Oxidized Low-Density Lipoprotein Promotes Mature Dendritic Cell Transition from Differentiating Monocyte

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Monocytes exit blood and enter tissues, where they can differentiate to macrophages or dendritic cells (DC). This differentiation occurs when monocytes cross the endothelium, but regulatory mechanisms controlling macrophage or DC generation in vivo are poorly understood. Some monocytes may only transiently traverse tissues and, under local inflammatory conditions, migrate to draining lymph node while acquiring DC phenotype and functions. Randolph et al. (1) showed that transendothelial transport and phagocytosis are critical events that promote monocyte-to-DC transition. Monocytes that reverse transmigrate, a process mimicking cellular migration from tissue to lymph, become DC while the others become macrophages. Monocyte-to-DC transition is greatly enhanced by a phagocytic stimulus provided by particulate material. Thus, endothelial tissues initiate differentiation of monocytes to DC, but efficient differentiation requires an additional stimulus provided in this study by foreign particulate material. In support of this is the in vivo injection of microspheres into the skin that induces phagocytic monocytes to migrate to the T cell area of draining lymph nodes where they express costimulatory and DC-restricted markers (2).

Upon entry into the tissues, monocytes also interact with environmental factors influencing their differentiation. For instance, IL-6 from fibroblasts promotes monocyte differentiation to macrophages (3). Of the many environmental factors that deserve to be analyzed, oxidized low-density lipoprotein (oxLDL) is particularly relevant because it is generated from native LDL trapped in the subendothelial space, it stimulates the expression of monocyte chemoattractant protein-1, M-CSF, and GM-CSF by endothelial cells, and it induces increased monocyte adhesion to and transmigration through the endothelial cell layer (4–8).

Lipoproteins are water-soluble spherical particles that transport nonpolar lipids. In humans, LDL is the major cholesterol transporter and consists of a hydrophobic core containing cholesterol ester molecules and a surface monolayer of polar lipids (primarily phospholipids) and apolipoprotein B. Plasma LDL is transported across the intact endothelium and becomes trapped in the extracellular matrix of the subendothelial space where it can be subjected to oxidative modifications (9, 10). Various cellular and biochemical mediators have been proposed to initiate or regulate LDL oxidation, but which enzymatic or nonenzymatic oxidative mechanisms are implicated in vivo is still the subject of debate and intensive study (11–16). oxLDL is a potent inducer of inflammatory molecules, but the substances within the oxLDL that are responsible for this effect are only partially known. This is mainly due to the complex mixture of various components in oxLDL, including lipid hydroperoxides, oxysterols, lysophosphatidylcholine, and aldehydes (17, 18).

Proinflammatory oxidative modification of LDL is under strict control by native LDL and high-density lipoprotein (HDL). Paraoxonase (PON1) and platelet-activating factor acetylhydrolase (PAF-AH) are two enzymes complexed with lipoproteins that prevent the accumulation of oxLDL (19–21). PON1 can inhibit LDL oxidation and destroy various bioactive oxidized phospholipids as well as hydrogen peroxide (22–24). In the model proposed by Watson et al. (20), oxidized phospholipids are first substrate for PON1 in HDL. If PON1 cannot stop the process, oxidized phospholipids undergo further modifications and become substrate for...

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oxLDL GENERATES MATURE DCs

PAF-AH. PAF-AH is a phospholipase A2 that hydrolyzes short-chain acyl groups and longer chain aldehyde esterified to the sn-2 position of phospholipids (21). Failure to inactivate all active compounds results in highly oxidized LDL production similar to those found in the necrotic core of atherosclerotic lesions. Under normal conditions, the two lines of control maintain the constant production of oxLDL below a critical threshold. During the acute phase response, HDL exhibits decreases in PON1 and PAF-AH and increases in the copper carrier ceruloplasmin (25). These changes alter HDL ability to inhibit LDL oxidation and anti-inflammatory HDL becomes proinflammatory during the acute phase response.

As the control of LDL oxidation is transiently lost during the acute phase response to tissue injury, we asked whether oxLDL could be an internal signal favoring the monocyte-to-DC transition initiated by endothelial tissue. Using GM-CSF and IL-4 to initiate monocyte differentiation, we investigated whether oxLDL could alter the phenotype and functional abilities of the cells at different stages in culture.

Materials and Methods

**LDL preparation**

LDL (1.025 ≤ d ≤ 1.055 g/ml) was isolated from human plasma of normolipemic healthy individuals by ultracentrifugation. The density of the plasma was raised to 1.025 g/ml using NaBr. After ultracentrifugation at 100,000 rpm at 4 °C for 4 h using a TL 100.4 rotor Beckman Instrument (Gagny, France), the light fraction containing chyloymyrcron, very low-density lipoprotein, and intermediate density lipoprotein was removed. The density was adjusted at 1.055 g/ml with NaBr and after ultracentrifugation in the same conditions, the light fraction containing LDL was collected, dialyzed extensively against 150 mM NaCl, 2.4 mM EDTA, pH 7.2 at 4 °C, filtered at 0.45 µm, and stored under nitrogen. The protein content of the fraction was estimated by Coomassie Protein MicroAssay procedure (Pierce, Rockford, IL) and its lipid composition was determined using Cholesterol STU, Triglycerides enzymatic PAP 150, and Phospholipids enzymatic PAP 150 kits from bioMéreux (Marcy l’Etoile, France). LDL was composed of 23 ± 1% protein (exclusively apolipoprotein B), 41 ± 4% cholesterol, 24 ± 1% phospholipids, and 13 ± 4% triglycerides. Endotoxin levels in LDL or oxLDL were less than 0.5 pg/ml in final concentration assessed by LPS-toxetax test (Sigma-Aldrich, St. Quentin-Fallavier, France).

**LDL oxidation**

LDL concentration was adjusted at 1 mg/ml of protein by dilution in PBS and was dialyzed at 4 °C against PBS to eliminate EDTA. Cu²⁺-mediated oxidation was conducted at 37°C for 24 h by dialysis against 5 µM CuSO₄/ PBS. The reaction was stopped by the addition of 40 µM butylated-hydroxy-toluene and extensive dialysis at 4 °C against PBS containing 1% phospholipids, and 13% 4% triglycerides. Endotoxin levels in LDL or oxLDL were less than 0.5 pg/ml in final concentration assessed by LPS-toxetax test (Sigma-Aldrich, St. Quentin-Fallavier, France).

**Monocyte-derived DC**

Mononuclear cells were isolated from human peripheral blood by density gradient centrifugation using Ficoll-Hyppaque at 400 x g for 20 min and then by centrifugation on a 50% Percoll solution at 400 x g for 20 min. Monocytes were purified by immunomagnetic depletion (Dynal Biotech, Oslo, Norway), using a mixture of mAbs anti-CD14 (4G7), anti-CD16 (3G8), anti-CD56 (NKH1), anti-glycoprotein A (11E4B7.6), and anti-CD14 (Ramos; all from Beckman Coulter), T lymphocytes were >95% pure as assessed by CD3 labeling. Primary MLRs were conducted in 96-well flat-bottom culture plates. APCs were treated or not with oxLDL, collected at day 6, extensively washed, and resuspended in RPMI/10% FCS. These cells were then cocultured with 2 x 10⁵ allogeneic or syngeneic T cells in 200 µl of complete culture medium at 1:5, 1:10, or 1:20 APC:responder T cells ratio. After 4 days, 50 µl of culture supernatant was frozen for IL-2 secretion assay and was replaced by 50 µl of fresh medium containing 1 µg of Lucifer Yellow Fluospheres of 0.45 µm (beads; Molecular Probes, Leiden, The Netherlands) for macrophocytosis. Internalization was stopped on ice with cold PBS containing 1% BSA and 0.05% NaN₃. Cells were washed three times at 4 °C in this buffer and were analyzed on a FACSCalibur (BD Biosciences).

**Mixed lymphocyte reaction**

Naive T lymphocytes were isolated from human peripheral blood. PBMC were isolated by density gradient centrifugation on Ficoll-Hyppaque after depletion of monocytes on Percoll gradient. PBL were recovered in the dense fraction. T lymphocytes were purified by immunomagnetic depletion using a mixture of mAbs anti-CD19 (4G7), anti-CD16 (3G8), anti-CD56 (NKH1), anti-glycoprotein A (11E4B7.6), and anti-CD14 (Ramos; all from Beckman Coulter). T lymphocytes were >95% pure as assessed by CD3 labeling. Primary MLRs were conducted in 96-well flat-bottom culture plates. APCs were treated or not with oxLDL, collected at day 6, extensively washed, and resuspended in RPMI/10% FCS. These cells were then cocultured with 2 x 10⁵ allogeneic or syngeneic T cells in 200 µl of complete culture medium at 1:5, 1:10, or 1:20 APC:responder T cells ratio. After 4 days, 50 µl of culture supernatant was frozen for IL-2 secretion assay and was replaced by 50 µl of fresh medium containing 1 µg of lucifer yellow Fluospheres of 0.45 µm (beads; Molecular Probes, Leiden, The Netherlands) for macrophocytosis. Internalization was stopped on ice with cold PBS containing 1% BSA and 0.05% NaN₃. Cells were washed three times at 4 °C in this buffer and were analyzed on a FACSCalibur (BD Biosciences).

Cytokine assay

Culture supernatants were aliquoted and stored at −80°C until they were analyzed for the presence of cytokines. IL-1α, IL-1β, IL-10, IL-12 p70, and TNF-α levels were determined using cytokine-specific ELISA kits purchased from Endogen (Woburn, MA).

**Statistical analysis**

Data are expressed as mean ± SD. Differences between means were examined by paired Student’s t test and were considered significant when p < 0.05. All experiments were repeated at least three times with different cell and lipoprotein preparations.

**Results**

oxLDL induces phenotypic and morphologic changes during monocyte differentiation to DC

The most common procedure to generate monocyte-derived DC is to expose CD14⁺CD1a⁻ monocytes to GM-CSF and IL-4 for 6–7 days to obtain CD14⁻CD1a⁺ immature DC (26, 27). DC express many accessory molecules that interact with receptors on T cells to enhance adhesion and signaling. These molecules are regulated upon exposure to various stimuli, including stress and microbial products. The maturation level of DC can thus be followed by several typical surface markers (in this study, CD14, CD1a, MHC class II, CD80, CD83, CD86, and CD40). To determine how the presence of oxLDL would affect monocyte differentiation, 10 µg/ml oxLDL was added at day 0 corresponding to the first day of culture with GM-CSF, IL-4, and FCS. Phenotype was monitored at day 6 and compared with phenotype of immature DC generated without oxLDL. Fig. 1A shows that oxLDL did not interfere with the differentiation of CD14⁺CD1a⁻ monocytes into CD14⁻CD1a⁺ DC, although CD1a labeling appeared consistently...
slightly weaker. As expected, control immature DC were CD86^−, but monocytes differentiated in the presence of oxLDL were mostly CD86^+. Other usual differentiation markers were not affected by oxLDL (data not shown). The addition of native LDL at day 0 of differentiation did not induce any phenotypic modifications of the cells (Fig. 1A).

Native lipoproteins contain enzymes that can destroy bioactive oxidized phospholipids. In addition, oxLDL are recognized by scavenger receptors (28–31), which bind a wide variety of ligands; some of which are able to bind native lipoproteins. To test the inhibitory action of native lipoproteins on oxLDL, monocytes were then differentiated in LPDS. Control culture without oxLDL showed that LPDS did not affect differentiation of monocytes into typical CD14^+CD1a^−CD86^− immature DC (Fig. 1B). Other markers showed the same labeling whether DC were generated in LPDS or FCS (data not shown). The effect of oxLDL was striking in LPDS. Cells obtained after a 6-day culture in LPDS supplemented with GM-CSF, IL-4, and oxLDL were all CD14^−CD1a^+ and expressed a high level of CD86 (Fig. 1B). Thus, CD1a and CD86 appeared to be two differentiation markers affected by oxLDL during the monocyte differentiation into DC. Under the same experimental conditions, native LDL did not induce phenotypic modifications (Fig. 1B). However, CD1a down-regulation and CD86 up-regulation induced by oxLDL was inhibited by an excess of native LDL (Fig. 1C).

Morphologic observation indicated that 30–40% of monocyte-derived DC obtained in LPDS and oxLDL had a bipolar elongated appearance at day 6 (Fig. 1D). Ten to 50 μg/ml native LDL alone did not induce any morphologic modification (Fig. 1D). As expected from the FACS analysis shown in Fig. 1C, DC obtained in LPDS with oxLDL and a 5-fold excess of native LDL (50 μg/ml) have a nearly normal morphology as compared with control LPDS (Fig. 1D) or FCS culture without oxLDL (data not shown). In addition, CD1a was down-regulated and CD86 was up-regulated by oxLDL in a dose-dependent manner (Fig. 1E). Weak induction of CD86 expression in FCS, dose-response activity of oxLDL, and competition with native LDL indicate that the effect of

Figure 1. Flow cytometric and morphologic evaluation of oxLDL-treated cells. A and B, Monocytes were cultured for 6 days in either FCS (A) or LPDS (B) medium containing GM-CSF and IL-4. Thin line, FCS or LPDS control conditions; dotted line, FCS or LPDS with 10 μg/ml native LDL; filled profiles, FCS or LPDS with 10 μg/ml oxLDL. Phenotype was analyzed at day 6. C, Competition of oxLDL by native LDL. Monocytes were cultured for 6 days in LPDS medium in control conditions (thin line), with 50 μg/ml LDL (dotted line), with 10 μg/ml oxLDL (filled profiles), or with 10 μg/ml oxLDL plus 50 μg/ml native LDL (bold line). Data are representative of more than five independent experiments. D, Morphologic changes induced by oxLDL. Monocytes were cultured in the presence of GM-CSF and IL-4 in LPDS, with 10 μg/ml oxLDL or with 10 μg/ml oxLDL plus 50 μg/ml native LDL (oxLDL and LDL). oxLDL with or without LDL was added on monocytes at day 0 and photographs were taken at day 6. E, Dose dependence of oxLDL on CD1a and CD86 markers. Cells were harvested at day 6 of differentiation in LPDS medium containing 10 μg/ml native LDL or varying doses of oxLDL: 0 (control LPDS), 2.5, 5, or 10 μg/ml. Mean fluorescence intensities of CD1a and CD86 for three independent experiments were normalized at 100 for the control LPDS. Mean ± SD is shown.
oxLDL can be weakened by endogenous competitors, including native lipoproteins. In the above experiments, cells were analyzed at day 6 and oxLDL was added concomitantly to GM-CSF and IL-4 at the first day of culture (day 0) without subsequent supplementation. We next asked whether the degree of monocyte differentiation changes its ability to respond to oxLDL. oxLDL (10 μg/ml) was added once at days 0, 3, 4, 5, or 6 (corresponding to immature DC) and CD86 expression was analyzed by FACS at days 6 or 7 when oxLDL was added at day 6. Fig. 2A shows that CD86 expression was always increased by oxLDL. However, CD86 expression was more important when oxLDL was added at day 4 or 5. As a control, the addition of native LDL under the same timing conditions never induced CD86 expression. Extended phenotypes are shown in Fig. 2B and they compare the consequences of oxLDL addition at days 0 and 5 of monocyte differentiation. Day 0-treated monocytes appeared to be CD14+CD1a−CD86−, but remained negative for other costimulatory molecules. In contrast, day 5-treated monocytes exhibited a fully mature DC phenotype except that these cells remained CD1a−. oxLDL added to immature DC (day 6 treatment) induced CD86 expression (Fig. 2A), but phenotype analysis revealed no induction of other costimulatory molecules or maturation markers after 24 or 48 h (data not shown). Therefore, phenotypic analysis indicated that oxLDL has a rapid and maximal effect during the late stage of monocyte differentiation and skews this differentiation directly toward mature DC. In contrast, immature DC have lost the ability to fully mature under the action of oxLDL.

oxLDL-treated cells display limited endocytic activity

Immature DC express a potent ability to uptake large amounts of external molecules essentially by two main mechanisms: receptor-mediated endocytosis and macropinocytosis (32, 33). This ability is lost when immature DC receive a maturation-inducing stimulus. Both pathways have been studied by flow cytometry to test whether oxLDL can down-regulate the capturing machinery like other classical maturation-inducing signals. FITC-Dextran has been used to measure mannose receptor-mediated endocytosis, fluorescent beads for macropinocytosis, and LY as nonspecific fluid phase markers. Fig. 3A shows that although oxLDL does not induce a fully mature DC phenotype when added early on monocytes, it still inhibits the mannose receptor pathway and LY uptake. Inhibition of macropinocytosis is less potent. oxLDL added at day 5 of monocyte differentiation, a condition that rapidly induces fully mature DC phenotype, results in a strong inhibition of both pathways. Cells treated with 10 μg/ml native LDL display the same endocytic activity as control cells differentiated in LPDS.

**oxLDL regulates IL-12p70 production**

We next analyzed whether or not oxLDL might induce release of cytokines by treated cells. Culture supernatants of control immature DC and monocytes treated at day 0 or 5 during the differentiation process were analyzed by ELISA to detect TNF-α, IL-1β, IL-10, and bioactive IL-12p70. TNF-α and IL-1β could not be detected in any of the culture conditions (data not shown). A very

**FIGURE 2.** Effect of oxLDL is dependent on the differentiation stage of the monocytes. A, Control is monocytes cultured in LPDS differentiation medium with or without LDL (10 μg/ml). oxLDL (10 μg/ml) was added at day 0, 3, 4, 5, or 6 of the differentiation. Cells were harvested at day 6 or 7 when oxLDL was added at day 6. CD86 expression was quantified by flow cytometry. Mean ± SD of three independent experiments is shown. B, Comparison of phenotypes of the cells obtained at day 6 of differentiation when oxLDL (10 μg/ml) was added at day 0 or 5.

**FIGURE 3.** Endocytic activity and cytokine production of oxLDL-treated cells. A, Inhibition of endocytosis by oxLDL. Monocytes were differentiated in LPDS medium with or without 10 μg/ml LDL (control) or with 10 μg/ml oxLDL added at day 0 (d0 + oxLDL) or day 5 (d5 + oxLDL). Cells were harvested at day 6. Cells were incubated at 37°C with 1 mg/ml FITC-Dextran or LY for 30 min or with fluorescent beads for 3 h. The amount of probe internalized was measured by flow cytometry. The mean fluorescent intensities of a representative experiment were normalized at 100% uptake in the control. B, Induction of IL-12p70 secretion by oxLDL. Monocytes were differentiated in LPDS medium in the presence or not of LDL (10 μg/ml) or oxLDL (10 μg/ml) added at day 0 (d0 + LDL or d0 + oxLDL) or day 5 (d5 + LDL or d5 + oxLDL). Culture supernatants were collected at day 6, and the amount of IL-10 and IL-12p70 was measured by sandwich ELISA. Mean values of four independent experiments for 10⁶ cells.
low quantity of IL-10 could be detected in supernatants of day 0- or 5-treated monocytes and immature DC (Fig. 3B). A major difference was observed for IL-12p70 production. Control immature DC and day 0-treated monocytes did not secrete a significant amount of IL-12p70. In contrast, day 5-treated monocytes were induced to produce a substantial amount of IL-12p70 by oxLDL, but not by native LDL (Fig. 3B). Thus, maturation of these cells promoted by oxLDL is associated with the production of the dominant cytokine in directing the development of Th1 cells.

**oxLDL-treated cells activate allogeneic and syngeneic T cells**

To further study the maturation level of these differentiating monocytes, we tested their capacity to stimulate allogeneic and syngeneic T cells, a functional characteristic of mature DC. As Fig. 4A shows, only oxLDL-treated monocytes, but not LDL-treated or untreated cells (immature DC), stimulated IL-2 secretion by allogeneic naive T cells. Cells treated at day 5 of differentiation, which display a mature DC phenotype, were better stimulators than cells treated at day 0, which express CD86, but no other activation markers. The allostimulatory capacity of these monocyte-derived cells was inhibited when an excess of native LDL was added concomitantly to oxLDL, an experimental procedure that also inhibits cell killing. The allostimulatory capacity of these monocyte-derived cells that have been treated with oxLDL at day 5 for 24 h also induced the proliferation of syngeneic T cells, indicating that these cells have the ability to present exogenous Ags from the medium (Fig. 4C). Induction of autologous T cell proliferation by monocytes treated at day 0 of differentiation varied from experiment to experiment.

**Discussion**

When crossing the endothelial barrier, monocytes differentiate in an environment containing unknown internal signals that contribute to determine whether monocytes progress along the DC or macrophage differentiation pathway. One such signal could be oxLDL, which accumulates in the subendothelial space under specific acute or chronic conditions (34, 35). Therefore, we examined how oxLDL could affect monocyte differentiation polarized toward DC by GM-CSF and IL-4.

oxLDL added at the first day of differentiation has a striking effect in LPDS, whereas only weak CD86 expression was induced in FCS, suggesting that native lipoproteins are endogenous inhibitors of oxLDL. In LPDS, all oxLDL-treated monocytes became CD14^+ CD1a^+ CD86^+. Although full maturation phenotype was not achieved under these conditions, these cells display some functional characteristics of mature DC. They have a reduced uptake capacity and can activate allogeneic T cells. In contrast, they do not secrete cytokines that are usually secreted by mature DC. Cells were more reactive when oxLDL was added at the late stages of differentiation. In 24 h, these cells acquired a fully mature DC

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**FIGURE 4.** Allogeneic and syngeneic T cell stimulation by oxLDL-treated cells. A. Control immature DC (LPDS) or monocytes treated at day 0 or 5 with 50 μg/ml native LDL (d0 + LDL or d5 + LDL), with 10 μg/ml oxLDL (d0 + oxLDL or d5 + oxLDL), or with 10 μg/ml oxLDL plus 50 μg/ml native LDL (d0 + oxLDL + LDL or d5 + oxLDL + LDL) were washed and cultured with allogeneic-purified T cells (2 × 10^5/well) at ratios ranging between 1:5 and 1:20 (DC:T) for 4 days. The amount of IL-2 in the supernatants of the coculture was measured by sandwich ELISA. Statistical significance was calculated with immature DC (LPDS) as control. *, p < 0.05; **, p < 0.01. B. Control immature DC (LPDS) or monocytes treated at day 5 with 50 μg/ml native LDL, 2.5, 5, or 10 μg/ml oxLDL, or with 10 μg/ml oxLDL and 50 μg/ml native LDL were cultured in triplicate with allogeneic-purified T cells (2 × 10^5/well) at the 1:10 DC:T cell ratio for 5 days. *, p < 0.005 compared with control LPDS cells. C. Control immature DC (LPDS) or monocytes treated at day 5 with 50 μg/ml native LDL, 10 μg/ml oxLDL, or 10 μg/ml oxLDL and 50 μg/ml native LDL were cultured in triplicate with syngeneic-purified T cells (2 × 10^5/well) at the 1:10 DC:T cell ratio for 5 days. [3H]Thymidine (1 μCi/well) was added for the last 16 h of culture, and incorporation was measured by a β-plate counter. *, p < 0.01 compared with control LPDS cells.
phenotype with a reduced uptake capacity, and they can stimulate allogeneic and syngeneic T cells. Importantly, these cells secreted bioactive IL-12, but very little IL-10. At day 6, once monocytes had become typical immature DC, oxLDL still induces CD86 expression, but does not promote full maturation, indicating that oxLDL is not a classical activator of immature DC. Rather, our data suggest that oxLDL could be an internal stimulus, inducing a polarized Th1 response by acting directly on monocytes during their differentiation to DC.

If oxLDL is an endogenous activator signal of the immune response, its delivery must be tightly controlled to allow reactivity when necessary and to prevent immunopathology. We showed that FCS containing native lipoproteins and LPDS supplemented with native LDL are efficient inhibitors of oxLDL activity. This strongly suggests that under normal conditions, oxLDL signals are not active because of endogenous native lipoproteins bearing appropriate enzymes hydrolyzing bioactive phospholipids. It has been reported that phospholipid liposomes prevent the biological activity of oxLDL by serving as a sink for the biologically active lipids (8). These two mechanisms might apply to our competition experiments where native LDL inhibited oxLDL activity. Thus, oxLDL may only express its biological activity when oxLDL level increases above a critical threshold. This would only happen under specific conditions, like acute phase response or atherosclerosis.

Although biologic activity of oxLDL is attributable to oxidized phospholipids and their derivatives, only a small fraction of these molecules has been identified. oxLDL and apoptotic cells share common oxidatively modified moieties that are recognized by macrophage receptors and mediate their phagocytosis (36, 37). As oxLDL activate APC, but removal of apoptotic cells usually does not, it is likely that the common structures of oxLDL and apoptotic cells are not essential for the generation of mature DC from differentiating monocytes (38). oxLDL interacts with scavenger receptors on phagocytic cells (28–31). These receptors belong to a large family, and they bind ligands that have domains with high negative charge like polyinosinic acid (39). This suggests that ligand binding is mediated by ionic interactions and that oxidized phospholipids are not likely to express their biological activity through a single receptor. The mechanism of action of oxLDL is further complicated by the fact that structurally similar oxidized phospholipids differentially regulate endothelial binding of monocytes (40).

Factors that govern the balance between immunity and tolerance remain largely unknown. So far, two types of signals that can activate DC have been identified. The first type of signal consists of conserved molecules of infectious agents that are recognized by pattern recognition receptors (41, 42). The second type of signal consists of endogenous activation signals released during infectious or ill-defined stressful events. DC can be activated by endogenous signals released by cells that are stressed, virally infected, or necrotic (38). The emerging consensus, pioneered by P. Matzinger (43, 44), is that the immune system is reacting to a dangerous situation by sensing internal signals. The observation that CD91 could sensor necrotic or tumoral cells argues in that direction (45). DC themselves might be able to sense these signals that provide the context of Ag presentation.

In reaction to infectious or noninfectious tissue injury, the host immediately executes wide ranging physiologic changes known as the acute phase response (46, 47). It is in the context of the acute phase response that Ag presentation first takes place. The acute phase response is a dynamic homeostatic reaction that involves the major biologic systems, in addition to the immune system, cardiovascular system, and CNS. It is characterized by a series of highly coordinated physiologic reactions and by rapid and transient changes in the level of a large number of plasma proteins. These changes are predominantly the result of alterations in the pattern of protein synthesis in the liver. Serum amyloid A proteins (SAA) circulate primarily with HDL and appear to be major acute phase reactants after a variety of stimuli such as surgery, infection and chronic arthritis (48). During the acute phase response, SAA in plasma is increased by up to 1000-fold. As a consequence, HDL exhibits a marked increase in SAA and a loss in apolipoprotein A (25, 49). Concomitantly, PAF-AH and PON1 are displaced from HDL, which loses its protective effect against LDL oxidation. HDL also exhibits increases in ceruloplasmin, which alters its ability to inhibit LDL oxidation. Therefore, one consequence of the acute phase response is that control of LDL oxidation by HDL is transiently lost, which results in enhanced monocyte chemoattractant protein-1 production by endothelial cells and increased monocyte transmigration in an environment that contains an elevated level of oxLDL. This would favor a rapid and transient generation of mature DC from monocytes.

An aberrant continuation of some aspects of the acute phase response can lead to chronic inflammation. Atherosclerosis is a chronic inflammatory disease developing in response to injury in the vessel wall. Most of the clinical events have been attributed to the arterial inflammatory responses initiated by the oxidation of LDL trapped in the extracellular space. Ludewig et al. (50) have shown that hypercholesterolemia, a high level of LDL associated with elevated oxLDL production, is a predisposing factor for inflammation and immunity in the vascular wall, and it contributes to the establishment of a chronic inflammatory state.

Environmental signals inducing costimulatory molecules on DC appear to be critical factors in discriminating between tolerance and reactivity. Our data suggest that by controlling oxidative modification of LDL, the interplay between HDL and LDL might be one factor regulating the balance between immune activation and tolerance induction. It can reasonably be speculated that the immune system does not react to an on/off signal provided by oxLDL, but rather that the immune status would be influenced by the degree and level of LDL oxidation. Besides the basic research observation that oxLDL promotes development of mature DC, the data presented in this study may have practical implications in the field of cell therapy. Treatment of differentiating monocytes with oxLDL or derivatives may facilitate and improve the production of DC in vitro for various therapeutic approaches.

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