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SCAVENGER LIPOPROTEIN RECEPTORS ARE MORE EFFECTIVE IN LIGAND INTERNALIZATION THAN LOW DENSITY LIPOPROTEIN RECEPTORS IN HUMAN MONOCYTE-MACROPHAGES

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SUMMARY. Human monocyte-macrophages in culture express specific receptors for low density lipoproteins (LDL receptor) and human acetylated LDL (AcLDL receptors or scavenger receptors). After 24 h in lipoprotein-deficient serum, the cells expressed 2-3 fold more AcLDL receptors than LDL receptors as measured by trypsin releasable radioactivity after exposure to ^{125}I-LDL or ^{125}I-AcLDL at 37°C. The efficiency of intracellular ligand delivery by the two receptors was evaluated as an internalization index (defined as intracellular + degraded/bound ligand). This index was several fold greater for ^{125}I-AcLDL than for ^{125}I-LDL, in the same cells exposed to either ligand under identical conditions. These results suggest that the scavenger receptors recycle more rapidly than do LDL receptors.

INTRODUCTION. Polypeptide-binding receptors that transport molecules into the cell for further metabolic processing have been categorized as class II receptors (for reviews see 1,2). One of the distinguishing features of receptors belonging to this class is that the receptor is reutilized multiple times during its lifetime. In the case of the well-studied low density lipoprotein (LDL) receptor in human cells, the efficiency of internalization of LDL has been shown to be directly proportional to the fraction of surface receptors that are associated with specialized cell surface invaginations called clathrin-coated pits (3).

The human macrophage has emerged recently as a cell of major importance in lipoprotein metabolism. One of the unique features of these cells is the presence of several high affinity binding sites on their cell surfaces that recognize certain naturally occurring and chemically-modified forms of lipoproteins. Distinct binding sites for LDL (4), negatively charged LDL (5,6) and β-VLDL (7)

ABBREVIATIONS. LDL, low density lipoprotein; AcLDL, low density lipoprotein modified by acetylation.

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have been demonstrated. In most studies, however, the degradation of ^{125}I-labeled LDL has been used as an indirect measure of receptor activity, and little is known about direct ligand/receptor interactions on these cells.

In this study we have addressed the question of the efficiency of intracellular ligand delivery by two distinct human monocyte-macrophage surface receptors, namely those for LDL and acetylated LDL (AcLDL).

MATERIALS AND METHODS

Cell preparation and culture. Blood was obtained from healthy normal volunteers after a 14-hour fast. Monocytes were separated from whole blood by method B of Fogelman et al. (8) as described in detail elsewhere (9). Cells were plated at a density of 4×10^5 cells/0.5 ml/16 mm diameter cluster well (Falcon Labware, Oxnard, CA) in RPMI 1640 medium (GIBCO, NY) supplemented with 20% (v/v) autologous serum . Cells were maintained for 13-17 days at 37°C in a humidified, 5% CO_2/air incubator and were fed twice weekly with fresh medium containing 20% autologous serum. This medium was changed to RPMI 1640 medium containing 10% (v/v) pooled human lipoprotein deficient serum 24 h before the cells were used for lipoprotein metabolism studies. This procedure was employed to up-regulate the relatively low LDL receptor activity the cells express after 2 weeks in culture (6,10).

Assays and chemical procedures. Human LDL (d=1.019-1.063 g/ml) and lipoprotein-deficient serum (density >1.25) were prepared by discontinuous gradient centrifugation (11) and preparative ultracentrifugation, and acetylation was performed by the method of Goldstein et al. (12). Native and acetylated LDL were iodinated with ^{125}I by the iodine monochloride method as modified for lipoproteins (13). To assay for LDL and AcLDL metabolism, cell layers received 0.5 ml of fresh RPMI 1640 medium containing lipoprotein-deficient serum to which ^{125}I-LDL or ^{125}I-AcLDL were added at the indicated concentrations, either in the absence or presence of 400 µg protein/ml unlabeled LDL or AcLDL, respectively. The cultures then were incubated for 4 hours at 37°C. After incubation, the medium was removed, and its content of non-iodide trichloroacetic acid-soluble radioactivity measured (14) (degradation). The cell layers were washed extensively at 4°C as described previously for skin fibroblasts and smooth muscle cells (15). The bound ^{125}I-lipoproteins were measured by incubating the washed cell layers at 37°C for 5-8 min in 0.05% trypsin/EDTA (GIBCO, NY) buffered with 20 mM Tris/HCl, pH 7.4. This medium was collected and the cell layers washed 3 times with phosphate buffered saline and the cells dissolved in 0.1 N NaOH for the measurement of trypsin-resistant radioactivity (intracellular) and of protein content (16). Cellular DNA contents were measured as described (17) and ^{3}H-leucine incorporation into proteins was measured after exposure of the cells to the tracer at 4 µCi/ml for 2 hours at 37°C and measuring the radioactivity precipitable by 10% trichloroacetic acid. Precipitates were washed and collected on Millipore filter discs (17).

RESULTS AND DISCUSSION. Purified human monocytes, seeded at the relatively high density of 4×10^5 cells/16 mm diameter well, resulted in partial covering of the growth surface after cellular attachment. During the next 2 weeks the cells grew dramatically in size; this caused the growth surfaces of the wells to be totally covered by cells. After 2 weeks in culture, the tightly packed cells

displayed a "cobblestone" appearance when viewed through phase contrast optics. The cells maintained this morphological appearance during all the procedures to assay ^{125}I-lipoprotein metabolism. It is important to note that the cells did not round up or become detached from the culture dish during the trypsin-treatment to remove surface bound material.

^{125}I-LDL and ^{125}I-AcLDL binding, intracellular content and rate of apoprotein degradation were compared after a 4 hour incubation at 37°C as a function of lipoprotein concentration (Fig. 1). At each concentration of ^{125}I-lipoprotein, duplicate culture dishes also received an excess of the corresponding unlabeled lipoprotein to control for non-specific, low affinity processes. The cells demonstrated relatively high affinities for the binding of both ligands and apparent half-maximal binding occurred at 13 μg protein/ml for ^{125}I-LDL and at 5.5 μg protein/ml for ^{125}I-AcLDL. Although maximum binding capacity was not reached with ^{125}I-LDL, extrapolation after linearization of the data yielded a 2-3 fold higher maximum capacity for ^{125}I-AcLDL than for ^{125}I-LDL. Similar kinetics were observed when ^{125}I-apoprotein degradation rates were assayed, indicating the validity of using degradation data as a measure for receptor activities. The intracellular content values obtained with ^{125}I-AcLDL indicated slightly lower affinities than the affinities obtained with the other two parameters in the experiment shown in Fig. 1. This was not a consistent observation and in other experiments good agreement between the three parameters was observed (see Fig. 2).

From the values illustrated above, it is clear that the total amounts of either lipoprotein internalized (intracellular + degraded) during the 4 hour incubation are several-fold higher than the corresponding amounts bound at any of the concentrations used. This could result from recycling of cell surface receptors or rapid synthesis of new receptors. In the case of the LDL receptor, evidence for receptor recycling has been well established in other human cells (3), and therefore is likely to occur in macrophages as well. However, to rule out rapid synthesis of the AcLDL receptor, the experiments were performed in the presence and absence of cycloheximide. Human macrophages in culture were much

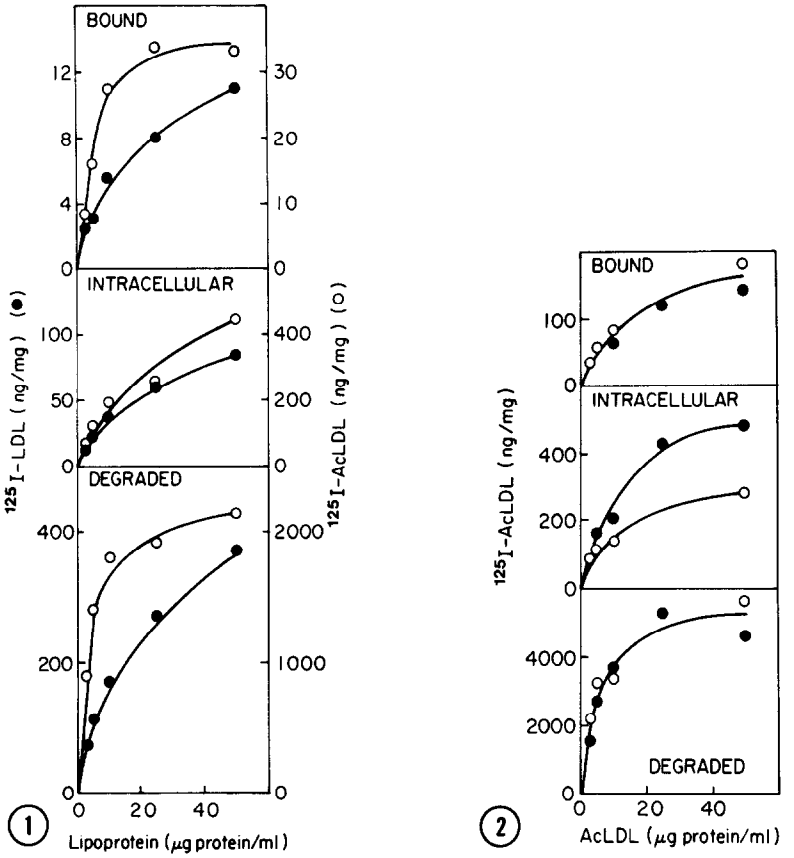

Figure 1: ^{125}I-LDL and ^{125}I-acetyl LDL metabolism by human monocyte-macrophages. Cells were treated, exposed to ^{125}I-lipoproteins and unlabeled lipoproteins, and analyzed as described in "Materials and Methods". Saturable activities, which represent the differences at each concentration between the values obtained in the absence and presence of the corresponding excess unlabeled lipoprotein, are presented. ^{125}I-LDL (●), ^{125}I-acetyl LDL (○). All values are the average of duplicate incubations carried out for 4 hours at each ^{125}I- or unlabeled lipoprotein concentrations.

Figure 2: The effect of cycloheximide on ^{125}I-acetyl LDL metabolism by human monocyte-macrophages. The experiment described in Fig. 1 was repeated in the absence (●) or presence (○) of 10 μM cycloheximide.

more sensitive to the general cytotoxic effects of this drug than other human cell types in culture. After exposure of human macrophages to 0, 10 or 25 μM cycloheximide for 24 hr, DNA concentrations were 1.55, 1.44 and 1.08 ug/well respectively, and ^{3}H-leucine incorporation into trichloroacetic acid-precipitates was inhibited by 0, 89 and 96%, respectively. At higher cycloheximide concentrations, the cytotoxic effects of the drug were easily apparent morphologically, and were confirmed by a dramatic reduction in DNA content of the wells. There-

fore, when cycloheximide was used to inhibit protein synthesis, a concentration of 10 μM was judged to be optimal.

The experiment shown in Fig. 2 was done in the presence or absence of 10 μM cycloheximide. The amount of ^{125}I-AcLDL bound and degraded was unaffected by this treatment, while the intracellular content was less in the cycloheximide-treated cases. Since the majority of ^{125}I-AcLDL internalized was degraded, this effect of cycloheximide on intracellular content had little influence on the net amount of lipoprotein internalized (i.e. intracellular + degraded).

To evaluate the efficiency of lipoprotein internalization, an internalization index (18) was calculated for ^{125}I-LDL and ^{125}I-AcLDL. It is clear that in each of three experiments (Table 1), the internalization index for AcLDL was much higher than that for LDL. The differences encountered in the absolute values between the three experiments are probably related to differences between cells isolated from different donors. The higher internalization index obtained with AcLDL compared to LDL indicated in the first place, more rapid rates of receptor internalization and surface replacement of AcLDL receptors. Furthermore,

Table 1: Internalization and Degradation Indices and Recycling Times of the LDL and AcLDL Receptors

Experiment # (Conditions)	Internalization Index $\left[\frac{\text{Intracellular + Degraded}}{\text{Bound}}\right]$		Degradation Index $\left[\frac{\text{Degraded}}{\text{Intracellular + Bound}}\right]$	
	^{125}I-LDL	^{125}I-AcLDL	^{125}I-LDL	^{125}I-AcLDL
1 (-Cycloheximide) n=5	39.0±1.3	84.4±9.0	4.3±0.3	8.6±1.5
2 (+Cycloheximide) n=4	12.6±2.0	50.2±8.1	3.3±0.5	16.7±1.9
(-Cycloheximide) n=5	ND*	50.2±4.5	ND*	11.1±1.1
3 (+Cycloheximide) n=3	8.5±2.0	65.9±5.3	1.9±0.2	9.4±0.0
(-Cycloheximide) n=3	8.5±4.7	52.3±2.4	1.4±0.8	8.0±0.3

*ND = not done

Values obtained at each lipoprotein concentration in the three experiments were calculated and are represented as the mean±standard error of the mean. Experiments 1 and 2 are those illustrated in Figures 1 and 2, respectively.

since the internalization index for both ligands was unchanged when protein synthesis was inhibited, we conclude that receptor recycling accounted for the replacement of both types of surface receptors. It appears likely that the more rapid internalization of AcLDL compared to LDL, reflects a more rapid recycling of the AcLDL receptor. On the other hand, the total recycling times for the two receptors, as opposed to the rates of internalization, could be similar if a large proportion of the AcLDL receptors were present intracellularly. Further studies are necessary to resolve this question. In Table 1 we also calculated values to assess lysosomal degradation, i.e. a degradation index. Since the differences between AcLDL and LDL assessed in this way were still apparent, we conclude that lysosomal function is not limiting for the degradation of the two ligands and that degradation follows directly the efficient intracellular delivery of ligands.

When LDL and AcLDL receptors have been compared in human monocyte-macrophages, higher receptor numbers and apparent affinities have been reported for the acetyl LDL receptor (5,6,10). We have confirmed these differences in the present study, and in addition, have demonstrated a remarkably higher efficiency of ligand internalization by the AcLDL receptor compared to the LDL receptor, analyzed at the same time, under identical conditions in the same cells. It is therefore clear that in reviewing all the receptor functions which will determine the rates of LDL and negatively charged LDL metabolism by human macrophages, the acetyl LDL or scavenger receptor is much more effective than the LDL receptor. In addition, as reported previously (6), auto-up-regulation of the acetyl LDL receptor also occurs in these cells. Thus, the human macrophage has the ability to rapidly and efficiently catabolize putative "altered" lipoproteins from their immediate environment. When this process is not balanced by an equally efficient removal system for cholesterol, cholesteryl ester accumulation and foam cell formation could ensue.

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REFERENCES

1. Kaplan, J. (1981) Science 212, 14-20.
2. Anderson, R.G., and Kaplan, J. (1983) Mod. Cell Biol. 1, 1-52.
3. Brown, M.S., Anderson, R.G., and Goldstein, J.L. (1983) Cell 32, 663-667.
4. Traber, M.G., and Kayden, H.J. (1980) Proc. Natl. Acad. Sci. USA 77, 5466-6570.
5. Shechter, I., Fogelman, A.M., Haberland, M.E., Seager, J., Hokom, M.M., and Edwards, P.A. (1981) J. Lipid Res. 22, 63-71.
6. Mazzone, T., and Chait, A. (1982) Arteriosclerosis 2, 487-492.
7. Van Lenten, B.J., Fogelman, A.M., Hokom, M.M., Benson, L., Haberland, M.E., and Edwards, P.A. (1983) J. Biol. Chem. 258, 5151-5157.
8. Fogelman, A.M., Seager, J., Hokom, M.M., and Edwards, P.A. (1979) J. Lipid Res. 20, 379-388.
9. Chait, A., Henze, K., Mazzone, T., Jensen, M., and Hammond, W. (1982) Metabolism 31, 721-727.
10. Fogelman, A.M., Haberland, M.E., Seager, J., Hokom, M.M., and Edwards, P.A. (1981) J. Lipid Res. 22, 1131-1141.
11. Chung, B.H., Wilkinson, T., Geer, J.C., and Segrest, J.P. (1980) J. Lipid Res. 21, 284-289.
12. Goldstein, J.L., Ho, Y.K., Basu, S.K., and Brown, M.S. (1979) Proc. Natl. Acad. Sci. USA 76, 333-337.
13. Langer, T., Strober, W., and Levy, R.I. (1972) J. Clin. Invest. 51, 1528-1536.
14. Bierman, E.L., Stein, O., and Stein, Y. (1974) Circ. Res. 35, 136-149.
15. Chait, A., Ross, R., Albers, J.J., and Bierman, E.L. (1980) Proc. Natl. Acad. Sci. USA 77, 4084-4088.
16. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 93, 265-275.
17. Fujimoto, W.Y., Teague, J., and Williams, R.H. (1977) In Vitro 13, 237-244.
18. Goldstein, J.L., Brown, M.S., and Stone, N.J. (1977) Cell 12, 629-641.