünberg B, Weber C, Vauti F, Aepfelbacher M, Siess W. Lovastatin inhibits receptor-stimulated Ca^{2+}-influx of retinoic acid differentiated U937 and HL-60 cells. Cell Signalling 1994; 7:735–42.
69. Giroux LM, Davignon J, Naruszewicz M. Simvastatin inhibits the oxidation of low-density lipoproteins by activated human monocyte-derived macrophages. Biochim Biophys Acta 1993; 1165:335–8.
70. Hrboticky N, Tang L, Zimmer B, Weber PC. Lovastatin increases arachidonic acid levels and stimulates thromboxane synthesis in human liver and monocytic cell lines. J Clin Invest 1994; 93:195–203.
71. Weber C, Erl W, Weber PC. Lovastatin induces differentiation of Mono Mac 6 cells. Cell Biochem Funct 1995; 13:273–7.
72. Jongkind JF, Verkerk A, Hoogerbrugge N. Monocytes from patients with combined hypercholesterolemia-hypertriglyceridemia and isolated hypercholesterolemia show increased adhesion to endothelial cells in vitro: influence of intrinsic and extrinsic monocyte binding. Metabolism 1995; 44:374–8.
73. Weber C, Erl W, Weber K, Weber PC. Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers. Circulation 1996; 93:1488–92.
74. Weber C, Erl W, Weber KSC, Weber PC. HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocytes to endothelium and reduce increased adhesiveness of monocytes isolated from hypercholesterolemic patients. J Am Coll Cardiol 1997; 30:1212–7.
75. Buyon JP, Abramson SB, Philips MR, Slade SG, Ross GD, Weissman G, *et al.* Dissociation between increased surface expression of Gp165/95 and homotypic neutrophil aggregation. J Immunol 1988; 140:3156–60.
76. Vedder NB, Harlan JM. Increased surface expression of CD11b/CD18 (Mac-1) is not required for stimulated neutrophil adherence to cultured endothelium. J Clin Invest 1988; 81:676–82.
77. Diamond MS, Springer TA. A subpopulation of Mac-1 (CD11b/CD18) molecules mediates neutrophil adhesion to ICAM-1 and fibrinogen. J Cell Biol 1993; 120:545–56.
78. Galis ZS, Sukhova GK, Kranzhofer R, Clark S, Libby P. Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases. Proc Natl Acad Sci USA 1995; 92:402–6.
79. Laudanna C, Campbell JJ, Butcher EC. Role of Rho in chemoattractant-activated leukocyte adhesion through integrins. Science 1996; 271:981–3.
80. Zhang Z, Vuori K, Wang H-G, Reed JC, Ruoslahti E. Integrin activation by R-ras. Cell 1996; 85:61–9.
81. Stragliotto E, Camera M, Postiglione A, Sirtori M, DiMinno G, Tremoli E. Functionally abnormal monocytes in hypercholesterolemia. Arterioscler Thromb 1993; 13:944–50.

82. Kimura M, Kurose I, Russell J, Granger DN. Effects of fluvastatin on leukocyte-endothelial cell adhesion in hypercholesterolemic rats. Arterioscler Thromb Vasc Biol 1997; 17:1521–6.
83. Kreuzer J, Bader J, Jahn L, Hautmann M, Kubler W, von Hodenberg E. Chemotaxis of the monocyte cell line U937: dependence on cholesterol and early mevalonate pathway products. Atherosclerosis 1991; 90:203–9.
84. Notabartolo A, Davi G, Averna M, Barbagallo CM, Giammarresi C, La Placa FP, *et al*. Inhibition of thromboxane biosynthesis and platelet function by simvastatin in type IIa hypercholesterolemia. Arterioscler Thromb Vasc Biol 1995; 15:247–51.
85. Bellosta S, Bernini F, Ferri N, Quarato P, Canavesi M, Arnaboldi L, *et al*. Direct vascular effects of HMG-CoA reductase inhibitors. Atherosclerosis 1998; 137 Suppl: S101–9.
86. Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. Circulation 1998; 97:1129–35.
87. Rossen RD. HMG-CoA reductase inhibitors: a new class of anti-inflammatory drugs? J Am Coll Cardiol 1997; 30: 1218–9.
88. Bellosta S, Via D, Canavesi M, Pfister P, Fumagalli, Paoletti R, *et al*. HMG-CoA reductase inhibitors reduce MMP-9 secretion by macrophages. Arterioscler Thromb Vasc Biol 1998; 18:1671–8.

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