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Vincent Lotteau

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Frédéric Coutant[*], Sophie Agaugué[*], Laure Perrin-Cocon[*], 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

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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

Abstract

Because of its oxidative modification during the acute phase response to an aggression, low density lipoprotein (LDL) can be regarded as a source of lipid mediators that can act both to promote and inhibit inflammation. This can be exemplified by the production of anti-inflammatory oxidized fatty acids and pro-inflammatory lysophosphatidylcholine (LPC) during LDL oxidation. We have shown previously that oxLDL plays an active role at the interface between innate and adaptive immunity by delivering instructive molecules like LPC which promotes mature dendritic cell (DC) generation from differentiating monocytes. It is shown here that LPC affects the signaling pathway of peroxisome proliferator-activated receptors (PPARs). LPC-induced DC maturation is associated with complete inhibition of PPARγ activity and up-regulation of the activity of an uncharacterized nuclear receptor that bind peroxisome proliferator response element. Oxidized fatty acids generated during LDL oxidation are natural ligands for PPARγ and inhibit oxLDL and LPC-induced maturation. Inhibition experiments with synthetic PPARγ ligands suggested a PPARγ-dependent and independent effect of LPC on DC maturation. Therefore, the relative amount of oxidized fatty acids and LPC influences the immunological functions of oxLDL on DC, in part by regulating the PPAR pathway. By sensing the biochemical composition of lipoprotein particles, the innate immune system may thus identify various endogenous signals that influence the immune response during the acute phase reaction. The therapeutic emulsion Intralipid also blocks LPC action on PPAR activity and DC maturation. Intralipid may thus be an alternative therapeutic strategy for some chronic inflammatory diseases.

Introduction

The acute phase response (APR)[3] to tissue injury is a series of reaction invoked by the liver to prevent tissue damage, favor pathogen clearance and activate the repair processes that are necessary to restore normal functions (1). APR is a rapid and transient deviation from homeostasis and is part of the systemic inflammatory arsenal of innate immunity. One of the consequences of APR is an oxidative modification of low density lipoprotein (LDL), the main transporter of cholesterol in humans (2, 3). During homeostasis, LDL is protected from oxidative modification by lipoprotein-associated enzymes which prevent the accumulation of biologically active modified lipids (4). During APR, acute phase reactants transiently integrate lipoprotein and modify LDL-associated enzymes activity which in turn lose their ability to control LDL oxidation (5). In recent studies, we showed that oxidized LDL (oxLDL) promotes mature dendritic cell (DC) generation from monocytes, therefore linking the nonspecific acute phase response to adaptive immunity (6). Mature DC generation induced by oxLDL was inhibited by native LDL and lipid emulsions. Based on these observations, it was hypothesized that the innate immune system could modulate the commitment of adaptive immunity to the host defense strategy by sensing the balance between native and oxidized LDL and the production of oxidized phospholipids.

As our understanding of LDL oxidation has evolved, it has become clear that oxLDL can act both to favor and inhibit inflammation depending on its own biochemical composition and cell type studied. Oxidation of LDL is a complex reaction generating various lipid mediators and the functional dualism of oxLDL is likely to reflect the relative level of these mediators in the particle. Among these lipid mediators, lysophosphatidylcholine (LPC) and oxidized fatty acids are of special interest because they display opposite effects on inflammation. High concentration of LPC appear to confer proinflammatory properties to oxLDL and a number of

reports have shown that LPC is an inducer of inflammation both *in vitro* and *in vivo* (7-9). LPC represents 1-5 % of the total phosphatidylcholine content of native LDL and its concentration is raised to 40-50 % upon LDL oxidation (10). LPC content is also increased in circulating LDL after LPS treatment (11). LPC is generated by oxidation and fragmentation of the polyunsaturated sn-2 fatty acyl residues of phosphatidylcholine followed by hydrolysis of the shortened fatty acyl residues (12). In previous reports, we have shown that the ability of oxLDL to promote mature DC generation was mediated by LPC through a G protein-coupled receptor. This function was modulated by native LDL and lipid nanoemulsion including the therapeutic emulsion Intralipid (13).

Other classes of oxidized lipids identified in oxLDL display anti-inflammatory properties. These lipids are peroxidation products of linoleic acid and arachidonic acid, the most abundant fatty acids in human LDL. LDL oxidation transforms linoleic acid and arachidonic acid to hydroperoxy derivatives which are converted to hydroxyoctadecadienoic acid (HODE) and hydroxyeicosatetraenoic acid (HETE) respectively (14). HODE and HETE are also products of lipoxygenases that can oxygenate free polyunsaturated fatty acids and phospholipids present in native LDL and in biomembranes (15). Under specific conditions, 9-HODE and 13-HODE can account for more than 60 % of all lipid peroxidation products found in oxLDL (14). 9-, 13-HODE and 11-, 15-HETE are activating ligands for the nuclear peroxisome proliferator-activated receptor γ (PPARγ) (16, 17). Ligands of PPARγ can interfere with monocyte and macrophage functions by inhibiting the production of inflammatory cytokines (18, 19). Moreover some ligands can have anti-inflammatory effects that are independent of PPARγ (20).

PPARs are important therapeutic targets in metabolic disorders (21, 22). The three PPARs isoforms (α, γ and δ) bind to the peroxisome proliferator response element (PPRE) as a heterodimer with the 9-cis retinoic receptor (RXR) and exhibit various functions relevant to

lipid and glucose metabolism (23). During the past few years, it has become apparent that PPARγ, in addition to its role in adipocyte differentiation and lipid metabolism, may also play a role in the regulation of immune responses (24). Recently, it has been reported that PPARγ activators affect negatively the maturation of DC in different experimental models (25-27) although a direct role of the receptors was not demonstrated.

Our previous work on oxLDL and LPC-induced generation of mature DC from monocytes suggested that the biochemical composition of APR LDL may signal the presence of a dangerous situation and favor the development of adaptive immunity. In this paper we asked whether the immunological function of oxLDL was controlled by its content in LPC and oxidized fatty acids and investigated the potential role of PPARγ ligands in modulating the DC response.

Materials and methods

Materials

L-α-Lysophosphatidylcholine (LPC) and ciglitizone were purchased from Sigma (St Quentin-Fallavier, France). 9(S)-hydroxyoctadecadienoic acid (HODE), 13(S)-HODE, 11(R, S)-hydroxyeicosatetraenoic acid (HETE) and 15(R, S)-HETE were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Intralipid 20 % was purchased from Fresenius Kabi (Sevres, France).

LDL preparation

LDL (1.025≤ d ≤1.055 g/ml) was isolated from human plasma of normolipidemic healthy individuals by ultracentrifugation as described previously (6). The protein content of LDL was estimated by Coomassie Protein Micro-Assay procedure (Pierce, Rockford, IL, USA) and its lipid composition was determined using Cholesterol RTU, Triglycerides enzymatic PAP 150 and Phospholipids enzymatic PAP 150 kits from bioMérieux (Marcy l'Etoile, France).

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 24h 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.

Monocyte-derived dendritic cells

Monocytes were isolated from human peripheral blood as described previously (6). Monocyte differentiation to immature DC was initiated with 40 ng/ml human recombinant GM-CSF and 250 U/ml human recombinant IL-4. After 6 days, more than 95 % of the cells were immature DC as assessed by CD1a labeling. Cultures were performed 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).

Treatment of differentiating monocytes

We have previously observed that direct generation of mature DC from differentiating monocytes was best achieved when oxLDL was added at day 5 of differentiation for 24h in lipoprotein deficient serum to prevent inhibition by native LDL (6). Differentiating monocytes were thus treated at day 5 as indicated and incubated for 24 hours at 37°C. When mentioned, PPARγ agonists 9-HODE or 13-HODE (25 µg/ml), 11-HETE or 15-HETE (12.5 µg/ml) or Ciglitizone (50 µM) were added 15 min prior to oxLDL (10 µg/ml) or LPC (40 µM). Intralipid 20 % (50 µg/ml phospholipids as described in (13)) was added concomitantly with LPC (40 µM). At the end of the differentiation (day 6), cell viability was superior to 90 %.

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, -CD86, -CD40 all from Beckman Coulter.

Mixed Lymphocyte Reaction

Naïve T lymphocytes were isolated from human peripheral blood as described (6). Primary MLRs were conducted in 96-well flat-bottomed culture plates. Antigen presenting cells were treated or not with LPC 40 µM or oxLDL 10 µg/ml in presence or not of PPARγ agonists as described above, collected at day 6, extensively washed and resuspended in RPMI / 10 % FCS. 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 was collected and analyzed for the presence of IFNγ using cytokine-specific ELISA kits purchased from Endogen (Woburn, MA, USA).

EMSA analysis

4 x 10^6 cells were treated at day 5 and incubated at 37°C for the indicated time. After treatment, the cells were washed twice with PBS, resuspended in 400 µl of ice-cold hypotonic buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 0.01 M DTT, 1.5 mM $MgCl_2$, and 1 x protease inhibitor cocktail (Sigma)) for 10 min, vortexed and centrifuged at 15000 x g for 30 s at 4°C. Nuclei were lyzed in 40 µl ice-cold saline buffer (20 mM HEPES (pH 7.9), 420 mM NaCl, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 25 % glycerol, and 1 x protease inhibitor mixture (Sigma)), at 4°C for 20 min, vortexed and centrifuged at 15000 x g for 5 min at 4°C. Protein concentrations were determined by Micro BCA Protein Assay Reagent (Pierce, Rockford, IL, USA). The sequence of the double-stranded oligonucleotide for detection of PPAR binding was: 5'-GGGGTCAGTAAGTCAGAGGCCAGGGA-3', according to Tontonoz *et al* (29). Oligonucleotide was end-labeled with [γ-^{32}P]ATP (Amersham Pharmacia Biosciences, Uppsala, Sweden) by T4 polynucleotide kinase (New England Biolab, Beverly, MA). Nuclear extracts (1 µg) were then mixed with 2 µg of poly (dI-dC) in a 20 µl reaction containing 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM DTT, 1 mM EDTA, 10 % glycerol and ^{32}P-labeled

oligonucleotide. After a 20 min incubation at room temperature, DNA-protein complexes were resolved on a 4 % polyacrylamide native gel in a 0.5 x Tris-Glycine EDTA buffer. Radioactive bands were visualized using a Typhoon PhosphorImager. For supershifts, nuclear extracts were incubated overnight at 4 °C with 4 µg of PPARγ rabbit anti-serum (Geneka Biotechnology, Rixensart, Belgium) before addition of labeled oligonucleotide.

Immunoblots

10^6 cells were treated at day 5 with 40 µM LPC for 2, 4 or 8 hours. Cells were washed with cold PBS and nuclear extraction was performed as described. Immunoblotting was performed with 3 µg of nuclear extract denatured in 8 M urea / 2 % SDS / 0.2 M Tris-HCL pH 8.0 / 100 mM dithiothreitol and boiled for 5 minutes. Nuclear extracts were then 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-PPARγ and anti-RXRα were from Geneka Biotechnology and Santa Cruz Biotechnology respectively. Detection was performed using HRP-conjugated anti-rabbit immunoglobulin (Amersham Pharmacia Biosciences) with the enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biosciences). The same immunoblotting conditions were applied to EMSA gels.

Results

OxLDL-induced DC functional maturation is inhibited by 9-HODE

The presence of oxLDL during monocyte differentiation yielded phenotypically and functionally mature DC (6). This effect is mediated by LPC generated during LDL oxidation (13). In addition to pro-inflammatory modified phospholipids, LDL oxidation also generates anti-inflammatory oxidized fatty acids like 9-HODE from linoleic acid. As oxLDL function is likely to depend on its relative content in modified phospholipids and fatty acids, we first asked whether an excess of HODE could affect the process of DC maturation induced by oxLDL. Monocytes differentiated to immature DC in GM-CSF and IL-4 in the absence of oxLDL expressed high levels of CD1a but no CD14, intermediate levels of HLA-DR and CD40 and were negative or low for CD86 and CD80 (Fig. 1A). Phenotype of 9-HODE-treated cells (25 μg/ml) was identical to control cells (data not shown). Cells treated with oxLDL (10 μg/ml) up-regulated HLA-DR, CD80, CD86 and CD40 but CD1a remained high as previously described (6). Addition of 9-HODE (25 μg/ml) together with oxLDL (10 μg/ml) did not prevent oxLDL-induced up-regulation of HLA-DR, CD86, CD80 and slightly reduced CD40 expression.

The ability of DC generated in the presence of oxLDL and 9-HODE to stimulate allogeneic T cells was further analyzed. As expected oxLDL-treated cells stimulated IFNγ secretion by allogeneic T cells while untreated (immature DC) or 9-HODE-treated cells did not (Fig. 1B). However, when DC were generated in the presence of both oxLDL and 9-HODE, allogeneic T cells were not stimulated to secrete IFNγ. Therefore, in the presence of additional 9-HODE, oxLDL induced phenotypical but not functional maturation of DC.

Effect of oxLDL on DC is inhibited by natural and synthetic PPARγ ligands

We then asked whether the functional effect of 9-HODE could be extended to other oxidized fatty acids generated during LDL oxidation. 13-HODE is another major oxidized metabolite of linoleic acid identified in oxLDL. Figure 2A shows that cotreatment with oxLDL and 13-HODE resulted in the generation of DC that do not stimulate IFNγ secretion by allogeneic T cells. The same results were obtained with the oxidized metabolites of arachidonic acid, 11-HETE and 15-HETE (Fig. 2B, C). Therefore, 9-HODE, 13-HODE, 11-HETE and 15-HETE inhibited the capacity of oxLDL to generate activating DC from differentiating monocytes. As all these lipids are ligands for PPARγ, inhibition of oxLDL-induced functional maturation of DC was tested using the specific synthetic agonist of PPARγ ciglitizone. As shown in Figure 2D, DC generated in the presence of oxLDL and ciglitizone lost their ability to stimulate IFNγ secretion by allogeneic T cells. As observed for 9-HODE, neither 13-HODE, HETE nor ciglitizone inhibited oxLDL-induced up-regulation of HLA-DR and costimulatory molecules except for a slight reduction of CD40 induction (data not shown). Overall these data suggest that PPARγ ligands can modulate the immunological function of oxLDL, some of these ligands being generated during the process of LDL oxidation in the form of oxidized fatty acids.

PPARγ agonists inhibit functional maturation of LPC-treated DC

LPC is a major component of oxidized LDL that can activate various cell types and is implicated in many aspects of the inflammatory response. In a recent study we showed that LPC mimicked oxLDL in that it was able to generate mature DC from differentiating monocytes (13). Experiments were thus conducted to test whether activation of PPARγ with ciglitizone could inhibit LPC action on DC as it did for oxLDL. As previously reported (13), LPC-treated cells up-regulated HLA-DR, CD86, CD40 and slightly CD80, while CD1a

remained high as for oxLDL. Addition of ciglitizone concomitantly with LPC did not affect up-regulation of HLA-DR and CD86 but inhibited CD80 and CD40 up-regulation (Fig. 3A). The ability of DC generated in the presence of LPC and ciglitizone to stimulate allogeneic T cells was then analyzed. As shown in Fig. 3B, LPC-treated cells stimulated IFNγ secretion by allogeneic T cells while ciglitizone-treated cells did not. The induction of IFNγ secretion was inhibited when DC were treated with both LPC and ciglitizone suggesting that PPARγ ligands can antagonize the LPC pathway. Similar results were observed with low concentration of Troglitazone and Pioglitazone (0.5 μM) and maximum inhibition was observed with high concentration of the drugs (5μM) (data not shown).

LPC inhibits the PPARγ pathway

The effect of LPC on the PPAR pathway was studied. PPARγ heterodimerizes with RXRα and, following ligand binding, can activate transcription by binding to PPRE of target genes. The core PPRE consists of an imperfect direct repeat of the consensus binding site for nuclear hormone receptors (TGACCT) separated by one nucleotide (DR-1). PPRE are not exclusive targets of PPARγ/RXRα and other transcription factors can bind to PPRE. Some of them, like the chicken ovalbumin upstream promoter transcription factor (COUP-TF) or PPARδ, have been reported to inhibit PPARγ transcriptional activity (28, 30). To evaluate the effect of LPC on the PPAR pathway, electrophoretic mobility shift assays (EMSA) were performed using nuclear extracts from LPC-treated cells (Fig. 4A). Three mobility shift bands were observed after EMSA, that could be blunted by competition with unlabeled probe in excess, confirming the binding specificity of these complexes (data not shown). The upper band (complex I) detected in untreated cells rapidly disappeared from nuclear extracts following LPC treatment. The two lower bands (complex II) migrated as doublet and progressively increased in

intensity following LPC treatment. Thus, complex I decreased and complex II increased upon LPC treatment.

EMSA were then performed with nuclear extracts of cells treated with the PPARγ ligand ciglitizone. This treatment resulted in an increase of complex I in nuclear extracts while complex II was unaffected, strongly suggesting that complex I represents activated PPARγ (Fig. 4B, lane 3). Inhibition of LPC by ciglitizone can be visualized at the level of these complexes. Indeed, while complex I disappeared upon LPC treatment, it is not affected when cells are treated concomitantly with LPC and ciglitizone (Fig. 4B, lane 4). In addition, complex II is strongly induced by LPC but this induction is inhibited by ciglitizone.

Further characterization of these complexes was performed by supershift and blotting experiments. The addition of a PPARγ-specific anti-serum supershifted the complex I in ciglitizone-treated extracts, confirming the presence of PPARγ in this complex (Fig. 4C). Anti-PPARα, anti-COUP-TF or anti-PPARδ antibodies had no effect on the mobility shift pattern of complex I (data not shown).

To further characterize complex I, nuclear extracts gel shift from control and LPC-treated cells (Fig. 5A) were subjected to membrane blotting. Immunoblotting with a PPARγ antibody confirmed the presence of PPARγ in complex I and its absence from complex II (Fig 5B). After total stripping, incubation of the same membrane with a RXRα specific antibody revealed that complex I but not complex II also contained RXRα. These experiments confirmed the identity of complex I as PPARγ/RXRα heterodimer. Supershift experiments with antibodies directed to various transcriptional factors including PPARα, PPARδ, RXRα, LXRα and COUP-TF suggested that PPARδ subunit may be present to some extend in complex II but this could not be confirmed by immunoblotting (data not shown). Complex II could not be characterized with available antibodies and remains to be identified.

To investigate the mechanisms responsible for the inhibition of PPARγ/RXRα binding activity by LPC, RXRα and PPARγ protein level was assessed by western blot in nuclear extracts of cells treated with LPC for different period of time (Fig. 5C). RXRα and PPARγ protein level was not significantly affected by LPC treatment indicating that inhibition of the DNA binding activity of PPARγ/RXRα does not result from the elimination of one or both subunits from the nucleus.

Intralipid modulates DNA binding activity of PPARs

We have shown previously that Intralipid can block the ability of LPC to induce functional maturation of DC (13). This ability to inhibit LPC-induced DC maturation is shared by ciglitizone which was shown above to block LPC action on PPARs. Therefore, we analyzed the effect of Intralipid on DNA-binding activities of the same transcription factors. In the presence of Intralipid, the effect of LPC on the DNA-binding activity of complex I and complex II was inhibited (Fig 6A). To further analyze the mechanism of action of Intralipid, cells were treated with Intralipid alone (50 μg/ml of phospholipids) for 1h to 24h and nuclear proteins were subjected to EMSA. Treatment with Intralipid resulted in a time dependent activation of complex I and a progressive decrease of complex II activity (Fig. 6B). Therefore, DNA-binding activity of PPARγ/RXRα was stimulated by Intralipid while that of complex II was inhibited, conferring a direct role for Intralipid in gene transcription.

Discussion

Continuous oxidation of LDL and accumulation of oxLDL in the subendothelial space are key factors of atherosclerosis which is a chronic inflammatory disorder (3, 31, 32). When transiently induced, alterations of LDL also appear to play an essential role in the link between innate and acquired immunity during the acute phase response to injury (6). We previously hypothesized that oxLDL and modified phospholipids such as LPC generated during APR could signal the presence of a dangerous situation to the immune system and showed that oxLDL and LPC favor the development of adaptive immunity by promoting mature dendritic cell generation (6, 13). The action of oxLDL and LPC on inflammation and immunity is temporally limited because of the transient aspect of the APR. The immunological function of oxLDL can also be regulated by its own biochemical composition. Indeed, although oxLDL are often considered as proinflammatory mediators, they have been newly regarded as inhibitors of inflammatory responses by several investigators (33, 34). This paradox is supported by the complexity of the oxidation process which can generate a mixture of components with inflammatory properties such as LPC or with anti-inflammatory properties such as the oxidized metabolites of linoleic and arachidonic acids, HODE and HETE respectively. Consistent with this idea, we show here that an excess of 9/13-HODE and 11/15-HETE dramatically affected the functional properties of oxidized LDL on DC. Addition of these lipids together with oxLDL had no significant effect on the induction of presentation or costimulatory molecules, except for CD40. These DC were unable to stimulate the secretion of IFNγ by allogeneic T cells. Our data suggest that the relative production of LPC and HODE/HETE during LDL oxidation may determine the immunological properties of oxLDL.

Since 9/13 HODE and 11/15 HETE are natural ligands for PPARγ, these data suggested that synthetic agonists of PPARγ could also inhibit the action of LPC and oxLDL on DC. Indeed, ciglitizone was shown to inhibit the functional maturation of DC treated with oxLDL or LPC. This PPARγ agonist blocked the up-regulation of CD80 and CD40 induced by LPC and these DC lost their ability to stimulate the production of IFNγ by allogeneic T lymphocytes. Whether this is due to the lack of CD40 or CD80 induction remains to be determined. In addition, no Th2 bias was observed under these experimental conditions as no increase in IL-4, IL-5, IL-10 or IL-13 secretion could be detected following activation of PPARγ (data not shown). At the molecular level, LPC induced a rapid decrease in the DNA binding activity of PPARγ/RXRα heterodimers associated with a concomitant increase in DNA binding of another complex. Neither PPARα, COUP-TF, LXRα or RXRα could be detected in this complex and additional experiments, including protein sequencing, are required to fully identify this nuclear receptor activated by LPC. Since inhibition of PPARγ/RXRα DNA binding activity could not be explained by protein degradation it may result either from post-transcriptional modifications of this complex or from increased competition by other transcription factors. Despite incomplete characterization of the molecular pathways it can be concluded that a pro-Th1 function of DC induced by LPC was associated with a down-regulation of PPARγ activity and an up-regulation of an unidentified complex that can bind PPRE. This effect on PPAR pathways was inhibited by ciglitizone and Intralipid. The effects of LPC on DC maturation were also inhibited by low concentration of other synthetic agonists of PPARγ, Troglitazone and Pioglitazone and higher concentrations of these drugs were better inhibitors of LPC (data not shown). Since high concentrations of PPARγ ligands have been reported to interfere with other signaling pathways, it is likely that the action of LPC on DC is both PPARγ-dependent and independent.

Interestingly, Intralipid alone appeared to stimulate the DNA binding activity of PPARγ/RXRα heterodimers. Activation of this anti-inflammatory arm of Intralipid may open therapeutic perspectives since Intralipid is an emulsion of lipids which is currently used in human, especially for parenteral nutrition. Our data with LPC and Intralipid are consistent with the different experimental models suggesting that PPARγ activation may impair the function of DC (25-27). Lysophosphatidic acid (LPA) is a transcellular PPARγ agonist which exerts multiple effects on immature and mature DC and inhibits their pro-Th1 potential (35, 36). Similarly, the prostaglandin 15-deoxy-Δ12,14-PGJ$_2$ is a PPARγ ligand which can redirect DC to a less stimulatory mode (26). Interestingly, cyclooxygenase 2 is implicated in both the onset and resolution of inflammation by generating both pro-inflammatory (PGE$_2$) and anti-inflammatory prostaglandins (15PGJ$_2$). If a parallel can be made, it is tempting to speculate that oxLDL also provides on (LPC) and off (HODE/HETE) signals of inflammation with opposite immunomodulatory properties.

Acting on these signals might be a strategy for some pathogenic microorganisms to escape the immune system. For instance, high concentration of HODE/HETE have been detected in red blood cells following *Plasmodium falciparum* infection and micromolar concentrations of HETE were estimated in monocytes after phagocytosis of parasitized red blood cells (37). It would be important to know whether the same could happen in DC and what would be the immunological consequences. Lipoxins are another class of anti-inflammatory lipids whose induction in response to microbial stimulation regulates DC function by suppressing IL-12 production (38). Thus, a number of lipid mediators whose production is highly controlled are progressively appearing as key elements in the regulation of innate and acquired immunity. Studies on LDL oxidation have highlighted the role of pro- and anti-inflammatory lipid mediators that can control the transition from innate to adaptive immunity during the APR.

Sensing biochemical composition of LDL by the innate immune system could be an essential

step in the detection of danger.

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Footnotes

[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; EMSA, electrophoretic mobility shift assay; GM-CSF, granulocyte macrophage-stimulating factor; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; LDL, low density lipoprotein; LPC, lysophosphatidylcholine; LPDS, lipoprotein deficient serum; oxLDL, oxidized low density lipoprotein; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; RXR, 9-cis retinoid receptor

Figure legends

Figure 1. 9-HODE blocks allostimulatory properties of oxLDL-generated DC. A, Phenotypic maturation of DC induced by oxLDL in presence or not of 9-HODE. Dotted lines, not treated control cells; filled profiles, oxLDL (10 µg/ml)-treated cells; thin lines, cells treated with oxLDL (10 µg/ml) and 9-HODE (25 µg/ml). B, Allogeneic T cell stimulation. Differentiating monocytes were treated or not at day 5 with oxLDL (10 µg/ml), 9-HODE (25 µg/ml) or oxLDL (10 µg/ml) and 9-HODE (25 µg/ml). At day 6, cells were washed and cultured in triplicate with allogeneic T cells (1/5 to 1/20 DC:T cell ratio) for 4 days. IFNγ in supernatants was measured by ELISA. Mean +/- SD of triplicates of one representative experiment out of 4.

Figure 2. Natural and synthetic PPARγ ligands inhibit functional maturation of oxLDL-generated DC. Differentiating monocytes were treated at day 5 with 10 µg/ml oxLDL, in the presence or not of 25 µg/ml 13-HODE (A), 12,5 µg/ml 11-HETE (B), 12,5 µg/ml 15-HETE (C), 50 µM ciglitizone (Cig.) (D). Cells were harvested at day 6, washed and cultured in triplicate with 2 x 10^5 allogeneic T cells (1/5 to 1/20 DC:T cell ratio) for 4 days. IFNγ in supernatants was measured by ELISA. Mean +/- SD of triplicates of one representative experiment out of 4.

Figure 3. Ciglitizone inhibits functional maturation of LPC-treated DC. At day 5, differentiating monocytes were treated or not (control) with 40 µM LPC in the presence or not of 50 µM ciglitizone (Cig.) for 24h. A, Phenotype of control cells (dotted lines), LPC-treated cells (filled profiles), and cells treated with LPC and ciglitizone (thin lines). B, Allogeneic T cell stimulation. Differentiating monocytes were treated at day 5 with 40 µM LPC, 50 µM

ciglitizone or 40 μM LPC plus 50 μM ciglitizone. At day 6, cells were washed and cultured with allogeneic T cells (1/5 to 1/20 DC:T cell ratio) for 4 days. IFNγ in supernatants was measured by ELISA.

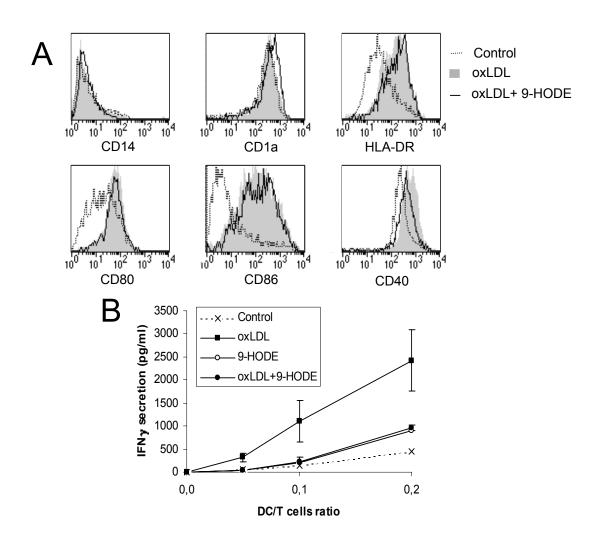**Figure 4.** LPC affects the PPAR signaling pathway. A, Nuclear proteins from differentiating monocytes treated at day 5 with 40 μM LPC for the indicated period of time were subjected to EMSA. EMSA led to three mobility shift bands: one upper band (complex I) and the two lower bands (complex II). B, Ciglitizone treatment increases complex I and inhibits LPC effect on PPAR. Radiolabeled PPRE oligonucleotides were incubated with nuclear extracts from control cells (lane 1) or cells treated for 2h with LPC (lane 2), ciglitizone (lane 3) or LPC and ciglitizone (lane 4). C, Complex I contains PPARγ. Supershift EMSA was performed with nuclear proteins from ciglitizone (50 μM) treated cells for 2h. The complex I is supershifted with a PPARγ anti-serum.
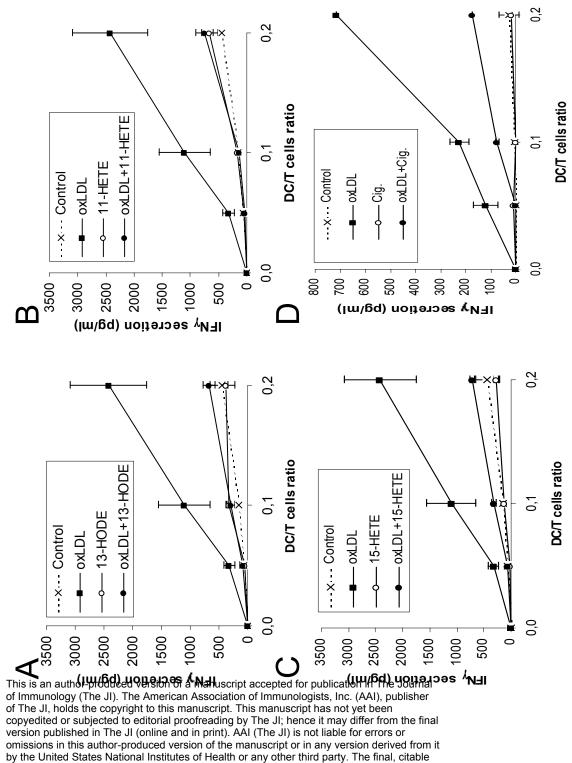
Figure 5. LPC inhibits PPARγ/RXRα binding activity. A, EMSA of nuclear extracts from untreated or LPC-treated cells for 2h. B, Immunoblotting analysis of the same EMSA gel with a PPARγ-specific antibody (left panel). The membrane was stripped, controlled and reprobed with RXRα antibody (right panel). C, Western blots of nuclear extracts from cells treated with LPC (40 μM) for the indicated period of time incubated with an anti-RXRα and anti-PPARγ.

Figure 6. Intralipid is acting on the PPAR pathway. A, At day 5, differentiating cells were stimulated or not for 2h with 40 μM LPC in presence or not of Intralipid (50 μg/ml phospholipids). B, Nuclear proteins were extracted from differentiating monocytes treated at day 5 with Intralipid alone (50 μg/ml of phospholipids) for the indicated period of time. A-B, Nuclear extracts were analyzed by EMSA as described.

A

Control
oxLDL
oxLDL+ 9-HODE

CD14   CD1a   HLA-DR

CD80   CD86   CD40

B

A

Control
LPC
LPC + Cig.

CD14   CD1a   HLA-DR

CD80   CD86   CD40

B

A

Time (hour)   0   2   4   8

Complex I ➔

Complex II ➔

B

1   2   3   4

LPC   -   +   -   +
Cig.   -   -   +   +

C

Cig.   +   +
α-PPARγ   -   +

Supershift ➔

Complex I ➔

A

LPC   −   +

B   LPC

α-PPARγ

−   +

α-RXRα

−   +

C   0   2   4   8   Time (hour)

RXRα

PPARγ

A
| LPC | - | + | + |
| Intralipid | - | - | + |

Complex I ➜

Complex II ➜

B

0   1   2   8   24   (h)

Complex I -

Complex II -