Role of PPAR-gamma in the Modulation of CD36 and FcgammaRII induced by LDL with Low and High Degrees of Oxidation During the Differentiation of the Monocytic THP-1 Cell Line

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Key Words

Macrophages • Oxidized LDL • Atherosclerosis • CD36 • FcgammaRII • PPARgamma

Abstract

Scavenger or Fcγ receptors are important for capture and clearance of modified LDL particles by monocytes/macrophages. Uptake via scavenger receptors is not regulated by intracellular levels of cholesterol and in consequence, macrophages develop into foam cells in the arterial intima. The levels of scavenger receptor CD36 are increased in atherosclerotic lesions and there is evidence that some components of oxLDL auto-regulate the expression of this receptor. Fcγ receptor expression is increased in cardiovascular diseases but it is not known whether their expression is regulated by oxLDL. The biological properties of oxLDLs vary depending on the degree of oxidation. In the present study we investigated the effect of LDL particles showing extensive or low oxidation (HoxLDL and LoxLDL) on the expression of CD36 and FcγRII in a human monocytic cell line (THP-1), differentiated or not to macrophage, and the involvement of PPARγ. It was found that both forms of oxLDL are able to increase the expression of CD36 and FcγRII and that this effect is dependent on the degree of oxidation

and of the stage of cell differentiation (monocyte or macrophage). We also showed that the increased expression of FcγRII is dependent on PPARγ whereas that of the CD36 is independent of PPARγ.

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Introduction

In the early stages of the development of atherosclerotic lesions, lipoproteins and their products are retained in the intima of the vascular wall. The low density lipoproteins, LDL, are prone to oxidative modifications and in this form they stimulate endothelial cells to express adhesion molecules [1] allowing infiltration of T lymphocytes and monocytes to the subendothelial space where they differentiate into macrophages. These cells recognize oxidatively modified LDL particles through scavenger receptors. The uptake of modified LDL by these receptors is not regulated by the intracellular levels of cholesterol, and this may lead to macrophage transformation into foam cells. This provides the pathological basis for atherosclerosis and the development of complex lesions [2-4]. The upregulated expression of scavenger receptors in atherosclerosis is suggested to be caused by autoregulation in response to components of the oxLDL

particle [5]. One of the scavenger receptors responsible for oxLDL uptake is the CD36, a class B scavenger receptor [6]. The relevance of this receptor in atherosclerosis was demonstrated by experiments using double, CD36 and ApoE *knock out* mice since the uptake of oxLDL by macrophages was greatly reduced as well as the intensity of the atherosclerotic lesions in comparison to the wild-type [7, 8].

Modification of LDL triggers the activation of the immune system, either inducing the production of antibodies against these particles [9], activating T-cells [2] or stimulating antigen-presenting cells [10]. The oxLDL particles bound to antibodies are taken up from the circulation via Fc γ II receptors present in cells from the mononuclear phagocytic system contributing to the inflammation in the vascular wall [11-14]. Involvement of these receptors in atherosclerosis has been recently demonstrated and decreased expression of Fc γ RII was found in patients with cardiovascular diseases [15]. It is not known if the expression of this receptor, like that of the CD36, is regulated by oxLDL.

The PPAR γ is a member of nuclear hormone superfamily involved in adipogenesis and lipid metabolism expressed constitutively in cells of the monocyte/macrophage lineage [16]. PPAR γ ligands such as thiazolidinedione drugs (TZDs), reduce atherosclerotic lesions suggesting that activation of these receptors have a protective role in the disease [17]. Although the uptake of oxLDL by macrophages via CD36 on its own increases PPAR γ and CD36 expression [18], more studies are needed to understand the role of PPAR γ in atherosclerosis as well as the co-regulation with other known receptors for modified LDL.

In the present study we investigated the effect of modified LDLs on the expression of CD36 and Fc γ RII in a human monocytic cell line (THP-1), differentiated or not to macrophages and the involvement of PPAR γ . Moreover, since the physiological, structural and biochemical properties of oxLDLs are known to vary depending on the degree of oxidation, we analyzed comparatively LDL particles showing extensive or low oxidation (HoxLDL and LoxLDL) with the non modified LDL.

Material and Methods

Materials

The medium RPMI-1640, Fetal Calf Serum, L-glutamine and HEPES-acid were purchased from Gibco Co-USA. PMA,

penicillin, streptomycin, aprotinin, leupeptin, benzamidin, chloramphenicol, PMSF and GW9662 were purchased from Sigma-Aldrich. The sterile culture plates and bottles were acquired from Corning Costar, Cambridge USA. The filters 0,22 and 0,45 μ m were purchased from Millipore (Berkford, USA). Rosiglitazone was acquired from Cayman-chemical (Ann Arbor, Michigam-USA). The labelled monoclonal antibodies to cytometry were purchased from BD-biosciences, USA.

Purification and oxidation of LDL

The blood was drawn from healthy human donors, and the plasma was obtained after centrifugation at 1000 g, 4 °C, for 15. Thereafter the plasma was supplemented with benzamidine (2 mM), gentamicin (0.5%), chloramphenicol (0.25%), PMSF (phenyl-methyl-sulfonyl-fluoride) (0.5 mM), and aprotinin (0.1 units/mL). The LDL was obtained by sequential ultracentrifugation at 100,000 x g, at 4 °C, using a 75 Ti rotor (Beckman Instruments). Briefly, the plasma density was adjusted to 1.019 g/mL adding KBr and centrifuged for 18 h. The supernatant containing VLDL was removed. After, the density of the infranant was adjusted to 1.063 g/mL and centrifuged for 18 h. The supernatant containing LDL was dialyzed at 4 °C against PBS pH 7.4, with EDTA 1 mM, filtered (0.22 μ m) and stored at 4 °C. The protein concentration was determined by BCA kit (Pierce, USA) using BSA as standard. The LoxLDL was obtained by dialysis of LDL 2 mg/mL against 2 μ M FeSO $_4$ · 7H $_2$ O in PBS pH 7.2 for 48 h at room temperature in the dark [19]. The HoxLDL was obtained by incubation of LDL 2 mg/mL with CuSO $_4$ 20 μ M for 18 h at 37 °C [9]. The oxidation of both protocols was terminated by addition of EDTA 1 mM.

Determination of TBARS and Conjugated Dienes

The degree of oxidation was determined by measuring the amount of tiobarbituric acid-reactive substances (TBARS). Shortly, 50 μ L of LDL, LoxLDL or HoxLDL were incubated with 200 μ L of TBARS reagent (tiobarbituric acid 1% w/v plus trichloroacetic acid 15 % w/v) warmed at 100 °C for 15 min. The samples were centrifugated for 5 min and the absorbance of supernatant was determined at 540 nm. The results were expressed as concentration of malondialdeide (MDA) per mg of protein. The presence of conjugated dienes was determined by absorbance at 234 nm and the results were expressed in optical density (O.D.).

Anion-exchange Chromatography

The lipoproteins, modified or not, were analysed by anion-exchange chromatography. Two solutions were used for elution: A, Tris-HCl 0,01 M, pH 7.4, and B, NaCl 1 M in buffer A. First, The concentration of the lipoprotein sample was adjusted to 1 mg/mL with Tris-HCl 0.01 M, pH 7.4, filtered through 0.45 μ m filter and injected via a 0.5 mL loop into an anion-exchange column (Resource-Q) and eluated at a flow rate of 1 mL/min by a linear gradient of 5-42% of buffer B during the first 47 min, followed by a 42-100% gradient of buffer B, from 47-52 min, a 100% gradient of Buffer B from 52-53 min, and 0% from 53-55 min. The effluent was analyzed with a diode-array detector (Shimadzu, Japan) using the Class VP program (Shimadzu).

Cell Culture

The monocytic cell line THP-1 cells were cultured in RPMI-1640 medium supplemented with FCS 5%, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamin, 15 mM HEPES, 11 mM sodium bicarbonate. Cell cultures were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C. In the experiments the suspensions were adjusted to 5 × 10⁵ cells/ml in RPMI with 1% FCS and plated in a volume of 500 µl/well into flat-bottom 24-well plate. The experiments were performed with monocytic THP-1 cells and after differentiation to macrophages. The differentiation of THP-1 monocytes into macrophages was induced by phorbol 12-myristate 13 acetate (PMA) 150 nM for 24 h. The non-adherent cells were removed by aspiration of supernatants and replacement with fresh medium and the experiments proceeded according to the protocol.

Flow cytometry

The THP-1 cells were treated with 20 µg/mL of LDL, LoxLDL or HoxLDL or with rosiglitazone (10⁻⁵; 10⁻⁶; 10⁻⁷ M) for 24 h. In another group of experiments, the cells were treated with GW9662, an antagonist of PPAR_γ, 30 min before. After incubation, the cells were centrifuged at 300 × g for 10 min, the supernatants were discarded and the pellets were resuspended in staining buffer (PBS, FCS 1%) containing the labelled monoclonal antibodies, PE-conjugated anti-human CD36; PE-CD11b/CD18 (mac-1) and FITC-conjugated anti-human CD32. The samples were evaluated using a flow cytometer (FACS calibur - Becton BD Biosciences) and the analysis was done using software Summit® V3.3 (DakoCytomation).

Statistical Analysis

Data are presented as mean ± standard error of at least three experiments. Each experiment was performed in triplicate. An analysis of variance (ANOVA), combined with the Student-Newman-Keuls test, was used to evaluate the statistical significance of the differences. *P* < 0.05 values were considered as significant.

Results

Anion exchange chromatography, TBARS and conjugated dienes of LDL with different degree of oxidation

This study was performed using modified LDL obtained by two different protocols. The oxidation process of the LDL particle is accompanied by increasing of negative charges in its chemical structure. According to increased retention time, we using an anion exchange column (Resource Q) to analyze the degree of oxidation of LDL particle, modified by iron or copper. The chromatograms obtained of LDL, LoxLDL and HoxLDL show an increase in negative charge according to retention time increased (Figure 1). The chromatogram profile of LDL shows peaks between 14 and 24 min., the peak

Fig. 1. Evaluation of the oxidation by anion-exchange chromatography. LDL, LoxLDL and HoxLDL were obtained according to material and methods. 500 µL of each sample was analyzed using an anion-exchange Resource Q column. As previously shown [9], oxidation leads to the appearance of peaks eluting at later times. The chromatograms are presented as follow: LDL (.....), LoxLDL (- - -) and HoxLDL (—).

Table 1. Thiobarbituric acid reactive substances (TBARS) and conjugated dienes.

	¹ TBARS	² Dienes
LDL	0.2	2.84
LoxLDL	7.5 ***	3.02
HoxLDL	53.1 ***	4.00 ***

are expressed as nmol of malondialdehyde /mg protein. ²Conjugated dienes are expressed as Optical Density of 1 mg/mL. The data are expressed in mean ± standard deviation of three independent experiments. ****p* < 0.001 vs LDL.

with higher protein concentration was eluted in 17 min. The LoxLDL shows peaks in the same retention time obtained for LDL, with two mean peaks eluted between in 18 and 26 min. In its turn, the HoxLDL shows an increase in the chromatogram retention time, the peaks were eluted between 20 and 27 min and the higher protein concentration peak was obtained in 23 min, showing a higher degree of oxidation than other LDL preparations. During the oxidation process occurs an increase of lipid peroxidation products, as MDA and conjugated dienes (Table 1). The LoxLDL showed a short increase in the MDA (7.5 nmol/mg protein) and conjugated dienes content (O.D. 3.02), compare to LDL. However, the HoxLDL showed a high concentration of MDA (53.1 nmol/mg protein) and conjugated dienes (O.D. 4.00) content. These data demonstrate the low and high degrees of oxidation to LoxLDL and HoxLDL respectively.

Expression of CD36, FcγRII and CD11b/CD18 (mac-1) in THP-1 cells

The THP-1 cells have a round shape and are maintained in suspension in cell culture medium. After

	Non-differentiated	differentiated
CD11b/ CD18 (mac-1)	13.00 ± 1.15	58.30 ± 7.40***
CD36	10.30 ± 1.20	86.60 ± 0.90***
FcγRII	73.60 ± 0.80	82.00 ± 0.60

Table 2. Expression of CD11b/CD18 (mac-1), CD36 and FcγRII in non-differentiated and differentiated THP-1 cells. Non-differentiated and PMA-differentiated THP-1 cells were labeled with specific antibodies and the expression of surface markers was evaluated by FACS. The data are expressed as mean ± standard error of three independent experiments. ***p<0.001 vs non-differentiated cells.

Fig. 2. Effect of modified LDL on the expression of CD36 and FcγRII in non-differentiated THP-1 cells. Non-differentiated THP-1 cells were treated with LDL, LoxLDL or HoxLDL (20 µg/mL) for 24 h. The graph 2A and histograms 2B, 2C, 2D show the expression of CD36 after the treatment with lipoproteins. The graph 2E shows the expression of FcγRII after the treatment with lipoproteins. The graphs represent the mean ± SEM of the Mean of Fluorescence Intensity (MFI) of three independent experiments performed in triplicate. The histograms show one representative experiment out of three independent experiments. *** p < 0.001 vs control untreated cells.

the treatment with 150 nM PMA for 24 h, they became adherent, express different surface antigens, and acquired macrophage-like phenotype. In this study we used as differentiated cells, only the adherent ones. The stage of differentiation of the THP-1 cells, was confirmed by the expression of the differentiation marker CD11b/CD18 (mac-1). As shown in Table 2, the PMA differentiated THP-1 cells presented an increase of about 5 fold of CD11b/ CD18 (mac-1) (58 ± 7.4) compared to the non-differentiated THP-1 cells (13 ± 1.15). The treatment of these cells with PMA resulted in a significant increase of CD36 expression (86.6 ± 0.9), around 8 fold compared to the non-differentiated THP-1 control cells (10.3 ± 1.2). The FcγRII expression was slightly increased in PMA differentiated THP-1 compared to the non differentiated cells (82 ± 0.67 vs 73.6 ± 0.8 respectively). The increased expression of CD11b/ CD18 (mac-1) and CD36 was found not only when the percentage of positive cells was analyzed as described above, but also when the amount of receptors per cell was calculated (increase of mean fluorescence intensity).

Effect of oxidized LDL on the expression of CD36 and FcγRII

A) Non differentiated THP-1 cells. The THP-1 cells were treated with LDL, LoxLDL and HoxLDL (20 µg/mL) and the expression of CD36 determined by flow

	Mean fluorescence	% of inhibition	% positive events
Control	10.3 ± 1.2	-	-
Rosiglitazone 10 ⁻⁵ M	138 ± 36.6***	-	63.1 ± 10.5**
Rosiglitazone + GW9662 (20 µM)	50.1 ± 15 ^{&}	63.7	20.9 ± 8.8 ^{&}

Table 3. Expression of CD36 induced by Rosiglitazone in THP-1 cells. The non-differentiated THP-1 cells were treated with Rosiglitazone. The expression of CD36 was evaluated 24 h later. Cells were pretreated with GW9662, at indicated concentration. The data are expressed in mean ± standard error of three independent experiments. **p<0.01, ***p<0.001 vs control. &p<0.05 vs Rosiglitazone 10⁻⁵ M.

cytometry. The treatment of non-differentiated THP-1 cells with LoxLDL or HoxLDL increased the CD36 expression compared to the untreated control cells (Fig. 2A, 2C and 2D). LoxLDL treatment caused an increase in CD36 expression, demonstrated by both, the mean fluorescence intensity (MFI: 27.3 ± 1.9) (Fig. 2A) and percentage of positive events (39.9% p < 0.001) (Fig. 2C). The CD36 expression was also increased by HoxLDL, with MFI of 24.9 ± 1.6 and 39% (p < 0.001) (Fig. 2A and 2D) of positive events, compared to the control cells (MFI 10.3 ± 1.2). Non-modified LDL did not increase significantly the expression of CD36 (Fig. 2A and 2B). The modified or unmodified LDLs did not affect the expression of FcγRII (Figure 2E). These results clearly demonstrate that in non differentiated THP-1 cells, oxidized LDLs, with low or high degrees of oxidation, upregulate the expression of the CD36 receptor but does not affect the expression of the Fcγ receptor.

Fig. 3. Effect of modified LDL on the expression of CD36 and FcγRII in PMA-differentiated THP-1 cells. The THP-1 cells were differentiated into macrophages by treatment with PMA, as described in material and methods. The THP-1 cells were treated with LDL, LoxLDL or HoxLDL (20 μg/mL) for 24 h. The graph 3A and histograms 3B, 3C, 3D show the expression of CD36 after the treatment with lipoproteins. The graph 3E and histogram 3F show the expression of FcγRII after the treatment with lipoproteins. The graphs represent the mean ± SEM of the Mean of Fluorescence Intensity (MFI) of three independent experiments performed in triplicate. The histograms show one representative experiment out of three independent experiments. *** p < 0.001 vs control untreated cells.

B) THP-1 differentiated cell. This set of experiments was performed using PMA differentiated THP-1 cells (THP1-PMA). After addition of PMA, the cells were treated with LDL, LoxLDL or HoxLDL as described before. Figures 3A, 3C and 3D show that both oxidized LDL increased the expression of CD36 compared to untreated control cells. The LoxLDL increased the percentage of positive events by 26.6% (Fig. 3C) with a MFI of 259 ± 71 (Fig. 3A) compared to the control 86 ± 2.9. Similar results were obtained with HoxLDL (34.7% of positive events with MFI of 227 ± 26.3) (Fig. 3D and 3A respectively). Differently from the results obtained with non-differentiated cells, in PMA differentiated THP-1 cells, the HoxLDL increased the FcγRII expression (74% positive events; MFI of 135 ± 2.08) (Fig. 3E and 3F) compared to the control (46% positive events; MFI 82 ± 0.5).

Fig. 4. Effects of PPARγ antagonist (GW9662) on CD36 and FcγRII expression. The THP-1 cells were differentiated into macrophages by treatment with PMA as described in material and methods. The THP-1 cells were pretreated with GW9662 (20 μM) for 30 min followed by stimulation with HoxLDL (20 μg/mL). The CD36 and FcγRII expression were measured after 24 h by FACS. The graph 4A and 4B show the effects of GW9662 on CD36 and FcγRII expression by THP-1 cells respectively. The histogram 4C shows the inhibitory effect of GW9662 on FcγRII expression inducible by HoxLDL. The graphs represent the mean ± SEM of the Mean of Fluorescence Intensity (MFI) of three independent experiments performed in triplicate. The histograms show one representative experiment out of three independent experiments. ** p < 0.01 HoxLDL + GW9662 vs HoxLDL.

Involvement of PPARγ in the expression of CD36 and FcγRII induced by modified LDLs

To investigate the involvement of PPARγ on the CD36 expression induced by modified LDLs, THP-1 cells were pretreated with a PPARγ antagonist, GW9662 (20 μM) for 30 min before addition of LoxLDL or HoxLDL. The results obtained showed that CD36 expression by LoxLDL (MFI: 112.6 ± 19.4) or by HoxLDL (100.6 ± 17.8) was not affected by GW9662 pre-treatment. Similar results were obtained when using THP1-PMA cells (Figure 4A). However, the pretreatment of THP1-PMA cells with GW9662 was able to inhibit the FcγRII

expression induced by HoxLDL (Figure 4B). This inhibition was 71.7% compared to the group untreated with GW9662 (MFI: 97 ± 10 vs 135 ± 2 , medium 82 ± 0.6). GW9662 also inhibited the positive events (60 ± 3.0 vs 75.0 ± 0.9 ; medium 45.6 ± 5.0). These data demonstrates that CD36 expression can be modulated by modified LDLs, but the mechanism involved in this process is independent of PPAR γ . However, the increased expression of Fc γ RII induced by the modified LDL with high degree of oxidation is PPAR γ -dependent. Furthermore, an agonist of PPAR γ , rosiglitazone, at 10^{-5} mol/L significantly increased CD36 expression (MFI: 138; 63.1 % positive events) (Table 3). This effect was inhibited by 20 μ M GW9662 (63.7%), confirming that the dose of the antagonist used in our experiments was effective. At lower doses rosiglitazone was no longer effective to increase CD36 expression.

Discussion

In the intima of the arterial wall, macrophages can interact with different species of oxidized lipoproteins resulting in their activation. In this study we used two different “species” of modified LDL, a) LoxLDL, obtained by the incubation of LDL with iron, and b) HoxLDL which was obtained by incubation of LDL with copper. The results obtained by TBARS, conjugated dienes and ion-exchange chromatography clearly showed the difference in the oxidation degree of the LDL particle with an increase in negative charges in HoxLDL in comparison with LoxLDL and LDL. Consistent with our results, it was shown that copper is much more potent than iron in mediating the LDL oxidation [20]. The LoxLDL is generated when the particle undergoes antioxidant depletion, oxidation of arachidonic acid-containing phospholipids, relatively less linoleic acid oxidation, and insignificant protein modification [19]. By contrast, during the HoxLDL formation, the phospholipids groups, triacylglycerol and cholesterol ester are transformed to hydroperoxides which react with ApoB, resulting in modification and fragmentation of amino acid side chains [21, 22]. It is assumed that LoxLDL is an early stage of HoxLDL generation, while HoxLDL is a fully oxidative stage of LDL. Apparently, these different characteristics lead to different biological effects, for instance some species of oxLDL may have more atherogenic properties than others [23, 24].

Several and different biological properties of the oxLDL have been described. Although the oxidative

modification of the LDL particle has been associated with pro-inflammatory mechanisms, the oxLDL is also able to inhibit gene expression induced by LPS [25]. Furthermore, compounds present in the oxLDL, such as sphingosine 1-phosphate (S1P) and oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (oxPAPC) were shown to inhibit TLR2 and TLR4 activation respectively [26, 27]. The complexity involved in this mechanism could be explained by interactions between different compounds formed after the oxidative process with different receptors present in macrophages, endothelial and smooth muscle cells, such as scavenger receptors A (SR-A) [2], Platelet-activating Factor (PAF) receptor [28, 29] and Toll-like receptor 4 (TLR-4) [30].

The CD36 expression in monocytes/macrophages is directly related to the increased uptake of the oxidized LDL particles [7, 18]. Here, we showed that LoxLDL and HoxLDL increased the expression of CD36 independently of their degree of oxidation. Our results confirm previous data from others [31] and further demonstrates that this increase occurs independently of the differentiation status of the cell: non-differentiated or differentiated THP-1 cells. These results suggest that CD36-dependent oxLDL uptake and further process of foam cell formation can start very early during atherogenesis, before differentiation of monocytes into macrophages and fatty streaks formation, and minimal modification in the LDL particle can be responsible for initiate this process. Several stimuli and/or downstream signals have been described to be involved in the over-expression of CD36 in monocyte/macrophages, including PPAR γ activation [32]. In this study, CD36 expression induced by LoxLDL and HoxLDL, in non-differentiated and differentiated cells, was not influenced by GW9662, a PPAR γ antagonist, while increased expression of CD36 induced by the PPAR γ agonist, rosiglitazone, was inhibited by GW9662 treatment. These findings clearly suggest that oxLDL-mediated over-expression of CD36 rather involves several different mechanisms than only PPAR γ activation.

The second part of our study focused on the effects of LoxLDL and HoxLDL in the expression of Fc γ RII. Activation of Fc γ R can trigger the uptake and degradation of immune complexes, antibody-dependent cellular cytotoxicity, production of superoxide anion, and secretion of many different biologically active molecules [33]. Less clear but also potentially relevant in atherogenesis is Fc γ R-mediated clearance of immune complexes of oxLDL that could enhance foam cell formation directly, or, as suggested by studies of lipoprotein immunization in animal models,

clearance could result in protection against lesion development [15]. After the differentiation, the THP-1 cells express more Fc γ RII in comparison with non-differentiated cells. Consistent with this, the up-regulation of Fc γ RII also can be found in human macrophages compared with monocytes [34]. These results show the efficiency of the differentiation protocol used in this study. The present study shows that HoxLDL, but not LoxLDL, increases the expression of Fc γ RII in differentiated THP-1 cells. It has been shown that PPAR γ expression is increased when human monocyte cell line is differentiated with PMA [35] as well as directly by oxLDL [31]. This directed our efforts towards study the influence of PPAR γ in the HoxLDL-mediated overexpression of Fc γ RII in differentiated THP-1 cells.

Finally, we observed that pre-treatment with GW9662 significantly reduced Fc γ RII expression suggesting that macrophages express different receptors for HoxLDL and also that the Fc γ RII-dependent uptake or activation and further foam cell formation occurs late during atherogenesis, possibly in stages where highly oxidized LDL and differentiated macrophages can be found in the lesion. Previous results [36] showed that PPAR γ agonists increase the phagocytosis mediated by the Fc γ receptor in alveolar macrophages. However, to the best of our knowledge this is the first time it has been demonstrated that the oxLDL-induced overexpression of Fc γ RII is dependent on PPAR γ activation.

The expression and genetic polymorphism of Fc γ RII have been associated with autoimmune diseases such as Systemic Lupus erythematosus and rheumatoid arthritis [37]. However, little is known about this receptor in the context of atherosclerosis which have been characterized as an autoimmune and inflammatory disease [38]. Our results show the complexity of effects caused by oxidized forms of LDL on cells from the monocyte/macrophage

lineage and points out to the difficulty to outline strategies for treatment of atherosclerosis. The increased expression of Fc γ RII induced by the HoxLDL would be expected to have a beneficial effect in atherosclerosis by helping to remove the oxLDL particles complexed with antibodies. However, its expression is associated with inflammatory diseases and further *in vivo* experiments should confirm if such effect is relevant in the atherosclerosis context. In this case, treatment with PPAR γ antagonist would not be desirable since it reverses this effect of HoxLDL. Moreover, rosiglitazone treatment is also not desirable because it increases the CD36 expression promoting foam cells formation. However, the molecular details for this mechanism remain to be elucidated.

In summary, our data demonstrated that LoxLDL and HoxLDL have different stimulatory properties on monocyte/macrophages. Moreover, the stage of cell differentiation seems also to play an important role in the outcome of cell activation by the different forms of LDL. We confirmed that oxLDL increases the expression of CD36, and besides that, we showed that this is independent of PPAR γ activation. Finally, we demonstrated that HoxLDL is able to increase the expression of Fc γ RII only on differentiated cells, and that this phenomenon involves the activation of PPAR γ .

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