HAL author manuscript
inserm-00136347, version 1

Journal of Immunology 2002;169(4):1688-95

Mature dendritic cell generation promoted by lysophosphatidylcholine[1]

Frédéric Coutant[*], Laure Perrin-Cocon[*], Sophie Agaugué[*], Thierry Delair[†], Patrice André[*] and Vincent Lotteau[*2]

[*]Centre d'Etude de Recherche en Virologie et Immunologie, Institut National de la Santé et de la Recherche Médicale U503, 21 Avenue Tony Garnier, 69007 Lyon, France

[†]UMR 2142 CNRS-bioMérieux, Ecole Normale Supérieure de Lyon, 46 Allée d' Italie, 69364 Lyon, France

Running title: Lysophosphatidylcholine generates mature dendritic cells

Corresponding author:

Vincent Lotteau

CERVI-INSERM U503

21 Avenue Tony Garnier

69007, Lyon, France

Tel : (33) 437 28 24 12

Fax : (33) 437 28 23 41

e-mail : lotteau@cervi-lyon.inserm.fr

Key words: Inflammation. Acute-phase reactants. Dendritic cells. Immunomodulators. Lipid Mediators.

This is an author-produced version of a manuscript accepted for publication in *The Journal of Immunology (The JI).* The American Association of Immunologists, Inc. (AAI), publisher of *The JI*, holds the copyright to this manuscript. This manuscript has not yet been copyedited or subjected to editorial proofreading by *The JI*; hence it may differ from the final version published in *The JI* (online and in print). AAI *(The JI)* is not liable for errors or omissions in this author-produced version of the manuscript or in any version derived from it by the United States National Institutes of Health or any other third party. The final, citable version of record can be found at www.jimmunol.org

HAL author manuscript inserm-00136347, version 1

Abstract

During the acute phase response the interplay between high density lipoproteins and low density lipoproteins (LDL) favors transient generation of oxidized LDL with proinflammatory activities. We hypothesized that oxidative modification of LDL is an endogenous signal for the immune system and have shown that oxidized LDL promotes mature dendritic cell transition from monocyte therefore linking the non specific acute phase response to adaptive immunity. Lysophosphatidylcholine (LPC) is a major lipid component of oxidized LDL with reported proinflammatory activities. We now report that LPC acts through G protein-coupled receptors on differentiating monocytes to generate mature dendritic cells with the ability to stimulate IL-2 and IFNγ production by allogeneic T lymphocytes. LPC is most effective in lipoprotein deprived serum and can be inhibited by an excess of native LDL reflecting normal plasma conditions. Therefore, by controlling the balance between native and oxidized lipoproteins and the resulting production of LPC, the acute phase reactants may provide a context of antigen presentation which is transiently favorable to immune activation. Intralipid, a therapeutic lipid emulsion for parenteral nutrition with unexplained immunomodulatory properties, also blocked LPC activity. This opens perspectives for the understanding and treatment of acute and chronic inflammatory diseases.

HAL author manuscript inserm-00136347, version 1

Introduction

The acute phase response (APR)[3] is a non specific physiological response of the body to injury, trauma or infection (1). One major biologic function of APR is to improve survival of the host by activating a variety of defense-related mechanisms such as tissue repair and limiting dispersal of pathogens before homeostasis can be restored. It is characterized by wide changes in concentration of a large number of plasma proteins called acute phase reactants (2). These modifications can be dramatic (up to a thousand fold) and are predominantly the result of alterations in the pattern of protein synthesis by the liver. Although measurement of some acute phase reactants concentration is currently used as diagnosis and prognosis tools, relatively little is known about their function in vivo.

Among other features, APR is accompanied by alterations in lipoprotein composition and is associated with oxidation of low density lipoprotein (LDL) (3, 4). Oxidative modification of LDL has been extensively studied for more than twenty years because it plays a central role in the pathogenesis of atherosclerosis (5). Epidemiologic studies have implicated infectious agents, in the induction and development of atheromas and many of the pathological features of this disease relate to dysregulated chronic inflammation. Interestingly, *Chlamydia pneumoniae* and chlamydial hsp60, an inflammatory antigen localized to atheromas, were found to induce cellular oxidation of LDL (6, 7). Increased levels of oxidized lipids in the serum and circulating LDL have been found in animal models of bacterial infection and inflammation (8). The host response to infection produces LDL that not only contains more oxidized lipids but is also more susceptible to further oxidation ex vivo (8). Metabolic changes that contribute to LDL oxidation in vivo are far from being understood but some clarification has been brought by comparative studies of high density lipoprotein (HDL) and LDL composition and function during homeostasis and APR. During homeostasis, LDL is protected from oxidative modification by HDL-associated enzymes, particularly paraoxonase, which destroys biologically active oxidized phospholipids (9). During APR, some acute phase reactants including serum amyloïd A, ceruloplasmin and apolipoprotein J transitory integrate HDL which in turn looses its ability to control oxidation of LDL and production of oxidized phospholipids (10). Thus, homeostatic HDL

HAL author manuscript inserm-00136347, version 1

maintains LDL in a non inflammatory state while acute phase HDL favors the conversion of native LDL to proinflammatory oxidized LDL (oxLDL) and production of oxidized phospholipids. APR can be truly acute as in the case of a viral or bacterial infection but it may also become chronic and result in inflammatory diseases like atherosclerosis. By controlling the transient generation of proinflammatory oxLDL and bioactive oxidized phospholipids, APR may thus be part of a nonspecific innate immune response. We have recently shown that oxLDL promotes mature dendritic cell transition from differentiating monocytes (11), therefore supporting the hypothesis that some oxidized phospholipids generated during APR may signal the presence of a dangerous situation and favor the development of adaptative immunity. OxLDL have also been studied on various cell types on which it exerts several proinflammatory effects, which include production of monocyte chemoattractant protein-1 (12), M-CSF and GM-CSF by endothelial cells (13), increased monocyte adhesion to and transmigration through the endothelial cell layer and lymphocyte chemotaxis (14-16).

LDL is the main source of blood cholesterol in humans. It is a spherical particle of 20 nm with a hydrophobic core containing triglycerides and cholesteryl ester molecules and a surface monolayer of polar lipids (primarily phospholipids) and apolipoprotein B. Lysophosphatidylcholine (LPC) represents 1 to 5 % of the total phosphatidylcholine content of LDL and is generated by oxidation and fragmentation of the polyunsaturated sn-2 fatty acyl residues of phosphatidylcholine, followed by the hydrolysis of the shortened fatty acyl residues by LDL-associated enzymes (17). Oxidative modification of LDL is associated with dramatic increased formation of LPC which can reach 40 to 50 % of the total phosphatidylcholine content (18). LPC content is also increased in circulating LDL after LPS treatment (8). LPC activates a wide range of cell types and is implicated in many aspects of the inflammatory response. It stimulates growth factor expression by endothelial cells (19, 20) and induces inflammation and leukocyte accumulation after intracutaneous injection in humans (21). LPC upregulates adhesion molecules on endothelial cells and their production of monocyte chemoattractant protein-1 and stimulates proinflammatory cytokines synthesis (22, 23). LPC is also one of the mediators required in the cytotoxic response of human NK cells to tumor cells

Supprimé : promotes a variety of inflammatory effects

(24). In addition to its proinflammatory activity, LPC displays some contrasting immunoregulatory activities by binding to its high affinity receptor G2A on T cells (25). Interaction of LPC with G2A is thought to limit T cell responses by increasing the threshold of lymphocyte activation and proliferation. The effect of LPC on antigen presenting cells such as dendritic cells (DC) has not been investigated, leaving the question of its global function in the immune system open. In this paper, we asked whether the ability of oxLDL to favor monocyte-to-mature DC transition was mediated by LPC and investigated the possibility of control of this activity by native LDL and lipid nanoemulsion.

Supprimé : including monocytes-macrophage activation and chemotaxis, production of various growth and differentiation factors as well as endothelial cell adhesion molecules.

Supprimé : LPC

HAL author manuscript inserm-00136347, version 1

HAL author manuscript inserm-00136347, version 1

Materials and methods

LDL preparation

LDL (1.025≤ d ≤1.055 g/ml) was isolated from human plasma of normolipidemic healthy individuals by ultracentrifugation. The density of the plasma was raised to 1.025 g/ml using NaBr. After ultracentrifugation at 100 000 rpm, 4°C for 4 h using a TL 100.4 rotor, the light fraction containing chylomycron, VLDL (very low density lipoprotein) and IDL (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.

LDL oxidation

LDL concentration was adjusted at 1 mg/ml of protein by dilution in PBS and dialyzed at 4°C against PBS to eliminate EDTA. Cu^{2+}-mediated oxidation was carried out at 37°C for 24 h by dialysis against 5 µM $CuSO_4$/PBS. The reaction was stopped by addition of 40 µM Butylated-Hydroxy-Toluene and extensive dialysis at 4°C against PBS containing 100 µM diethylendiamine pentaacetic acid (DTPA). The oxidation degree was assessed by malonyl-dialdehyde (MDA) production measured by the LPO-586 assay (Oxis, Portland, OR, USA), and hydrogen peroxide content was measured by PeroxOQuant™ quantitative peroxide assay (Pierce Chemicals, Rockford, IL, USA). Typically, native LDL preparations contained < 1.5 ± 0.3 nmol MDA/mg total protein and 230 ± 82 nmol peroxides/mg total protein. After 24 h oxidation, oxLDL contained 16.7 ± 6.6 nmol MDA/mg total protein and 896 ± 295 nmol peroxides/mg total protein. Endotoxins in LDL or oxLDL were less than 0.6 pg/ml in final concentration as assessed by E-toxate test (Sigma, St Quentin-Fallavier, France).

HAL author manuscript inserm-00136347, version 1

Extraction of lipid and aqueous phases from lipoproteins

400 µl of methanol was added to 100 µl of LDL or oxLDL (1 mg/ml). After mixing, 100 µl of chloroform was added and samples were vortexed. 300 µl of water was added for phase separation, samples were vortexed vigorously and centrifuged for 1 min at 9000 g. The upper phase (defined as the aqueous phase) was collected and dried in speed vacuum. The lower phase was mixed with 300 µl of methanol and centrifuged for 2 min at 9000 g to pellet the proteins. Supernatant (defined as the lipid phase) containing the lipophilic molecules extracted in chloroform was collected and dried in speed vacuum. Dried products of both phases were resuspended in 100 µl of 150 mM NaCl/1 mM EDTA/10 mM Tris pH 7.4.

Lipid emulsions

Nanoemulsions were prepared by the solvent displacement method as described (26). 1-palmitoyl-2-arachidonoyl-phosphatidylcholine and L-α-lysophosphatidylcholine (Sigma, St Quentin-Fallavier, France) were diluted in ethanol (100 mg in 2 ml of solvent). 350 mg in 375 µl of Medium Chain Triglycerides (Société Française des Oléagineux, St. Laurent-Blagny, France) constituting the oily core of the emulsion were added. This ethanolic solution was subsequently diluted to 15 ml with acetone. Solution was dropped to 30 ml of an aqueous solution containing 84 mg of Pluronic F68, a non ionic surfactant triblock copolymer (poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) BASF, Ludwigshafen, Germany). The resulting mixture turned milky as a result of nanoemulsion formation. After stirring for 1 hour, organic solvents were evaporated under reduced pressure at 40°C and the solution concentrated to 5 ml. Mean diameters of prepared emulsions and Intralipide® 20 % (Fresenius Kabi, Sevres, France) were determined by photon correlation spectroscopy using a Malvern Zetasizer 3000HS (Malvern Instruments, Worcs, UK). Composition and size of these particles are reported in Table I.

Protein and lipid assays

HAL author manuscript inserm-00136347, version 1

The protein content of LDL, Intralipide® 20 %, LPC or PC emulsions was estimated by Coomassie Protein Micro-Assay procedure (Pierce) and lipid composition was determined using Cholesterol RTU, Triglycerides enzymatic PAP 150 and Phospholipids enzymatic PAP 150 kits from bioMérieux (Marcy l'Etoile, France).

Differentiation and treatment of monocyte-derived dendritic cells

PBMC were isolated from human peripheral blood by standard density gradient centrifugation on Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden), then mononuclear cells were separated from PBL by centrifugation on a 50 % Percoll solution. Monocytes were purified by immunomagnetic depletion (Dynal, Oslo, Norway), using a cocktail of mAbs anti-CD19 (4G7 hybridoma, provided by Dr Ron Levy), anti-CD3 (OKT3, American Type Culture Collection, Rockville, MD, USA) and anti-CD56 (NKH1, Beckman Coulter, Fullerton, CA, USA). Recovered monocytes were more than 90 % pure as assessed by CD14 labeling. Monocytes were differentiated to immature DC during 6 days with 40 ng/ml human recombinant GM-CSF and 250 U/ml human recombinant IL-4 in RPMI 1640 (Life Technologies, Rockville, MD, USA) supplemented with 2 mM glutamin (Life Technologies), 10 mM hepes (Life Technologies), 40 ng/ml gentamycin (Life Technologies) and 10 % lipoprotein-deficient fetal calf serum (LPDS, Sigma).

Differentiating monocytes were treated at day 5 (d5) by addition of lipoproteins, phospholipids or lipid emulsions to the medium at the last day of differentiation. 2 x 10^6 cells were treated with 10 µg/ml oxLDL or LDL (total lipoprotein), with 20 µl of lipid or aqueous phases extracted from oxLDL or LDL, or with 40 µM LPC or PC either dissolved in ethanol or emulsified with triglycerides (LPC or PC emulsion). To block Gαi proteins, d5 differentiating monocytes were pretreated for 3 hours with 100 ng/ml Pertussis Toxin (Biomol, Plymouth Meeting, PA, USA), then the medium was changed and cells were further incubated with 40 µM LPC for 24 hours as previously indicated. When mentioned, a PAF receptor antagonist (BN52021; Biomol) was added 15 min prior to LPC. Competition experiments were performed by concomitant adjunction of oxLDL (10 µg/ml) or LPC (40 µM) with native LDL (50 µg/ml of proteins

HAL author manuscript inserm-00136347, version 1

corresponding to 50 µg/ml of phospholipids) or Intralipide® 20 % (50 µg/ml of phospholipids). At the end of the differentiation (day 6), cells were harvested and analyzed. Cell viability was superior to 90 %. Endotoxin contamination of LPC was excluded by lack of polymixin B (Sigma) inhibition.

Phenotype

Phenotype was analyzed by flow cytometry on a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ, USA) using FITC-conjugated anti-CD14, -HLA-DR, -CD80 and PE-conjugated anti-CD1a, -CD83, -CD86, all from Beckman Coulter.

Endocytosis

Differentiated cells, treated or not with 40 µM LPC as described above, were harvested at day 6 (d6) and resuspended in 10 % FCS medium and incubated at 37°C for 30 min with 1 mg/ml Lucifer Yellow (LY) (Sigma) or 1 mg/ml FITC-T70-Dextran (Sigma) or for 3 h with carboxylate-modified yellow-green FluoSpheres of 0.45 µm (Beads) (Molecular Probes, Leiden, The Netherlands). Internalization was stopped on ice with cold PBS containing 0.1 % BSA and 0.05 % NaN_3. Cells were washed three times at 4°C in this buffer and their content in fluorescent probe was analyzed on a FACSCalibur (Becton Dickinson).

HAL author manuscript inserm-00136347, version 1

Mixed Lymphocyte Reaction

Naïve T lymphocytes were isolated from human peripheral blood. PBMC were isolated by density gradient centrifugation on Ficoll-Hypaque. After depletion of monocytes on Percoll gradient, PBL were recovered in the dense fraction. T lymphocytes were purified by immunomagnetic depletion using a cocktail of mAbs anti-CD19 (4G7), anti-CD16 (3G8), anti-CD56 (NKH1), anti-glycophorin A (11E4B7.6) and anti-CD14 (RMO52) all from Beckman Coulter. T lymphocytes were more than 95 % pure as assessed by CD3 labeling. Primary MLRs were conducted in 96-well flat-bottomed culture plates. DC were treated or not at d5 with 40 µM LPC or 10 µg/ml oxLDL in presence or not of competitors as described above, collected at d6, extensively washed and resuspended in RPMI 1640 supplemented with 2 mM glutamine, 10 mM hepes, 40 ng/ml gentamycin and 10 % FCS (BioMedia, Boussens, France). These cells were then co-cultured with 2 x 10^5 allogeneic T cells in 200 µl complete culture medium at 1/5, 1/10 or 1/20 DC/T cells ratio. After 4 days, 150 µl of culture supernatant were analyzed for the presence of IL-2 and IFNγ using cytokine-specific ELISA kits purchased from Endogen (Woburn, MA, USA).

Receptor analysis by RT-PCR

Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Courtaboeuf, France). 1 µg of total RNA was reverse transcribed using Thermoscript™ RT-PCR system (Life Technologies). cDNA was then used for PCR amplification of G2A receptor, platelet-activating factor receptor (PAF-R), GPR4, lectin-like oxLDL receptor (LOX-1) and ribosomal protein S12. The following primers were used to amplify G2A (5'-CGGTGGTTGTCATCTTCCTA-3' and 5'-TTAGCGGCCGCTCAGCAGGACTCCTCAA-TCAG-3') (27), GPR4 (5'-TAATGCTAGCGGCAACCACACGTGGGAG-3' and 5'-TCCAGTTGTCGTGGTGCA-3') (28), PAF-R (5'-CTCGGGGTCATTGCTAATG-3' and 5'-TTCAGTGACCGTATCCGTG-3') (29), LOX-1 (5'-GGCTTGCTGCGACTCTAGG-3' and 5'-AGTGGGGCATCAAAGGAG-3') (30) and S12 (5'-GGAGGTGTAATGGACGTTA-3' and 5'-CTGAGACTCCTTGCCATAG-3') (29). The amplified products

were analyzed by gel electrophoresis (414 bp for G2A, 258 bp for GPR4, 698 bp for PAF-R, 569 bp for LOX-1, 311 bp for S12).

Phosphorylation of extracellular signal-regulated kinase (ERK)

10^7 d5 differentiating monocytes were starved for 1 hour in RPMI 1640 medium without serum containing 0.3 % BSA (Sigma) before treatment with 40 µM LPC for 2, 5, 10, or 15 min. Cells were washed with cold PBS and the pellet was lyzed in 8 M urea / 2 % SDS / 0.2 M Tris-HCL pH 8.0 / 100 mM dithiothreitol and boiled for 5 minutes. After centrifugation, the supernatant was analyzed on a 10 % SDS-PAGE and proteins were transferred on Immobilon-P (Millipore Corporation, Bedford, MA, USA). Blots were saturated with 5 % fat free milk / TBS / 0.1 % Tween 20 for 1 h. Incubations with primary and secondary antibodies were carried out in TBS / 0.1 % Tween 20 / 5 % BSA. Anti-phospho-ERK and total-ERK were from Cell Signaling Technologies (Berverly, MA, USA). Detection was performed using HRP-conjugated anti-mouse antibody (Amersham Biosciences, Uppsala, Sweden) with the enhanced chemiluminescence kit ECL (Amersham Biosciences).

HAL author manuscript inserm-00136347, version 1

Results

CD86 expression induced by lipophilic molecules of oxLDL

We have previously shown that the presence of oxidized LDL during monocyte differentiation to DC yielded phenotypically and functionally mature DC (11). Optimal reactivity of monocytes was obtained when oxLDL was added during late stages of differentiation in GM-CSF and IL-4. Direct generation of mature DC from differentiating monocytes was best achieved when oxLDL was added at d5 of differentiation for 24 h. We also showed that FCS containing native lipoproteins and native LDL added to lipoprotein-deficient serum (LPDS) were efficient inhibitors of oxLDL activity, indicating that oxLDL are active in inflammatory conditions when the level of native LDL is reduced. Here we used the same experimental procedure to screen for bioactive molecules on differentiating monocytes cultured in LPDS to avoid inhibition by native LDL. As strong upregulation of CD86 is a constant feature induced by oxLDL, this marker was used as a first screening read-out to determine which component of oxLDL was responsible for dendritic cell activation. Differentiating monocytes were treated for 24 h with aqueous or lipid phases from native LDL or oxLDL. The oxidation process generates various components including oxysterols and modified phospholipids which are concentrated in the lipid phase, whereas the aqueous phase may contain polar oxidized free fatty acids. Neither total native LDL nor phases from native LDL could induce CD86 expression (Fig. 1A-C). In contrast, lipid phase from oxLDL strongly upregulated CD86 expression, as did total oxLDL (Fig. 1A-B). The aqueous phase from oxLDL had no effect on CD86 expression (Fig. 1C). Phosphatidylcholine (PC) is the major phospholipid component of native LDL and its derivative LPC can constitute as much as 50 % of the total phospholipids of oxLDL. Therefore, the effect of both PC and LPC was tested on d5 differentiating monocytes. PC and LPC were also emulsified with triglycerides to form particles of 300 to 400 nm constituted of triglycerides covered with a monolayer of PC or LPC. Composition of these emulsions is shown in Table I. Both LPC and LPC emulsion induced strong upregulation of CD86 expression whereas PC and PC emulsion did not (Fig.1D-E). CD86 upregulation by LPC was dose-dependent with optimal induction for 40µM LPC under these experimental conditions (data not shown).

HAL author manuscript inserm-00136347, version 1

HAL author manuscript inserm-00136347, version 1

Thus, LPC appeared to be one of the bioactive molecules of oxLDL and its activity could be vehicled by LDL-like structures.

LPC promotes generation of mature DC

As expected, monocytes differentiated to immature DC in GM-CSF and IL-4 in the absence of LPC (control) expressed high level of CD1a but no CD14, intermediate level of HLA-DR but no CD80, CD83 or CD86 (Fig. 2A). Cells treated with LPC upregulated HLA-DR, CD83 and CD86 but CD80 was only weakly induced. CD14 was not expressed but CD1a remained high and CD40 was upregulated as it did after oxLDL treatment (11, data not shown). Thus, LPC treatment of differentiating monocytes induced phenotypical characteristics of mature DC. Cell mortality measured by incorporation of propidium iodide was not increased by LPC treatment (data not shown).

Reduced ability to capture exogenous antigens is an early event of DC maturation (31, 32). Therefore, the internalization capacities of LPC treated cells were analyzed by flow cytometry using specific fluorescent probes. Lucifer Yellow was used to measure non-specific fluid phase pinocytosis, FITC-dextran for mannose receptor-mediated endocytosis and fluorescent beads for macropinocytosis. As shown in figure 2B, LPC treated cells show a significant decrease in their endocytosis activities compared to untreated cells corresponding to immature DC (from 30 % reduction for macropinocytosis to 50 % for pinocytosis and receptor-mediated endocytosis).

The capacity to stimulate allogeneic T cells is another characteristic of mature DC. Therefore, the functional properties of LPC treated cells were further investigated in mixed leukocyte reaction (MLR). In contrast to control untreated cells (immature DC), LPC treated cells could activate allogeneic naïve T cells inducing the release of IL-2 (Fig. 2C). Thus, addition of LPC during the late stage of monocyte differentiation gave rise directly to mature DC supporting allogeneic T cell stimulation.

LPC action through G protein-coupled receptors

HAL author manuscript inserm-00136347, version 1

It has been recently shown that LPC is a high affinity ligand for G2A receptor expressed on lymphocytes (25). LPC can also bind with low affinity to GPR4 receptor which has a wider expression (28). Partial sensitivity to PAF receptor antagonists indicated that some LPC effects could be mediated by the PAF receptor in various cell types (33, 34). Finally, LOX-1, the lectin-like oxLDL receptor which belongs to the scavenger receptor family can mediate LPC-induced oxLDL uptake in smooth muscle cells (35). Expression of these LPC receptors was tested by RT-PCR. Total mRNA was extracted from freshly isolated human monocytes, from differentiating monocytes at d5 and from immature DC at d6 of culture in GM-CSF and IL-4. Total mRNA was also extracted when differentiating monocytes were treated at d5 with 40 μM LPC for 24 h. RT-PCR amplification yielded a DNA fragment of the expected size for G2A, PAF-R and GPR4 in undifferentiated monocytes as well as in differentiating monocytes at d5 and in immature DC at d6 (Fig. 3A). mRNA levels of G2A, PAF-R and GPR4 remained constant throughout the differentiation and was unchanged by LPC treatment. In contrast, LOX-1 mRNA was undetectable in undifferentiated monocytes but was present at d5 of differentiation and in immature DC. Although quantitative RT-PCR was not performed, LOX-1 mRNA seemed to be further induced by LPC treatment (Fig. 3A).

G2A, GPR4 and PAF-R belong to the G protein-coupled receptor family. Gαi proteins are sensitive to pertussis toxin (PTX) which blocks Gαi-coupled signaling. Figure 3B shows that upregulation of CD86 by LPC was totally inhibited when cells were preincubated with 100 ng/ml PTX for 3h. Others phenotypical modifications induced by LPC treatment and shown in Fig. 2A were also inhibited by PTX (data not shown). These data indicated that all LPC activity was mediated through G protein-coupled receptors therefore excluding scavenger receptors like LOX-1.

Figure 3C shows that upregulation of CD86 by LPC was reduced by 40 % when cells were preincubated for 15 min with the PAF receptor antagonist BN52021 prior to stimulation with LPC. No specific antagonist for G2A or GPR4 are currently available but it has been shown that phosphorylation of the extracellular signal-related kinases (ERK) upon LPC treatment was dependent on G2A or GPR4 expression (25). As shown in figure 3D, western blot analysis of differentiating monocytes exposed to LPC at d5 revealed a strong and

HAL author manuscript inserm-00136347, version 1

transient increase in phospho-ERK occuring within 2 min after LPC adjunction and decreasing rapidly to return to its basal level after 15 min.

Th1 oriented response initiated by oxLDL and LPC and its regulation by lipids

The ability of maturing DC to initiate Th1-type versus Th2-type responses depends on the microenvironmental conditions of their development. The question to what extent the inflammatory mediators oxLDL and LPC polarize Th cell development was addressed by analyzing the cytokines secreted by allogeneic naïve T cells in MLR. Figure 4 shows that both oxLDL and LPC treated cells stimulated the production of IFNγ by T cells whereas no IL-4 could be detected (data not shown). This indicated that oxLDL and LPC may instruct DC to initiate Th1-biased responses.

LPC can reach high concentration (100 µM) in the serum of healthy individuals (36). Because LPC can also be present in albumin and lipoprotein-bound forms (37), the functionally available concentration of LPC is difficult to investigate. It is likely, however, that endogenous inhibitors present in the serum protect circulating monocytes from the proinflammatory activities of LPC. We have previously shown that native LDL can antagonize DC maturation induced by oxLDL (11). We thus compared the phenotype and allostimulatory ability of DC obtained after oxLDL or LPC treatment in the presence or absence of purified native LDL (50 µg/ml). Composition of purified LDL is shown in Table III. In the presence of native LDL, both oxLDL and LPC-induced CD86 upregulation were strongly inhibited (73 % and 87 % respectively) (Table II). Inhibition of DC maturation correlated with the inhibition of DC ability to stimulate IFNγ secretion by allogeneic T cells. Therefore, as for oxLDL, native LDL is an endogenous inhibitor of LPC.

In order to understand how native LDL can block LPC and oxLDL, native LDL were replaced by Intralipid. Intralipid is a lipid emulsion currently used for parenteral nutrition in humans. It is composed of large particles of triglycerides and phospholipids which contain no protein (Table III). This emulsion strongly antagonized the phenotypic and functional maturation of DC induced by LPC or oxLDL, strongly inhibiting

CD86 upregulation and IFNγ secretion by allogeneic T cells (Table II). This suggests that lipid composition of native LDL may determine its inhibitory potential.

HAL author manuscript inserm-00136347, version 1

HAL author manuscript inserm-00136347, version 1

Discussion

Growing lines of evidence suggest that oxidation of lipoproteins is a critical event of inflammation and host response to infection (6, 8, 38, 39). We have shown recently that oxLDL promotes mature dendritic cell transition from differentiating monocytes (11) and postulated that modification of lipoprotein composition may be a hitherto unrecognized control mechanism linking innate to adaptative immunity. To characterize the active components within the oxidized LDL, the particles were separated into aqueous and lipid phase. The strong effect elicited by the lipid phase led us to test the effect of LPC, one of the major component of oxLDL. The data demonstrate that LPC-treated differentiating monocytes display phenotypical and functional properties of mature DC. Their internalization capacities are reduced whereas they can efficiently stimulate allogeneic T cells and IFNγ production. Thus, LPC is one of the oxLDL components that can promote mature DC generation. Interestingly, LPC treated cells did not appear to secrete high level of IL12 after a 24 hour treatment and relative contribution of cytokines to T cell stimulation remains to be studied in a kinetic analysis. IFNγ production during T-DC interaction indicated that LPC is a Th1-promoting innate stimulus.

Although LPC is an important lipid mediator of inflammation, its initial mechanism of action is still poorly understood. GPR4 and G2A, respectively low and high affinity receptors for LPC, have recently been described (25, 28). In addition to these two receptors, LPC action through the PAF receptor and LOX-1 has also been suggested (33-35, 40). LOX-1 is a scavenger receptor with a role in defense against $Gram^+$ and $Gram^-$ bacteria (41). This receptor however could be excluded because experiments using pertussis toxin as an inhibitor of Gαi showed that LPC action was mediated through a G protein-coupled receptor. The PAF receptor is strongly implicated in inflammatory reaction and is directly stimulated by lipoteichoic acid, a lipopolysaccharide of the Gram-positive cell wall (42). The PAF receptor antagonist BN52021 partially inhibited LPC action indicating that this receptor is involved in LPC action. Our preliminary data suggest that this pathway may not be initiator of LPC effect but may rather amplify LPC action through other receptors (Coutant. F, unpublished data). There is no specific inhibitor or blocking antibody for GPR4 or

G2A. However, it has been shown that ERK MAP kinase activation by LPC was dependent of GPR4 or G2A expression (25). Here we confirmed that LPC treatment of differentiating monocytes resulted in ERK activation therefore strengthening the involvement of GPR4 or G2A in LPC-induced DC maturation. Further studies are needed to address the possible functional redundancy or complementarity among these three receptors. It is tempting to speculate on the role of lysophospholipid receptors and LPC in the control of innate and adaptive immunity. LPC appears to favor the antigen-specific response by promoting mature DC generation. LPC also appears to increase the threshold for proliferation and activation of T cells (25). Therefore, it is reasonable to assume that the highly controlled release of LPC could be one mechanism to improve efficiency and safety of immunity by increasing antigen presentation to naïve T cells while limiting the extend of the response and avoiding non-specific activation of lymphocytes.

The high concentration of serum LPC (up to 100 µM) during homeostasis suggests that the proinflammatory activities of LPC are blocked by circulating activators. Albumin appears to be one of these inhibitors as it binds to LPC and decreases some of its effects in vitro. Our results shown in Table II indicate that native LDL is another inhibitor of LPC explaining why LPC is more efficient in LPDS than in FCS. Inhibition of LPC by native LDL can be mimicked by Intralipid, a stable emulsion of triglycerides and phospholipids. Although the inhibition of LPC by Intralipid remains to be further addressed, this support the notion that the ratio of phospholipids to lysophospholipids in the direct environment of DC may influence its maturation. Intralipid is used as a source of energy to avoid wasting during septic shock. Our data suggest that it may also help to limit inflammation.

Sensing the danger is a crucial function of antigen-presenting cells, especially for dendritic cells which become activated and initiate primary immune responses (43, 44). DC can be activated by exogenous pathogen-derived molecules or endogenous signals released by cells that are stressed, infected or necrotic (45, 46). Our data suggest that oxidation of lipoproteins and production of LPC may be one inducible endogenous signal of danger that is released during the APR. The concentration of albumin drops during the APR whereas oxidation of native LDL is actively promoted. As a consequence, the concentration of LPC is

HAL author manuscript inserm-00136347, version 1

HAL author manuscript   inserm-00136347, version 1

increased while its two inhibitors are rendered less effective. Thus, the functionally available concentration of LPC is tightly controlled by the acute phase reactants in response to tissue injury. How DC and T cells integrate LPC signal through a family of G protein-coupled receptors to promote the initiation of an immune response while limiting the extent of the response may thus be a major issue for the understanding and control of inflammatory and autoimmune diseases.

Besides the basic research observation that LPC promotes development of mature DC, the data presented in this study may have practical implications in the field of cell therapy. Moreover, identification of blocking molecules in Intralipid and native LDL may prove valuable for the design of lipid nanoemulsions with anti-inflammatory properties.

HAL author manuscript inserm-00136347, version 1

Aknowledgments

We thank Anne-Kim Isnard for technical assistance.

HAL author manuscript inserm-00136347, version 1

References

1. Baumann, H., and J. Gauldie. 1994. The acute phase response. *Immunol Today 15:74*.
2. Gabay, C., and I. Kushner. 1999. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med 340:448*.
3. Cabana, V. G., J. N. Siegel, and S. M. Sabesin. 1989. Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. *J Lipid Res 30:39*.
4. Steinberg, D. 1997. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem 272:20963*.
5. Witztum, J. L., and D. Steinberg. 1991. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest 88:1785*.
6. Kalayoglu, M. V., B. Hoerneman, D. LaVerda, S. G. Morrison, R. P. Morrison, and G. I. Byrne. 1999. Cellular oxidation of low-density lipoprotein by Chlamydia pneumoniae. *J Infect Dis 180:780*.
7. Kalayoglu, M. V., Indrawati, R. P. Morrison, S. G. Morrison, Y. Yuan, and G. I. Byrne. 2000. Chlamydial virulence determinants in atherogenesis: the role of chlamydial lipopolysaccharide and heat shock protein 60 in macrophage- lipoprotein interactions. *J Infect Dis 181 Suppl 3:S483*.
8. Memon, R. A., I. Staprans, M. Noor, W. M. Holleran, Y. Uchida, A. H. Moser, K. R. Feingold, and C. Grunfeld. 2000. Infection and inflammation induce LDL oxidation in vivo. *Arterioscler Thromb Vasc Biol 20:1536*.
9. Mackness, M. I., S. Arrol, and P. N. Durrington. 1991. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Lett 286:152*.
10. Van Lenten, B. J., S. Y. Hama, F. C. de Beer, D. M. Stafforini, T. M. McIntyre, S. M. Prescott, B. N. La Du, A. M. Fogelman, and M. Navab. 1995. Anti-inflammatory HDL becomes pro-inflammatory

HAL author manuscript inserm-00136347, version 1

during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. *J Clin Invest 96:2758*.

11. Perrin-Cocon, L., F. Coutant, S. Agaugue, S. Deforges, P. Andre, and V. Lotteau. 2001. Oxidized low-density lipoprotein promotes mature dendritic cell transition from differentiating monocyte. *J Immunol 167:3785*.

12. Navab, M., S. S. Imes, S. Y. Hama, G. P. Hough, L. A. Ross, R. W. Bork, A. J. Valente, J. A. Berliner, D. C. Drinkwater, H. Laks, and et al. 1991. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J Clin Invest 88:2039*.

13. Rajavashisth, T. B., A. Andalibi, M. C. Territo, J. A. Berliner, M. Navab, A. M. Fogelman, and A. J. Lusis. 1990. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature 344:254*.

14. Berliner, J. A., M. C. Territo, A. Sevanian, S. Ramin, J. A. Kim, B. Bamshad, M. Esterson, and A. M. Fogelman. 1990. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. *J Clin Invest 85:1260*.

15. Frostegard, J., J. Nilsson, A. Haegerstrand, A. Hamsten, H. Wigzell, and M. Gidlund. 1990. Oxidized low density lipoprotein induces differentiation and adhesion of human monocytes and the monocytic cell line U937. *Proc Natl Acad Sci U S A 87:904*.

16. Frostegard, J., R. Wu, A. Haegerstrand, M. Patarroyo, A. K. Lefvert, and J. Nilsson. 1993. Mononuclear leukocytes exposed to oxidized low density lipoprotein secrete a factor that stimulates endothelial cells to express adhesion molecules. *Atherosclerosis 103:213*.

17. Steinbrecher, U. P., and P. H. Pritchard. 1989. Hydrolysis of phosphatidylcholine during LDL oxidation is mediated by platelet-activating factor acetylhydrolase. *J Lipid Res 30:305*.

HAL author manuscript inserm-00136347, version 1

18. Parthasarathy, S., U. P. Steinbrecher, J. Barnett, J. L. Witztum, and D. Steinberg. 1985. Essential role of phospholipase A2 activity in endothelial cell-induced modification of low density lipoprotein. *Proc Natl Acad Sci U S A 82:3000*.

19. Kume, N., and M. A. Gimbrone, Jr. 1994. Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. *J Clin Invest 93:907*.

20. Nakano, T., E. W. Raines, J. A. Abraham, M. Klagsbrun, and R. Ross. 1994. Lysophosphatidylcholine upregulates the level of heparin-binding epidermal growth factor-like growth factor mRNA in human monocytes. *Proc Natl Acad Sci U S A 91:1069*.

21. Ryborg, A. K., B. Deleuran, H. Sogaard, and K. Kragballe. 2000. Intracutaneous injection of lysophosphatidylcholine induces skin inflammation and accumulation of leukocytes. *Acta Derm Venereol 80:242*.

22. Kume, N., M. I. Cybulsky, and M. A. Gimbrone, Jr. 1992. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J Clin Invest 90:1138*.

23. Takahara, N., A. Kashiwagi, H. Maegawa, and Y. Shigeta. 1996. Lysophosphatidylcholine stimulates the expression and production of MCP- 1 by human vascular endothelial cells. *Metabolism 45:559*.

24. Whalen, M. M., R. N. Doshi, B. W. Bader, and A. D. Bankhurst. 1999. Lysophosphatidylcholine and arachidonic acid are required in the cytotoxic response of human natural killer cells to tumor target cells. *Cell Physiol Biochem 9:297*.

25. Kabarowski, J. H., K. Zhu, L. Q. Le, O. N. Witte, and Y. Xu. 2001. Lysophosphatidylcholine as a ligand for the immunoregulatory receptor G2A. *Science 293:702*.

26. Fessi, H., F. Puisieux, J. P. Devissaguet, N. Ammoury, and S. Benita. 1989. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *International Journal of Pharmaceutics 55:R1*.

HAL author manuscript inserm-00136347, version 1

27. Weng, Z., A. C. Fluckiger, S. Nisitani, M. I. Wahl, L. Q. Le, C. A. Hunter, A. A. Fernal, M. M. Le Beau, and O. N. Witte. 1998. A DNA damage and stress inducible G protein-coupled receptor blocks cells in G2/M. *Proc Natl Acad Sci U S A 95:12334*.

28. Zhu, K., L. M. Baudhuin, G. Hong, F. S. Williams, K. L. Cristina, J. H. Kabarowski, O. N. Witte, and Y. Xu. 2001. Sphingosylphosphorylcholine and lysophosphatidylcholine are ligands for the G protein-coupled receptor GPR4. *J Biol Chem 276:41325*.

29. Dichmann, S., H. Rheinen, E. Panther, Y. Herouy, W. Czech, C. Termeer, J. C. Simon, P. J. Gebicke-Haerter, and J. Norgauer. 2000. Downregulation of platelet-activating factor responsiveness during maturation of human dendritic cells. *J Cell Physiol 185:394*.

30. Yoshida, H., N. Kondratenko, S. Green, D. Steinberg, and O. Quehenberger. 1998. Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. *Biochem J 334:9*.

31. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med 182:389*.

32. Steinman, R. M., and J. Swanson. 1995. The endocytic activity of dendritic cells. *J Exp Med 182:283*.

33. Ogita, T., Y. Tanaka, T. Nakaoka, R. Matsuoka, Y. Kira, M. Nakamura, T. Shimizu, and T. Fujita. 1997. Lysophosphatidylcholine transduces Ca2+ signaling via the platelet- activating factor receptor in macrophages. *Am J Physiol 272:H17*.

34. Huang, Y. H., L. Schafer-Elinder, R. Wu, H. E. Claesson, and J. Frostegard. 1999. Lysophosphatidylcholine (LPC) induces proinflammatory cytokines by a platelet-activating factor (PAF) receptor-dependent mechanism. *Clin Exp Immunol 116:326*.

35. Aoyama, T., M. Chen, H. Fujiwara, T. Masaki, and T. Sawamura. 2000. LOX-1 mediates lysophosphatidylcholine-induced oxidized LDL uptake in smooth muscle cells. *FEBS Lett 467:217*.

HAL author manuscript inserm-00136347, version 1

36. Subbaiah, P. V., C. H. Chen, J. D. Bagdade, and J. J. Albers. 1985. Substrate specificity of plasma lysolecithin acyltransferase and the molecular species of lecithin formed by the reaction. *J Biol Chem 260:5308*.

37. Croset, M., N. Brossard, A. Polette, and M. Lagarde. 2000. Characterization of plasma unsaturated lysophosphatidylcholines in human and rat. *Biochem J 345 Pt 1:61*.

38. Khovidhunkit, W., R. A. Memon, K. R. Feingold, and C. Grunfeld. 2000. Infection and inflammation-induced proatherogenic changes of lipoproteins. *J Infect Dis 181 Suppl 3:S462*.

39. Hajjar, D. P. 2000. Oxidized lipoproteins and infectious agents: are they in collusion to accelerate atherogenesis? *Arterioscler Thromb Vasc Biol 20:1421*.

40. Aoyama, T., H. Fujiwara, T. Masaki, and T. Sawamura. 1999. Induction of lectin-like oxidized LDL receptor by oxidized LDL and lysophosphatidylcholine in cultured endothelial cells. *J Mol Cell Cardiol 31:2101*.

41. Shimaoka, T., N. Kume, M. Minami, K. Hayashida, T. Sawamura, T. Kita, and S. Yonehara. 2001. LOX-1 supports adhesion of Gram-positive and Gram-negative bacteria. *J Immunol 166:5108*.

42. Lemjabbar, H., and C. Basbaum. 2002. Platelet-activating factor receptor and ADAM10 mediate responses to Staphylococcus aureus in epithelial cells. *Nat Med 8:41*.

43. Matzinger, P. 1994. Tolerance, danger, and the extended family. *Annu Rev Immunol 12:991*.

44. Matzinger, P. 1998. An innate sense of danger. *Semin Immunol 10:399*.

45. Janeway, C. A., Jr., and R. Medzhitov. 2002. Innate immune recognition. *Annu Rev Immunol 20:197*.

46. Gallucci, S., M. Lolkema, and P. Matzinger. 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat Med 5:1249*.

HAL author manuscript inserm-00136347, version 1

Footnotes

[1] This work was supported by ANRS (Agence Nationale de Recherche sur le Sida) (HC EP 05), INSERM (institut national de la santé et de la recherche médicale) and bioMérieux SA. F. Coutant is a recipient of an ANRS doctoral scholarship. S. Agaugé is a recipient of a Ligue Nationale Française contre le Cancer doctoral scholarship. L. Perrin-Cocon is a recipient of a bioMérieux SA postdoctoral fellowship.

[2] Correspondence address: Dr. Vincent Lotteau, CERVI-INSERM U503, 21 Avenue Tony Garnier, F-69007 Lyon, France. E-mail address : lotteau@cervi-lyon.inserm.fr

[3] Abbreviations used in this paper: APR, acute phase response; DC, dendritic cell; ERK, extracellular signal-related kinase; GM-CSF, granulocyte macrophage-stimulating factor; HDL, high density lipoprotein; LDL, low density lipoprotein; LPC, lysophosphatidylcholine; LPDS, lipoprotein deficient serum; LOX-1, lectin-like oxLDL receptor; oxLDL, oxidized low density lipoprotein; PAF-R, platelet-activating factor receptor; PC, phosphatidylcholine; PTX, pertussis toxin

HAL author manuscript inserm-00136347, version 1

Table I. *Size and composition of LPC and PC emulsions* [a].

	Proteins (µg/ml)	Phospholipids (µg/ml)	Triglycerides (µg/ml)	Cholesterol (µg/ml)	Diameter (nm)
LPC emulsion	0	25	72	0	323
PC emulsion	0	35	48	0	394

[a] The table displays final concentrations when these emulsions provide 40 µM of LPC or PC.

Table II. *Native LDL and Intralipid antagonize oxLDL and LPC* [a].

Inducer	Competitor	CD86 expression (% of inhibition)	IFNγ secretion (% of inhibition)
LPC	Native LDL	87 ± 14	90 ± 20
oxLDL	Native LDL	73 ± 15	75 ± 23
LPC	Intralipid	86 ± 12	76 ± 20
oxLDL	Intralipid	88 ± 10	85 ± 29

[a] At day 5, differentiating monocytes were stimulated with 10 µg/ml oxLDL or 40 µM LPC in presence or not of LDL or Intralipid (both used at 50 µg/ml of phospholipids). At day 6, cells were harvested and washed. The expression of CD86 was analyzed by flow cytometry. The secretion of IFNγ was measured by sandwich ELISA in the supernatant of MLR experiments where 4 x 10^4 DC/well were co-cultured with 2 x 10^5 naïve allogeneic T cells for 4 days. Mean ± S.D. of 3 independent experiments is shown.

Table III. *Size and composition of native LDL and Intralipid* [a].

HAL author manuscript inserm-00136347, version 1

	Proteins (µg/ml)	Phospholipids (µg/ml)	Triglycerides (µg/ml)	Cholesterol (µg/ml)	Diameter (nm)
Native LDL	50	51 ± 9	25 ± 12	79 ± 19	20
Intralipid	0	50	1440	0	335

[a] The table shows final concentrations when these particles are used as competitors at 50 µg/ml phospholipid for Intralipid or 50 µg/ml protein for native LDL. Mean values ± S.D. of lipid concentrations for LDL preparations from 8 different donors are shown.

HAL author manuscript inserm-00136347, version 1

Figure legends

Figure 1. Bioactive molecules contained in the lipid phase of oxLDL.

Monocytes were differentiated in LPDS medium containing GM-CSF and IL-4. Cells were treated at day 5 and expression of CD86 was monitored by flow cytometry at day 6. (A-C)- CD86 upregulation by lipid phase extracted from oxLDL. (A)- Cells were treated with 10 µg/ml total native lipoprotein (thin line) or 10 µg/ml total oxidized lipoprotein (filled profile). (B-C)- Cells were treated with lipid phase (B) or aqueous phase (C) isolated from native LDL (thin line) or from oxLDL (filled profile). (D-E)- CD86 upregulation by LPC. Cells were treated with 40 µM LPC (D) or 40 µM PC (E) solubilized in ethanol (thick line) or emulsified with triglycerides (filled profile). Thin line shows CD86 expression by untreated control cells corresponding to immature dendritic cells.

Figure 2. Mature DC generation induced by LPC. Monocytes were differentiated in LPDS medium supplemented with GM-CSF and IL-4, with or without LPC (40µM) added at day 5. Cells were harvested and analyzed at day 6. (A)- Phenotype of LPC-treated cells (filled profiles) or untreated control cells (thin line). (B)- Reduced endocytic activity of LPC-treated cells. Cells were incubated at 37°C with 1 mg/ml LY or FITC-Dextran for 30 min or with fluorescent beads for 3h. The amount of probe internalized was measured by flow cytometry. Mean fluorescent intensities of one representative experiment out of three were normalized at 100 % uptake in untreated control cells. (C)- Allogeneic T cell stimulation by LPC-treated cells. Differentiating monocytes were treated or not by LPC at day 5 for 24 h, washed and cultured with allogeneic T cells (2 x 10^5 /well) at ratios ranging between 1/5 and 1/20 (DC/T cells) for 4 days. The amount of IL-2 in the supernatants of the co-culture was measured by sandwich ELISA.

Figure 3. (A)- Expression of G2A, PAF-R, GPR4 and LOX-1 in monocytes and dendritic cells. Lane 1, undifferentiated monocytes; lane 2, differentiating monocytes at day 5; lanes 3, differentiating monocytes at

HAL author manuscript inserm-00136347, version 1

day 6 (corresponding to immature DC); lane 4, differentiating monocytes treated with 40 µM LPC at day 5 and harvested at day 6. (B)- Involvement of G protein-coupled receptors in CD86 upregulation by LPC. (PTX)- Differentiating monocytes at day 5 were treated for 3 hours with PTX (100 ng/ml) and analyzed at day 6. (LPC)- Differentiating monocytes at day 5 were treated with 40 µM LPC for 24 h. At day 6, mean fluorescence intensity of CD86 was normalized at 100 %. (LPC + PTX)- Differentiating monocytes at day 5 were preteated for 3 hours with PTX (100 ng/ml) before treatment with 40 µM LPC for 24 h. Means ± S.D. of three independent experiments are shown. (C)- Involvement of the PAF receptor in LPC-induced CD86 expression. (BN52021)- Differentiating monocytes at day 5 were treated with the PAF receptor antagonist BN52021 (100 µM) and analyzed at day 6. (LPC)- Differentiating monocytes at day 5 were treated with 40 µM LPC for 24 h. At day 6, mean fluorescence intensity of CD86 was normalized at 100 %. (LPC + BN52021)- Differentiating monocytes at day 5 were treated with 40 µM LPC for 24 h in presence of the PAF receptor antagonist BN52021 (100 µM). Means ± S.D. of three independent experiments are shown. (D)- Time course of ERK phosphorylation induced by LPC. Day 5 differentiating monocytes were treated with LPC (40 µM) for the indicated period of time. Phosphorylated ERK (phospho-ERK) and total-ERK were detected by western blot analysis of cell lysates.

Figure 4. OxLDL and LPC treated cells induced a Th1-biased response. Differentiating cells were treated or not (control) at day 5 with 10 µg/ml oxLDL or 40 µM LPC. Cells were harvested at day 6, washed and cultured in triplicate with allogeneic purified T cells (2 x 10^5 /well) at ratios ranging between 1/5 and 1/20 (DC/T cells) for 4 days. The amount of IFNγ in the supernatants of co-culture was measured by sandwich ELISA. Results of one representative experiment out of three.