Expression of Fractalkine (CX3CL1) and its Receptor, CX3CR1, Is Elevated in Coronary Artery Disease and Is Reduced During Statin Therapy

Jan K. Damås, Agnes Boullier, Torgun Wæhre, Camilla Smith, Wiggo J. Sandberg, Simone Green, Pål Aukrust, Oswald Quehenberger

Objective—Recent data derived primarily from studies in animal models suggest that fractalkine (CX3CL1) and its cognate receptor, CX3CR1, play a role in atherogenesis. We, therefore, hypothesized that enhanced CX3CL1/CX3CR1 expression may promote atherogenesis in patients with coronary artery disease (CAD).

Methods and Results—We examined the plasma levels of CX3CL1 and CX3CR1 expression in peripheral blood mononuclear cells (PBMC) in various CAD populations (30 patients with previous myocardial infarction, 40 patients with stable angina, 40 patients with unstable angina, and a total of 35 controls) and used various experimental approaches to characterize CX3CL1-mediated leukocyte responses. We found that the plasma levels of CX3CL1 are greatly increased in CAD, particularly in unstable disease. The parallel increase of CX3CR1 expression in PBMC was predominantly attributable to an expansion of the CX3CR1+CD3+CD8+ T cell subset and was associated with enhanced chemotactic, adhesive, and inflammatory responses to CX3CL1. Statin therapy for 6 months reduced the expression of CX3CL1 and CX3CR1, reaching statistical significance for both parameters only during aggressive (atorvastatin, 80 mg qd) but not conventional (simvastatin, 20 mg qd) therapy. Consequently, the functional responses of the PBMC to CX3CL1 including migration, adhesion, and secretion of interleukin-8 were attenuated by the treatments.

Conclusion—Our results suggest that the CX3CL1/CX3CR1 dyad may contribute to atherogenesis and plaque destabilization in human CAD. (Arterioscler Thromb Vasc Biol. 2005;25:2567-2572.)

Key Words: atherosclerosis ■ coronary artery disease ■ cytokines ■ immune system ■ leukocytes

Besides lipid deposition and vascular smooth muscle cell (VSMC) proliferation, the infiltration of monocytes and T cells is a cardinal feature of atherosclerotic plaques of all stages.1,2 This persistent recruitment of mononuclear cells into the vessel wall is a critical step in atherogenesis, and growing evidence suggests that chemokines are involved in these processes by activating and directing leukocytes into atherosclerotic lesions.3,4

Fractalkine (CX3CL1) is the unique member of the CX3 chemokine subfamily. In contrast to other chemokines, it exists in two forms, each mediating distinct biological actions.5 The membrane-anchored protein, which is expressed primarily on the inflamed endothelium, serves as an adhesion protein promoting the retention of monocytes and T cells.6 The soluble form resembles more a conventional chemokine and strongly induces chemotaxis. Both chemotaxis and adhesion are mediated by the G protein–coupled receptor CX3CR1.7,8 Based on these chemotactic and adhesive properties, CX3CL1 has been suggested to play an important role in inflammation and accumulating evidence indicates that CX3CL1/CX3CR1 are involved in the pathogenesis of various inflammatory disorders.9,10

Recently, two independent reports showed that the targeted deletion of CX3CR1 decreased atherosclerosis in mice, implicating a role of this chemokine system in atherogenesis.11,12 Moreover, Lucas et al and Greaves et al have recently shown expression of CX3CL1 and CX3CR1 in human coronary atherosclerotic plaques,13,14 and polymorphisms in the CX3CR1 gene have been shown to be associated with coronary events.15-17 However, the role of the CX3CL1/CX3CR1 dyad in human coronary artery disease (CAD), and particularly in unstable disease, remains unclear. The present study was designed to further elucidate the potential role of CX3CL1/CX3CR1 in human CAD by examining the expression of CX3CL1 and its receptor in CAD patients, including patients with stable and unstable angina. We also investigated the ability of 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitors (statins) to modulate CX3CL1/CX3CR1 levels in these patients.
Methods

Patients and Controls
In the angina study we included 40 patients with unstable angina (defined as ischemic chest pain at rest preceding 48 hours, transient ST-T-segment depression, and/or T-wave inversion but with no evidence of myocardial necrosis by enzymatic criteria) and 40 patients with stable angina (defined as effort angina >3 months and a positive exercise test) (Table I, available online at http://atvb.ahajournals.org). The diagnosis of CAD was confirmed by coronary angiography. For the statin study, we had available to us human plasma and leukocytes obtained for an unrelated study described previously. Briefly, 30 patients with previous myocardial infarction without prior statin treatment were included and randomized to simvastatin 20 mg qd (n=15) or atorvastatin 80 mg qd (n=15) therapy for 6 months in an open study (Table II, available online at http://atvb.ahajournals.org), representing both conventional and aggressive statin therapy, respectively. Exclusion criteria in both studies were myocardial infarction or thrombolytic treatment within the previous 6 weeks, severe concomitant disease (eg, infections, connective tissue disease, or malignancies), congestive heart failure, and the use of medications other than aspirin with known antiinflammatory effects. For comparison, blood was collected from 35 healthy controls (20 in the angina study and 15 in the statin study) matched for age, sex, and smoking habits (Tables I and II). The controls were health care workers, consecutively recruited from the same area of Norway as the patients (eastern part). Although asymptomatic CAD cannot be totally excluded, all the controls were evaluated as healthy based on clinical examination, disease history, and analyses of C-reactive protein (CRP) and lipid parameters. The regional ethical committee approved all parts of the study, and all individuals gave their informed consent.

Isolation of Cells and Plasma
Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by Isopaque–Ficoll gradient centrifugation, and separation of monocytes and CD3+ T cells from freshly isolated PBMC was performed as described elsewhere. For details, please see the expanded online Methods, available online at http://atvb.ahajournals.org.

Cell Culture
Thawed cryopreserved PBMC and primary human umbilical vein endothelial cells (HUVEC) were cultured as described previously (please see the expanded online Methods).

Chemotaxis and Adhesion Assays
CX3CL1-mediated chemotactic activities and adhesion were examined with thawed cryopreserved PBMC or freshly isolated T cells and monocytes (please see the expanded online Methods).

Real-Time Quantitative Reverse Transcriptase–Polymerase Chain Reaction
mRNA levels of CX3CL1 and CX3CR1 were quantified by real-time RT-PCR and data were normalized to β-actin gene expression. Horizontal lines represent the median values.

Expression of CX3CL1 and CX3CR1 in Angina Patients
As shown in Figure 1, both stable (756±72 pg/mL) and unstable (1199±112 pg/mL) angina patients had significantly increased plasma levels of CX3CL1, compared with healthy controls (423±25 pg/mL). The increase was highly significant and was particularly pronounced in unstable disease (P<0.001 versus stable angina). Analyses of PBMC transcripts showed that CX3CR1 expression was also substantially higher in the angina patients and, like CX3CL1, was particularly elevated in patients with unstable disease (P<0.01 versus stable angina; Figure 1). In fact, 32 of the 40 patients with unstable angina had higher plasma levels of CX3CL1 than the highest concentration in healthy controls, and as for CX3CR1 expression there was no overlap between unstable angina patients and controls. The unstable patients with the highest CX3CL1 levels (>median) were not significantly different from the other individuals in this group with regard to sex, smoking habits, diabetes, or plasma cholesterol levels. Moreover, there was no correlation between CRP levels and either CX3CL1 or CX3CR1 expression within the angina patients (data not shown).

Reduction of CX3CL1 and CX3CR1 Expression by Statin Therapy
To examine the effect of statin treatment on CX3CL1 and CX3CR1 levels, a group of 30 CAD patients was examined before and after therapy. Before the start of the statin therapy, the patients exhibited significantly elevated plasma levels of CX3CL1 (P<0.01) and enhanced PBMC expression of CX3CR1 (P<0.001) compared with controls (Figure 2). More detailed analysis by flow cytometry showed that although the CX3CR1 expression on monocytes was similar between patients and controls, the fraction of CX3CR1-expressing CD3+ T cells was significantly increased in the CAD group (P<0.001), which was mainly attributable to an expansion of the CX3CR1-expressing CD3+CD8+ T cell

Figure 1. A, Plasma levels of CX3CL1 in 40 patients with stable and 40 patients with unstable angina pectoris (AP) and 20 healthy controls. B, mRNA levels of CX3CR1 in PBMC from 20 stable and 20 unstable AP patients (randomly selected from the angina population) and 20 healthy controls. The mRNA levels were quantified using real-time RT-PCR and data were normalized to β-actin gene expression. Horizontal lines represent the median values.

1), the nonparametric Kruskal–Wallis test was used. If a significant difference was found, Mann–Whitney U test was used to calculate the difference between each pair of groups. For comparison within the same individuals, the Wilcoxon matched-pairs test was used. Coefficients of correlation were calculated by the Spearman rank test. Data are given as mean±SEM unless otherwise stated. Probability values are 2-sided and considered significant when <0.05.

Results

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Correlations Between LDL Cholesterol and CX3CL1/CX3CR1 Expression

Analysis of the lipid parameters and CX3CL1/CX3CR1 levels indicated a significant correlation between CX3CR1 expression in circulating PBMC and LDL cholesterol levels in CAD patients at baseline ($r=0.44$, $P<0.05$; Figure 3). In addition, the decrease in CX3CR1 mRNA was also significantly correlated with the decrease in LDL cholesterol during statin therapy ($r=0.51$, $P<0.05$; both groups were combined, $n=30$). Such correlations with LDL levels at baseline were also seen when CX3CR1 expression was assessed on CD3$^+$ T cells by flow cytometry ($r=0.56$, $P<0.05$), although the correlations did not reach statistical significance for changes in these parameters during statin therapy. In contrast, no significant correlation was found between plasma levels of CX3CL1 and LDL cholesterol either at baseline or during statin treatment.

Inhibition of Endothelial CX3CL1 Release by Statins

To further examine whether the statins exert the CX3CL1/ CX3CR1 related effects at the cellular or at a systemic level through lowering plasma concentrations of cholesterol, we tested the ability of atorvastatin to modulate the release and gene expression of CX3CL1 in HUVEC. To mimic the in vivo situation in CAD, the cells were stimulated for 20 hours with IL-1$\beta$, an inflammatory cytokine known to be upregulated in CAD. Atorvastatin potently reduced the IL-1$\beta$-stimulated gene expression and release of CX3CL1 in HUVEC (Figure II, available online at http://atvb.ahajournals.org). This inhibition was reversed by the addition of mevalonate, suggesting that this effect of statin may be operative at the cellular level. In contrast, neither atorvastatin nor mevalonate had any effect on the IL-1$\beta$-stimulated expression of CX3CR1 in PBMC from healthy controls ($n=5$) under identical experimental conditions (data not shown).

CX3CR1-Mediated Chemotaxis and Adhesion

To elucidate the functional implications of enhanced CX3CR1 expression, we next examined the chemotactic and adhesive properties of PBMC from CAD patients and healthy controls. CX3CL1 induced chemotaxis in a dose-dependent manner in cells from both CAD patients and controls; however, the response was higher in PBMC from CAD patients (Figure 4). Although the number of migrated cells in the CAD group at baseline appears somewhat dispersed, there was no correlation between the chemotactic activity and plasma levels of CX3CL1 within the group. Statin therapy attenuated the chemotactic response to levels characteristic for cells from healthy controls (Figure 4). Cell adhesion was also higher with the PBMC from the CAD patients. Again, statin therapy significantly decreased the adhesive property to CX3CL1; however, the effect was more modest compared with chemotaxis (Figure 4). The extent of the reductions was similar for both atorvastatin and simvastatin and the data were, therefore, combined.

To substantiate the results obtained with cryopreserved cells, we performed chemotaxis and adhesion assays using freshly purified CD14$^+$ monocytes and CD3$^+$ T cells from 7 CAD patients and 7 healthy controls. Compared with the cells...
from healthy controls, the freshly isolated CD3\(^+\) T cells from the CAD patients showed significantly increased adhesion to CX3CL1 (25.0±2.7 versus 9.6±1.6 cells per field, \(P<0.05\)) and migration (38.1±2.5 versus 15.2±2.2 cells per field, \(P<0.05\); 10 nmol/L CX3CL1). In contrast, no such difference was found with freshly isolated CD14\(^+\) monocytes.

**Effect of CX3CL1 on Chemokine Levels in PBMC Supernatants**

Exposure of leukocytes to inflammatory conditions can trigger the secretion of cytokines that may lead to cell activation via paracrine or autocrine mechanisms. Because IL-8 is critically involved in atherogenesis and plaque destabilization,\(^4\) we examined the efficacy of CX3CL1 to stimulate the secretion of this chemokine. As shown in Figure III (available online at http://atvb.ahajournals.org/), the IL-8 secretion was significantly higher in PBMC from CAD patients compared with controls, and this elevated level was further increased by exogenous CX3CL1. Interestingly, although the CX3CL1 chemokine domain by itself was sufficient to induce chemotaxis (see above), it did not stimulate the expression of IL-8 (data not shown). For effective stimulation of IL-8 secretion, the full-length extracellular domain of CX3CL1 was required. The reason for this is not clear, but some cellular responses may entail specific modes of interaction with CX3CR1 that involve the mucine stalk of the chemokine.\(^22\) The effect of CX3CL1 on chemokine secretion seemed to be specific for IL-8, and no changes in the other chemokines including MCP-1 and MIP-1\(\alpha\) were observed (data not shown). As with adhesion and chemotaxis, statin treatment markedly attenuated this CX3CL1-stimulated IL-8 release (Figure III). Atorvastatin and simvastatin were similar in their efficacy and the data were, therefore, combined.

**Discussion**

In the present study, we show that the plasma levels of CX3CL1 are greatly increased in CAD patients, particularly in those with unstable disease. Concomitantly, the CX3CR1-expressing leukocytes, primarily CD3\(^+\)CD8\(^+\) T cells, were significantly expanded in these patients. Although continuous or repeated stimulation of G protein–coupled receptors by their agonists may reduce their responsiveness (eg, desensitization),\(^23\) the changes in CX3CL1 plasma levels did not impair receptor function and the CX3CR1-expressing leukocytes displayed enhanced chemotactic, adhesive, and other inflammatory responses. Notably, statin therapy reduced the expression of CX3CL1 and CX3CR1 in CAD patients and attenuated the functional responses of the circulating leukocytes to CX3CL1, including chemotaxis and adhesion as well as inflammatory responses such as IL-8 secretion.

Although atherosclerosis was traditionally considered a simple lipid disorder, the inflammatory component of the disease has gained appreciation in recent years. Consistent with this, increased expression of CX3CR1 and CX3CL1 has
that CX3CR1+ T cells are terminally differentiated effector phenotypes with enhanced cytotoxic properties.25 Moreover, the excessive activation of cytotoxic T cells has been associated with incidental vascular and tissue damage.26 Therefore, the disproportional increase in CX3CR1+CD3+CD8+ T cells in CAD may be of particular importance for disease initiation and progression. The ability of CX3CL1/CX3CR1 to enhance the effector function of CD8+ T cells27 may further support a pathogenic role for CX3CR1-expressing CD8+ T cells in atherosclerosis. Nevertheless, a recent study with human atherosclerotic tissues found no colocalization of CX3CR1 with CD3+ T cells.13 These findings are inconsistent with our results; however, the differences can be explained by the phenotypic changes T cells may undergo. The expression of CX3CR1 on CD3+ lymphocytes is negatively regulated by T cell activation,7 and the low expression of CX3CR1 on CD3+ T cells in lesions may reflect downregulation that occurred after cell recruitment in response to local activation signals. Consistent with an important role in the recruitment process and inflammation, CX3CR1 was found to be preferentially expressed in Th1 cells compared with Th2 cells and has been suggested to play an important role in Th1-dominated inflammatory disorders including atherosclerosis.28

Experimental as well as some clinical data indicate that statins may confer cardiovascular benefits in addition to the lipid lowering activity.29 These pleiotropic effects of statins function in part by modulating the inflammatory arm of atherosclerosis. In the present study, we show that besides lowering plasma lipid levels, statin treatment also attenuated the expression of CX3CR1 and CX3CL1 and reduced some of the excessive CX3CL1-mediated inflammatory responses in CAD patients. Although some of these responses appear to be more pronounced in the atorvastatin group, caution should be exercised when comparing the two statin groups because of the relatively low number of patients that were studied. The differences between the aggressive (atorvastatin 80 mg qd) and conventional (simvastatin 20 mg qd) treatment group are small and may be attributable to slight differences in the lipid-lowering capacity rather than class differences. Consistent with this, we found a significant correlation between the plasma levels of LDL cholesterol and CX3CR1 gene expression in PBMC, predominantly T cells, both before and during statin treatment. Furthermore, ex vivo treatment of PBMC with statin had no effect on CX3CR1 expression. The statin-mediated effect on T cells may not be specific for CX3CR1. For example, statins have been shown to suppress also interferon-γ-expressing T cells and to downregulate markers of T cell activation (eg, HLA-DR and CD38) in CAD.30,31 In addition, we found that statin therapy significantly attenuated the release of sCD25 from PBMC, a widely used marker of T cell activation (data not shown). These results imply that the conditions of CAD cause a global stimulation of T cell programs and are suggestive of a more general systemic modulation of the inflammatory status of T cells by statins.

In contrast to CX3CR1, no statistical correlation was found between plasma levels of lipids and CX3CL1, and more importantly, treatment of cytokine-activated endothelial cells with statin markedly reduced the CX3CL1 secretion in vitro. Although we have no direct data addressing specifically the proteolytic cleavage of CX3CL1 from the cell surface, the
observed decrease at both the mRNA and protein level suggests that their suppressive effect on the IL-1β-mediated CX3CL1 release from endothelial cells does not merely reflect attenuated proteolytic cleavage of membrane-bound CX3CL1. These findings indicate that the property of the statins on CX3CL1 secretion may be independent of plasma lipid levels and may be operative at the cellular level. Similar antiinflammatory benefits of statins have also been reported in non-CAD patients (eg, multiple sclerosis), further supporting that the immunomodulatory effects of statins are not merely a secondary phenomenon to lipid lowering.

In the present study we have shown that the plasma levels of CX3CL1 and T cell expression of CX3CR1 are elevated in CAD patients. We have further shown that statin therapy attenuated the expression of these inflammatory mediators as well as the inflammatory response of the PBMC to CX3CL1. Our findings suggest that the CX3CL1/CX3CR1 chemokine system may constitute a therapeutic target in the care of CAD patients including those with unstable disease.

Acknowledgments

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References

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SUPPLEMENTAL DATA:

Methods:

Isolation of cells and plasma
Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by Isopaque-Ficoll gradient centrifugation (Lymphoprep; Nycomed, Oslo, Norway). Cells were immediately cryopreserved\textsuperscript{18} for subsequent use in cell culture and for flow cytometry, or stored in liquid nitrogen as pellets for RNA isolation. Separation of monocytes (anti-human CD14-labeled magnetic beads; MACS, Miltenyi Biotec, Bergish Gladbach, Germany) and CD3\textsuperscript{+} T-cells (negative selection by monodisperse immunomagnetic beads; Dynal, Oslo, Norway) from freshly isolated PBMC was performed as described elsewhere.\textsuperscript{19,20} The selected T-cells consisted of >90\% CD3\textsuperscript{+} cells and the isolated monocytes of >95\% CD14\textsuperscript{+} cells (flow cytometry). Plasma was collected and stored as previously described.\textsuperscript{18} For practical reasons, PBMC could not been obtained from all patients, and the patients selected for PBMC studies were randomly selected from the total study population.

Cell culture
Thawed cryopreserved PBMC were cultured (2x10\textsuperscript{6} cells/mL) in 96-well trays (Costar, Cambridge, MA) in RPMI 1640 containing 10 \% FCS and 2 mmol/L L-glutamine (Gibco, Paisley, UK). Primary human umbilical vein endothelial cells (HUVEC) were isolated from human umbilical cord veins and cultured as described previously.\textsuperscript{21} To examine the secretion and gene expression of CX3CL1 in HUVEC and the gene expression of CX3CR1 in PBMC, cells were stimulated for 6 and 20 hours with 10 ng/mL interleukin (IL)-1\textbeta (R&D Systems, Minneapolis, MN) with or without atorvastatin (gift from Pfizer) and mevalonate (Sigma). To ensure internalization of atorvastatin and mevalonate before IL-1\textbeta activation, the cells were incubated with these compounds for 1 hour before cytokine activation. To examine the spontaneous and stimulated release of chemokines by the PBMC, the cryopreserved cells were incubated for 20 hours in the presence or absence of 50 nmol/L CX3CL1 (extracellular domain; R&D Systems).
**Chemotaxis assay**

Chemotactic activities were estimated in 24-well Transwell plates (Costar) using polycarbonate membranes with 8 µm pore size (Falcon; Becton Dickinson Labware, Bedford, MA). The lower chamber contained 800 µL RPMI 1640 with 0.5% BSA, 20 nmol/L HEPES, pH 7.4 (buffer A) and human CX3CL1 (chemokine domain; R&D Systems) at concentrations known to be chemotactic for T-cells and monocytes. To estimate random migration, the chemokine was omitted in negative control experiments. Thawed cryopreserved PBMC or freshly isolated T-cells and monocytes (2.5x10⁵/200 µL buffer A) were loaded into the upper chamber, incubated at 37°C for 90 minutes and the cells attached to the side of the polycarbonate membrane in contact with the cell suspension were removed. After fixation with 1% glutaraldehyde, the migrated cells adhering to the underside of the membranes were stained with crystal violet and counted in 5 high-power fields under an inverted microscope.

**Adhesion assay**

96-well plates (Costar) were coated overnight at 4°C with anti poly-histidine (His) antibody (Sigma) diluted in PBS (1:1000). After two washes with PBS, the wells were blocked with buffer A for 1 hour at room temperature and histidine tagged human CX3CL1 (extracellular domain; R&D Systems), was then added in buffer A at a final concentration of 30 nmol/L and incubated for one additional hour at room temperature. Unbound CX3CL1 that was not attached to the anti-His antibody was removed by two washes with buffer A. Thawed cryopreserved PBMC or freshly isolated T-cells and monocytes (2x10⁴/100 µL buffer A) were added to each well and incubated for 30 minutes at 37°C. Non-adhering cells were washed off (2xPBS) and the adherent cells were counted in 5 high-power fields under an inverted microscope. In control experiments, wells not coated with CX3CL1 were used.

**Real-Time quantitative RT-PCR**

Total RNA was extracted from PBMC and HUVEC using RNeasy columns (Qiagen; Hilden, Germany), subjected to DNase I treatment (RQI DNase; Promega, Madison, WI) and stored in RNA storage solution (Ambion; Austin, TX) at -80°C. Primers were designed with the Primer Express software, version 2.0 (Applied Biosystems; Foster City, CA).
City, CA) and the sequences were for CX3CR1: forward primer; 5’-AAT-GCC-TGG-CTG-TCC-TGT-GT-3’, reverse primer; 5’-GCC-TGC-TCC-TTT-GTG-ATT-CAG-3’, and TaqMan probe; 5’-CGC-TCA-GTC-CAC-GTT-GAT-TTC-TCC-TCA-3’, and for CX3CL1: forward primer; 5’-GCA-AAC-GCG-CAA-TCA-TCT-T-3’, reverse primer; 5’-GCG-GTC-CAG-ATG-CTG-CAT-3’. Quantification of mRNA was performed using the ABI Prism7000 (Applied Biosystems). Gene expression of the housekeeping gene β-actin (Applied Biosystems) was used for normalization.

**Flow cytometry**

Flow cytometry analyses of thawed cryopreserved PBMC were performed with the use of allophycocyanin (APC)-conjugated anti-CD14 (clone M5E2), APC-conjugated anti-CD8 (clone RPA-T8), APC-conjugated anti-CD4 (clone RPA-T4), phycoerythrin (PE)-conjugated anti-CD3 (clone UCHT1) from Becton Dickinson (San Jose, CA) and fluorescein isothiocyanate (FITC)-conjugated anti-CX3CR1 (clone 2A9-1) from Medical & Biological Laboratories (Woburn, MA). Dead cells (∼20%) were identified with propidium iodine and were gated out. Nonspecific IgG isotypes were used as negative controls. Flow cytometry was performed using a FACSCalibur instrument with CellQuest software (Becton Dickinson Bioscience; San Jose, CA). List mode files were collected for 50,000 cells from each sample.
### TABLE I. Characteristics of the Angina Study Groups

<table>
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<th>Controls (n=20)</th>
<th>Stable angina (n=40)</th>
<th>Unstable angina (n=40)</th>
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<td>Age, years</td>
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<td>Warfarin</td>
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<td>Total cholesterol, mmol/L</td>
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<td>LDL cholesterol, mmol/L</td>
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<td>HDL cholesterol, mmol/L</td>
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Data are presented as mean±SD. Statins, hydroxymethylglutaryl coenzyme A reductase inhibitors. P, differences between stable and unstable angina patients.
<table>
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<th></th>
<th>Controls (n=15)</th>
<th>Atorvastatin Group (n=15)</th>
<th>Simvastatin Group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 Months</td>
<td>Baseline</td>
</tr>
<tr>
<td>Age, y</td>
<td>60.2±6.5</td>
<td>58.3±7.1</td>
<td>...</td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>6/9</td>
<td>5/10</td>
<td>...</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>20</td>
<td>27</td>
<td>...</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.21±0.3</td>
<td>2.27±0.4</td>
<td>2.13±0.3</td>
</tr>
<tr>
<td>Medication, %</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>0</td>
<td>100</td>
<td>...</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0</td>
<td>93</td>
<td>...</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>0</td>
<td>7</td>
<td>...</td>
</tr>
<tr>
<td>Warfarin</td>
<td>0</td>
<td>20</td>
<td>...</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.3±0.7</td>
<td>6.0±1.2</td>
<td>3.5±0.9*</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.7±0.5</td>
<td>4.1±1.3</td>
<td>1.8±0.6*</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.3±0.3</td>
<td>1.1±0.4</td>
<td>1.2±0.4*</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4±0.5</td>
<td>2.0±1.1</td>
<td>1.2±0.4*</td>
</tr>
</tbody>
</table>

Data are mean±SD unless otherwise indicated. *P<0.05 vs. baseline. There were no significant differences between the baseline characteristics in the atorvastatin and simvastatin groups. The patients discussed here were participants of an earlier study and most of the pertinent patient characteristics were presented previously (reference 18).
Figure I. Flow cytometry analysis of CX3CR1 in PBMC from controls and patients with coronary artery disease (CAD) at baseline and after 6 months (mo) of statin therapy. Figures indicate percentages of CX3CR1^+ cells of the total amount of CD3^+CD8^+ cells in one CAD patient before and after treatment and one control, and are representative of samples from fourteen CAD patients and eight healthy controls. Also mean fluorescence intensity of CX3CR1 decreased in CAD patients during statin therapy (253±20.5 at baseline vs. 193±30.5 after 6 mo, p<0.05) and was significantly increased in patients comparing controls (133±27.4, p<0.01).
Figure II. Effect of atorvastatin on the mRNA levels and secretion of CX3CL1 from HUVEC. A primary culture of HUVEC was treated with the indicated concentrations of atorvastatin (10 µmol/L for A) and the effects on CX3CL1 mRNA levels (A) and protein secretion (B) were examined after stimulation with IL-1β (10 ng/mL) for 6 and 20 hours, respectively. To allow for internalization, atorvastatin and mevalonate were added to the cell culture 1 hour prior to the addition of IL-1β. The mRNA levels were quantified using real-time RT-PCR and data were normalized to β-actin gene expression. CX3CL1 levels were analyzed by enzyme immunoassays. Data are mean±SEM (n=5). *p<0.05 versus unstimulated; # p<0.05 versus IL-1β alone.
Figure III. Protein levels of IL-8 in supernatants from unstimulated and CX3CL1-stimulated (50 nmol/L) cryopreserved PBMC from healthy controls (n=8) and CAD patients (n=16) before and after 6 months (mo) of statin therapy (simvastatin, 20 mg qd, n=8 and atorvastatin, 80 mg qd, n=8). Both drugs had similar effect on IL-8 production and the results were combined. PBMC were cultured for 20 hours and chemokines levels were analyzed by enzyme immunoassays. Data are mean±SEM. *p<0.05 and **p<0.01 versus healthy controls; # p<0.05 and ## p<0.01 after versus before statin treatment.