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Phosphatidylinositol turnover in human monocyte-derived macrophages by native and acetyl LDL

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Monocyte-derived macrophages play a key role in pathogenesis of atherosclerosis. However, the mechanism of activating macrophages in atheromatous lesions has not been fully investigated. This report describes the contribution of phosphatidylinositol turnover to the uptake of low density lipoproteins (LDL) by macrophages. Both native and acetyl LDL stimulated inositol 1,4,5-triphosphate (IP_3) formation in a dose-dependent manner at concentrations of 0–70 μg/ml. The potency of IP_3 formation by acetyl LDL (0.44 nmol/mg protein) was 2-fold higher than that by native LDL (0.21 nmol/mg protein). Time course studies showed that a maximal effect of IP_3 formation by acetyl LDL at concentrations of 30 μg/ml was observed at 3 min. Longer incubation diminished IP_3 formation. Oxidized LDL also stimulated IP_3 formation with a similar efficiency to acetyl LDL. It was indicated that chemically modified LDL which were taken up through the scavenger receptor pathway activated the macrophages by mediating the phosphatidylinositol hydrolysis and IP_3 formation.

LDL; Macrophage; Phosphatidylinositol turnover

1. INTRODUCTION

It has been well established that activated macrophages are essential in many pathological conditions, such as wound repair, inflammation and immune response [1]. Monocyte-derived macrophages are identified in atheromatous lesions and considered to play a key role in pathogenesis of atherosclerosis [2]. As well as other pathological conditions, macrophages in atheromatous lesions might be activated. The process of lipid accumulation in macrophages has been well investigated and two receptor pathways of lipid accumulation are proposed. One is LDL receptor, which binds to native LDL and is taken up into macrophages [3]. The other is scavenger receptor, which binds to chemically modified LDL, such as acetyl LDL, before internalization [4]. The properties of scavenger receptor are the large number of the receptors [5], high internalization index [5], and lack of control by cell cholesterol content [6,7]. The characterization of LDL receptor and scavenger receptor made the mechanism of lipid uptake by macrophages clear, however, the mechanism of activating the macrophages remains to be clarified.

IP_3 is well known as a second messenger produced by a receptor-mediated inositol phospholipid hydrolysis and plays a central role in intracellular signal transduction by releasing calcium from the endoplasmic reticulum store [8,9]. This communication describes the contribution of phosphatidylinositol turnover to native and chemically modified LDL uptake by human monocyte-derived macrophages.

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Abbreviations: LDL, low density lipoprotein(s); IP_3, inositol 1,4,5-triphosphate; TCA, trichloroacetic acid

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2. MATERIALS AND METHODS

2.1. *Macrophage preparation*

Human monocyte-derived macrophages were separated as described by Bøyum [10]. Briefly, heparinized venous blood from a normolipemic healthy volunteer was obtained after overnight fasting. The lymphocyte fraction was separated by Ficoll-Paque (Pharmacia) density-gradient centrifugation from the blood. The mononuclear cell layer was washed three times with calcium-free phosphate-buffered saline (PBS) to remove platelets. The platelet-free mononuclear cells were suspended in RPMI 1640 containing sterile 10% autologous serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at the final cell concentration of 10^7/ml approx., seeded on 35 mm culture dishes and incubated at 37°C for 2 h. The non-adherent cells were removed by rinsing the dish with RPMI 1640. The adherent cells were cultured for 5 days at 37°C in CO_2 incubator, changing the medium every 2 days. On the 5th day of culture, the medium was changed to serum-free RPMI 1640 and incubated for 2 days.

2.2. *LDL preparation*

Native LDL (d = 1.020–1.060 g/ml) was isolated by sequential ultracentrifugation by the method of Havel et al. [11]. Protein was determined as described by Bradford [12], utilizing bovine serum albumin as the standard. Acetyl LDL was prepared as described by Basu et al. [13]. Copper-oxidized LDL was prepared by incubating native LDL with 5 μM $CuSO_4$ in calcium-free PBS at 37°C for 24 h [14].

2.3. *IP_3 assay*

A human monocyte-derived macrophage culture, which was incubated in RPMI 1640 containing 10% autologous serum for 5 days and in serum-free RPMI 1640 for 2 days, was rinsed with warm balanced salt solution (BSS) consisting of 130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$ and 20 mM Hepes buffer at pH 7.4, and incubated for 15 min in warm BSS containing 10 mM glucose and 10 mM LiCl. The cells were incubated with the indicated doses of each LDL for 0–10 min at 37°C. The reaction was terminated by placing the dishes on ice and changing the medium immediately to ice-cold 15% TCA. The culture dishes were kept on ice for 30 min and the cells were scraped. The extract was transferred to the test tube and TCA was removed by adding diethyl ether. This extract was neutralized by 0.1 N NaOH. IP_3 was assayed using an IP_3 assay kit (Amersham).

2.4. *Agarose gel electrophoresis*

Agarose gel electrophoresis was performed in barbital buffer at pH 8.6 in agarose gel as described by Noble [15], using an electrophoresis system (Ciba Corning).

3. RESULTS

Agarose gel electrophoresis revealed that chemically modified LDL were negatively charged in various degrees. As shown in fig.1, both acetyl and oxidized LDL moved to the anode side faster than native LDL. The degree of migration of

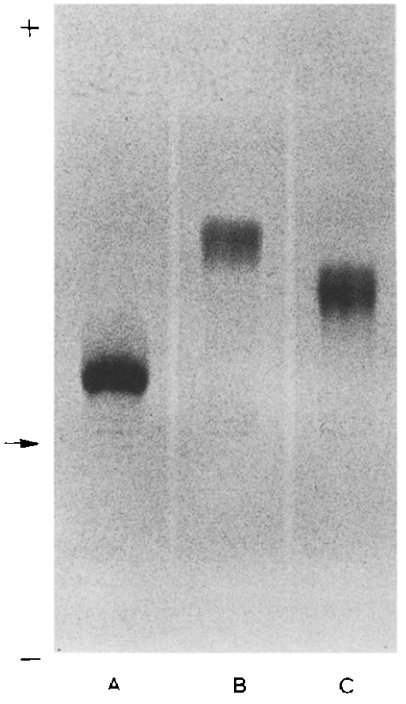

Fig.1. Electrophoretic mobility of native and chemically modified LDL preparations. 10 μg of protein of each LDL preparation was subjected to electrophoresis in agarose at pH 8.6, after which the gel was stained with Fat Red 7B. The arrow indicates the origin. (A) Native LDL; (B) acetyl LDL; (C) oxidized LDL.

chemically modified LDL varied from each other: acetyl LDL migrated, to some extent, faster than oxidized LDL.

Fig.2 shows the time course study of IP_3 formation in human monocyte-derived macrophages stimulated by 30 μg/ml of native and acetyl LDL. Acetyl LDL induced rapid IP_3 formation in human monocyte-derived macrophages, and the maximal effect was observed at 3 min. Longer incubation diminished IP_3 formation probably due to its faster degradation than its formation. Native LDL of this dose showed only slight IP_3 formation. To confirm the maximal effect of acetyl LDL on IP_3 formation and to find out whether native LDL stimulates IP_3 formation at higher doses, we investigated IP_3 formation in human monocyte-derived macrophages stimulated by various doses of native and acetyl LDL. As shown in fig.3, both native and acetyl LDL stimulated IP_3 formation in a dose-dependent manner at concentrations of 0–70 μg/ml. The amount of IP_3 in human

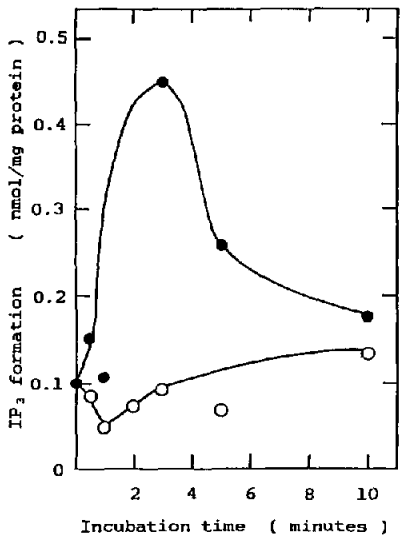

Fig.2. Time course study for IP_3 formation in human monocyte-derived macrophages. 30 μg/ml of LDL was added to macrophage monolayers (20–40 μg cell protein/dish). The reaction was terminated by ice-cold 15% TCA. IP_3 was assayed as described in section 2. (●) Acetyl LDL; (○) native LDL.

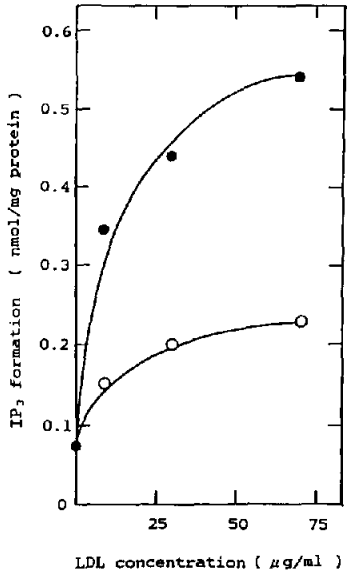

Fig.3. IP_3 formation in human monocyte-derived macrophages stimulated by various concentrations of acetyl and native LDL. Various concentrations of LDL were added to macrophage monolayers (20–40 μg cell protein/dish). After 3 min incubation, the reaction was terminated by ice-cold 15% TCA. IP_3 was assayed as described in section 2. (●) Acetyl LDL; (○) native LDL.

Table 1

IP_3 formation in macrophages stimulated by various LDL

	IP_3 formation	
	nmol/mg protein	%
Acetyl LDL	0.44	100
Oxidized LDL	0.41	93.1
Native LDL	0.20	45.4
None	0.079	17.9

30 μg/ml of various LDL were added to each culture dish containing human monocyte-derived macrophages (20–40 μg cell protein/dish), incubated for 3 min. The reaction was terminated by ice-cold 15% TCA. IP_3 was assayed as described in section 2

monocyte-derived macrophages stimulated by acetyl LDL was 2-fold greater than that by native LDL.

Table 1 shows the potency of each LDL to produce IP_3 in human monocyte-derived macrophages. IP_3 formation was evaluated under the condition of 30 μg/ml of each LDL and 3 min of incubation. This condition was considered to produce a nearly half-maximal effect on IP_3 formation. Acetyl, oxidized and native LDL induced 0.44, 0.41 and 0.20 nmol/mg protein, respectively. Oxidized LDL stimulated IP_3 formation with a similar efficiency to acetyl LDL (93.1% of that produced by acetyl LDL).

4. DISCUSSION

In this study, we showed that both native and acetyl LDL induced IP_3 formation in human monocyte-derived macrophages. However, IP_3 formation stimulated by acetyl LDL was 2-fold greater than that by native LDL. The potency of LDL to stimulate IP_3 formation seemed to be proportional to the degree of the negative charge of each LDL (fig.1 and table 1). Block et al. [16] described that native LDL, at concentrations high enough for receptor binding but not high enough to saturate the receptor, induced the activation of phosphatidylinositol turnover in human platelets, fibroblasts, lymphocytes, and rat smooth muscle cells. It is well known that phosphatidylinositol hydrolysis is one of the important mechanisms of intracellular signal transduction and cell activation, which plays a crucial role in growth control

and secretion [8,9]. IP_3 formation in human monocyte-derived macrophages stimulated by native and acetyl LDL indicates that these LDL activate macrophages. Beg et al. [17] reported that 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), a key enzyme in cholesterol synthesis, was regulated by protein kinase C in hepatocytes. Cyclic AMP was reported to induce a decrease in HMG-CoA reductase activity in the human hepatoma cell line HepG2 [18]. Cholesterol synthesis in macrophages is not so active as in hepatocytes, however, it is likely that the activation of macrophages by LDL results in the metabolic regulation or the release of biologically active substances toward cell proliferation. Indeed, activated macrophages have been reported to release macrophage-derived growth factor and stimulate non-lymphoid mesenchymal cell proliferation [19]. This study demonstrated that modified LDL, which are thought to form foam cells, induced IP_3 formation 2-fold more than native LDL. This result may correspond to the phenomenon that macrophages are activated especially under pathological conditions. IP_3 formation might play a crucial role in activating macrophages in the initial step of atherosclerosis. Future studies might investigate how macrophages respond to the stimulation by native and acetyl LDL after IP_3 formation.

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