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Studies on the proteins involved in the interaction of high-density lipoprotein with isolated human small intestine epithelial cells

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Treatment of ^{125}I-labelled high-density lipoprotein ([^{125}I]HDL_3) with monospecific polyclonal antibodies against apolipoproteins A-I and A-II resulted in a dose-dependent inhibition of the [^{125}I]HDL_3 binding to isolated human small intestine epithelial cells by 25% and 50%, respectively. Both antibodies also inhibited intracellular degradation of [^{125}I]HDL_3 by 80%. Treatment of enterocytes with polyclonal antibody against apolipoprotein A-I binding protein, a putative HDL receptor, inhibited both binding and degradation of [^{125}I]HDL_3 by these cells by 50%. Antibodies to apolipoprotein A-I, A-II and apo A-I-binding protein also inhibited [^{125}I]HDL_3 binding to cholesterol-loaded cells.

Apolipoprotein A-I; Apolipoprotein A-II; High-density lipoprotein receptor; Human enterocyte

1. INTRODUCTION

Putative high-density lipoprotein (HDL) receptors have been demonstrated on several types of human and animal cells [1] including small intestine epithelial cells (enterocytes) [2–4]. Despite attempts to identify which component(s) of HDL interact with its receptor, the exact nature of the ligand remains controversial. Although most evidence supports the role of apolipoproteins as ligands [5,6], conflicting data still exist regarding which protein is involved in the interaction, and different laboratories reported that apolipoproteins (apo) A-I (apo A-I) [6], apo A-II [5], A-IV [6] and C [6] may be determinants of HDL binding.

Proteins which specifically bind apo A-I and HDL have been isolated and partially characterized from a number of cells. These proteins may represent the putative HDL receptor or parts thereof [7–10]. One of these proteins, isolated from human placenta by affinity chromatography, specifically bound to apo A-I and was designated as apo A-I-binding protein (apo A-I-BP) [9].

In the present study we have used antibody against apo A-I, apo A-II and apo A-I-BP to further investigate which proteins are involved in the interaction of HDL with human enterocytes.

Abbreviations: apo A-I, apolipoprotein A-I; apo A-II, apolipoprotein A-II; apo A-I-BP, apolipoprotein A-I-binding protein; HDL, high-density lipoprotein; LDS, lipoprotein-deficient serum; MEM, minimum essential medium.

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2. MATERIALS AND METHODS

2.1. *Cells*

A segment of middle jejunum was taken at autopsy within 1 h after death. Small intestine epithelial cells (enterocytes) were isolated and maintained as previously described [3,4].

2.2. *Lipoproteins*

High-density lipoproteins (HDL_3) (d = 1.125–1.216 g/cm^3) were isolated from the blood plasma of healthy donors by sequential preparative ultracentrifugation [11]. Iodination was performed according to Bilheimer et al. [12].

2.3. *Antibodies*

The preparation and characterization of polyclonal anti-apo A-I and anti-apo A-II antibodies [13], and antibody against apo A-I-BP [9] has been described previously.

2.4. *Binding assay*

To determine the effect of anti-A-I-BP antibodies on HDL interaction with enterocytes the incubation mixture containing 0.5×10^6 cells, 50 μl lipoprotein-deficient serum (LDS), 250 μg non-immune rabbit IgG, indicated concentrations of anti-apo A-I-BP antibody, and minimum essential medium (MEM, Flow, Ayrshire, UK) in a total volume of 250 μl, was incubated in the wells of 24-well plates for 1 h at 37°C in a CO_2 incubator (5% CO_2, 95% air) with shaking in an orbital shaker at 60 rpm. After pre-incubation with antibody [^{125}I]HDL_3 was added to the incubation mixture (final concentration 5 μg/ml). To determine non-specific binding, a 10-fold excess of HDL_3 was added in parallel incubations. The mixture was incubated for a further 2 h under the same conditions. The amount of bound and degraded [^{125}I]HDL_3 was determined as previously described [3,4].

To determine the effect of anti-apo A-I and anti-apo A-II antibodies on [^{125}I]HDL_3 interaction with cells [^{125}I]HDL_3 (2.5 μg) was incubated with indicated concentrations of antibodies or 250 μg non-immune rabbit IgG (control incubations) in MEM (total volume 250 μl) for 1 h at room temperature with shaking at 100 rpm. After pre-incubation, cells (0.5×10^6), LDS (50 μl) and MEM were added (total volume 0.5 ml) and the mixture was incubated for a further 2 h under the same conditions. The amount of bound and degraded radioactivity was determined as described above. To test the effect of antibodies on

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[^{125}I]HDL$_3$ binding to cholesterol-loaded cells, cholesterol in the form of 1% ethanolic solution was added to the pre-incubated mixture to a final concentration of 60 μg/ml, and the mixture was incubated for 1 h at 37°C. After pre-incubation cells were washed with 10 ml of MEM containing 1 mg/ml bovine serum albumin, and binding experiments were performed as described above.

3. RESULTS

3.1. *Effect of anti-apo A-I and anti-apo A-II on the interaction of [^{125}I]HDL$_3$ with enterocytes*

To study the effect of antibodies specific for two major apolipoproteins of HDL$_3$ on [^{125}I]HDL$_3$ interaction with human enterocytes [^{125}I]HDL$_3$ was pre-incubated with each antibody (50–300 μg/ml) or a mixture of the two. Treatment of [^{125}I]HDL$_3$ with anti-apo A-I and anti-apo A-II resulted in a dose-dependent inhibition of [^{125}I]HDL$_3$ binding to enterocytes by 20–30 and 50%, respectively (Fig. 1). The degradation of [^{125}I]HDL$_3$ was reduced by 80%, i.e. down to the level of non-specific degradation. The non-specific binding and degradation accounted for 20–30% of the total and did not change after treatment of [^{125}I]HDL$_3$ with antibodies. Treatment of [^{125}I]HDL$_3$ with non-immune rabbit IgG (1 mg/ml) had no effect on its binding and degradation. Treatment of [^{125}I]HDL$_3$ with the mixture of both antibodies (50 μg/ml each) had no additional effect on its binding and degradation (not shown).

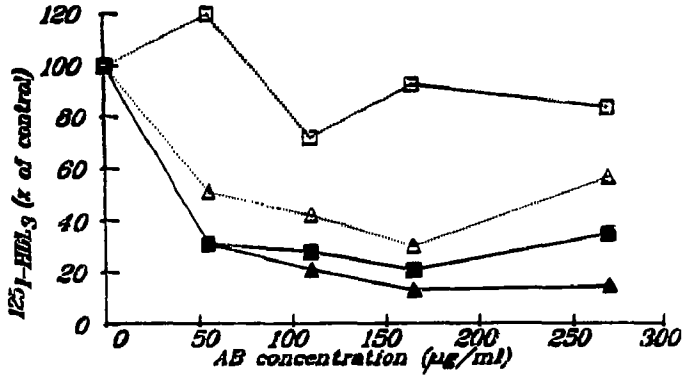

Fig. 1. Effect of anti-apo A-I and anti-apo A-II antibodies on [^{125}I]HDL$_3$ interaction with human enterocytes. [^{125}I]HDL$_3$ (2.5 μg) was pre-incubated with indicated concentrations of anti-apo A-I (□,■) or anti-apo A-II (△,▲) or 250 μg/ml non-immune rabbit IgG (controls) for 1 h at room temperature. After pre-incubation lipoprotein was added to cells (0.5 × 10^6, final volume 0.5 ml) and samples were incubated for a further 2 h at 37°C with shaking at 60 rpm. Amount of bound (open symbols) and degraded (filled symbols) [^{125}I]HDL$_3$ was determined as described in section 2. Each point is the mean of two (△,▲) or three (□,■) independent experiments; each experiment was done in duplicate.

Table I

Effect of anti-apo A-I, anti-apo A-II and anti-apo A-I-binding protein antibodies on [^{125}I]HDL$_3$ interaction with human enterocytes

Additions	[^{125}I]HDL$_3$ binding (ng/mg cell protein)	
	Non-cholesterol-loaded cells	Cholesterol-loaded cells
Experiment I		
None	54.5 ± 3.1	69.0 ± 3.3*
Anti-apo A-I-BP	30.7 ± 3.1**	48.1 ± 5.5***
Anti-apo A-II	25.2 ± 9.1**	22.1 ± 7.3***
Experiment II		
None	252.0 ± 15.0	355.0 ± 64.0*
Anti-apo A-I	210.0 ± 10.0**	147.0 ± 31.0**
Anti-apo A-II	38.0 ± 5.0***	43.0 ± 12.0***

Human enterocytes (0.5 × 10^6) were pre-incubated with no additions or with apo A-I-BP antibody (final concentration 200 μg/ml) in the presence or absence of cholesterol (final concentration 60 μg/ml) for 1 h at 37°C with shaking at 60 rpm. Simultaneously, [^{125}I]HDL$_3$ was pre-incubated with no additions or with anti-apo A-I antibody (final concentration 100 μg/ml) or anti-apo A-II (final concentration 100 μg/ml) for 1 h at room temperature. After pre-incubation cells were washed by centrifugation at 500 × *g* at room temperature for 10 min and resuspended in 0.5 ml of media containing 10% LDS, pre-incubated [^{125}I]HDL$_3$ (final concentration 5 μg/ml) and corresponding antibody in a final concentration identical to the pre-incubation mixture. The mixture was incubated for 2 h at 37°C with shaking at 60 rpm. Amount of bound [^{125}I]HDL$_3$ was determined as described in section 2. Specific binding (i.e. total minus non-specific, measured in the presence of a 10-fold excess of unlabelled particles) is presented. Each value is the mean ± S.E.M. of quadruplicate determinations of a representative experiment. *$P < 0.01$ (vs. corresponding value with non-cholesterol-loaded cells); **$P < 0.01$ (vs. no additions); ***$P < 0.001$ (vs. no additions).

3.2. *Effect of anti-apo A-I-BP antibody on the interaction of [^{125}I]HDL$_3$ with enterocytes*

To study the effect of antibody against apo A-I-BP on [^{125}I]HDL$_3$ interaction with enterocytes cells were pre-incubated with different concentrations of these antibodies (5–200 μg/ml). To exclude non-specific effects of IgG excess of non-immune rabbit IgG (250 μg/ml) was added to the incubation mixtures (including controls). Comparison of control incubations with and without added IgG demonstrated that non-immune rabbit IgG (250 μg/ml) caused 40% inhibition of [^{125}I]HDL$_3$ binding but had no effect on its degradation. Treatment of human enterocytes with anti-apo A-I-BP antibody reduced [^{125}I]HDL$_3$ binding and degradation by about 50% in a dose-dependent manner (Fig. 2). Non-specific binding and degradation did not change after treatment of cells with anti-apo A-I-BP antibody.

3.3. *Effect of anti-apo A-I-BP and anti-apo A-I and apo A-II antibodies on [^{125}I]HDL$_3$ binding to cholesterol-loaded enterocytes*

It was demonstrated previously that loading of enterocytes with cholesterol upregulates [^{125}I]HDL$_3$ binding to these cells [4]. Treatment of cholesterol-loaded and non-cholesterol-loaded enterocytes with apo A-I-BP antibody decreased [^{125}I]HDL$_3$ binding by 30 and 44%, respectively (Table I). Treatment of [^{125}I]HDL$_3$ with anti-apo A-I and anti-apo A-II antibody also decreased its binding to both cholesterol-loaded and non-cholesterol-loaded cells (Table I).

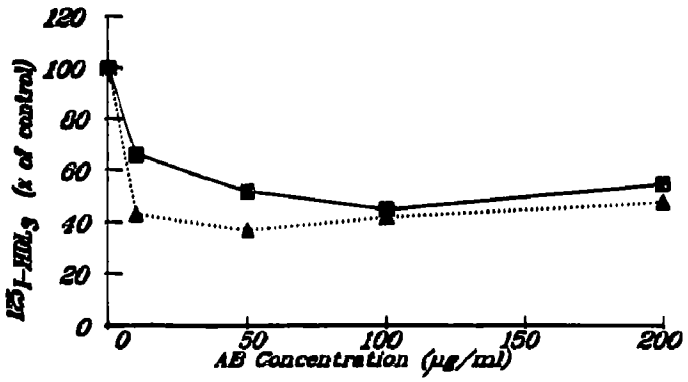

Fig. 2. Effect of anti-apo A-I-binding protein antibody on [^{125}I]HDL$_3$ interaction with human enterocytes. Human enterocytes were pre-incubated with 1 mg/ml of rabbit non-immune IgG, 10% LDS and indicated concentrations of anti-apo A-I-BP for 1 h at 37°C with shaking at 60 rpm. Then, [^{125}I]HDL$_3$ was added to a final concentration of 5 μg/ml and incubated for a further 2 h at 37°C with shaking at 60 rpm. Amount of bound (■) and degraded (▲) [^{125}I]HDL$_3$ was determined as described in section 2. Specific binding and degradation (i.e. total minus non-specific, measured in the presence of 10-fold excess of unlabelled particles) are presented. Each point is the mean of two independent experiments; each experiment was done in duplicate.

4. DISCUSSION

If the association between HDL and cells includes a receptor–ligand interaction this implies that both lipoprotein and receptor possess specific recognition sites. Several attempts have been undertaken to identify determinants in both HDL [5] and its putative receptor [7–10]. These studies have, however, not resulted in a consensus for the identities of specific determinants involved in this interaction. In the present work we have tried to address this question using antibodies against two potential ligands, apo A-I and apo A-II, and also against a potential receptor, an apo A-I-BP from human placenta [9].

We have shown that treatment of [^{125}I]HDL$_3$ with antibodies against apo A-I and apo A-II inhibits [^{125}I]HDL$_3$ binding to both cholesterol-loaded and non-cholesterol-loaded human enterocytes. The effect of antibodies on [^{125}I]HDL$_3$ degradation was more pronounced than on its binding. This may be due to the interference of the binding of [^{125}I]HDL$_3$–antibody complex to FC receptors on the enterocytes or to the formation of aggregates containing several [^{125}I]HDL$_3$ particles bound to the antibodies. Both anti-apoA-I and -apo A-II antibodies inhibited interaction of [^{125}I]HDL$_3$ with human enterocytes. This could mean that both apo A-I and apo A-II may be determinants of HDL receptor. The distribution of hydrophobic, acidic and basic amino acids in the 11-mer, which is the main internal repeat unit of both apo A-I and apo A-II, shows a high degree of homology between these apolipoproteins [14]. However, the possibility cannot be excluded that binding of antibody to [^{125}I]HDL$_3$ may inhibit its interaction with the receptor due to steric interference. Our data are consistent with those reported by Fidge and Nestel [5].

Treatment of enterocytes with anti-apo A-I-BP inhibited [^{125}I]HDL$_3$ binding and degradation by 50%. Moreover, in experiments with cholesterol-loaded enterocytes only half of the additionally appearing binding sites were inhibited by treatment with antibody. This suggests that either apo A-I binding protein is only a part of the HDL receptor, or that antibodies have not fully masked the epitopes involved in binding.

REFERENCES

[1] Bierman, E.L. and Oram, J.F. (1987) Am. Heart J. 113 (part II), 549–550.
[2] Suzuki, N., Fidge, N., Nestel, P. and Yin, J. (1983) J. Lipid Res. 24, 253–264.
[3] Sviridov, D.D., Safonova, I.G., Gusev, V.A., Talalaev, A.G., Tsibulsky, V.P., Ivanov, V.O., Preobrazensky, S.N., Repin, V.S. and Smirnov, V.N. (1986) Metabolism 35, 588–595.
[4] Sviridov, D.D., Safonova, I.G., Tsybulsky, V.P., Talalaev, A.G., Preobrazensky, S.N., Repin, V.S. and Smirnov, V.N. (1987) Biochim. Biophys. Acta 919, 266–274.
[5] Fidge, N.H. and Nestel, P.J. (1985) J. Biol. Chem. 260, 3570–3575.
[6] Mitchel, Y.B., Rifici, V.A. and Eder, H.A. (1987) Biochim. Biophys. Acta 917, 324–332.
[7] Fidge, N. (1986) FEBS Lett. 199, 265–268.
[8] Graham, D.L. and Oram, J.F. (1987) J. Biol. Chem. 262, 7439–7442.
[9] Keso, L., Lukka, M., Ehnholm, C., Baumann, M., Vihko, P. and Olkinuora, M. (1987) FEBS Lett. 215, 105–108.
[10] Kambouris, A.M., Roach, P.D. and Nestel, P.J. (1988) FEBS Lett. 230, 176–180.
[11] Lindgren, F.T. (1975) In: Analysis of Lipids and Lipoproteins (Perkins, E.G. ed.) pp. 204–224, American Oil Chemists Society, New York.
[12] Bilheimer, D.W., Eisenberg, S. and Levi, R. (1972) Biochim. Biophys. Acta 260, 212–221.
[13] Huttunen, J.K., Lansimies, E., Voutilainen, E., Ehnholm, C., Hietanen, E., Penttila, I., Snitonen, O. and Rauramma, R. (1979) Circulation 60, 1220–1229.
[14] Li, W.-H., Tanimura, M., Luo, C.-C., Datta, S. and Chan, L. (1988) J. Lipid Res. 29, 245–271.