Potential involvement of dissociated apoA-I in the ABCA1-dependent cellular lipid release by HDL

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Abstract Helical apolipoproteins of high density lipoprotein (HDL) remove phospholipid and cholesterol from cells and generate HDL particles being mediated by ATP binding cassette transporter A1 (ABCA1). In murine macrophage cell line RAW264 cells, cAMP induced expression of ABCA1, release of cellular phospholipid and cholesterol by apolipoprotein A-I (apoA-I), and reversible binding of apoA-I to the cell. The apoA-I-dependent lipid release was directly proportional to the cAMP-induced binding of apoA-I, and was inhibited 70% by a monoclonal antibody selective to lipid-free apoA-I, 725-1E2. In contrast, apparent cellular cholesterol release to HDL was substantial even without ABCA1 induction, and it was increased only by 27% after the cAMP treatment. The antibody inhibited this increment by 70%. Lipid-free apoA-II liberated apoA-I from HDL by displacement and thereby markedly expanded the cAMP-induced part of the cholesterol release to the HDL-containing medium, and the antibody inhibited this part also by 70%. Binding experiments of the double-labeled reconstituted HDL showed that cAMP induced reversible binding of apoA-I but not the association of cholesteryl ester with the cells. The effect of the antibody on the cellular cholesterol release to the reconstituted HDL was similar to that of the HDL-mediated release. The data implicated that the ABCA1-dependent cholesterol release to HDL is mediated by apoA-I dissociated from HDL.—Okuhira, K-i., M. Tsujita, Y. Yamauchi, S. Abe-Dohmae, K. Kato, T. Handa, and S. Yokoyama. **Potential involvement of dissociated apoA-I in the ABCA1-dependent cellular lipid release by HDL. 2004.** ***J. Lipid. Res.*** 45: **645–652.**

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High density lipoprotein (HDL) is a mediator of cholesterol transport from peripheral cells to the liver for its catabolism. Two distinct mechanisms are involved in the initial step of this transport system (1–3): *1*) nonspecific exchange of cholesterol between cell surface and HDL, and *2*) assembly of cellular lipids to form new HDL particles by lipid-free helical apolipoproteins. The latter reaction was found to be defective in the cells of the patients with Tangier disease and genetic HDL deficiency (4), and mutations were identified in the gene of ATP-binding cassette transporter A1 (ABCA1) in this disease (5–7). In addition, disruption of this gene resulted in HDL deficiency in mice (8, 9). Therefore, this reaction is thought to be essential for production of plasma HDL. Mechanisms by which apolipoproteins interact with ABCA1-expressing cells and generate HDL are still unknown. Some authors indicate apolipoprotein interaction directly with ABCA1 (10–12), while others may suggest an indirect interaction (13–15).

Lipid-free or lipid-very-poor apolipoproteins were reportedly identified in human blood plasma and in intercellular fluid (16), but its physiological concentration may not be well established. Thus, one of the main questions is how the helical apolipoproteins interact with cells. HDL apoproteins do not directly interact with cells when it is bound to lipid microemulsion surface (17). However, helical apolipoproteins are present in equilibrium between lipid-bound and lipid-free forms (18), and certain reactions may enhance dissociation of apolipoproteins, especially apoA-I (apolipoprotein A-I) (19, 20). On the basis of these facts, in vivo recycle of apoA-I between the lipid-bound and free forms has been proposed (21), and lipid-free apoA-I thus generated would interact with cells (2).

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Abbreviations: apoA-I, apolipoprotein A-I; CO, cholesteryl oleate; DF medium, DMEM/F12 medium; PBS, phosphate buffered saline; rHDL, reconstituted HDL.

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Our recent finding of ABCA1 stabilization by free apolipoproteins but not by those in the HDL-bound form (22) supported the idea that free apolipoproteins interact with ABCA1 in their free forms. We therefore undertook the experiments in the attempt to provide more direct evidence that apoA-I dissociates from HDL to interact with cells and generate new HDL particles.

MATERIALS AND METHODS

Materials

RAW 264 cells were obtained from Riken Gene Bank (Tsukuba, Japan). ProapoA-I cDNA was kindly provided by Research Laboratory of Mitsubishi Pharma Corporation (Yokohama). TCM serum replacement was purchased from ICN. [^{3}H]acetate, [^{3}H]amino acid mixture, and [^{14}C]cholesteryl oleate ([^{14}C]CO) were purchased from Amersham Pharmacia Biotech Co. CO and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) were purchased from Sigma Chemical Co. Several monoclonal antibodies against human apoA-I were kindly provided by Daiichi Pure Chemicals (Tokyo).

Cellular lipid release

HDL was isolated from fresh human plasma of a healthy donor as a density range of 1.063–1.21 g/dl. HDL_2 and HDL_3 were further isolated as density ranges of 1.063–1.125 g/dl and 1.125–1.21 g/dl, respectively. ApoA-I and apoA-II were isolated from human plasma as described elsewhere (23, 24). ProapoA-I was produced in *E. coli* JM109 by introducing a recombinant plasmid with its cDNA and isolated as described previously (25). RAW 264 cells were premaintained in DMEM/F12 (DF) medium containing 10% fetal calf serum or in 2% TCM replacement for 3 days. After preincubation for 16 h with 300 μM dibutyryl cAMP in DF medium containing 0.1% BSA, cells were incubated for 24 h with or without human apoA-I or recombinant proapoA-I, also in the presence of cAMP (26). The lipid mass in the medium was measured by using enzymatic methods (26).

Probing lipid-free apoA-I

Several monoclonal antibodies were raised against human apoA-I and provided by Daiichi Pure Chemicals (27). BALB/c mice were immunized by apoA-I isolated from human plasma, and the spleen cells were extracted and fused with murine myeloma cells (SP2/0-Ag14). The clones that produce anti-apoA-I antibody were isolated. Among those, an antibody 725-1E2 was found reactive to lipid-free apoA-I but not to HDL as being characterized in our laboratory by immunoprecipitation. The monoclonal antibody 725-1E2, 5 μg, was absorbed to 20 μg of protein G-sepharose and incubated for 16 h with lipid-free apoA-I, apoA-II and HDL_3. Immunoprecipitated apoA-I with protein G-sepharose was detected by immunoblot analysis by using goat anti-human apoA-I.

Displacement of apoA-I by apoA-II from HDL

HDL_3 as 1 μg protein was incubated with apoA-II, 0, 0.5, 1, and 2 μg in phosphate buffered saline (PBS) at 4°C for 16 h. Displacement of apoA-I was examined by immunoprecipitation of lipid-free apoA-I by using the antibody 725-1E2, and by electrophoresis after separating free protein and HDL_3 by ultracentrifugation at a density of 1.21 g/dl.

Binding of apolipoprotein and lipoprotein to the cells

ProapoA-I was homogeneously labeled with tritium as it was produced in the presence of [^{3}H]amino acid mixture and isolated (25) with the highest specific radioactivity of 1.9×10^{15} dpm/mole. The cells were treated or untreated with cAMP and incubated with the labeled proapoA-I medium for 2 h at 4°C. The bound protein was displaced by 100 μg/ml of unlabeled proapoA-I for 4 h at 4°C. Then the cells were washed with PBS, lysed in 0.1 N NaOH, and a radioactivity in aliquot of the lysate was counted.

Effect of free apoA-I specific antibody on cellular cholesterol release

The cellular lipid was labeled by incubating with [^{3}H]acetate for 16 h. After washing, the cells were incubated with lipid-free apoA-I, HDL_2, and HDL_3 for 4 h in the presence of 725-1E2 or mouse IgG. ApoA-II was preincubated with HDL_3 for 16 h prior to the incubation with cells. Released [^{3}H]cholesterol in the medium was detected after lipid was extracted and separated by thin-layer chromatography.

Reconstituted HDL preparation

For lipoprotein binding study, reconstituted HDL (rHDL) was prepared. ProapoA-I or apoA-I (5 mg), CO (2.5 mg), and POPC (7 mg) in 15 ml of PBS were sonicated for 30 min at 4°C in a KUBOTA Insonator 201M at 200 watts, and lipoprotein particles generated were isolated by ultracentrifugation as a density range of 1.065–1.17 g/ml. After the dialysis in PBS, rHDL was characterized by nondenaturing gradient gel electrophoresis (5% to 20% polyacrylamide) (28) and by electron micrograph in negative staining (29). The binding experiment was carried out in the same manner as free proapoA-I, with cAMP-stimulated and unstimulated RAW264 cells, by using rHDL prepared with [^{3}H]-labeled proapoA-I and [^{14}C]CO. Cholesterol release from RAW264 cells was determined by measuring its mass in the medium in the presence of apoA-I-rHDL, and the effect of the antibody 725-1E2 was observed.

RESULTS

Mouse monocytic leukemia cell RAW264 was used as a model system to distinguish ABCA1-dependent and -independent cellular cholesterol release, as ABCA1 expression is induced by cAMP, and the apolipoprotein-mediated cellular lipid release was induced from zero to substantial and measurable levels (26, 30). **Figure 1** shows release of cholesterol and phospholipid by apoA-I isolated from human plasma HDL and by recombinant human proapoA-I after the cells are stimulated by cAMP (Fig. 1A). Concentration dependency profiles were indistinguishable between these two proteins (Fig. 1A). ProapoA-I, which represents 4–8% of circulating apoA-I (31) and is known to have similar physicochemical and biochemical properties (32–34) to those of mature apoA-I, thus was shown to be the same as apoA-I, also for cellular lipid release. These results were not influenced by using TCM serum replacement in order to avoid any potential influence of serum components (26), so that TCM was applied for further experiments. A weight ratio of cholesterol to phospholipid was always 1:1 in the released lipid (Fig. 1B, C) that is to form preβ HDL particles (1). Induction of ABCA1 expression by cAMP was confirmed by the immunoblot analysis (26, 35) (Fig. 1D).

ProapoA-I was uniformly labeled with the tritiated amino acids to avoid potential interference with the binding study by attaching large molecules such as ^{125}I or fluo-

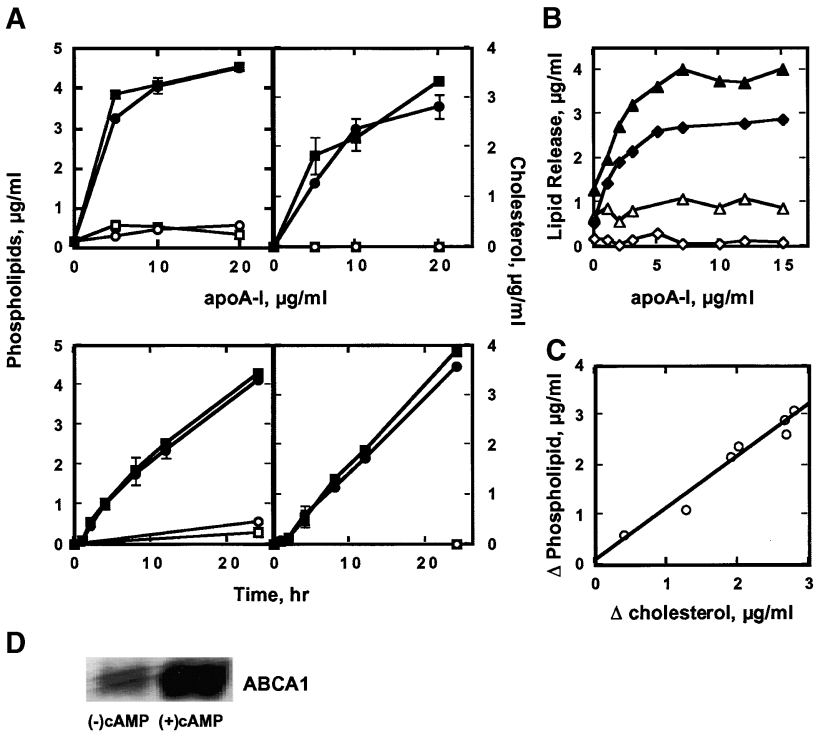

Fig. 1. Release of lipids by human plasma apolipoprotein A-I (apoA-I) and recombinant human proapoA-I from RAW264 cells. RAW264 cells were premaintained in DMEM/F12 medium containing 10% fetal calf serum (A) or in 2% TCM replacement (B). After preincubation for 16 h with 300 mM dibutyryl cAMP, cells were incubated for 24 h with or without human apoA-I or recombinant proapoA-I also in the presence of dibutyryl cAMP. A: Release of choline-phospholipid (left panels) and cholesterol (right panels) from cAMP-stimulated (closed squares and circles) and unstimulated (open squares and circles) RAW264 cells, in the presence of various amounts of plasma apoA-I (closed and open circles) or recombinant proapoA-I (closed and open squares) for 24 h (upper panels), and in the presence of 20 μg/ml apoA-I up to 24 h (lower panels). B: Release of choline-phospholipid (closed and open triangles) and cholesterol (closed and open diamonds) from cAMP-stimulated (closed triangles and diamonds) and cAMP-unstimulated (open triangles and diamonds) cells by proapoA-I was measured for 24 h. C: The data of panel B were plotted as the increment of phospholipid release by the cAMP treatment against the increase of cholesterol release by the cAMP. D: ATP binding cassette transporter A1 expression demonstrated by immunoblotting in the cAMP-stimulated or unstimulated RAW264 cells. Each data point in the panels of A represents the average ± SD for more than three samples. The data points in the panels of B and C represent single point assays.

rescence compounds. **Figure 2A** demonstrates binding of [^{3}H]-labeled proapoA-I to the cells stimulated by cAMP. Reversible binding of apoA-I was estimated by subtracting the binding after displacement by the unlabeled apoA-I (Fig. 2A, inset). The release of cholesterol and phospholipid were both directly proportional to this reversible binding (Fig. 2B). No reversible binding was observed when the cells were not treated with cAMP (data not shown).

A monoclonal antibody raised against human apoA-I, 725-1E2, was shown to recognize lipid-free apoA-I molecule, but hardly the one bound to HDL (at most 2–3% of free apoA-I) (**Fig. 3A**). The very weak reactivity of the HDL solution may represent free apoA-I dissociated in equilibrium or poor partial recognition of the lipid-bound apoA-I. The effect of 725-1E2 is shown in Fig. 3B on the release of cholesterol by lipid-free apoA-I and by HDL from RAW264. Without cAMP induction, there was no cholesterol release by apoA-I. When ABCA1 was induced by cAMP, cholesterol release by apoA-I was inhibited by the antibody by 74%. In contrast, a substantial amount of cholesterol release was observed by HDL even without induction of ABCA1, and it was increased only 26% by cAMP. The antibody 725-1E2 did not influence the cholesterol release from the cells without ABCA1 expression. When the cells were treated with cAMP, it inhibited 20% of the total release, and this inhibition accounted for 75% of the portion increased by cAMP for HDL_2 and 51% of that for HDL_3, both similar extents of the inhibition of the free-apoA-I-mediated release by the antibody. The antibody did not influence the cholesterol content when added to the conditioned medium of lipid-free apoA-I (data not shown). The results thus indicated that the antibody inhibits the activity of apoA-I to release cellular cholesterol, but not cholesterol exchange between HDL and cells. In other words, an ABCA1-dependent portion of cholesterol release to HDL was inhibited by this antibody.

Thus, it is likely that ABCA1-HDL interaction requires liberation of apoA-I from HDL. To confirm this view by expanding apoA-I dissociation from HDL, displacement of

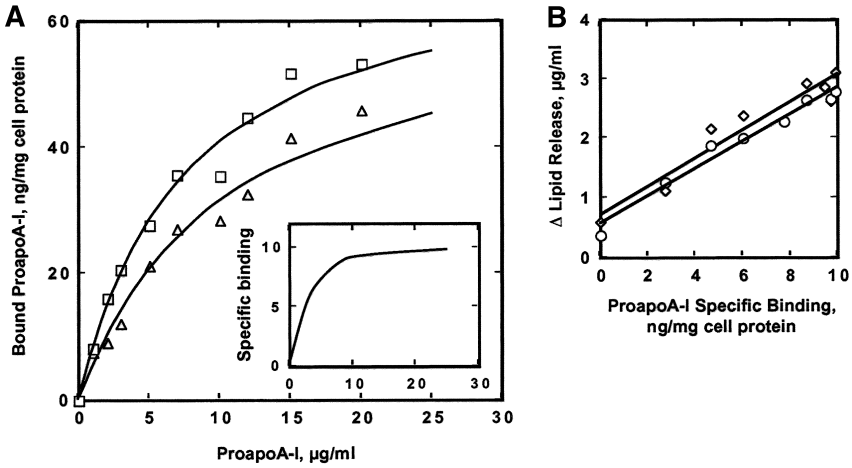

Fig. 2. Binding of proapoA-I to RAW264 cells. ProapoA-I was homogeneously labeled with tritium and the binding study was carried out as described under Materials and Methods, with the cells treated or untreated with cAMP. A: Binding of proapoA-I, before (open squares) and after (open triangles) displacement. Solid lines represent binding curves in equilibrium calculated by a least square regression of each data set. Specific binding was calculated by subtracting the binding after the displacement from that of before the displacement. K_d.cell was obtained by fitting the specific binding data to a saturable equilibrium binding model. B: Release of phospholipid (open circles) and cholesterol (open diamonds) are plotted against specific binding of proapoA-I.

apoA-I by apoA-II was attempted. When apoA-II was incubated with HDL, apoA-I was displaced and released from HDL by apoA-II as previously observed (36–39) (**Fig. 4A**, B). Figure 4A shows that lipid-free apoA-I recognized by 725-1E2 is increased by adding apoA-II to HDL, as the HDL-bound apoA-I decreased and the HDL-bound apoA-II increased (Fig. 4B). In the condition that all the added apoA-II was bound to HDL (up to 1:1 weight ratio of apoA-I/HDL protein according to Fig. 4B), the cAMP-inducible release of cellular cholesterol was increased by 5 to 6 times while the ABCA1-independent cholesterol release to HDL was not changed (Fig. 4C). The antibody, 725-1E2, inhibited this increment by 60% (Fig. 4C). Cholesterol release was induced by free apoA-II (26), but this was not influenced by the antibody (data not shown), so that the increase of the cAMP-inducible part was due to liberation of apoA-I. Therefore, ABCA1-dependent cholesterol release to HDL is likely to involve apoA-I dissociated from HDL. Inhibition by the antibody of the cAMP-inducible cholesterol release was slightly less than that without apoA-II. This may indicate the effect of a trace amount of unbound apoA-II in the medium. In order to examine a mode of interaction of lipid-bound apoA-I with cells, reconstituted HDL was prepared by sonicating POPC, [^{14}C]CO, and [^{3}H]proapoA-I, isolated by ultracentrifugation, and characterized by nondenaturing gel

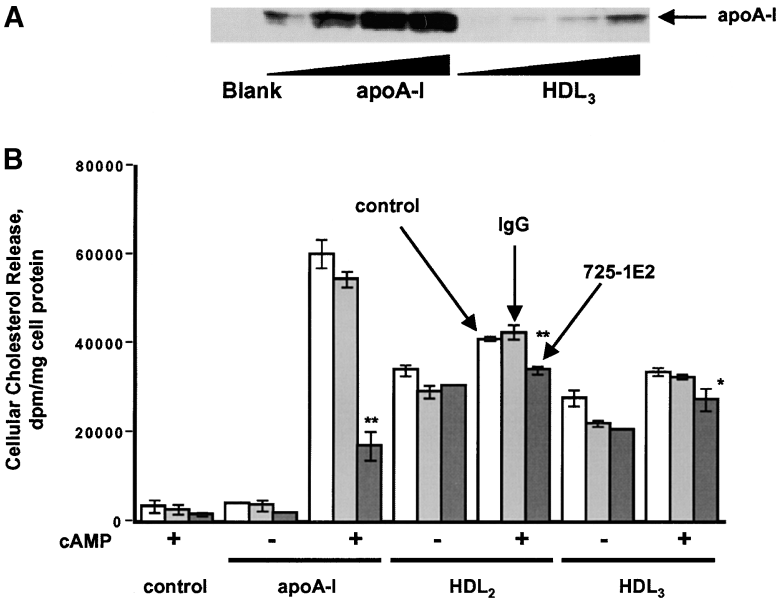

Fig. 3. A: Immunoprecipitation of apoA-I by the lipid-free apoA-I-specific monoclonal antibody, 725-1E2. The monoclonal antibody, 5 μg, was absorbed to 20 μg of protein G-sepharose and incubated with 0, 0.25, 0.5, 0.75, and 1 μg of lipid-free apoA-I and high density lipoprotein 3 (HDL_3) (as protein). Immunoprecipitated apoA-I with protein G-sepharose was detected by immunoblot analysis using goat anti-human apoA-I. B: Effect of 725-1E2 on the release of cholesterol from RAW264 by apoA-I and by HDL. The cellular lipid was labeled with [^{3}H]acetate. Cells were incubated with lipid-free apoA-I (10 μg in 1 ml medium), HDL_2, and HDL_3 (10 μg protein) for 4 h in the presence of 200 μg 725-1E2 or mouse IgG. Release of [^{3}H]cholesterol into the medium was detected. Each datum represents the average ± SD for more than three measurements. A single asterisk indicates the difference between the data with IgG with $P < 0.05$, and double asterisks with $P < 0.01$.

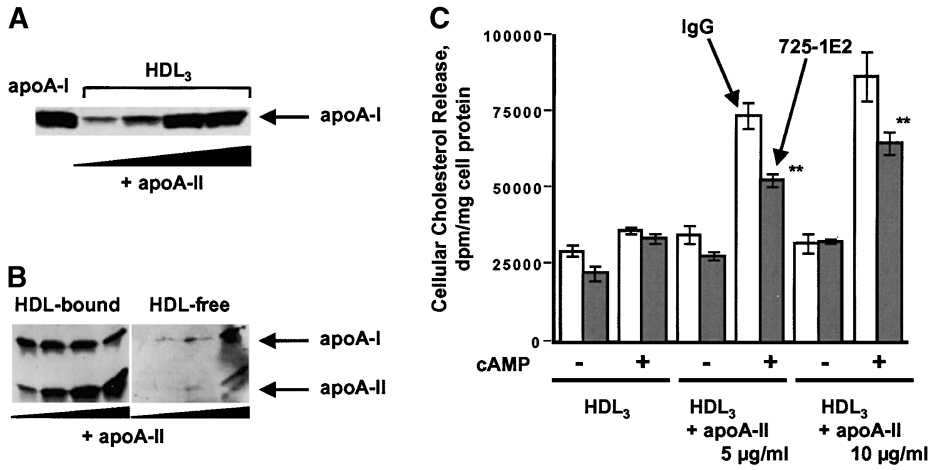

Fig. 4. Effect of apoA-II on the release of cholesterol from RAW264. A: Displacement of apoA-I by apoA-II from HDL. HDL_3 (1 μg protein) was incubated with apoA-II (0, 0.5, 1, and 2 μg) in 100 μl for 16 h at room temperature. Free apoA-I was probed by a free apoA-I-specific monoclonal antibody, 725-1E2, by using an immunoprecipitation technique as described for Fig. 3A. B: Displacement of apoA-I by apoA-II from HDL. After incubation of HDL_3 with apoA-II as above, HDL-bound and -free proteins were separated by ultracentrifugation at a density of 1.21 g/dl. Proteins were analyzed in electrophoresis in 10% polyacrylamide and by staining with Coomassie Brilliant Blue. C: Cholesterol release from RAW264 cells was examined in the presence and absence of dibutyryl cAMP, and inhibition by the antibody was attempted. The cells in 1 ml medium were incubated with HDL_3 (10 μg protein), which was preincubated with apoA-II (0, 5, and 10 μg), for 4 h to measure the cholesterol release. Each datum represents the average ± SD for more than three measurements. Double asterisks indicate the difference between the data with IgG and $P < 0.01$.

electrophoresis (**Fig. 5A**) and by negatively-stained electronmicroscopy (Fig. 5B, C). The particles appear homogeneous, having an average diameter of 11.9 ± 3.0 nm and 12.3 ± 4.3 nm, respectively. When the reconstituted HDL was incubated with the cells, specific reversible binding of proapoA-I was induced by cAMP, while no apparent association of CO was induced (**Fig. 6**). The results indicated that specific binding induced by cAMP is not applied for the whole lipoprotein particles, but only for the lipid-free apoA-I that may dissociate from the particles.

Finally, the release of cholesterol to rHDL was examined. The similar results to those with natural HDL were demonstrated with rHDL. The cAMP-inducible cholesterol release was 120% of the nonspecific basal release, and the antibody inhibited about 60% of this portion (**Fig. 7**).

DISCUSSION

ApoA-I is a major protein component of HDL and forms this lipoprotein complex in plasma based on its multisegment structure of amphiphilic α-helix (40). ApoA-I and other helical apolipoproteins in their free form interact with cells and generate HDL by removing cellular lipid when ABCA1 is present in the cells. This reaction does not

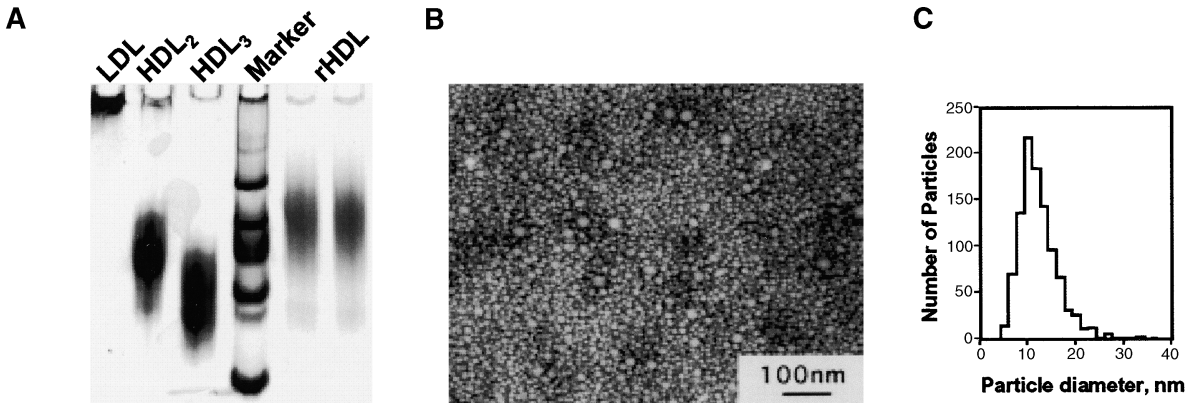

Fig. 5. Characterization of reconstituted HDL (rHDL). A: Profile of rHDL in nondenaturing gradient gel electrophoresis (5% to 20% polyacrylamide). B: Electronmicrograph in negative staining. C: Histogram of the diameter of 1,000 rHDL particles measured on the electronmicrogram. Chemical composition of the particle was protein/phosphatidylcholine/cholesteryl oleate (CO) (1:1.56:0.43; w/w/w).

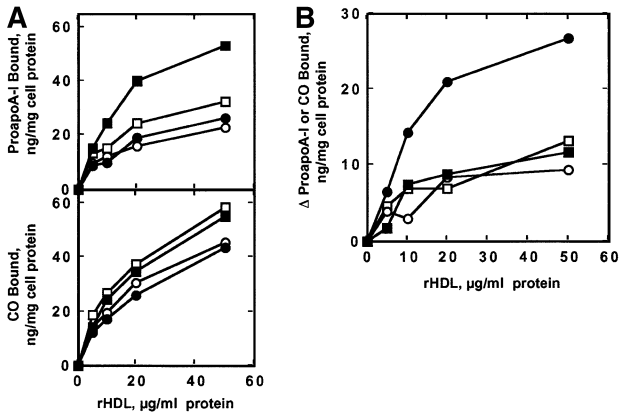

Fig. 6. Binding of rHDL to RAW264 cells. rHDL was prepared with [^{3}H]-labeled proapoA-I and [^{14}C]cholesteryl oleate ([^{14}C]CO) (New England Nuclear). The binding experiment was carried out in the same manner as free proapoA-I with cAMP-stimulated (closed squares and circles) and unstimulated (open squares and circles) RAW264 cells. Displacement was carried out by 100 μg/ml of the unlabeled rHDL. A: Binding of proapoA-I and CO was measured by counting ^{3}H (upper panel) and ^{14}C (lower panel), respectively. Reversible binding was calculated by subtracting the binding after displacement (closed and open circles) from total binding (closed and open squares) for protein and lipid, respectively. B: Reversible binding of apoA-I (closed and open circles) and CO (closed and open squares). While cAMP induced the proapoA-I binding, binding of CO was not induced by cAMP.

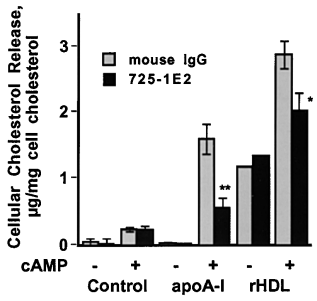

Fig. 7. Effect of 725-1E2 on the cholesterol release by rHDL. After the cAMP treatment, the cells were incubated with apoA-I (10 μg) or rHDL (10 μg protein) in the presence of either mouse IgG or 725-1E2 for 24 h. Cholesterol mass in the medium was determined as described in the method. Each datum represents the average ± SD for more than three measurements. Double asterisks indicate the difference between the data with IgG and $P < 0.01$.

take place when they are in the lipid-bound form (1, 17). This observation is of physiological relevance because the association of helical apolipoproteins with HDL is reversible (41), and the presence of lipid-free apoA-I is implicated in blood plasma (21). However, more direct evidence is needed to establish the hypothesis that apoA-I actually dissociates from HDL to interact with cells.

The results presented in this paper are summarized as follows: *1*) ABCA1-dependent release of cellular lipid by apoA-I is proportional to its reversible binding to the cell; *2*) a free apoA-I-selective monoclonal antibody effectively inhibits this reaction but shows no effect on ABCA1-independent cholesterol exchange between HDL and cells; *3*) the ABCA1-dependent portion of cell cholesterol release to HDL is inhibited by the antibody to the same extent as the inhibition of the apoA-I-mediated release, including the condition that apoA-I is forced to dissociate by apoA-II-induced displacement; and *4*) the ABCA1-dependent binding is only apoA-I, but not lipoprotein particles, when examined by using reconstituted HDL.

The proportion of the dissociated apoA-I in the HDL preparation was estimated at most 2% to 3% by the antibody (Fig. 3) and 1% to 2% by the enteropeptidase (enterokinase E-0585, Sigma Chemical Co.) (data not shown), the enzyme that recognizes only lipid-free apoA-I (42). Inasmuch as HDL-apoA-I concentration is in the order of 10^{-5} M in these experimental conditions, its dissociation constant for HDL (K_d.HDL) can be estimated around 10^{-8}–10^{-9} M. This is much lower than the K_d values previously reported for helical apolipoproteins to interact with lipid surface, in the order of 10^{-7} M (18, 43, 44). However, these values were obtained by measuring the binding of apolipoproteins to the lipid surface with lower curvature than HDL or using the protein labeled with a large probe molecule such as iodine or a fluorescent compound. Therefore, actual dissociation of apoA-I from a natural HDL particle can be less than the experimentally measured dissociation because the protein is more integrated into the particles due to its higher surface curvature and contributes more to its structural stability. The cAMP-inducible cholesterol release from RAW264 cell by HDL was 26% of the basic release and 120% by rHDL (Fig. 3), which may reflect more unstable lipid-protein interaction in rHDL. The data are not inconsistent with the results, with fibroblasts of the patients with Tangier disease, that more than half of the cholesterol release to HDL was nonspecific and ABCA1-independent (4).

The reaction model above was validated by kinetic analysis of the data by using the assumptions that *1*) only the lipid-free apoA-I is in equilibrium with the cell-bound apoA-I from the data in Fig. 2, and *2*) apoA-I is also in equilibrium between the free form and the HDL-bound form. From the data in Figs. 2 and 6, K_d.cell/K_d.app is 0.09 ($7.4 \times 10^{-8}/8.0 \times -10^{-7}$ M/M), and [B_f.HDL] is far below 10^{-7} M, so that K_d.HDL should be below 10^{-8} M (see Appendix). Therefore, the experimental observation was consistent with this reaction model. Granted that apoA-I binds to HDL with such high affinity, apoA-I can still be transferred from HDL to cell surface to interact with ABCA1 to generate new HDL without assuming a further specific mechanism.

Cholesterol release from RAW264 cells stimulated by cAMP for ABCA1 expression was directly proportional to the apoA-I reversible binding to the cell. Phillips and his colleagues implicated the unparalleled relationship between apoA-I binding and cholesterol efflux by showing different concentration dependency on the apoA-I of these two reactions (45). Their K_m for cholesterol efflux was similar to ours, but K_d for apoA-I binding was higher than our result. Apparent binding affinity of fluorescence-labeled apoA-I was also higher than the present data in our own previous work by using the same cell line (26).

This difference may be due to the labeling of apoA-I with large molecules such as ^{125}I or a fluorescent compound (46). More work is required to identify the site and nature of apoA-I binding, and further kinetic analysis should provide more suggestive information about this issue.

We propose that when HDL removes cell cholesterol, specific interaction with the cell takes place not as the whole particle but with the dissociated apoA-I. The hypothesis is consistent with our recent finding that helical apolipoproteins protect ABCA1 from its proteolytic degradation only in their lipid-free forms but not in the HDL-bound forms (22). Release of cholesterol and phospholipid were both proportional to specific binding of apoA-I, so that the HDL assembly is likely to be mediated by physical interaction of lipid-free apoA-I with the cells. In vivo, the reactions that involve cholesteryl ester transfer reaction in the presence of lipolysis (19) and phospholipid transfer reaction (20) would promote the release apoA-I from HDL, so that lipid-free apoA-I could be available for the ABCA1-mediated HDL assembly reaction more than expected by dissociation in equilibrium.

APPENDIX

When P_f is lipid-free apoA-I: B_f.cell is unsaturated cellular specific binding site for apolipoprotein A-I (apoA-I); P_b.cell is the cell-bound apoA-I; K_d.cell is a dissociation constant of apoA-I for the interaction with the cell; [B_f.HDL] is unsaturated HDL binding site for apoA-I; [P_b.HDL] is the HDL-bound apoA-I; and K_d.HDL is a dissociation constant of apoA-I with HDL, [P_f] × [B_f.cell] = K_d.cell × [P_b.cell], and [P_f] × [B_f.HDL] = K_d.HDL × [P_b.HDL], and then

$$K_d.HDL \times [P_b.HDL] \times [B_f.cell] = K_d.cell \times [P_b.cell] \times [B_f.HDL] \qquad (Eq.\ A1)$$

From an apparent binding profile of apoA-I as the whole HDL particle in Fig. 6, an apparent dissociation constant K_d.app is described in an empirical equation,

$$([P_f] + [P_b.HDL]) \times [B_f.cell] = K_d.app \times [P_b.cell],$$

which can be abbreviated by assuming [P_f] << [P_b.HDL] as

$$[P_b.HDL] \times [B_f.cell] = K_d.app \times [P_b.cell] \qquad (Eq.\ A2)$$

Consequently, equation 1 can empirically be abbreviated as

$$[K_d.HDL] \times [(K_d.cell)/(K_d.app)] = [B_f.HDL]$$

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