Oxidized LDL Induces Monocytic Cell Expression of Interleukin-8, a Chemokine With T-Lymphocyte Chemotactic Activity

Robert Terkeltaub, Carole L. Banka, Joell Solan, Denise Santoro, Korbinian Brand, Linda K. Curtiss

Abstract T lymphocytes, macrophages, and oxidized low-density lipoprotein (Ox-LDL) are colocalized in early atherosclerotic lesions. Using a low-endotoxin in vitro system, we observed that Ox-LDL but not native LDL induced the production, by both freshly adherent human peripheral blood monocytes and human monocytic THP-1 cells, of the α chemokine interleukin (IL)-8, a potent chemoattractant for T lymphocytes. Marked IL-8 induction by Ox-LDL did not require IL-1β generation in THP-1 cells. Ox-LDL-induced chemokine production was selective, as Ox-LDL did not stimulate the production by THP-1 cells of the T-lymphocyte chemotactic β chemokine macrophage inflammatory protein (MIP)-1α. IL-8 induction increased in proportion to the extent of oxidation of LDL as measured by the content of lipid oxidation end products. To identify potentially active components of Ox-LDL, we treated monocytes/macrophages with superoxide dismutase, an arachidonic acid metabolite, or a phospholipase A2 inhibitor. We also released cell-free constituents from Ox-LDL by dialysis, some IL-8–inducing activity was released into the dialysate. To address the nature of the LDL particle modification required to induce IL-8, acetylated or malondialdehyde–treated native LDL particles were monitored for activity. Neither procedure rendered LDL capable of inducing IL-8. However, phospholipid A2–treated LDL induced THP-1 cell expression of IL-8. Thus, Ox-LDL induced a chemokine in monocytic THP-1 cells by a mechanism that did not absolutely require IL-1β, appeared to be predominantly mediated by particle-associated and nondiffusible end products of lipid degradation, and could be reproduced by phospholipase A2 treatment of LDL. The Ox-LDL–induced generation from monocyte/macrophages of selected chemokines that chemoattract T lymphocytes may contribute to atherogenic and inflammation-regulating properties of Ox-LDL. (Arterioscler Thromb. 1994;14:47-53.)

Key Words • oxidized LDL • chemokines • interleukin-8 • macrophage inflammatory protein-1α • interleukin-1 • T lymphocytes • atherosclerosis

The cellular components of certain atherosclerotic lesions include T lymphocytes, which are not usually present within the normal arterial wall. Within early atherosclerotic lesions (ie, intimal thickening and fatty streaks), T cells and macrophages are colocalized in significant numbers. T lymphocytes also comprise up to 20% of the cells in the fibrous cap.

The majority of atherosclerotic plaque T cells are memory cells, many of which are in a state of late or chronic activation. Lesional T cells express an increased display of the low-molecular-weight isoform of CD45 (CD45RO), the integrin VLA-1, CD26, and HLA-DR. This lymphocyte phenotype is compatible with a capacity for cytokine production and suggestive of recruitment and/or retention of activated peripheral blood T cells (and/or local antigenic stimulation of T cells).

Activated T cells are believed to play regulatory roles in the pathogenesis of atherosclerosis. Interferon gamma, a product of certain activated T cells, is expressed in atherosclerotic lesions. Interferon gamma inhibits smooth muscle proliferation in the process of vascular repair, promotes nitric oxide production by smooth muscle cells, inhibits lipoprotein lipase expression, suppresses enzymes involved in fatty acid biosynthesis, inhibits macrophage degradation of modified or acetylated low-density lipoprotein (LDL), and inhibits macrophage secretion of apolipoprotein (apo) E. Furthermore, interferon gamma is protective against atherosclerosis in an animal model system. Activated T cells can also elaborate a monococyte chemotactic factor, suggesting that T cells in the subendothelial intimal space could play a key role in maintaining the macrophage within the lesion.

Leukocyte recruitment is a complex process that includes cell rolling, firm cell adhesion on the endothelium, and directed migration in response to chemotactic gradients. Certain mechanisms that potentially mediate T-cell recruitment in atherosclerotic lesions have been described. First, upregulation of the surface activity of selected leukocyte integrin ligands (including intercellular adhesion molecule–1 and vascular cell adhesion molecule–1 [VCAM-1]) on the endothelium and on other cells in atherosclerotic lesions is believed to be critical for T-cell adhesion and retention. Second, the general adhesiveness of leukocytes to endothelial cells and the directional migration of T lymphocytes are stimulated by oxidized LDL (Ox-LDL), which is...
detected within atheromas and exerts several other proatherogenic effects.\textsuperscript{19} Ox-LDL--derived lipids and their degradative end products can also be released from Ox-LDL particles and generate biological effects that have the potential to mediate T-cell recruitment.\textsuperscript{19} In this regard, lysophosphatidylcholine (lyso-PC) mediates macrophage and T-lymphocyte chemotaxis to Ox-LDL.\textsuperscript{18,20} and lyso-PC upregulates endothelial expression of VCAM-1, which mediates T-cell adherence.\textsuperscript{21} In addition, both Ox-LDL and 9-hydroxyoctadecadienoic acid (9-HODE), an oxidative breakdown product of the major LDL polyunsaturated fatty acid linoleate, can induce interleukin (IL)-1, which can chemoattract T cells.\textsuperscript{19,22}

The induction of expression of members of the chemokine family of low-molecular-weight polypeptide (8 to 11 kDa) cytokines is an important event in immune-mediated inflammatory reactions. Chemokines modulate leukocyte transmigration through the endothelium and amplify directed leukocyte movement to sites of inflammation and wound repair.\textsuperscript{13,23} The chemokine family tree has two major branches, which are based on the structure of the first pair of cysteine residues (C-X-C, \( \alpha \) chemokines; and C-C, \( \beta \) chemokines).\textsuperscript{23} Ox-LDL was recently shown to induce expression by endothelial cells and monocytes of the \( \beta \) chemokine monocyte chemotactic and activating factor (MCAF)/monocyte chemoattractant protein-1 (MCP-1), which is chemotactic and activating for monocytes/macrophages.\textsuperscript{24-26} Two \( \beta \) chemokines (macrophage inflammatory protein [MIP]--1\( \alpha \) and MIP-1\( \beta \)) and two \( \alpha \) chemokines (IL-8 and interferon--induced protein--10), which are produced by monocytes, can chemoattract T lymphocytes.\textsuperscript{27-29} IL-8 also chemoattracts neutrophils, but is chemotactic for T lymphocytes at concentrations up to 10-fold lower than the concentrations at which it chemoattracts neutrophils.\textsuperscript{27}

In this study, we tested the hypothesis that Ox-LDL has the capacity to induce the expression of chemokines that attract T lymphocytes. We established that under low endotoxin conditions, Ox-LDL induces expression of IL-8 in an IL-1--independent manner, and we addressed the specific modifications and components of Ox-LDL that mediate this activity.

**Methods**

**Reagents**

EDTA, 2-thiobarbituric acid (TBA), butylated hydroxyltoluene (BHT), E-Tox-Clean, and cupric acetate were purchased from Sigma Chemical Co, St Louis, Mo. Low-endotoxin, high-glucose Dubecco's modified Eagle's medium (DMEM) was prepared in the media kitchen at The Scripps Research Institute. Low-endotoxin Ham's F-10 medium was purchased from Whittaker M.A. Bioproducts, Walkersville, Md. 9-HODE and related lipids were purchased from Cayman Chemicals, Ann Arbor, Mich, and malondialdehyde (MDA) was from Aldrich, Milwaukee, Wis. Human recombinant IL-1\( \beta \) was purchased from R&D Systems, Minneapolis, Minn, and phorbol myristate acetate (PMA) was from Sigma. Lipo-polysaccharide (LPS; \textit{Escherichia coli} O111:B4) was obtained from Calbiochem, La Jolla, Calif. Sterile water was obtained from Baxter, Deerfield, Ill.

**Isolation and Modification of LDL**

LDL (d=1.019 to 1.063 g/mL) was isolated from normal human plasma by sequential ultracentrifugation and stored for no longer than 4 weeks in DMEM and 0.3 mmol/L EDTA.\textsuperscript{30} LDL was oxidized at a concentration of 200 \( \mu \)g/mL in Ham's F-10 medium by exposure to 10 mmol/L cupric acetate for 20 to 24 hours. The oxidation was terminated by addition of BHT to a final concentration of 40 mmol/L. Precautions taken to prevent endotoxin contamination during isolation and oxidation included the use of pyrogen--free sterile water for all reagents. All glassware was washed with 1% E-Tox-Clean in sterile water, rinsed with sterile water, and baked at 185°C for 5 hours. Endotoxin contamination of all media, reagents, and lipoproteins was monitored with the chromogenic \textit{Limulus} lysate assay (Whittaker Bioproducts) using \textit{E coli} O111.B4 endotoxin supplied with the kit for the standard curve.

Phospholipase A\( _2 \) (PLA\( _2 \))--treated LDL was generated by adding 50 mU PLA\( _2 \) (\textit{Naja naja} venom; Sigma) per 75 \textmu mol/L LDL in a solution of 100 mmol/L tris(hydroxymethyl)aminomethane and 2 mmol/L CaCl\( _2 \), pH 9.0, for 2 hours at 22°C.\textsuperscript{20} PLA\( _2 \)--treated LDL was resolubilized by potassium bromide density gradient flotation and dialyzed against Ham's F-10 containing 40 mmol/L BHT before use. More dense fractions of the potassium bromide gradient containing PLA\( _2 \) were recovered, dialyzed, and diluted by the same factor as the LDL fractions before addition to medium containing THP-1 cells.

Acetylated LDL was prepared by treatment with acetic anhydride\textsuperscript{24} and stored in 0.15 mol/L NaCl containing 10 mmol/L BHT. Effectiveness of the acetylation was verified by assessing the capacity of acetylated LDL to promote cholesterol ester loading of PMA-differentiated THP-1 cells.\textsuperscript{30} MDA modification of LDL was performed as described by Haberland et al.\textsuperscript{31} Malondialdehyde bis(dimethyl acetal) (Aldrich) (0.1 to 0.2 mmol/L) was hydrolyzed to MDA with HCl, adjusted to pH 6.4, and used immediately by mixing equal volumes with LDL (5 mg/mL in Ham's F-10 medium). The reaction was terminated by dialysis lasting from 30 minutes through 3 hours.

**TBARS Assay**

The thiobarbituric acid--reactive substance (TBARS) content of the lipoproteins was used as a measure of lipid peroxidation and was done according to a modification of the method of Steinbrecher et al.\textsuperscript{33} Briefly, 0.2 mL of sample containing 40 \( \mu \)g lipoprotein and 40 \( \mu \)mol/L BHT was tested by the addition of 1.0 mL of 20% trichloracetic acid followed by 1.0 mL TBA (0.67% in 0.05N NaOH). Samples were heated for 30 minutes at 90°C, cooled, and centrifuged, and the absorbance at 532 nm (A\textsubscript{532}) was monitored.

**Lipoprotein Electrophoresis**

Ox-LDL was examined for apo B fragmentation by sodium dodecyl sulfate--polyacrylamide gel electrophoresis (SDS-PAGE) using the Phast System (Pharbi. LKB Biotechnology, Uppsala, Sweden). LDL and Ox-LDL were separated on 4% to 15% polyacrylamide gradient gels and stained with both silver and Coomassie Blue R.

**Monocyte and THP-1 Cell Culture**

Peripheral blood mononuclear cells were isolated by Ficoll--Hypaque as described.\textsuperscript{34} Monocytes were isolated from peripheral blood mononuclear cells by using Sepacell-MN (Sepatech Corp, Oklahoma City, Okla) on fibronectin-coated tissue-culture plates (Collaborative Research Inc, Bedford, Mass). The purity of the monocyte preparation was more than 90% as determined by nonspecific esterase staining. The monocytes were cultured in low-endotoxin RPMI 1040 containing 5% fetal calf serum (FCS), 20 mmol/L N-2-hydroxyethylpiperazin-N'-2-ethanesulfonic acid (HEPES), and 10 \( \mu \)g/mL gentamicin at a density of 1 x 10\textsuperscript{6} cells in 1 mL per well in 24--well tissue-culture dishes. Monocytes were exposed to agonists at approximately 2 hours after adhesion-mediated isolation from peripheral blood. Viability of cells was monitored by trypan blue exclusion.

THP-1 cells (passages 30 through 50) were a gift of Dr Dario Altieri, The Scripps Research Institute. They were maintained in...
of cellular sensitivity to extremely low levels of contaminating endotoxin in media and reagents). When freshly adherent monocytes were incubated with copper-catalyzed Ox-LDL (10 to 40 µg/mL), we observed a potent, dose-dependent induction of IL-8 activity (17.5 to 31.3 ng/mL) (Table 1). Ox-LDL did not induce expression of TNF-α but induced a potent IL-1β response (more than 6 ng/mL) in the peripheral blood monocytes.

To further define the mechanism of Ox-LDL-induced monocyte cell activation, we used THP-1 cells, a human myeloid leukemia cell line with a mature monocyte phenotype that can be induced to express proinflammatory cytokines. Undifferentiated THP-1 cells were used in this study because such cells generally express little IL-1 unless treated with phorbol esters or a limited number of other agonists. Ox-LDL (25 to 150 ng/mL) induced THP-1 cells to produce nanogram amounts of IL-8 (Table 2). In contrast to the observations with monocytes, THP-1 cell induction of IL-8 by Ox-LDL was not accompanied by IL-1β induction (Table 2). Furthermore, the undifferentiated THP-1 cells did not upregulate IL-8 production in response to nanogram amounts of IL-1 (Table 3). Thus, Ox-LDL–induced IL-8 generation in monocyctic cells did not necessarily require IL-1β induction.

### Table 2. Oxidized LDL–Induced IL-8 but Not IL-1β Production In Human Monocytic THP-1 Cells

<table>
<thead>
<tr>
<th>Agonist</th>
<th>IL-8, ng/mL</th>
<th>IL-1β, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>2.8±0.6</td>
<td>&lt;150</td>
</tr>
<tr>
<td>LDL, 25 µg/mL</td>
<td>2.7±2.1</td>
<td>ND</td>
</tr>
<tr>
<td>LDL, 75 µg/mL</td>
<td>3.0±0.5</td>
<td>&lt;150</td>
</tr>
<tr>
<td>LDL, 150 µg/mL</td>
<td>3.2±0.3</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Ox-LDL, 25 µg/mL</td>
<td>17.0±4.2</td>
<td>ND</td>
</tr>
<tr>
<td>Ox-LDL, 75 µg/mL</td>
<td>24.0±5.6</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Ox-LDL, 150 µg/mL</td>
<td>55.7±4.5</td>
<td>&lt;150</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; IL, interleukin; TNF, tumor necrosis factor; Ox-LDL, oxidized LDL; LPS, lipopolysaccharide, and ND, not determined. Human LDL was isolated and oxidized under stringent low-endotoxin conditions and added to human peripheral monocytes for 24 hours at 37°C as described in "Methods." Release of IL-8 and IL-1β into conditioned medium and generation of TNF-α in cell pellets were measured as described in "Methods." Results are representative of four separate experiments performed in triplicate.

### Results

**Ox-LDL Induces IL-8 Expression in Both Human Monocytes and Monocytic THP-1 Cells**

Because LPS is a frequent contaminant of LDL preparations, we tested monocyte and THP-1 cell responsiveness to Ox-LDL under low-endotoxin conditions that were rigorously established and evaluated (K. Brand et al, unpublished data, October 1993). All LDL and Ox-LDL preparations used in the experiments described contained less than 0.8 pg endotoxin/µg LDL. Furthermore, as described in a separate study (K. Brand et al, unpublished data, October 1993), the preparations of LDL and Ox-LDL were verified as incapable of inducing the expression of tissue factor in freshly adherent human monocytes (a particularly sensitive indicator of cellular sensitivity to extremely low levels of contaminating endotoxin in media and reagents). When freshly adherent monocytes were incubated with copper-catalyzed Ox-LDL (10 to 40 µg/mL), we observed a potent, dose-dependent induction of IL-8 activity (17.5 to 31.3 ng/mL) (Table 1). Ox-LDL did not induce expression of TNF-α but induced a potent IL-1β response (more than 6 ng/mL) in the peripheral blood monocytes.

To further define the mechanism of Ox-LDL–induced monocyte cell activation, we used THP-1 cells, a human myeloid leukemia cell line with a mature monocyte phenotype that can be induced to express proinflammatory cytokines. Undifferentiated THP-1 cells were used in this study because such cells generally express little IL-1 unless treated with phorbol esters or a limited number of other agonists. Ox-LDL (25 to 150 ng/mL) induced THP-1 cells to produce nanogram amounts of IL-8 (Table 2). In contrast to the observations with monocytes, THP-1 cell induction of IL-8 by Ox-LDL was not accompanied by IL-1β induction (Table 2). Furthermore, the undifferentiated THP-1 cells did not upregulate IL-8 production in response to nanogram amounts of IL-1 (Table 3). Thus, Ox-LDL–induced IL-8 generation in monocyctic cells did not necessarily require IL-1β induction.
The β chemokine MIP-1α produced by macrophages is a potent attractant for T lymphocytes. As expected, both IL-8 and MIP-1α were induced by LPS-stimulated THP-1 cells (Table 4). However, Ox-LDL failed to induce MIP-1α in THP-1 cells under conditions in which IL-8 production was markedly stimulated (Table 4). Therefore, the ability of Ox-LDL to induce chemokine expression was selective.

**Effects of Selected LDL-Free Lipid Modification End Products**

The LDL constituents most susceptible to oxidation are the polyunsaturated fatty acyl chains of the LDL lipids. Polyunsaturated fatty acid oxidation is complex and can produce a variety of compounds, including epoxides, aldehydes, fatty acyl hydroperoxides, and fragmentation products such as aldehydes, alkanes, and cyclic compounds. The arachidonate oxidation end product MDA and other end products of lipid oxidation generated within Ox-LDL are capable of diffusion from the LDL particle. For this reason, we first studied the effects of certain soluble compounds known to be generated within Ox-LDL.

Free MDA (≤30 μmol/L) consistently demonstrated the capacity for only modest IL-8 induction (never greater than 1 ng/mL). IL-8 was released over background (Table 5). 9-HODE, an oxidative breakdown product of the major LDL polyunsaturated fatty acid linoleate that activates monocytes, induced IL-8, though THP-1 cells responded in a variable manner to 9-HODE (Table 6). To better define the activity of 9-HODE, several different subclones of THP-1 cells were screened, and the subclone that responded best to 9-HODE was studied further. In this subclone, 9-HODE induced IL-8 without a significant upregulation of IL-1 (Table 6). Furthermore, IL-8 induction was observed with both racemic 9-HODE and 9(S)-HODE, but not with cholesterol-esterified 9(S)-HODE (Table 6).

Thus, more than one diffusible end product known to be generated in LDL oxidative modification was able to induce IL-8 production. We next tested the effects of selected modifications of the LDL particle.

**Effects of Modifications of the LDL Particle**

MDA and certain other lipid-oxidation end products (eg, 4-hydroxynonenal) generated in Ox-LDL can covalently modify LDL-apoB lysine ε-amines and contribute to changes in apob that, if sufficiently extensive, promote enhanced recognition of Ox-LDL by macrophages and a loss of recognition by the receptor for native LDL.

**Table 3. Recombinant IL-1 and Absence of IL-8 Induction in Undifferentiated THP-1 Cells**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>IL-8, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>PMA</td>
<td>32.9±1.4</td>
</tr>
<tr>
<td>IL-1</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

Human recombinant interleukin (IL)-1β (1 ng/mL), or as a positive control, phorbol myristate acetate (PMA) (30 ng/mL), was added to THP-1 cells (1 x 10⁶ cells in 0.5 mL) in triplicate for 24 hours at 37°C as described in "Methods." Supernatants were assayed for IL-8. This experiment was repeated once with a similar absence of IL-8 induction by IL-1.

**Table 4. Ox-LDL and Induction of MIP-1α and IL-8**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>IL-8, ng/mL</th>
<th>MIP-1α, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>&lt;0.5</td>
<td>&lt;150</td>
</tr>
<tr>
<td>LPS</td>
<td>60.3±1.3</td>
<td>&gt;3500</td>
</tr>
<tr>
<td>Ox-LDL</td>
<td>10.4±2.5</td>
<td>&lt;150</td>
</tr>
</tbody>
</table>

Ox-LDL indicates oxidized low-density lipoprotein; MIP, macrophage inflammatory protein; IL, interleukin; and LPS, lipopolysaccharide. Ox-LDL or LPS was added to THP-1 cells for 24 hours at 37°C. The release of IL-8 and MIP-1α into conditioned medium was measured as described in "Methods." Results are representative of three experiments with similar results.

Ox-LDL, 150 μg/mL 26.1±1.5 <150

**Table 5. IL-8 Induction by MDA**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>IL-8, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>MDA, 1 μmol/L</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>MDA, 10 μmol/L</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td>MDA, 30 μmol/L</td>
<td>1.0±0.2</td>
</tr>
</tbody>
</table>

THP-1 cells were incubated for 24 hours with malondialdehyde (MDA). Conditioned media were collected and assayed as described in "Methods." Results are representative of four separate experiments in which interleukin-8 (IL-8) release was never greater than 1 ng/mL at up to 30 μmol/L MDA.
TABLE 6. Variable Responsiveness of THP-1 Cells to 9-HODE

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Agonist</th>
<th>IL-8, ng/mL</th>
<th>IL-1, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffer</td>
<td>6.0±4.9</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>9-HODE (racemic)</td>
<td>34.1±11</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>Buffer</td>
<td>0.1±0.1</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>9-HODE (racemic)</td>
<td>0.4±0.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

TABLE 7. Malondialdehyde Treatment of LDL and IL-8 Expression in THP-1 Cells

<table>
<thead>
<tr>
<th>Agonist</th>
<th>TBARS, nmol/L</th>
<th>IL-8, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0</td>
<td>0.4±0.7</td>
</tr>
<tr>
<td>MDA-LDL</td>
<td>0.82</td>
<td>1.2±0.7</td>
</tr>
<tr>
<td>Sham MDA-treated</td>
<td>&lt;0.1</td>
<td>1.6±0.6</td>
</tr>
<tr>
<td>LDL</td>
<td>ND</td>
<td>1.7±0.8</td>
</tr>
<tr>
<td>Ox-LDL</td>
<td>0.375</td>
<td>10.3±2.8</td>
</tr>
</tbody>
</table>

Low-density lipoprotein (LDL) was nonoxidatively modified by treatment with malondialdehyde (MDA) or sham treatment with no MDA as described in "Methods." MDA-LDL preparations routinely contained 10 to 20 nmol thiobarbituric acid-reactive substance (TBARS) per mg LDL. Oxidized LDL (Ox-LDL) preparations routinely contained 5 nmol TBARS/mg LDL. THP-1 cells were incubated with LDL preparations (all at 75 μg/mL) for 24 hours, at which time interleukin (IL)-8 release into the conditioned medium was determined. Results are representative of four separate experiments. ND indicates not determined.

Discussion

We observed that Ox-LDL activates the production of the chemokine IL-8 from monocytes under low-endotoxin conditions. Furthermore, Ox-LDL induced IL-8 in THP-1 cells that did not produce a detectable rise in IL-1 in response to IL-8 (Table 2) and did not detectably upregulate IL-8 in response to purified IL-1 (Table 3). Thus, IL-8 induction by Ox-LDL did not appear to absolutely require IL-1, which has the capacity to directly upregulate IL-8.23

Ox-LDL stimulated freshly adherent human peripheral monocytes but not monocyte THP-1 cells to produce IL-1 (Tables 1 through 3). Previous investigation of Ox-LDL–induced activation of cells of the monocyte/macrophage lineage has similarly demonstrated Ox-LDL–induced IL-1 expression in freshly adherent monocytes.22 However, mature macrophages do not produce IL-1 in response to Ox-LDL, and IL-1 generation (and TNF-α generation) stimulated by other agonists can actually be inhibited by coculture with Ox-LDL in these cells.5,41 The basis for these differences between monocytes and macrophages has been suggested to be due to receptor-mediated Ox-LDL internalization,41 but this was not confirmed in a separate study42 and thus requires more investigation. Importantly, the demonstration in this study of IL-1–independent IL-8 induction by Ox-LDL suggested a potential mechanism whereby Ox-LDL can amplify cell recruitment irrespective of the capacity of monocyte lineage cells to produce IL-1 or the IL-1 receptor antagonist.43 Such a mechanism may be significant because the maturation of monocytes to macrophages is associated with a marked downregulation (50% or more) in their ability to produce IL-1/β and a large upregulation in their production of the IL-1 receptor antagonist.43

We analyzed the structural requirements for the capacity of Ox-LDL to activate monocytes by using IL-8 induction in THP-1 cells as a model system. Oxidative modification of LDL involves multiple events that generate complex products.19,38 Our results suggested that the monocyte-activating constituents of Ox-LDL are heterogeneous. Certain end products of lipid degradation known to be generated within Ox-LDL are heterogeneous. Certain end products of lipid degradation known to be generated within Ox-LDL (Tables 5 and 6), and some of the stimulating activity was diffusible from the Ox-LDL particle (Figure). However, the bulk of the IL-8–inducing activity of Ox-LDL appeared nondialyzable (Figure). Furthermore, selective modification of the LDL particle (by extrinsic PLA2 but not by MDA treatment or acetylation) rendered the lipoprotein stimulatory (Tables 7 and 8). An important future area of study will be to ascertain whether this activity of Ox-LDL is attributable to protein constituents (eg, monocyte-activating, degraded, soluble apoB peptides) and/or to lipid constituents (eg, oxidative lipid degradation end products, lysophospholipids, or free fatty acids that remain conjugated to constituents of the Ox-LDL). A newly described paradigm suggests that both of these possibilities may be relevant and testable.44 Specifically, lipid hydroperoxides appear to react with free amino groups in polypeptides and in phosphatidylethanolamine.
Ox-LDL can induce not only the α chemokine IL-8 but also the β chemokine MCAF/MCP-1.\textsuperscript{24,26} However, Ox-LDL–induced THP-1 cell activation produced selective chemokine production, as the T-lymphocyte–chemoattracting β chemokine MIP-1α\textsuperscript{23,29} was not induced (Table 4). Thus, Ox-LDL–induced chemokine induction may require specific membrane and intracellular signals; these will be valuable to define in further studies.

MCAF/MCP-1 is identified within macrophage-derived foam cells in atherosclerotic lesions that appear to bear Ox-LDL.\textsuperscript{25} The potential presence in similar atherosclerotic lesions of IL-8 (and other chemokines that recruit T lymphocytes) merits similar evaluation for several reasons. Specifically, IL-8 is active at picomolar concentrations as a selective T-lymphocyte chemoattractant,\textsuperscript{27} and IL-8 preferentially attracts T lymphocytes enriched in CD45RO\textsuperscript{45}; as discussed above, CD45RO-enriched T cells are the predominant phenotype in atherosclerotic lesions.\textsuperscript{4} Thus, the potential generation of IL-8 by monocytes/macrophages (and endothelial and smooth muscle cells\textsuperscript{66}) in atherosclerotic lesions could help modulate the colocalization of activated T cells and macrophages. Because IL-8 appears to be more potent as a T-cell chemotactant than a neutrophil chemotactant,\textsuperscript{27} it is possible that under certain conditions, the production of small amounts of IL-8 might not produce concomitant neutrophil influx; in this regard, the paucity of neutrophils in atherosclerotic lesions is well documented.\textsuperscript{1}

IL-8 is also an extremely potent angiogenic factor and a chemotactant for endothelial cells\textsuperscript{88}; neovascularization is a common feature in atherosclerotic plaques and may predispose to further plaque injury and thrombosis.\textsuperscript{87} Fragmented atherosclerotic plaques stimulate angiogenesis,\textsuperscript{90} and the potential contribution of IL-8 generation to this activity merits investigation.

Finally, IL-8, in contrast to many other nonchemo-


tactants (eg, leukotriene B4), has a partic-


tually prolonged functional half-life that is conferred by its relative resistance to proteolytic degradation.\textsuperscript{25} Thus, the mechanism that mediates Ox-LDL–induced IL-8 induction may be the prototype for generating long-lived mediators that recruit T lymphocytes to sites where monocytes/macrophages encounter Ox-LDL.

### Acknowledgments

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