Electronegative LDL induces Fas and modifies gene expression in mononuclear cells

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1. ABSTRACT

Electronegative LDL (LDL(-)) is a minor modified LDL subfraction that promotes cytokine release by human mononuclear cells. The aim of the current study was to evaluate changes in gene expression induced by LDL(-) versus native LDL in lymphocytes and monocytes. Therefore, mononuclear cells were incubated with these LDL subfractions and their effects on expression in human whole genome were analyzed by gene array. Differential expression of the genes was quantified by real-time RT-PCR. LDL(-) altered the gene expression pattern, particularly of inflammatory genes. LDL(-) down-regulated CD36 and colony-stimulating factor 1 receptor (CSF1R) genes and up-regulated Fas expression and Fas protein on cellular membrane. LDL(-) seemed to promote the alterations in these genes by activation of NF-kB and inhibition of AP1 and PPARG. In conclusion, LDL(-) induced changes in gene expression in monocytes and lymphocytes. Fas up-regulation suggests a proinflammatory action; however, CSF1R and CD36 down-regulation could decrease monocyte differentiation and activation. Therefore, LDL(-) promoted not only inflammatory effects but also counteracting actions in circulating mononuclear cells.

2. INTRODUCTION

LDL(-) is an electronegative LDL subfraction that exists in plasma circulation and presents atherogenic properties. LDL(-) proportion is increased in some diseases associated with increased atherosclerotic risk, such as diabetes mellitus or familial hypercholesterolemia.(1, 2) It has also been widely reported that LDL(-) promotes inflammation,(3) cytotoxicity(4) and apoptosis(5) in endothelial cells. However, similar studies in other cell types related to atherosclerosis, such as mononuclear cells, are scarce. We recently reported the effect of LDL(-) on monocytes and lymphocytes, in which it induced cytokine expression and release, including interleukins 6 , 8 and 10 (IL6, IL8, IL10), monocyte chemoattractant protein (MCP1) and growth-related oncogenes β and γ (GROβ and GROγ). (6) Most of these molecules are inflammatory to cells; however, our data indicated that IL10 modulated the release of pro-inflammatory cytokines induced by LDL(-), thereby counteracting its atherogenic action. (6) Therefore, the behavior of LDL(-) appears to be complex and it is feasible that several of the molecules related to inflammation could be activated or inhibited by LDL(-) in mononuclear leukocytes.
LDL(−) modifies gene expression in leukocytes

This study was conducted to determine genes differentially expressed by LDL(−) in monocytes and lymphocytes. To achieve this goal, a preliminary genomic-wide screening was performed with microarrays of human whole genome for analysis of gene expression profiles, which permits the simultaneous evaluation of a wide range of genes and comparison of gene expression profiles in different biological conditions. This technique has already been used to evaluate the LDL(−) effect on endothelial cells,(7) and the authors found that LDL(−) induced several chemokine expression, thereby corroborating some of our previous results, and also the vascular cell adhesion molecule (VCAM).

In the current study, after the first approach by microarrays, a selection of the differentially-expressed genes which were considered of interest and some transcription factors or proteins related to the expression of these genes were assessed by real-time RT-PCR.

Fas (CD95) was one of the genes up-regulated by LDL(−), particularly in lymphocytes, and the protein concentration induced by LDL(−) compared to LDL(+) was also quantified. This molecule was chosen for its biological importance, since Fas is a cell surface membrane protein involved in apoptosis (8) and cytokine induction, regardless or not of apoptosis, in different types of cells, including monocytes (9) and lymphocytes. (10)

3. MATERIALS AND METHODS

3.1. Lipoprotein and cell isolation

Plasma samples from healthy normolipemic subjects (total cholesterol < 5.2 mmol/L, triglyceride < 1 mmol/L) were drawn into EDTA-containing Vacutainer tubes. Total LDL was isolated and subfractioned in native LDL (LDL(+)) and LDL(−) by preparative anion-exchange chromatography, as described. (6) In all experiments, LDL(−) proportion and the main characteristics of both LDL subfractions were similar to those previously reported. (3)

Peripheral blood of human volunteers was collected; mononuclear cells were isolated according to density, as described. (6) The appropriate number of cells for each experiment was seeded (8x10⁶ cells for gene arrays, 3x10⁶ cells for real-time RT-PCR studies and 1x10⁶ cells for ELISA experiments) and incubated with 150 µg apoB/L LDL subfractions.

3.2. CodeLink™ microarrays for gene expression profile analysis

Cells were incubated alone (blank or basal expression), with LDL(+) or LDL(−) for 4 hours. Two independent experiments were performed; in each experiment, monocytes and lymphocytes isolated from 2 volunteers were used and processed independently until microarray analysis. RNA was isolated with the RNAspin mini RNA isolation kit (GE Healthcare) and its integrity was assessed using an Agilent 2100 Bioanalyzer. Microarrays, CodeLink™ Human Whole Genome (GE Healthcare), containing approximately 35,000 gene probes derived from annotated mRNA sequences and ESTs, were used to analyze gene expression profiles. Labeling and hybridization were performed using the CodeLink™ iExpress assay reagent kit according to the manufacturer’s instructions at the Genomic core facility of the Instituto de Investigaciones Biomédicas (Madrid, Spain). After hybridization and washing, slides were scanned in an Axon GenePix Scanner and analyzed using CodeLink™ Expression Analysis Software (GE Healthcare). Data were median-normalized, log2-transformed and exported to the MeV software (TIGR, Rockville, MD) (11)

The LDL(−)/LDL(+) ratio of gene expression, with or without basal expression subtraction, was calculated. To assess only the most important changes in expression promoted by LDL(−) and limit to a reasonable number of genes, we established the following criteria to be met in all experiments and with at least one of the probes of the gene. Up-regulated genes: the expression promoted by LDL(−) was higher than blank and LDL(+) and the LDL(−)/LDL(+) ratio reached a value higher than 3 after blank subtraction and higher than 1.4 without considering blank. Down-regulated genes: blank and/or LDL(+) expression was higher than with LDL(−) and the LDL(−)/LDL(+) ratio was lower than 0.6.

3.3. Real-time RT-PCR

A selection of the genes up- or down-regulated differently by LDL(−), according to microarray experiments, and genes related to their transcription were selected for analysis by real-time RT-PCR. After incubation with LDLs for 4 and 20 hours, cells were collected and frozen. RNA isolation, reverse transcription and real-time RT-PCR were performed as described, (6) human β-actin (Applied Biosystems) was used as internal control (assay ID Hs9999903-m1).

The IL10 effect on Fas expression in mononuclear cells was evaluated by comparing the RNA copies induced by LDL(−) alone or co-incubated with IL10 (5 µg/L) (Sigma) by real-time RT-PCR.

3.4. ELISA experiments

Fas protein was quantified from cell supernatants and membrane protein extracts by ELISA kit, Diacalone (BioNova) for soluble Fas (sFas) and Fas ligand (FasL), and RayBio cell lysate (Tebu-bio) for membrane-bound Fas.

After incubation of cells with samples, culture medium supernatants were collected and frozen at −80°C. To extract membrane proteins, cells were scraped and centrifuged, and the cellular pellet was incubated with 0.1 mL of buffer (solubilization buffer: Tris-HCl 20 mM, EDTA 1mM, Triton x-100 0.2 % and protease cocktail 1%, pH=7.4) at 4°C with gentle shaking. Proteins were centrifuged and their concentration was measured by BCA method (Pierce), and protein samples (0.5 g/L) were frozen.

3.5. Statistical analysis

Data were analyzed by Wilcoxon's t-test using the SPSS statistical package. A p value <0.05 was considered significant.
LDL(-) modifies gene expression in leukocytes

Table 1. Expression of the selected genes (CD36, CSF1R and Fas) evaluated by microarrays

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>ACCN#</th>
<th>Probe name</th>
<th>Blank</th>
<th>LDL(+)</th>
<th>LDL(-)</th>
<th>Ratio LDL(-)/LDL(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MONOCYTES</td>
<td>CD36</td>
<td>NM001001547</td>
<td>GE58184</td>
<td>38.10±16.78</td>
<td>16.15±0.58</td>
<td>11.00±0.41</td>
</tr>
<tr>
<td></td>
<td>CSFIR</td>
<td>NM005211</td>
<td>GE59653</td>
<td>19.8±0.71</td>
<td>18.25±1.13</td>
<td>1.07±0.14</td>
</tr>
<tr>
<td></td>
<td>FAS</td>
<td>NM000043</td>
<td>GE59899</td>
<td>1.69±0.71</td>
<td>3.58±0.90</td>
<td>1.22 (1.52)</td>
</tr>
<tr>
<td>LYMPCYOTES</td>
<td>CD36</td>
<td>NM001001547</td>
<td>GE58184</td>
<td>4.68±5.05</td>
<td>2.39±2.21</td>
<td>0.60 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>CSFIR</td>
<td>NM005211</td>
<td>GE59653</td>
<td>4.99±5.75</td>
<td>1.56±1.76</td>
<td>1.05 ± 0.95</td>
</tr>
<tr>
<td></td>
<td>FAS</td>
<td>NM000043</td>
<td>GE59899</td>
<td>1.00±0.53</td>
<td>3.73±0.25</td>
<td>1.00 ± 0.32</td>
</tr>
</tbody>
</table>

Results are presented as mean±SD. In the column of LDL(-)/LDL(+) ratio, the ratio after blank subtraction is indicated in parenthesis, in the case of up-regulation.

Table 2. Expression of the selected genes related to transcription factors evaluated by microarrays

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>ACCN#</th>
<th>Probe name</th>
<th>Blank</th>
<th>LDL(+)</th>
<th>LDL(-)</th>
<th>Ratio LDL(-)/LDL(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MONOCYTES</td>
<td>BATF</td>
<td>NM006399</td>
<td>GE54141</td>
<td>4.61±0.18</td>
<td>19.11±19.36</td>
<td>1.26 (1.38)</td>
</tr>
<tr>
<td></td>
<td>PPARG</td>
<td>NM005037</td>
<td>GE60128</td>
<td>15.42±0.70</td>
<td>4.24±1.65</td>
<td>0.88 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>TANK</td>
<td>NM004180</td>
<td>GE61485</td>
<td>7.11±0.87</td>
<td>22.71±26.60</td>
<td>2.02 (2.48)</td>
</tr>
<tr>
<td></td>
<td>FAS</td>
<td>NM000043</td>
<td>GE59899</td>
<td>1.00±0.53</td>
<td>3.73±0.25</td>
<td>1.00 ± 0.32</td>
</tr>
<tr>
<td>LYMPCYOTES</td>
<td>BATF</td>
<td>NM006399</td>
<td>GE54141</td>
<td>10.52±0.70</td>
<td>17.92±13.30</td>
<td>1.60 (2.27)</td>
</tr>
<tr>
<td></td>
<td>PPARG</td>
<td>NM005037</td>
<td>GE60128</td>
<td>3.06±0.93</td>
<td>2.80±2.62</td>
<td>1.00 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>TANK</td>
<td>NM004180</td>
<td>GE61485</td>
<td>1.09±0.33</td>
<td>0.55±0.47</td>
<td>1.00 ± 0.32</td>
</tr>
</tbody>
</table>

Results are presented as mean±SD. In the column of LDL(-)/LDL(+) ratio, the ratio after blank subtraction is indicated in parenthesis, in the case of up-regulation.

4. RESULTS

4.1. Human whole genome array

Two independent experiments were performed, each from two subjects, for analysis of the LDL(+) and LDL(-) effect on gene expression profile in the human whole genome in mononuclear cells. A large number of genes presented an altered expression when incubated with LDL(-) compared to LDL(+) or blank. Table 1 (monocytes) and Table 2 (lymphocytes), in the on-line supplement data, show the list of up-regulated (A) or down-regulated (B) genes by LDL(-) according to the criteria explained in Methods. The mean of the 2 experiments for each condition and the LDL(-)/LDL(+) ratio are indicated. The ratio was calculated in up-regulated genes with and without subtracting blank (basal expression), and in down-regulated genes without subtraction, since basal expression was often lower than that induced by LDL(+) and LDL(-).

The genes up- or down-regulated by LDL(-) included those related to cytokines, growth factors, receptors, enzymes, membrane proteins, signaling pathway proteins, transcription factors, proteins related to metabolism and others. According to the established criteria, 19 genes were up-regulated and 48 down-regulated in monocytes, and 17 up-regulated and 19 down-regulated in lymphocytes. Three of these genes, marked and underlined in on-line Tables, were chosen for their relationship with atherosclerosis to be analyzed by real-time RT-PCR. These genes were the scavenger receptor CD36, colony-stimulating factor 1 receptor (CSF1R), and Fas, also named CD95. The expression of these selected genes in both cell types, marked in bold when fitted to the criteria of different expression, is shown in Table 1. LDL(-) inhibited CD36 transcription in lymphocytes, which was slightly lower in monocytes. CSF1R was down-regulated in both cell types by LDL(-), but only in monocytes did the ratio reach the threshold according to the criteria. Both genes presented greater transcription in monocytes, in contrast to Fas whose expression was higher in lymphocytes and up-regulated by LDL(-).

4.2. Real-time RT-PCR

Expression of the selected genes, CD36, CSF1R and Fas, was evaluated by real-time RT-PCR experiments at 4 hours (as in array experiments) and 20 hours to ascertain whether expression differed with longer incubation times.

The number of RNA copies of CD36 (A), CSF1R (B) and Fas (C) quantified by real-time RT-PCR (n=6) is shown in Figure 1. Regarding CD36, results concurred with those of the microarray, since cells treated with LDLs presented a lower CD36 expression than blank and in all cases LDL(-) presented a more inhibitory effect than LDL(+). Moreover, at 4 hours of incubation, the LDL(-) effect was greater in lymphocytes than in monocytes.

LDL(-) inhibited the number of RNA copies of CSF1R versus blank in monocytes and lymphocytes after 4 hours of incubation. LDL(-) inhibited CSF1R expression more potently than LDL(+), but only in lymphocytes at 4 hours did it decrease significantly.

Fas gene expression was up-regulated by LDL(-) compared to LDL(+), but only in lymphocytes at 4 hours’ incubation was a significant difference found. On the other hand, IL10 effect on Fas gene expression was also evaluated by real-time RT-PCR and it was observed that, at the concentration used (5µg/L), IL10 inhibited the number of RNA copies of Fas induced by LDL(+). This effect is particularly great in lymphocytes, in which IL10 inhibited Fas expression by almost 60% (Figure 2).

Some genes codifying for proteins related to transcription of CD36, CSF1R and Fas were also evaluated by real-time RT-PCR. According to microarray data, differential expression of these genes was promoted by
LDL(-) modifies gene expression in leukocytes

Figure 1. Number of RNA copies evaluated by RT-PCR of CD36, CSF1R and Fas in monocytes and lymphocytes incubated with LDL(-) vs LDL(+). A selection of the genes differently induced by LDL(-) was chosen for their relationship with inflammation, including CD36, CSF1R and Fas. Monocytes and lymphocytes were incubated with LDLs (150 mg/L), RNA was extracted after 4 or 20 hours and real-time RT-PCR was performed. Probe names (Applied Biosystems): CD36 Hs01567186-m1, CSF1R Hs00234622-m1 and Fas Hs00236330-m1. Data are the mean±SD of 6 experiments. Above the LDL(-) bar, the LDL(-)/LDL(+) ratio is indicated. Monocyte results are expressed in white bars, and lymphocyte results in gray bars. # p<0.05 LDL versus blank of cells and * p<0.05 LDL(-) versus LDL(+) .
LDL(-) modifies gene expression in leukocytes

Figure 2. Number of RNA copies evaluated by RT-PCR of Fas in monocytes and lymphocytes incubated with LDL(+), LDL(-) or LDL(-) plus IL10. Monocytes and lymphocytes were incubated with LDLs (150 mg/L) or LDL(-) plus IL10 (5 pg/L); after 4 hours, RNA was extracted and real-time RT-PCR was performed. Data are the mean±SD of 3 experiments. Monocyte results are expressed in white bars, and lymphocyte results in gray bars.

LDL(-) versus LDL(+) (Table 2), although it did not fit the criteria considered for inclusion in on-line Tables 1 or 2. The study of these genes included BATF (basic leucine zipper transcription factor, ATF-like), Fos (subunit of activator protein 1 or AP1), peroxisome proliferator-activated receptor gamma (PPARG) and TANK (TRAF-associated activator of NF-kB).

Real-time RT-PCR results of these transcription factor expression at 4 hours’ incubation (Figure 3) (n=4) concur with array data results (Table 2). Figure 3 shows the up-regulation of BATF and TANK by LDL(-) compared to LDL(+) and blank, particularly in lymphocytes. In contrast, Fos and PPARG expression was down-regulated by LDLs, but LDL(-) inhibited gene expression more than LDL(+) in both cell types. Regarding Fos, its inhibition by LDL(-) compared to LDL(+) was more remarkable in monocytes. Real-time RT-PCR data at 20 hours of incubation were similar to those found at 4 hours (data not shown).

Regarding PPARs, PPARA was also checked, and microarray and RT-PCR ruled out different induction by LDL(-) versus LDL(+), (ratio LDL(-)/LDL(+) between 0.8-1.1).

4.3. ELISA experiments

Fas gene up-regulation by LDL(-) was considered of great interest for its relationship with inflammation and apoptosis. For these reasons, sFas and cell membrane-bound Fas protein from monocytes and lymphocytes incubated with LDL(+) or LDL(-) for 4 and 20 hours were analyzed. In all cases, more protein release was observed at 20 hours of incubation than at 4 hours.

Figure 4A showed that, in lymphocytes, LDL(-) induced significant membrane Fas protein, subtracting Fas basal expression, compared to LDL(+) at 4 hours of incubation and even to a greater degree at 20 hours. Nevertheless, in monocytes no significant effect by LDL(-) was observed, at 20 hours of incubation, LDL(-) promoted values of 336.1±265.24 ng/L compared to LDL(+) which induced 286.1±233.88 ng/L. OxLDL also induced Fas on membrane at a similar level to LDL(-) or slightly greater.

Both Fas protein release from cell supernatant and FasL, which can be released in some inflammatory situations, were analyzed. In Figure 4B, it can be observed that LDL(-) did not induce the release of more sFas than LDL(+) or blank at 20 hours of incubation. The same result was obtained for sFasL: LDL(-) promoted 24.3±6.8 ng/L in monocytes and 63.9±43.3 in lymphocytes versus 25.4±4.1 and 58.9±39.9 by LDL(+).

5. DISCUSSION

LDL(-) induces the release of several inflammatory cytokines by endothelial cells and mononuclear cells. In monocytes and lymphocytes, apart from inflammatory molecules, LDL(-) also induces anti-inflammatory IL10, which seems to modulate the release of pro-inflammatory cytokines induced by LDL(-), thereby counteracting its atherogenic action.(6) Therefore, the behavior of LDL(-) appears to be complex and it is feasible that LDL(-) induces the expression of several inflammatory/anti-inflammatory molecules in mononuclear leukocytes.

Microarrays for analysis of gene expression profiles have previously been used to evaluate the effect of LDL(-) on endothelial cells.(7) We applied this technique to make a first approach to the LDL(-) effect on gene expression profile in the human whole genome in mononuclear cells. A large number of genes presented an altered expression when incubated with LDL(-) compared to LDL(+) or blank. Three of these genes were chosen for
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Figure 3. Number of RNA copies evaluated by RT-PCR of proteins related to transcription (BATF, TANK, Fos and PPARG) in monocytes and lymphocytes incubated with LDL(-) vs LDL(+). Monocytes and lymphocytes were incubated with LDLs (150 mg/L); after 4 hours, RNA was extracted and real-time RT-PCR was performed. Probe names (Applied Biosystems): BATF Hs00232390-m1, TANK Hs00370305-m1, Fos Hs00170630-m1 and PPARG Hs01115513-m1. Data are the mean±SD of 4 experiments. Above the LDL(-) bar, the LDL(-)/LDL(+) ratio is indicated. Monocyte results are expressed in white bars, and lymphocyte results in gray bars. # p<0.05 LDL versus blank of cells and * p<0.05 LDL(-) versus LDL(+).

their relationship with atherosclerosis: scavenger receptor CD36, involved in foam cell formation, colony-stimulating factor 1 receptor, CSF1R, related to monocyte differentiation, and Fas, also named CD95, involved in apoptosis and cytokine induction. These genes were analyzed by real-time RT-PCR, since the microarray technique is not a quantitative technique and the number of experiments performed was low. In general, an overall concordance was found in the up/down-regulation of the genes by both methods, arrays and real-time RT-PCR, and also in the abundance of a specific gene in one cell type versus the other. In some cases, discrepancy existed owing to the different sensitivity and detection limits of both methods and the use of different detection probes.

Previous data established that LDL(-) plays an important role in the early events of atherosclerosis since it promotes mononuclear cell attraction to endothelium by inducing chemokine secretion. However, it seems that LDL(-) would not promote monocyte differentiation to macrophages since it decreased CSF1R and CD36 expression. CSF1R mediates the biological effects of CSF1 and is required for macrophage differentiation. The inhibition of CSF1R by LDL(-) would be similar to the action of some macrophage activators, such as lipopolysaccharide (LPS) or tumor necrosis factor α (TNFα), which down-regulate the surface expression of CSF1R, thereby hindering CSF1 action(12) and avoiding an excessive cell activation. Regarding CD36, its expression in monocytes is increased by oxLDL,(13) and this contributes to its uptake which leads to foam cell formation. Nevertheless, CD36 expression has been reported to be downregulated in inflammation(13) by several inflammatory mediators, such as LPS. Therefore, down-regulated CD36 expression by LDL(-) could reflect a proinflammatory milieu around monocytes/lymphocytes promoted by this LDL subfraction.

Fas is expressed at low but detectable levels on the surface of resting cells, but expression is enhanced after lymphocyte activation(14) and soluble Fas levels also rise in inflammatory states.(15) Interestingly, LDL(-) induced Fas gene expression and also membrane-bound Fas protein on the lymphocyte surface, but did not promote a greater Fas release, behavior that differs from oxidized LDL. It is reported that when Fas binds to FasL, an intracellular signal is activated and apoptosis is triggered.(8) According to the microarray results, FasL expression was not increased by LDL(-), but an increased Fas level would lead to greater activation of the Fas/FasL system or make cells more susceptible to this pathway. Whether this activation could be related to the increased apoptotic activity of LDL(-) in endothelial cells remains to be established.(5) On the other hand, it has been reported that Fas is also involved in

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Figure 4. Evaluation of Fas protein induction in cells after incubation with 150 mg/L of LDL(+) or LDL(-). Figure 4A. Membrane Fas protein evaluated by ELISA of protein membrane extracts from lymphocytes incubated with LDL(+) or LDL(-) for 4 or 20 hours. Values are expressed in ng/L after blank subtraction and are the mean±SD of 6 experiments. Above the LDL(-) bar, the LDL(-)/LDL(+) ratio is indicated. * p<0.05 LDL(-) versus LDL(+) . Figure 4B. Soluble Fas protein released by monocytes and lymphocytes after incubation with LDL(+) or LDL(-) for 20 hours, evaluated by ELISA. Monocyte results are expressed in white bars, and lymphocyte results in gray bars. Results are expressed in ng/L and are the mean±SD of 4 experiments.

cytokine induction, regardless or not of apoptosis, in several cell types, including monocytes,(9) macrophage-like cell lines (16) and lymphocytes.(10) Therefore, it could be hypothesized that LDL(-) could induce cytokine release in part by Fas activation. On the other hand, although there is discrepancy regarding the effect of IL10 on Fas expression,(17, 18) IL10 in our studies inhibited the number of RNA copies of Fas induced by LDL(-). Concerning Fas upregulation by LDL(-), more experiments should be performed to delve further into the putative role of Fas and the mechanisms activated by LDL(-).

Regarding studies of transcription factors involved in LDL(-) effect, to consider gene expression of the transcription factor itself is limited since other regulatory mechanisms, such as phosphorylation or nuclear translocation, are usually required in the activation of a transcription factor. Gene transcription is a complex system and several proteins are involved in the activation/inhibition of a transcription factor. Therefore, some genes codifying for proteins related to transcription of CD36, CSF1R and Fas were also evaluated by real-time RT-PCR.

AP1 plays an important role in CSF1R gene expression.(19) In mononuclear leukocytes, the decrease in AP1 promoted by LDL(-) could be involved in CSF1R inhibition. The results suggest that LDL(-) did not induce AP1, since LDL(-) caused Fos inhibition and BATF induction. Fos dimerizes with Jun to form AP1, and BATF belongs to the AP1/ATF superfamily and forms transcriptionally inhibitory heterodimers with Jun proteins.(20) These results disagree with previous data in endothelial cells, in which an AP1 activation by LDL(-) was observed.(21) However, the LDL(-) effect probably differs among different cell types.
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In the current work, a decreased PPARG expression promoted by LDL(-) was observed. PPARG, a negative regulator of proinflammatory gene expression in monocytes and macrophages,(22) is induced by oxLDL and other modified LDL(13) and is involved in CD36 up-regulation and monocyte differentiation.(23) PPARG down-regulation by LDL(-), together with inhibition of CSF1R and CD36, suggests that LDL(-) would not promote monocyte differentiation. On the other hand, PPARG presents antiinflammatory properties, in part by antagonizing the activity of some transcription factors such as NF-kB.(23) Therefore, PPARG decreased expression by LDL(-) could lead to NF-kB inflammatory pathway activation.

NF-kB develops an important role in the Fas/FasL system signaling pathway(24) and in Fas promoter activation.(25) Moreover, NF-kB has been reported to be involved in the inflammatory effect of LDL(-) on endothelial cells.(21) These data appear to support an activation of NF-kB by LDL(-) in mononuclear cells. Furthermore, TANK expression, an NF-kB activator,(26) was increased by LDL(-), particularly in lymphocytes in which Fas gene up-regulation was greater than in monocytes. Nevertheless, although some data exist suggesting an involvement of NF-kB in Fas activation, more studies should be conducted to ascertain the mechanisms by which LDL(-) leads to the expression of Fas and other inflammatory molecules in mononuclear cells.

In conclusion, the current study analyzed the changes in gene expression induced by LDL(-) in monocytes and lymphocytes. Fas up-regulation suggests a proinflammatory action; however, Fas expression can be modulated by IL10, which is also released by the effect of LDL(-). CSF1R and CD36 down-regulation by LDL(-) suggests decreased monocyte differentiation and activation, which would represent an antiinflammatory effect. As a consequence, LDL(-) promoted not only inflammatory effects but also counteracting actions in circulating mononuclear cells. Nevertheless, further studies will be required to define the complex role of LDL(-) in mononuclear leukocytes.

6. ACKNOWLEDGMENTS

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**Abbreviations:**

AP1: activator protein 1; BATF: basic leucine zipper transcription factor, ATF-like; CSF1R: colony-stimulating factor 1 receptor; FasL: Fas ligand; sFas: soluble Fas; GRO: growth-related oncogene; IL: interleukin; LDL(-): electronegative low density lipoprotein; LPS: lipopolysaccharide; TNFalpha: tumor necrosis factor alpha; MCP1: monocyte chemoattractant protein; NF-kB: nuclear factor kappa B; RT-PCR: real-time polymerase chain reaction; PPAR: peroxisome proliferator-activated receptor; TANK: TRAF-associated activator of NF-kB; VCA: vascular cell adhesion molecule

**Key Words:** LDL(-), Monocytes, Lymphocytes, Gene Expression Array, real-time RT-PCR, Fas, CD36, CSF1R, Transcription Factors

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