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Low-density lipoproteins interact with liposome-binding sites on the cell surface

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Under physiological conditions significant amounts of low-density lipoprotein LDL particles ar taken up by cells independently of specific high-affinity LDL receptors (apo-B receptors). Previously it was established that some cells contain surface sites capable of binding liposomes. We proposed that liposome-binding sites could contribute to LDL interaction with the cell surface via phospholipid molecules of LDL particles. To check this hypothesis we studied the competitive interaction of human LDL and DPPC liposomes with mouse embryo fibroblasts depleted of apo-B receptors by preliminary incubation with LDL. We have found that after removal of the liposome-binding sites from cell lamellae these areas of the cell surface lose their ability to bind LDL.

Low-density lipoprotein; Liposome

1. INTRODUCTION

The concentration of low-density lipoproteins (LDL) in plasma depends to a large extent on the interaction of LDL with specific high-affinity LDL receptors (apo-B receptor) on the cell surface [1]. However, under physiological conditions significant amounts of LDL particles are taken up by cells 'non-specifically' [2,3] or perhaps via sites different from the apo-B receptors.

Earlier it was found that cells contain surface sites capable of binding liposomes [4–6]. Since more than one-half of the LDL surface consists of phospholipids it was reasonable to suppose that liposome binding sites could contribute to LDL binding to the cell surface.

To test this hypothesis we studied the interaction of human LDL with mouse embryo fibroblasts depleted of apo-B receptors. We report here that removal of the liposome-binding sites from certain parts of cell surface results in the loss of the ability of these parts to bind LDL.

2. MATERIALS AND METHODS

Mouse embryo fibroblasts were isolated and cultured as sparse cultures, as described earlier [6]. Liposomes were made from dipalmitoyl-PC (Sigma) and tagged with perylenoyl-labelled PC (a generous gift of Dr Y.G. Molotkovsky [7]) as described earlier [5,6]. LDL were isolated by centrifugation of blood plasma from healthy donors [8]. The density of the particles was between 1.19 and 1.63 g/cm. To deplete LDL-specific high-affinity receptors the cells were pre-incubated for 20 h with LDL (50 μg/ml) [9].

The affinity of apo-B-receptor-depleted cells to: (i) liposomes, (ii) LDL and (iii) LDL after liposome treatment was studied by fluorescent, scanning and transmission electron microscopy of cell replicas. A liposome suspension was added to the cells at concentration 5 mg of lipid/ml for 10 min at 37°C. After washing the cells were incubated with LDL (0.1–1 mg/ml for 10 min at 37°C). LDL particles were labelled on the cell surface with rabbit antibodies to human LDL and visualized with sheep or goat anti-rabbit antibodies conjugated with either fluorescein isothiocyanate [10] or colloidal gold particles (20–40 nm diameter) [11]. Control experiments showed that the label reflects the distribution of LDL. Preparing for platinum replica of cell surface and preparation of specimens for fluorescent and electron microscopy see [12].

Abbreviations: LDL, low-density lipoproteins; PC, phosphatidylcholine; DPPC, dipalmitoyl PC

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3. RESULTS

We have studied the ability of LDL particles to bind to those parts of the apo-B-depleted cell surface which were cleared from liposome-binding sites due to their migration together with the bound liposomes to the epinuclear regions of the cell surface ('capping').

3.1. *Liposome binding (control)*

Liposomes labeled with fluorescent phosphatidylcholine were bound diffusely to the entire cell surface if the cells were incubated at 4°C or fixed with formaldehyde before liposome treatment (Fig. 1a). When living cells were incubated with fluorescent liposomes at 37°C the initial distribution changed almost immediately. Liposomes migrated rapidly from the cell periphery

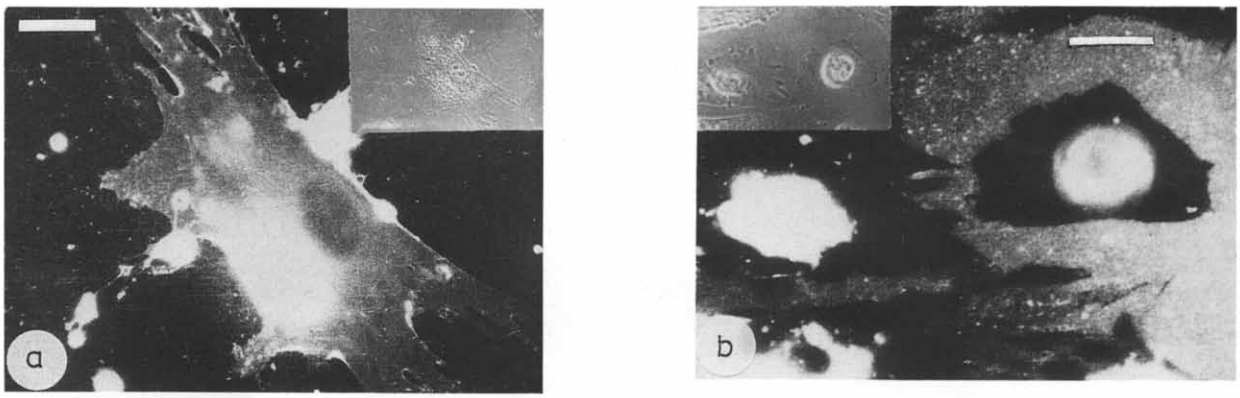

Fig. 1. Binding of fluorescent liposomes to the cell surface (fluorescent microscopy). Insert, phase-contrast image of the microscopic field. (a) Control pre-fixed cells. Note that liposomes bind to the entire cell surface. (b) Living cells. Dark parts of the cell represent lamellae which are cleared of fluorescent liposomes. Fluorescent liposomes are concentrated on the epinuclear parts of the cell surface (capped). Bar = 20 μm.

to the central parts of the cells ('capped'). New liposomes from the media did not adhere to the peripheral areas of the cell surface cleared of liposomes for at least 20 min (Fig. 1b).

3.2. *LDL binding (control)*

Double immuno staining with fluorescent antibodies revealed that LDL adhere to the entire surface of the cells (Fig. 2a). The distribution of LDL over the cell

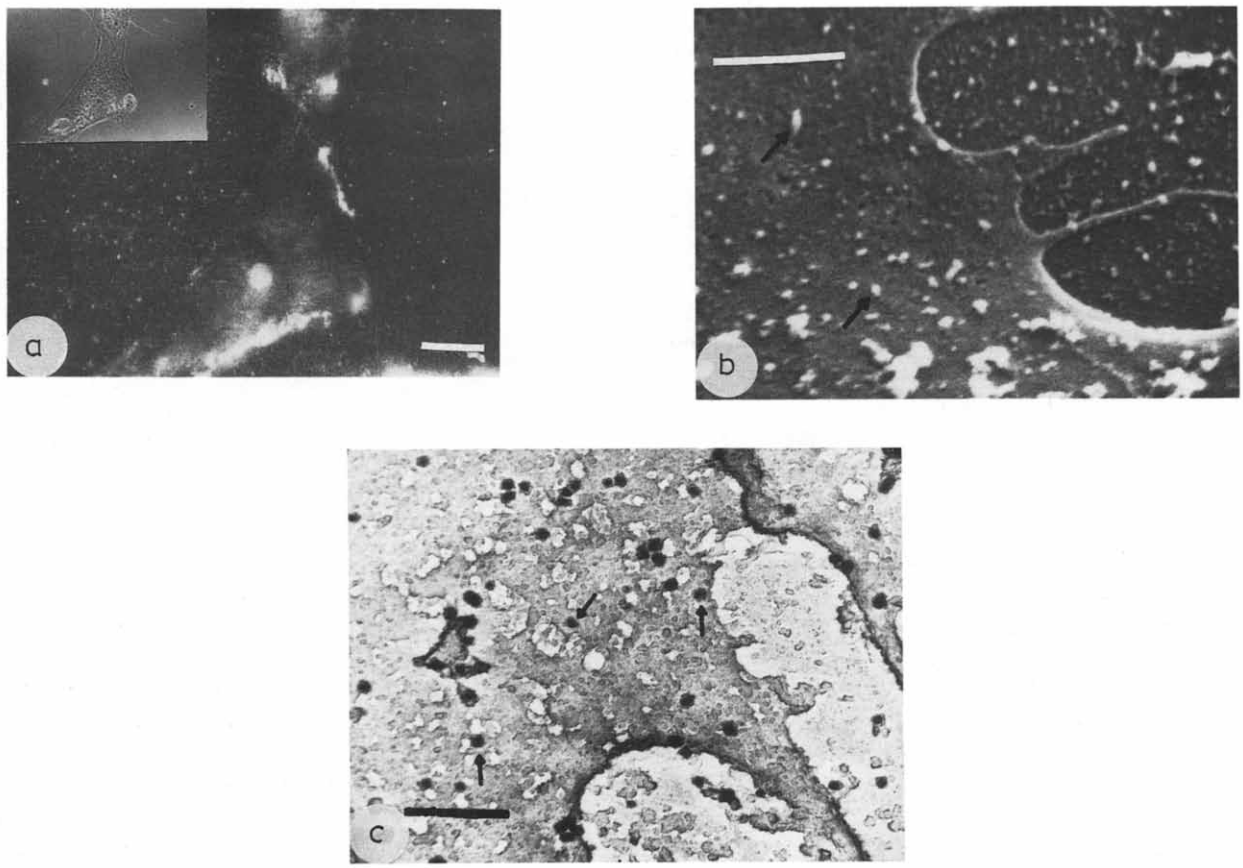

Fig. 2. Distribution of LDL over the surface of mouse embryo fibroblasts. LDL are visualized with double immunomicroscopic methods with rabbit anti-human LDL antibodies and goat anti-rabbit antibodies conjugated with: (a) FITC (fluorescent microscopy). Insert, phase contrast image of the same fields. Bar = 20 μm. (b) Golden particles (scanning electron microscopy of platinum replica). Lamellar part of a single cell. Golden particles (arrows) correspond to LDL-binding sites. Bar = 5 μm. (c) Golden particles (transmission electron microscopy of platinum replica). Lamellar part of the surface of a single cell. The golden particles (arrows) correspond to the bound LDL particles. Bar = 0.5 μm. Note in all three cases that LDL particles bind to the entire cell surface, including lamellae.

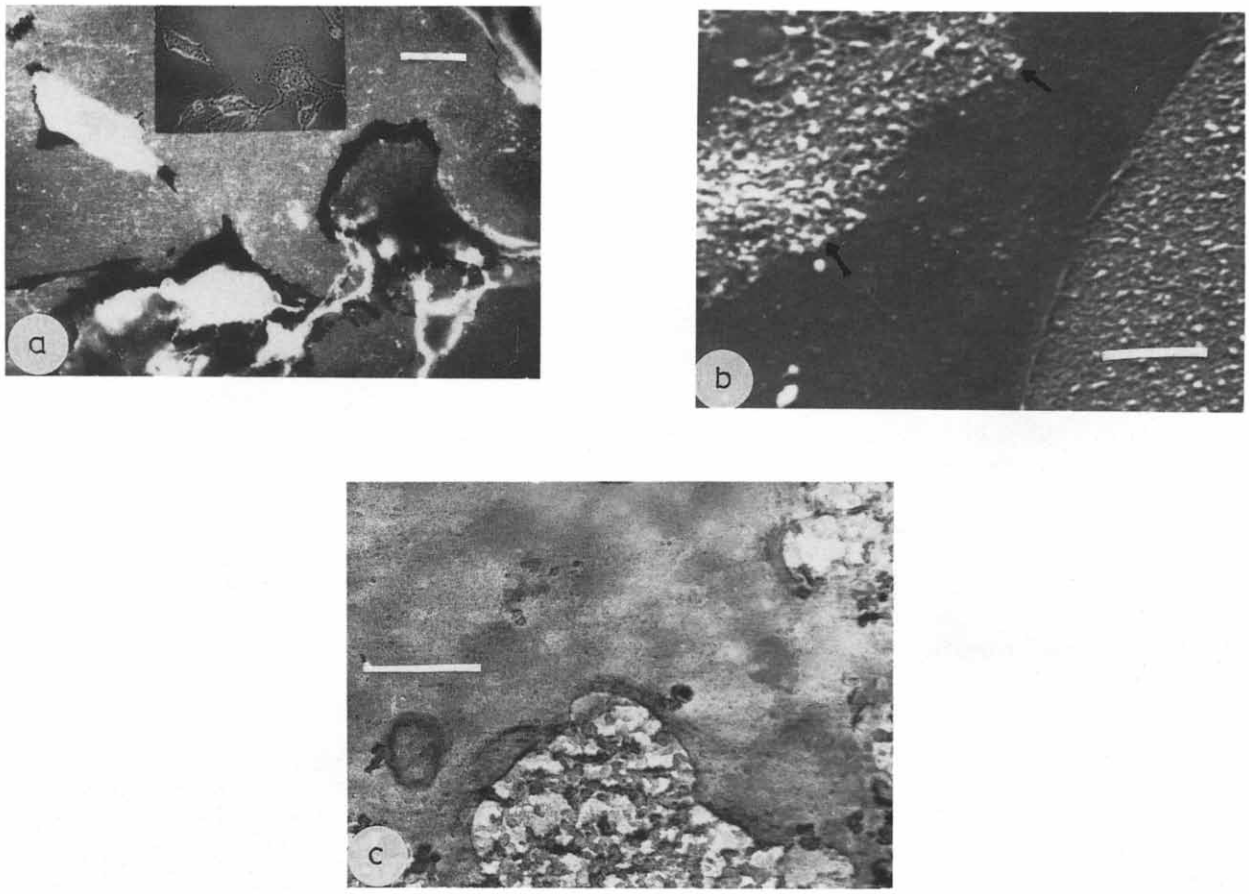

Fig. 3. LDL binding to cells pre-treated with non-labelled liposomes. LDL are visualized with double immunomicroscopic methods with rabbit anti-human LDL antibodies and goat anti-rabbit antibodies conjugated with: (a) FITC (fluorescent microscopy). Insert, phase-contrast image of the same fields. Fluorescence corresponds to the parts of the cell surface which bind LDL (compare with control, Fig. 2a). Bar = 20 μm. (b) Golden particles (scanning electron microscopy of platinum replica). Note the lack of golden particles on the cell lamellae (compare with the control, Fig. 2b). Bar = 5 μm. (c) Golden particles (transmission electron microscopy of platinum replica). Part of the lamellar surface of a single cell, pre-treated with liposomes. Note the lack of golden particles (compare with the control, Fig. 2c). Bar = 0.5 μm. Note in all three cases the absence of LDL on the lamellar part of the cell, pre-treated with liposomes.

surface was studied more precisely with antibodies labelled with colloidal gold using scanning electron microscopy (Fig. 2b) and transmission electron microscopy of platinum replicas of the cell surface (Fig. 2c). LDL particles bind to the entire cell surface, including lamellar parts.

3.3. *Binding of LDL after liposome treatment*

In these experiments the cells were first treated with non-labelled liposomes and then incubated with LDL. When the cells were pre-incubated with liposomes at 37°C LDL particles did not bind to areas of the cell surface cleared of liposomes (Fig. 3). This observation was verified by immunofluorescent microscopy (Fig. 3a), scanning electron microscopy of whole cells (Fig. 3b) and by transmission electron microscopy of cell replicas (Fig. 3c). LDL particles were concentrated in the epinuclear parts of the cells to which liposomes had migrated.

4. DISCUSSION

To study whether LDL can bind to liposome-binding sites of the cell surface we used a heterologous system of human LDL–mouse fibroblasts in which the receptor-mediated pathway of LDL uptake was preliminarily exhausted.

In principle the question whether LDL particles interact with liposome-binding sites on the cell surface could be answered by standard competition experiments between liposomes and LDL. However direct experiments would be misleading because LDL particles could bind to cell-associated liposomes rather than to the cell surface.

The cell association of phosphatidylcholine liposomes of different fluidity was studied previously [6]. It was shown that in contrast to fluid liposomes gel-state liposomes remain on the cell surface after binding. The binding of gel-state liposomes was saturable and

trypsin-sensitive [4,6] and thus resembled the interaction of a ligand with its specific receptor. Like other cell-bound ligands, liposome–receptor complexes migrated from the cell periphery to the central cell regions ('capped') and the spread lamellae were cleared of liposomes and remained unable to bind liposomes for at least 20 min.

In order to test the hypothesis that LDL–cell interaction could involve liposome-binding sites, we used the phenomenon of cell capping. We found that after liposome capping, cell lamellae which we cleared of liposome-binding sites ceased to bind LDL. We conclude that the liposome-binding sites of the cell surface participate in the binding of LDL.

However the actual mode of such an involvement is not quite clear. LDL and liposomes could bind to different surface sites and then co-capped (co-migration of two types of receptors after occupation of one receptor type has been described [13]). It can be supposed that liposome treatment of cells may induce such a modification of the cell lamellae that the cells lose their ability to bind LDL. However this is hardly the case since the cleared regions still retained their ability to bind Concanavalin A in a specific receptor-mediated manner.

The experiments presented show that an additional way of LDL–cell interaction exists, which is based on interaction with liposome-binding sites on the cell membrane. This interaction could represent a part of the so-called 'non-specific' cell–lipoprotein interaction. The physiological significance of this pathway remains to be studied.

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