Enhanced Levels of Oxidized Low-Density Lipoprotein Prime Monocytes to Cytokine Overproduction via Upregulation of CD14 and Toll-Like Receptor 4 in Unstable Angina


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Enhanced Levels of Oxidized Low-Density Lipoprotein Prime Monocytes to Cytokine Overproduction via Upregulation of CD14 and Toll-Like Receptor 4 in Unstable Angina


Objectives—The purpose of this study was to establish whether oxidized low-density lipoprotein (oxLDL) contributes to cytokine overproduction via upregulation of CD14 and toll-like receptor-4 (TLR-4) expression on circulating monocytes of unstable angina (UA) patients.

Methods and Results—Expression of CD14 and TLR-4 on circulating monocytes, and the concentration of plasma oxLDL, (interleukin [IL]-6, IL-1 beta, IL-8, tumor necrosis factor (TNF)-alpha, monocyte chemoattractant protein-1 (MCP-1) were measured in 27 control (C) subjects, 29 patients with stable angina (SA), and 27 with UA. CD14 and TLR-4 expression on monocytes and circulating IL-6, IL-1 beta, and oxLDL were higher in UA than in SA and C subjects ($P<$0.001). In in vitro experiments, oxLDL increased CD14 and TLR-4 expression ($P<$0.001) in control monocytes as well as IL-6, IL-1 beta, and at a lower extent TNF-$
\alpha$ and MCP-1 levels in the supernatant ($P$ from $<$0.05 to $<$0.001). The preincubation of sera derived from UA patients but with control monocytes also induced a significant increase of CD14 and TLR-4 expression ($P<$0.001) and of IL-6 and IL-1 beta production ($P<$0.001) in the supernatant.

Conclusions—In UA patients oxLDL may contribute to monocyte overproduction of some cytokines by upregulating CD14 and TLR-4 expression. (Arterioscler Thromb Vasc Biol. 2007;27:1991-1997.)

Key Words: oxidized LDL ■ IL-6 ■ IL-1 beta ■ CD14 ■ toll-like receptors ■ monocytes ■ unstable angina

Recent findings suggest that inflammation may play an important role in the pathogenesis of acute coronary syndromes. It is known that monocytes/macrophages participate in several critical aspects of coronary artery disease and that the magnitude of the acute-phase response may also be the result of the behavior of circulating inflammatory cells. Circulating monocytes have been found to be activated in patients with unstable angina (UA). These monocytes are characterized by upregulation of adhesion molecules, increased production of cytokines, and procoagulant substances and activation of nuclear factor-kB. Furthermore, these cells have been demonstrated to exhibit an enhanced production of IL-6 in response to low-dose lipopolysaccharide (LPS).

A markedly increased expression of CD14 and Toll-like receptor 4 (TLR-4) has recently been reported in the monocytes of UA patients. CD14 is the receptor that binds LPS. It lacks a transmembrane and cytoplasmic domain and therefore it is unlikely to have direct signaling capabilities. Signaling associations occur with member of the TLR family. This receptor family is made up of at least 9 members, but TLR-4 has been described as the likely receptor in LPS recognition and activity.

oxLDL levels have been shown to be elevated in acute coronary syndromes and in particular in UA and to upregulate the in vitro expression of CD14 and TLR-4 in monocytes/macrophages. CD14/TLR-4 complex may therefore provide a potential pathophysiological link between lipids and infection/inflammation and atherosclerosis. However it has to be emphasized that studies done with TLR-4, the adaptor molecule myeloid differentiation factor-88, TLR-2, and CD14-deficient mouse models showed that all except CD14 contributed to atherosclerotic disease.

This study was aimed to evaluate whether in UA patients oxLDL contributes to monocyctic overproduction of cytokines via upregulation of TLR-4 and CD14.

Methods

Study Population
The study was approved by the hospital ethical committee and informed consent was obtained from all the patients before their enrolment.
Major requirements for the enrolment in all the groups were: absence of infectious or acute/chronic inflammatory diseases, known malignancy, absence of acute/chronic renal failure, or hepatic failure. Three groups of patients were studied: control (C) subjects (patients without clinical history of CAD, who underwent coronary angiography for a presurgical evaluation, because affected by valvular or congenital heart disease), patients with stable angina (SA), and patients with UA. The subjects were enrolled on the basis of previously established criteria.6,17

For both SA and UA patients exclusion criteria were: prior coronary artery bypass grafting (CABG), recent (<6 months) myocardial infarction (MI), recent (<6 months) percutaneous coronary intervention (PCI), congestive heart failure (CHF), coronary tree free of significant coronary artery disease (CAD: defined as at least one stenosis with minimal luminal diameter >70% of the arterial lumen by visual estimate) detected at coronary angiography.

The following data were obtained from all the patients: age, sex, presence of CAD risk factors (hypertension, cigarette smoking, diabetes mellitus, hypercholesterolemia, family history of CAD), use of medications, previous MI, previous revascularization (PCI or CABG).

Blood Samples

Venous blood samples were obtained from SA patients and C subjects the morning after the day of admission together with the routine laboratory tests. In UA patients samples were drawn within 24 hours from the index event (the last episode of anginal pain or admission if angina occurred previously). Blood was collected from each patient and drawn into pyrogen-free blood collection tubes without additives. Multiple aliquots of serum were placed into sterile 1-mL screw-capped polypropylene vials and stored at −80°C in plastic bags. Samples were kept frozen for no longer than 30 days with an average of 21 days. For thawing, serum tubes were placed overnight in a refrigerator at 2 to 8°C. The samples were frozen and thawed only once.

Total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, triglycerides, glucose, white blood cell (WBC) count, were measured with standard methods. IL-6, IL-1 beta, TNF-α, IL-8, and MCP-1 were measured with commercial assay kits (Quintikine, R&D System). CRP was measured by a high sensitivity C-reactive protein (CRP) assay (Quantikine, R&D System). CRP was measured by a high sensitivity method.

Peripheral Blood Mononuclear Cells and Monocyte Isolation

Peripheral blood mononuclear cells (PBMCs) were separated as previously described.18 A detailed description of the method is available in the supplemental materials (available online at http://atvb.ahajournals.org).

Stimulation of Whole Blood With LPS and Measurement of IL-6 and IL-1 Beta

Aliquots of 1-mL of heparinized whole blood (with or without LPS) were placed in sterile 1.5-mL centrifuge tubes and placed on a rotator and incubated under sterile conditions at 37°C in an atmosphere containing 5% CO₂. The samples were treated with 1 ng/mL of LPS (Sigma Chemical) for 4 hours. The supernatant was then removed and stored at −80°C for the measurement of IL-6 and IL-1 beta.

Stimulation of Monocytes With LPS and Measurement of IL-6 and IL-1 Beta

Freshly isolated monocytes (5×10⁶) were incubated for 4 hours at 37°C in RPMI-1640 supplemented with 8% fetal calf serum, 50 U/mL penicillin, 50 μg/mL streptomycin, and 0.2 mmol/L L-glutamine with or without LPS (1 ng/mL). Then the culture supernatant was removed and stored at −80°C for the measurement of IL-6 and IL-1 beta.

Flow Cytometry and Western Blotting Analysis

CD14 and TLR-4 protein expression was analyzed in blood samples and in pools of separated monocytes by flow cytometry and Western blotting analysis. A detailed description of the 2 methods is available in the supplemental materials.

Circulating Plasma oxLDL Measurement

Circulating plasma oxLDL levels were measured with the enzyme-linked immunosorbent assay Mercodia Oxidized LDL ELISA (Merckodia AB) as previously described.6 Cu²⁺-modified LDL ranging from 50 to 500 ng/mL was used as a standard solution.

LDL Isolation and Oxidation

LDL and Cu²⁺-modified LDL were prepared as reported.6

Real-Time Quantitative RT-PCR Analysis and Protein Expression of CD14 and TLR-4 in Separated Monocytes

A detailed description of the method is available in the supplement materials.

oxLDL-Dependent Expression of CD14/TLR-4 in Monocytes and Production of IL-6, IL-1 Beta, TNF-α, and MCP-1

Purified monocytes (3×10⁶/mL, 200 μL/well) from healthy donors were cultured in RPMI 1640 with L-glutamine (GIBCO) for 20 hours at 37°C with increasing amounts of oxLDL (from 20 to 40 μg/mL medium), CD14 and TLR-4 expression and IL-6, IL-1 beta, TNF-α, and MCP-1 concentration in the supernatant were measured as described. In some experiments, blocking anti-CD14 monoclonal antibody (mAb; 20 μg/mL) or control mouse IgG (50 μg/mL) were also added.

Serum-Dependent Expression of CD14/TLR-4 in Monocytes and Production of IL-6, IL-1 Beta, TNF-α, and MCP-1

Purified monocytes (3×10⁶/mL, 200 μL/well) from healthy volunteers were cultured in RPMI 1640 with L-glutamine (GIBCO) for 20 hours at 37°C with 40% serum from: (1) 10 UA patients with the highest oxLDL levels, (2) 10 UA patients with the lowest oxLDL levels, (3) 10 C subjects with the lowest oxLDL levels, and (4) 10 C subjects with the highest oxLDL levels. For processing of serum, 80 μL of serum from each patient or C subject was added to monocyte culture immediately after thawing, at the start of the culture period. As a further control, monocytes were also incubated with the corresponding UA lipoprotein-deprived serum (LPDS) in which all the lipoproteins were taken away by ultracentrifugation at a density >1.21 g/mL.6 CD14 and TLR-4 expression and IL-6, IL-1 beta, TNF-α, and MCP-1 concentration in the supernatant were measured as described. In some experiments anti-CD14 mAb (20 μg/mL) or control human IgG (50 μg/mL) were also added.

Endotoxin contamination of cell cultures involving the use of oxLDL, serum, or LPDS was routinely excluded with the chromogenic Limulus amebocyte lysate assay (Sigma).

Statistical Analysis

A detailed description of the statistical methods is available in the supplement materials.

Results

Baseline Characteristics of the Patients

Along a period of 14 months, 102 patients were enrolled in the study; of these only 82 (27 C, 29 with SA, and 27 with UA) fully satisfied the enrolling criteria. Baseline clinical characteristics of the patients are described in supplemental Table I.
Data on Cytokines and Chemokines in the Three Groups of Patients

<table>
<thead>
<tr>
<th></th>
<th>C (n=27)</th>
<th>SA (n=29)</th>
<th>UA (n=26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6, pg/ml</td>
<td>0.72 (0.0-1.2)</td>
<td>1.18 (0.82-4.4)</td>
<td>7.22 (4.21-31.1)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-1 beta, pg/ml</td>
<td>0.14 (0.04-0.25)</td>
<td>0.21 (0.12-0.42)</td>
<td>0.62 (0.22-1.11)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-alpha, pg/ml</td>
<td>4.97 (2.61-1.241)</td>
<td>4.11 (1.96-10.52)</td>
<td>4.19 (2.90-10.21) NS</td>
<td></td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>141.2 (82.3-181.4)</td>
<td>153.3 (96.6-204.6)</td>
<td>145.8 (102.3-202.7) NS</td>
<td></td>
</tr>
<tr>
<td>IL-8, pg/ml</td>
<td>15.88 (2.44-40.58)</td>
<td>18.42 (7.32-48.53)</td>
<td>17.92 (3.47-39.62) NS</td>
<td></td>
</tr>
<tr>
<td>IL-6 response to LPS in blood, pg/ml</td>
<td>634 (186-1430)</td>
<td>938 (189-1942)</td>
<td>3457 (1510-8609)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6 response to LPS in monocytes, pg/ml</td>
<td>447 (126-1094)</td>
<td>655 (189-1751)</td>
<td>2318 (998-4601)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-1 beta response to LPS in blood, pg/ml</td>
<td>659 (82-1211)</td>
<td>905 (156-1600)</td>
<td>2103 (609-4311)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-1 beta response to LPS in monocytes, pg/ml</td>
<td>190 (80-742)</td>
<td>382 (120-1091)</td>
<td>1612 (620-1950)*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Data are presented as median (ranges).

Laboratory Data

Data on total, LDL, HDL cholesterol, triglycerides, glucose plasma levels, and on WBC number and CRP are shown in supplemental Table II. Total WBC number and CRP were higher in UA patients than in SA or C patients (P<0.001). Data on plasma IL-6, IL-1 beta, TNF-alpha, IL-8, and MCP-1 and on the concentrations of IL-6 and IL-1 beta stimulated in blood and in separated monocytes with LPS are indicated in the Table. There were no significant differences in TNF-alpha, IL-8, and MCP-1 plasma levels; both basal and stimulated IL-6 and IL-1 beta levels were higher in UA patients than in SA or C patients (P<0.001).

CD4 and TLR-4 Expression on Circulating Monocytes

Figure 1A shows the mean expression of CD14 and TLR-4 in peripheral blood monocytes of the 3 groups of patients as measured by flow cytometry. CD14 and TLR-4 were higher in UA patients than in SA and in C subjects (P<0.001). A representative histogram of the flow cytometry analysis is presented in supplemental Figures I and II. Data on TLR-4 were confirmed by Western blotting analysis on separated monocytes (Figure 1B).

Circulating Plasma oxLDL

oxLDL differed significantly among groups (Figure 2A). In particular, post-hoc tests revealed that oxLDL levels were higher in UA than in SA and C subjects and in SA than in C subjects (P from <0.001 to <0.01).

In the whole population of subjects studied, a statistically significant nonlinear sigmoidal regression (r²=0.983; P<0.001) was detected between plasma oxLDL and TLR-4 (Figure 2B). oxLDL levels also correlated linearly with CD14 (r=0.81, P<0.001; data not shown).

oxLDL-Dependent Expression of CD14 and TLR-4 and Cytokine Production in Monocytes Derived From Healthy Volunteers

Different amounts of Cu²⁺-oxLDL but not of native-LDL (n-LDL) dose-dependently increased cell mRNA and protein expression of CD14 and TLR-4 as evaluated by flow-cytometry and Western analysis (data on n-LDL not shown) in monocytes derived from healthy volunteers (P<0.001; supplemental Figure IIIA through IIIC).

Similarly increasing amounts of oxLDL but not of n-LDL dose-dependently increased the production of IL-6, IL-1 beta, TNF-alpha and MCP-1 (P<0.001; Figure IVA through IVD). The effect on oxLDL on TNF-alpha and MCP-1 was much lower than on IL-6 and IL-1 beta (P<0.001 for any amount of oxLDL). On average with the highest dose of oxLDL (40 μg/mL), IL-6 and IL-1 beta increased respectively 23.3±3.1 and 10.2±1.8 times whereas TNF-alpha and MCP-1 respectively 2.6±0.31 and 3.2±0.41 times when compared with control. The presence of anti-CD14 blocking mAb significantly reduced the oxLDL-dependent increase of IL-6, IL-1 beta, TNF-alpha, and MCP-1 (P from <0.01 to <0.001; supplemental Figure IVA through IVD).

Serum-Dependent Expression of CD14 and TLR-4 and Cytokine Production in Monocytes

Monocytes from healthy volunteers were evaluated for CD14 and TLR-4 expression after they were cultured for 20 hours in medium supplemented with either 40% serum from UA patients with the highest (n=10) and lowest (n=10) oxLDL plasma concentrations (respectively 36.4±3.25 and 26.4±3.21 μg/mL) or 40% serum from C subjects with the highest (n=10) and lowest (n=10) oxLDL levels (respectively 17.7±2.9 and 9.73±3.1 μg/mL). The incubation of monocytes with the sera derived from UA patients with the highest and lowest oxLDL levels induced a significant increase in the expression of CD14 and TLR-4 compared with the corresponding sera derived from the C subjects (Figure 3A and 3B as evaluated by flow cytometry and Western analysis respectively; P<0.001). The incubation of monocytes with the LPDS of UA patients with the highest oxLDL levels (oxLDL undetectable) induced a reduced increase in CD14 and TLR-4 when compared with the corresponding sera in toto (Figure 3A and 3B; P<0.001). The concentrations of IL-6, IL-1 beta, TNF-alpha, and MCP-1 were significantly higher in the monocytes incubated with the sera of UA patients with highest and lowest oxLDL levels than in those incubated with the corresponding sera of C subjects (P<0.001; Figures 4A, 4B, 5A, and 5B). The effect on TNF-alpha and MCP-1, however, was negligible, and post-hoc test revealed that the changes of IL-6 and IL-1 beta were...
much higher than those of TNF-α and MCP-1 (P<0.001).

The presence of anti-CD14 blocking mAb significantly reduced the sera-dependent increase of IL-6 and IL-1 beta (P<0.001), but not of TNF-α and MCP-1 (Figures 4A, 4B, 5A, and 5B). In the LPDS-stimulated monocytes, only the production of IL-6 and IL-1 beta was lower than that induced by UA sera in toto (P<0.001; Figures 4A, 4B, 5A, and 5B).

**Discussion**

The results of this study show that during unstable phases of angina, UA patients exhibited a greatly enhanced concentration of IL-6 and IL-1 beta in plasma as well as in whole blood and separated monocytes after LPS stimulation. The mechanisms leading to this rather selective increase of cytokines and to the abnormal production of IL-6 and IL-1 beta after LPS challenge in UA patients are unclear. Here we considered the possibility that oxLDL may contribute to the cytokine overproduction by upregulating CD14 and TLR-4 expression in circulating monocytes of UA patients.

In our study there was an expansion of CD14- and TLR-4-positive monocytes in UA patients and very interestingly statistically significant correlations were found between the plasma levels of circulating oxLDL and the circulating concentration of CD14 and TLR-4 in the whole population of subjects studied (r=0.983, P<0.001) (B). Results on oxLDL are the means±SD of measurements performed in triplicate. *P<0.001 vs SA and C subjects; †P<0.01 vs isotype control Ab.

![Figure 1](image1.png)

*Figure 1.* CD14 and toll like receptor-4 (TLR-4) protein expression in peripheral blood cells as evaluated by flow cytometry (A) and TLR-4 protein expression as evaluated by Western analysis (B) in separated monocytes of unstable UA and stable angina (SA) patients and in control subjects (C). Results of flow cytometry are expressed as mean fluorescence intensity and are the means±SD of measurements performed in triplicate. As controls isotypes of TLR-4 and CD14 antibodies were used. *P<0.001 vs SA, C, and isotype control antibody (Ab); †P<0.001 vs isotype control Ab. Results of Western analysis were expressed as density in arbitrary units (AU), as determined by densitometric analysis, and are the means±SD of measurements performed in triplicate. *P<0.001 vs SA and C subjects; †P<0.01 vs isotype control Ab.

and separated monocytes after LPS stimulation. The mechanisms leading to this rather selective increase of cytokines and to the abnormal production of IL-6 and IL-1 beta after LPS challenge in UA patients are unclear. Here we considered the possibility that oxLDL may contribute to the cytokine overproduction by upregulating CD14 and TLR-4 expression in circulating monocytes of UA patients.

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![Figure 2](image2.png)

*Figure 2.* Concentrations of plasma oxLDL in unstable (UA) and stable angina (SA) patients and in control subjects (C) (A) and nonlinear sigmoidal regression between plasma oxLDL and circulating concentration of toll-like receptor-4 (TLR-4) in the whole population of subjects studied (r=0.983, P<0.001) (B). Results on oxLDL are the means±SD of measurements performed in triplicate. *P<0.001 vs C; †P<0.01 vs SA.
The fact that the presence of blocking anti-CD14 mAb reduced the cytokine concentration in the supernatant of monocytes, may indicate that the cytokine increase and above all that of IL-6 and IL-1 beta is, at least partially, related to the binding of oxLDL to this receptor and therefore to TLR-4 engagement. This conclusion is in agreement with previous results showing that oxLDL induced actin polymerization and spreading of macrophages by binding to CD14 and signaling.

Figure 3. Expression of CD14 and toll-like receptor-4 (TLR-4) as evaluated by flow cytometry (A) and of TLR-4 as evaluated by Western analysis (B) in monocytes derived from healthy volunteers incubated with serum of unstable angina (UA) patients with the highest (UA highest, n=10) and lowest (UA lowest, n=10) oxLDL levels and controls (C) with the highest (C highest, n=10) and lowest (C lowest, n=10) oxLDL levels. Monocytes were also incubated with lipoprotein deprived serum (LPDS) derived from UA patients with the highest oxLDL levels (LPDS UA highest; n=10). Results of flow cytometry are expressed as mean fluorescence intensity and are the means±SD of experiments performed in triplicate. As controls, isotypes of TLR-4 and CD14 antibodies were used. *P<0.001 vs C lowest; †P<0.01 vs UA lowest; ‡P<0.001 vs C highest; §P<0.001 vs LPDS UA highest. Results of Western analysis are expressed as density in arbitrary units (AU), as determined as densitometry analysis and are the means±SD of experiments performed in triplicate. *P<0.001 vs C lowest; †P<0.001 vs anti-CD14 Ab; ‡P<0.001 vs UA lowest; §P<0.001 vs C highest; |P<0.001 vs LPDS UA highest.

Figure 4. IL-6 (A) and IL-1 beta (B) production induced by serum of unstable angina (UA) patients with the highest (UA highest, n=10) and lowest (UA lowest, n=10) oxLDL levels and controls (C) with the highest (C highest, n=10) and lowest (C lowest, n=10) oxLDL levels in monocytes derived from healthy volunteers. Monocytes were also incubated with lipoprotein-deprived serum (LPDS) derived from UA patients with the highest oxLDL levels (LPDS UA highest; n=10). In some experiments, anti-CD14 antibody (Ab; 20 µg/mL) or control IgG (50 µg/mL) were also added to cell culture. Results are the means±SD of experiments performed in triplicate. *P<0.001 vs C lowest; †P<0.001 vs anti-CD14 Ab; ‡P<0.001 vs UA lowest; §P<0.001 vs C highest; |P<0.001 vs LPDS UA highest.

The fact that the presence of blocking anti-CD14 mAb reduced the cytokine concentration in the supernatant of monocytes, may indicate that the cytokine increase and above all that of IL-6 and IL-1 beta is, at least partially, related to the binding of oxLDL to this receptor and therefore to TLR-4 engagement. This conclusion is in agreement with previous results showing that oxLDL induced actin polymerization and spreading of macrophages by binding to CD14 and signaling.
through the LPS receptor complex composed of TLR-4 and MD-2. At variance with these results, Walton et al20 showed that CD14 did not appear to mediate the action of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (ox-PAPC) on IL-8 transcription in endothelial cells because it was not blocked by anti-CD14 neutralizing antibodies. These differences may be attributable to the fact that oxLDL is a mixture of possible ligands, including various proteins, cholesterol, and oxidized phospholipids, whereas ox-PAPC only contains oxidized phospholipids. Our conclusion is also in contrast with the results of Miller et al21 who found that the induction of IL-6 by oxLDL was not dependent on TLR-4. These contrasting results may be attributable to the different oxLDL preparations or alternatively to differences in cell types.

Starting from these results we then evaluated the effect of sera derived from UA patients and C subjects on CD14 and TLR-4 expression in monocytes derived from healthy volunteers. The results show that the sera of UA patients increased at a much higher extent the expression of CD14 and TLR-4 in monocytes derived from healthy volunteers than in those of C subjects. Similarly to the results obtained with oxLDL where there was a prevalent increase of IL-6 and IL-1 beta, the overexpression of CD14 and TLR-4 caused by sera of UA patients was associated with a clear-cut increase of IL-6 and IL-1 beta whereas the rise of TNF-α and MCP-1 was negligible. The reasons why oxLDL and sera of UA patients with the highest oxLDL circulating levels produced slight variations of TNF-α and MCP-1 are unclear. However, it is known that TLR engagement results in activation of mitogen-activated protein kinase (MAPKs), which, together with the NF-kB pathway, transduces extracellular signals to cellular responses.22 Negative regulation of MAPK activity is affected primarily by MAPK phosphatase-1 (MKP-1), a member of the MAPK phosphatase family, which has been recently reported to regulate the temporal response of pro- and antiinflammatory cytokines by TLRs.24 oxLDL has been demonstrated to activate MKP-1,25 and even though its role in TLR-mediated immune responses in vivo is far from being fully elucidated, a tentative explanation of our results could be that in our experimental conditions MKP-1 may have repressed to a greater degree some cytokines than others. Of course on the basis of present results we cannot exclude that other suppressing mechanisms that are normally evoked by TLR activation26 may have contributed to the different cytokine production. Furthermore different serum components may have reduced the secretion of TNF-α and MCP-1 induced by the sera of UA patients. For instance, a C-C chemokine called regulated on activation normally T-cell express and secreted (RANTES), which has been reported to be high in acute coronary syndrome, has been shown to modulate the TLR4-induced cytokine secretion in human peripheral blood monocytes.28 These hypothetical counteracting mechanisms and others unknown may have therefore depressed not all but just some cytokines/chemokines and in particular of TNF-α may have contributed to the results.29

Because a number of circulating agents could potentially have increased monocytic CD14 and TLR-4 expression as well cytokine production, the LPDS of UA patients was then used to evaluate the magnitude of lipoprotein effect. In these conditions, ie, in absence of lipoproteins and with no detectable oxLDL, the increase in monocytic expression of CD14/
TLR-4 and in the concentration of supernatant IL-6 and IL-1 beta were significantly lower than that obtained with the complete sera. These results of course have limitations because they have not been shown for oxLDL specifically because in LPDS all the lipoproteins and not only oxLDL were removed. Furthermore, because a considerable amount of TLR4/CD14 expression and of IL-6 and IL-1 beta production was not abolished by the absence of lipoproteins in the sera, it is likely that many other parameters and not just oxLDL or other lipoproteins may differ between the 2 groups and contribute to the results. These unknown parameters may have also been important in determining the slight increase of TNF-α and MCP-1 because there was no difference in the results obtained with sera in toto and LPDS.

Taken together these results suggest that the increased plasma levels of IL-6 and IL-1 beta in patients with UA may be at least in part related to the effect of circulating oxLDL on monocyte expression of CD14 and TLR-4 and provide new insights into the inflammatory origin of atherosclerosis.

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**Disclosures**

None.

**References**