**Immunomodulating effect of low density lipoprotein on human monocytes**

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**SUMMARY**

Low density lipoprotein (LDL) isolated from sera of healthy volunteers in 50 μg protein/ml concentration induced an early adenylate cyclase activation in human monocytes followed by elevation of cGMP level. In addition, a rapid 45Ca²⁺ influx was also detected on addition of 25–100 μg protein/ml concentrations. The monocyte activating effect of LDL under in vitro circumstances was characterized by an enhanced O₂ consumption, H₂O₂ generation and by the increased release of lysosomal enzymes such as β-glucuronidase and elastase like protease (ELP). On the other hand, LDL diminished markedly the Fcy receptor (FcyR) mediated rosette formation, phagocytosis and the antibody dependent cellular cytotoxicity (ADCC) of monocytes without a significant decrease in the IgG binding capability of cells. High levels of serum LDL may play a significant role in the arterial wall injury by elastase like protease as well as biologically active oxygen species released from monocytes of patients suffering from arteriosclerosis.

**Keywords** low density lipoprotein monocytes respiratory burst Fcy receptors

**INTRODUCTION**

A close correlation probably exists between the lipid metabolism and the immune system (Hui & Harmony, 1980; Cuthbert & Lipsky, 1984; Antonaci et al., 1984). On one hand, the immune processes affect the lipid metabolism as follows:

(a) The so called 'scavenger-receptors' located on the surface of macrophages are responsible for the disappearance of the low density lipoproteins (LDL) from the circulation (Brown & Goldstein, 1983; Traber, Kallman & Kayden, 1983; van der Schroeff et al., 1983).

(b) Macrophages having been 'activated' through the scavenger receptors secrete apoprotein E being a prerequisite of the high density lipoprotein (HDL) generation (Brown & Goldstein, 1983; Takemura & Werb, 1984).

(c) Some immunostimulants (lymphokines) inhibit the secretion of apoprotein E by the macrophages (Takemura & Werb, 1984; Driscoll & Getz, 1984).

(d) The uptake and storage of LDL by the macrophages leads to the formation of the so called 'foam-cells' in arterio-sclerosis, taking part in the formation of the atheromatotic plaques (Agel et al., 1984).

(e) A great amount of H₂O₂ and other biologically active oxygen species (BAOS) as well as intralysosomal elastase are liberated from the circulating phagocytic cells, and these components

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may play a causal role in the early endothelial damages (Schwartz, 1983; Ager & Gordon, 1984; Tsan et al., 1985).

On the other hand, the serum lipoproteins undoubtedly affect the immunological events through the specific receptors localized on the surface of lymphocytes (Bilheimer et al., 1978) and monocytes (Wang-Iverson et al., 1985). For example, the LDL is able to suppress the lectin-induced lymphoblast transformation (Morse et al., 1977; Waelti, Glueck & Hess, 1981), and the anti-tumour cytotoxicity of both lymphocytes and macrophages (Hibbs, Chapman & Weinberg, 1980; Yamazaki et al., 1977; Antonaci et al., 1984).

The present work was aimed to clarify whether the human monocytes circulating in the blood and possessing specific LDL receptors can be activated in vitro by LDL.

The monocytes were exposed to LDL, and the levels of cAMP, cGMP, the uptake of $^{45}$Ca, as well as the intensity of the oxidative processes and release of lysosomal enzymes were determined. According to our results, the monocytes were activated by LDL, whereas the Fcγ receptor mediated functions (rosette-formation, phagocytosis and ADCC) were inhibited after a 60 min incubation.

**MATERIALS AND METHODS**

**Monocytes.** Monocytes were separated from heparinized venous blood of healthy young (20–25 year old) male subjects by Ficol-Hypaque gradient centrifugation and by subsequent purification according to the method of Kumagai et al. (1978) on fetal calf serum treated plastic petri dishes.

**Isolation of LDL.** Isolation of LDL was carried out by saline density gradient ultracentrifugation in a Janetzky VAC 602 type ultracentrifuge (DDR) in 6 x 85 ml fixed angle rotor as previously described (Cornwell et al., 1961; Szondy et al., 1983). Sera for LDL separation were pooled from healthy male volunteers. LDL concentrations were expressed as μg protein/ml determined by method of Lowry et al. (1951).

**Experimental conditions.** The cell suspension as well as solutions were prepared in RPMI-1640 medium (Gibco, Grand Island, NY, USA). For the enzyme assays isotonic Hanks' balanced salt solution (HBSS) was applied. The incubations were carried out in an ASSAB CO2 incubator (CO2 5%, air 95%, humidity 95%) at 37°C.

**cAMP and cGMP determination.** Intracellular cAMP and cGMP levels in monocytes were determined at the beginning and in the 15th, 30th, 60th and 120th min of incubation. The incubations were performed in RPMI-1640 and the applied cell suspension contained 2 x 10⁶ monocytes/ml. For cyclic nucleotide determination, cells were prepared as described by Stabinsky et al. (1980). The determinations were made according to the instructions enclosed in the radioimmunoassay kit (Amersham, UK).

$^{45}$Ca²⁺ influx. $^{45}$Ca²⁺ influx was measured on monocyte monolayers prepared in Nunclon petri dishes (N-1420) with 30 mm diameter. Each monolayer contained about 10⁶ cells per dishes. All incubations and dilutions were made in HBSS containing 140 mM Na⁺, 5 mM K⁺, 1.4 mM Ca²⁺ and 1.2 mM Mg²⁺. Before determinations monocyte-monolayers were incubated for 15–20 min in HBSS for pre-equilibration. To each monolayer 1.0 ml HBSS containing LDL and 1.0 μCi $^{45}$CaCl₂ (Isotope Institute of the Hungarian Academy of Sciences, 96-37 GBq/h) was added and after 5, 15 and 30 min incubation the monolayers were washed rigorously with ice-cold buffer containing 4 mM LaCl₃. Finally, monolayers were dried by vacuum-aspiration and lysed with 0.2% of sodium dodecyl sulphate (SDS) solution. After 60 min the appropriate dilution was prepared with distilled water and 50 μl aliquots were placed in a scintillation cocktail.

**Oxygen consumption.** Oxygen consumption was measured with a Clark type oxygen electrode in 3 ml suspension containing 2 x 10⁶ cells under constant stirring at 37°C. The preincubation time to achieve equilibration with air was 30 min. The method was carried out as described by Tanabe, Kobayashi and Usui (1983). To determine the non-mitochondrial respiration of monocytes the experiments were performed also in the presence of 360 μm antimycin A.

**Determination of H₂O₂ production.** Measurement of H₂O₂ production was carried out according to the method of Pick and Keisari (1980), which is based on the H₂O₂ mediated and horseradish
LDL-induced changes in monocyte functions

peroxidase dependent oxidation of phenol red. For the determination 2 x 10^6 monocytes were used in the presence of different concentrations of LDL. The determinations were carried out also in the presence of 360 μm antimycin A (Sigma Chemical Co., St Louis, MO, USA).

Determination of elastase activity. Elastase activity was measured according to the method of Hornebeck et al. (1983) with slight modification. Briefly, monocytes were incubated for 60 min with 25, 50 and 100 μg/ml concentrations of LDL. After centrifugation to 1.0 ml of the 1.4 dilution of supernatants 5 μg N-succinyl (L-alanyl) p-nitroanilide was added in 20 μl volume. After 15 h incubation the change in absorbance at 410 nm was determined.

Lactate dehydrogenase (LDH) assay. LDH assay was carried out according to the method of Dioguardi et al. (1963) with slight modification.

Determination of β-glucuronidase activity. β-glucuronidase activity was assessed according to the method of Brittinger et al. (1968) using phenolphthalein β-glucuronide as substrate. The amounts of phenolphthaleine were determined spectrophotometrically after 18 h incubation.

Preparation of ^51^Cr-human red blood cells. Type O, Rh-positive human erythrocytes (10^9 cells) were incubated for 120 min with 100 μCi sodium chromate (Radioisotope Centre, Swierk, Poland) at 37°C. After washing the final cell suspension was made up in RPMI-1640.

Measurement of phagocytosis through Fcγ receptors. The method has been described elsewhere (Fülöp et al., 1985). Briefly, monocyte monolayers containing approximately 7 x 10^6 cells were prepared in petri dishes (Nunclon, N-1420). The accurate cell density of monolayers was determined microscopically. The monolayers were incubated for 60 min with different concentrations of LDL, and after washing, with anti-D human IgG-coated (National Institute of Haematology and Blood Transfusion) ^51^Cr-human red blood cells (approximately 5 x 10^7) suspended in 1.0 ml of RPMI-1640, were added to the monocytes. After 60 min incubation at 37°C, tracer erythrocytes involved in rosette-formation were lysed with distilled water, whereas engulfed erythrocytes were removed together with monocytes using 0.2% SDS. On the basis of the specific activity of labelled human red blood cells the number of attached and/or engulfed erythrocytes per monocyte was calculated.

Measurement of monocyte-ADCC activity. The method was carried out as in the case of phagocytosis, however, the monolayers were incubated with 7, 14, 28 and 56 x 10^6 tracer erythrocytes. After 4 h incubation the radioactivity of the supernatants was determined. After subtraction of spontaneous lysis the number of erythrocytes lysed by monocytes were calculated.

Binding of ^125^I-IgG. The method described earlier (Dezső & Föris, 1981) was used with slight modifications. Monocyte monolayers containing about 7 x 10^6 cells were prepared in petri dishes and incubated for 120 min with different concentrations of LDL before applying the labelled IgG (250 μg/ml protein; 310 Bq/μg). After washing, the accurate cell count of the monolayers were determined microscopically. The cells were lysed with 0.2% SDS and the radioactivities were assessed in a Gamma NK-350 scintillation counter.

^125^I-IgG preparation. IgG was separated from pooled sera of healthy subjects according to the method of Baumstark, Lattin and Bardawill (1964). The IgG was labelled with Bolton-Hunter reagent (Bolton & Hunter, 1973) and purified on a Bio-Gel P-10 column. Unlabelled identical IgG was added to reach the desired specific activity.

RESULTS

In the first series of experiments, the effect of 50 μg/ml LDL was studied on the cyclic nucleotide levels (Fig. 1). The main finding was that cAMP increased quickly from the beginning, and this was followed by an increase of cGMP level after 30 min of incubation. On the other hand, 25, 50 or 100 μg/ml LDL resulted in a quick initial influx of ^45^Ca into the monocytes (Fig. 2). The comparison of the data of Figs 1 and 2 show unanimously that the effects of the LDL-receptor complex secured through the secondary messengers.

The activating influence of LDL was further supported by the observation of an increased O₂ consumption and H₂O₂ generation as shown in Table 1.

The O₂ consumption was also detected in the presence of antimycin A which blocked the
Fig. 1. LDL-induced alteration in cyclic nucleotide level in human monocytes. In experiments 50 µg protein/ml LDL was applied. (●) cAMP; (O) cGMP. Each point represents the mean ± s.e.m. of four experiments.

Fig. 2. The effect of LDL on the 45Ca2+ uptake by human monocyte monolayers. Each point represents the mean of five experiments ± s.e.m. As controls both spontaneous and Ca ionophore A 23187 induced 45Ca2+ uptake by monocytes is demonstrated.

Table 1. Stimulating effect of LDL on the oxidative processes in monocytes

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>O2 consumption nmol/30 min/10^6 cells</th>
<th>Produced H2O2 nmol/30 min/10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.65 ± 1.16</td>
<td>1.45 ± 0.16</td>
</tr>
<tr>
<td>+ AA</td>
<td>10.28 ± 0.67</td>
<td>1.57 ± 0.18</td>
</tr>
<tr>
<td>LDL 25 µg/ml</td>
<td>81.95 ± 8.48*</td>
<td>8.08 ± 0.97*</td>
</tr>
<tr>
<td>+ AA</td>
<td>34.66 ± 1.15†</td>
<td>7.93 ± 0.52†</td>
</tr>
<tr>
<td>LDL 50 µg/ml</td>
<td>78.05 ± 2.34*</td>
<td>7.61 ± 0.63*</td>
</tr>
<tr>
<td>+ AA</td>
<td>28.70 ± 1.74†</td>
<td>7.48 ± 1.11†</td>
</tr>
<tr>
<td>LDL 100 µg/ml</td>
<td>97.86 ± 3.25*</td>
<td>4.98 ± 0.36*</td>
</tr>
<tr>
<td>+ AA</td>
<td>19.63 ± 0.86†</td>
<td>5.36 ± 0.23†</td>
</tr>
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</table>

* Data differ significantly from the control values (P<0.01).
† Data differ significantly from the values of treated control groups (P<0.01). Each value represent the mean ± s.e.m. of three experiments.
AA Antimycin A.
Table 2. The LDL induced enzyme release from monocytes

<table>
<thead>
<tr>
<th>Incubation (60 min)</th>
<th>Lactate dehydrogenase iu/10^6 cells</th>
<th>β-glucuronidase nmol phenolphthaleine/19 h/10^6 cells</th>
<th>nmol pNA/18 h/10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total activity</td>
<td>6.2 ± 0.08</td>
<td>158.3 ± 6.8</td>
<td>228.6 ± 8.7</td>
</tr>
<tr>
<td>Control (medium)</td>
<td>&lt;0.3</td>
<td>23.8 ± 0.8</td>
<td>18.8 ± 0.9</td>
</tr>
<tr>
<td>LDL 25 μg/ml</td>
<td>0.52 ± 0.03</td>
<td>60.2 ± 2.5*</td>
<td>92.1 ± 1.2*</td>
</tr>
<tr>
<td>LDL 50 μg/ml</td>
<td>0.64 ± 0.03</td>
<td>81.8 ± 2.1*</td>
<td>114.5 ± 1.1*</td>
</tr>
<tr>
<td>LDL 100 μg/ml</td>
<td>0.51 ± 0.05</td>
<td>78.3 ± 4.1*</td>
<td>104.5 ± 0.9*</td>
</tr>
</tbody>
</table>

* Data differ significantly from the control values (P < 0.001). Each value represents mean ± s.e.m. of three experiments.

Table 3. The LDL induced inhibition of Fcγ receptor mediated rosette-formation, phagocytosis and antibody dependent cellular cytotoxicity of monocytes

<table>
<thead>
<tr>
<th>Preincubation (60 min)</th>
<th>Number of ⁵¹Cr-HRBC/monocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Involved in EA rosettes</td>
</tr>
<tr>
<td>Control</td>
<td>4.58 ± 0.18</td>
</tr>
<tr>
<td>LDL 25 μg/ml</td>
<td>3.82 ± 0.17</td>
</tr>
<tr>
<td>LDL 50 μg/ml</td>
<td>1.48 ± 0.11*</td>
</tr>
<tr>
<td>LDL 100 μg/ml</td>
<td>1.86 ± 0.11*</td>
</tr>
</tbody>
</table>

* Data differ significantly from the control values (P < 0.001). Each value represents mean ± s.e.m. of three experiments.

Table 4. The effect of LDL after 120 min incubation on the ¹²⁵I-IgG binding capacity of monocyte-monolayers

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>¹²⁵I-IgG bound (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.0 ± 7.8</td>
</tr>
<tr>
<td>LDL 25 μg/ml</td>
<td>91.2 ± 8.1</td>
</tr>
<tr>
<td>LDL 50 μg/ml</td>
<td>86.4 ± 7.0</td>
</tr>
<tr>
<td>LDL 100 μg/ml</td>
<td>78.8 ± 7.1*</td>
</tr>
</tbody>
</table>

* Data differ significantly from the control values (P < 0.01). Each value represents mean ± s.e.m. of three experiments.

mitochondrial respiration. Our data show that antimycin A markedly diminished O₂ consumption, whereas it had no effect upon stimulated H₂O₂ generation. Furthermore, O₂ consumption was increased by LDL, also in the presence of antimycin A, suggesting that both respiratory burst and mitochondrial respiration are stimulated through LDL receptors.
Our further experiments revealed the effects of LDL on the lysosomal enzyme release. The data of Table 2 show that a significant release of \( \beta \)-glucuronidase and elastase occurs after 60 min incubation period. The release of the cytoplasmic LDH was also studied as a control test; this, however, did not exceed 10% of the total LDH activity of the monocytes.

In the second part of our experiments, the Fc\( \gamma \) receptor-dependent functions of monocytes were studied subsequent to 60 min incubation with LDL. Our results indicate that LDL-treatment dramatically reduced rosette-formation, phagocytosis and ADCC activity (Table 3). The \( ^{125} \)I-IgG binding capacity of the monocyte monolayers remained practically unaltered, apart from a slight decrease observed when 100 \( \mu \)g/ml dose was used (Table 4).

**DISCUSSION**

The LDL-induced alterations in the monocyte functions cannot be attributed to the cytotoxic effects of the LDL, as suggested by some authors (Evensen, Galdal & Nilsen, 1983; Morel, Hessler & Chisom, 1983; van Hinsbergh, 1984), since in our experimental system the applied concentrations of the LDL resulted in practically no increase of the LDH release.

Furthermore, pyrogen (endotoxin) contamination of the applied LDL could not be involved in the reaction, because endotoxin in \( 10^{-6} \)–\( 10^{-7} \) \( \mu \)g/ml concentration was not able to produce within 60 min ‘respiratory burst’ or suppression of Fc\( \gamma \)R activity (unpublished data).

The most intriguing question regarding the monocyte activating effect of LDL is whether this effect is realized through the specific receptors. Our experiments with \( ^{125} \)I-LDL (unpublished data), in line with those of Goldstein and Brown (1974) revealed that 20–25% of the intracellular LDL degradation (if LDL is applied in 25–100 \( \mu \)g/ml concentration range) takes place through non-specific pathways. This type of non-specific pathway observed \textit{in vitro} involves an endocytosis of LDL as in the case of liposomes of high cholesterol content (Hibbs, Chapman & Weinberg, 1980). It may play a role \textit{in vivo} presumably only, if the specific LDL receptors are damaged or missing, as in familiar hypercholesterinemas, and an increased serum cholesterol level is also present (Goldstein & Brown, 1974; Bilheimer et al., 1978). The two different ways of LDL uptake represent quite different consequences in the intracellular space: namely, the incorporation through the specific receptors decreases the 3-Hydroxy-methyl-glutaryl Coenzyme A (HMG-CoA) activity being responsible for the cholesterol synthesis, inhibits the \textit{de novo} synthesis of the LDL receptors, and increases the cholesterol esterification (Brown & Goldstein, 1979). On the other hand, the fraction of LDL incorporated through the non-specific pathway (called also ‘low affinity’ uptake by some authors) (Goldstein & Brown, 1974; Johnston, Robson & Melnykovych, 1983), becomes decomposed and increases the intracellular cholesterol content at least temporarily, since it cannot activate the regulatory mechanisms depending on the specific receptors.

In our experiment, a considerable part of the LDL is certainly taken up by the so-called non-specific pathway into the monocytes, whereas another part of it binds to the specific receptors and induces all the processes characterizing the activated state, like the ‘respiratory burst’ the increase of mitochondrial respiration and the release of lysosomal enzymes. It should be noted that if the above processes are regulated really by the specific LDL receptors, the signalization system of the LDL receptors must be completely the same as that activated by the Fc\( \gamma \) receptors during phagocytosis (Ögmundsdottir & Weir, 1978). A transient increase of the cAMP level followed by a progressive increase of the cGMP concentration, as well as an initial high Ca\(^{2+}\)-influx were observed also during the first period of phagocytosis.

The suppression of the Fc-receptor-mediated monocyte functions (rosette formation, phagocytosis, ADCC) in the 60th minute of the LDL activation may be a natural consequence of some membrane alterations following the cellular activation, as well as of the non-specific endocytosis of the LDL. In both cases one has to take into account an increase in the cholesterol to phospholipid ratio in the cell membrane (Hibbs \textit{et al}., 1980). The membrane rigidity increased in this way results in an inhibition of the lateral mobility of the Fc receptors being related to the receptor-aggregation (Traill & Wick, 1984). This may be one of the explanations why in our experiments the \( ^{125} \)I-IgG binding of the monocytes was only slightly altered, meanwhile the phagocytosis and the ADCC significantly decreased.
Although it is still too early to draw any definitive conclusion from our results, due to the in vitro character of the experiments, we believe that the pathologically altered high LDL levels may play a role in the release of biologically active oxygen species and elastase from the phagocytic cells, which may be harmful for the blood vessel wall, and in the suppression of the phagocytic system.

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REFERENCES


PICK, E. & KEISARI, Y. (1981) Superoxide anion and


