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CHOLESTEROL EFFLUX FROM CULTURED ADIPOSE CELLS IS MEDIATED BY LpA_I PARTICLES BUT NOT BY LpA_I:A_{II} PARTICLES

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Cholesterol efflux was studied in cultured adipose cells after preloading with LDL cholesterol. Long-term exposure to LpA_I and LpA_I:A_{II} particles isolated from the HDL fraction showed that LpA_I particles only were able to promote cholesterol efflux. Liposomes containing different $ApoA_I/ApoA_{II}$ molar ratios were tested : the larger the proportion of $ApoA_I$, the faster the ability to remove cholesterol from Ob1771 cells. Dose-response curves showed that LpA_I particles were active within a physiological range of concentrations, whereas LpA_I:A_{II} particles had no effect at all concentrations. The results are in favour of LpA_I particles being the active components of the HDL fraction for the promotion of cholesterol efflux and suggest that LpA_I particles and LpA_I:A_{II} particles represent distinct metabolic entities.

It has been observed by several authors that HDL can promote cholesterol efflux from a variety of cells (1). Cultured mouse adipose cells have been shown recently to bind ApoE-free HDL isolated from mouse or human plasma. $ApoA_I$ and $ApoA_{II}$, when inserted into liposomes, were shown to be the ligands for cell surface binding sites. Long-term exposure of adipose cells to LDL cholesterol as a function of LDL concentration led to an accumulation of cellular unesterified cholesterol. This process was saturable and reversible as a function of time and concentration by exposure to HDL_3 or $ApoA_I$-containing liposomes, whereas $ApoA_{II}$-containing liposomes did not

ABBREVIATIONS:

HDL, high density lipoproteins ; **LDL**, low density lipoproteins ; **ApoE, $ApoA_I$ and $ApoA_{II}$**, apolipoproteins E, A_I and A_{II}, respectively ; **DMPC**, dimyristoyl phosphatidylcholine ; **DME** medium, Dulbecco's modified Eagle's medium ; **FBS**, fetal bovine serum ; **PBS**, phosphate-buffered saline ; **T_3**, triiodothyronine.

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promote any cholesterol efflux (2). A striking feature was the higher affinity of binding sites for $ApoA_{II}$ as compared to $ApoA_I$. These findings led us to propose that cholesterol efflux involved the recognition of $ApoA_I$ by specific binding sites and that $ApoA_{II}$ behaved as an antagonist. In regard to human lipoprotein metabolism, this observed phenomenon is of great interest. Indeed, it is now recognized that $ApoA_I$ is distributed within HDL between two types of lipoprotein particles. One type contains both $ApoA_I$ and $ApoA_{II}$ ($LpA_I:A_{II}$ particles) whereas $ApoA_{II}$ is absent from the other one (LpA_I particles) (3-7). These two types of lipoprotein particles have been recently well characterized (6). The levels of LpA_I and $LpA_I:A_{II}$ particles have been shown to vary differently upon drug treatment (5) or alcohol consumption (8). Actually, it has been suggested that LpA_I particles might represent the "anti-atherogenic" fraction of HDL as their plasma levels are significantly lower in subjects suffering from an angiographically defined coronary artery disease than in healthy patients (9). Altogether, the data would indicate that LpA_I and $LpA_I:A_{II}$ particles might have different metabolic functions. The purpose of the present study was to compare the effects of human LpA_I and $LpA_I:A_{II}$ particles on the promotion of cholesterol efflux from cultured mouse adipose cells. Liposomes containing $ApoA_I$ and $ApoA_{II}$ at different molar ratios were also tested.

MATERIALS AND METHODS

Clonal line and cell culture

The characterization of Ob1771 preadipocyte clonal line has been previously reported (10). Cells were plated at $2x10^3$ cells/cm^2 in 35-mm dishes and grown until confluence in DME medium supplemented with 10% FBS, 200 units/ml penicillin, 50 µg/ml streptomycin, 33 µM biotin and 17 µM pantothenate (defined as standard medium). After confluence (4 to 5 days after seeding), this medium was replaced by the differentiation medium, i.e. standard medium supplemented with 17 nM insulin, 2 nM T_3, 10 µM methylglyoxal bis(guanylhydrazone) and 100 µM putrescine. Under these conditions, most Ob1771 cells differentiated into adipose cells within 10 days. The medium was changed every other day. The experiments reported below were performed on 10-day post-confluent cells.

Isolation of lipoproteins and quantitation of apolipoproteins

Human LDL and HDL were separated by sequential ultracentrifugation of normolipemic human plasma (11). Their purity was assessed by 10% polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate (12). The LDL preparation (d=1.006-1.063) contained no detectable apolipoproteins in addition to ApoB, whereas the HDL preparation (d=1.065-1.21) contained $ApoA_I$ and A_{II} but no detectable ApoE ; however, ApoE could be detected in the HDL fraction by using more sensitive enzyme immunoassays (vide infra).

Isolation of LpA_I and $LpA_I:A_{II}$ particles by immunoaffinity chromatography

Immunoaffinity columns were prepared as follows : IgG fractions from rabbit immunserum directed against human $ApoA_I$ or $ApoA_{II}$ were first isolated by chromato-

graphy on protein A Sepharose 4B column. Specific antibodies against $ApoA_I$ or $ApoA_{II}$ were isolated by passing each IgG fraction over a cyanogen bromide-activated Sepharose 4B column to which $ApoA_I$ or $ApoA_{II}$ had been previously coupled. Anti-$ApoA_I$ and anti-$ApoA_{II}$ immunoadsorbents were prepared by coupling the specific antibodies (100 mg) to cyanogen bromide-activated Sepharose 4B column (5 mg/g of gel, ref.13). After antibody coupling, immunoadsorbers were equilibrated with PBS pH 7.4 containing 0.01% EDTA.

For isolation of $LpA_I{:}A_{II}$ particles from the HDL fraction (d=1.065-1.21), 5 ml of HDL (2 mg protein/ml) were run over the immunoaffinity column specific for $ApoA_{II}$. Samples were applied at a slow rate to an amount of gel sufficient to bind quantitatively all $ApoA_{II}$-containing particles. The gel was then washed extensively with the same buffer as above, and non-specifically bound material was eluted with 0.5 M sodium chloride. The unretained fraction was collected as LpA_I particles : they were used for isolation by immunoaffinity chromatography on anti-$ApoA_I$ column (see below). In order to dissociate and collect the retained fraction, 20 ml of 3 M NaSCN were applied to the anti-$ApoA_{II}$ column. To minimize contact between $ApoA_{II}$-containing particles and the dissociating agent, immunosorbers were constructed with a layer of Sephadex G25 below the immunosorber portion. This bottom layer of Sephadex G25 allowed immediate separation of the particles from the dissociating agent.

For isolation of LpA_I particles, the unretained fraction on anti-$ApoA_{II}$ immunosorber was checked for absence of $ApoA_{II}$ by enzyme immunoassay, and subsequently incubated with anti-$ApoA_I$ immunosorber. The retained fraction was collected as described above for the isolation of $LpA_I{:}A_{II}$ particles.

After collection, the fraction containing LpA_I or $LpA_I{:}A_{II}$ particles was extensively dialyzed against PBS, pH 7.4 and concentrated under reduced pressure. $ApoA_I$, A_{II}, B, C_{III} and E were measured in both fractions using specific enzyme immunoassays (14).

Preparation of apolipoprotein-containing liposomes

$ApoA_I$- and $ApoA_{II}$-containing liposomes were prepared as previously described, at an initial DMPC to protein molar ratio of 625 to 1 (2). DMPC liposomes containing various proportions of human $ApoA_I$ and $ApoA_{II}$ were prepared by adding a solution of $ApoA_I$ and $ApoA_{II}$ (molar ratio 1/2 to 2/1) to a sonicated preparation of DMPC liposomes, at a total protein concentration of 0.5 mg/ml. Under these conditions, as indicated by high-pressure liquid chromatography with a TSK-3000 GW column, neither $ApoA_I$ nor DMPC remained free in solution. Since the conditions to prepare apolipoprotein-containing liposomes were identical to those described by Roth et al. (15), it is assumed that their Stoke radius was between DMPC liposomes (> 120 Å) and that of ApoE-containing DMPC liposomes (< 150 Å).

Cholesterol influx and efflux

Cholesterol loading of the cells was performed at 37°C via LDL cholesterol (2). The promotion of cholesterol efflux was initiated at 37°C on differentiated, cholesterol-preloaded Ob1771 cells. The concentration of the remaining cellular cholesterol was determined by HPLC as previously described (2).

Materials

Culture media and fetal bovine serum were products of Flow Laboratories (Bethesda, U.S.A.). All other products were purchased from Sigma Chemical Co. (St.Louis, U.S.A.).

RESULTS

Preliminary observations indicated that LpA_I and $LpA_I{:}A_{II}$ particles were as effective competitors as human $ApoA_I$-containing liposomes for the binding of ^{125}I-$ApoA_I$-containing liposomes to differentiated Ob1771 cells (not shown). Therefore, differentiated Ob1771 cells were exposed at 37°C to LpA_I and $LpA_I{:}A_{II}$ particles and apolipoprotein-containing liposomes in order to study cholesterol efflux. Exposure of

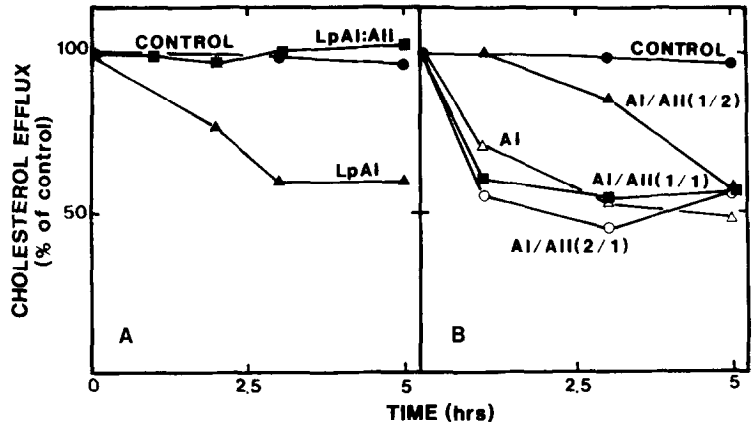

Fig.1 . Kinetics of cholesterol efflux in differentiated Ob1771 cells
Cholesterol-loaded cells were obtained as previously described (2). **(A)**, differentiated cells were exposed (▲, ■) or not (●) to both types of particles present at 100 µg/ml of protein ; **(B)**, differentiated cells (same series as in A) were exposed (▲, ■, △, ○) or not (●) to $ApoA_I$:$ApoA_{II}$-containing liposomes present at 100 µg/ml of protein. At the indicated times, cells from duplicate dishes were carefully washed and assayed separately for cholesterol content (2). The mean values from duplicate dishes are reported (± 10% from the mean) ; they are representative of two independent experiments performed on two different series of cells. Note that the same HDL fraction was used to prepare LpA_I and LpA_I:A_{II} particles. 100% correspond to 38 µg cholesterol/mg cell protein.

cholesterol-preloaded cells to LpA_I particles promoted a cholesterol efflux. A rapid decrease in the cellular cholesterol content occurred and reached a minimal value within 3 h (Fig.1A). This minimal value was identical to that determined in control cells before exposure to LDL cholesterol (ref.2 and not shown). No efflux was observed in the absence of LpA_I particles or in the presence of LpA_I:A_{II} particles (Fig.1A). When compared to the effect of LpA_I particles, the lack of effect of LpA_I:A_{II} particles was not due to possible differences in their affinity for specific binding sites since no cholesterol efflux was observed when varying the concentration of LpA_I:A_{II} particles from 25 to 200 µg/ml (Fig.2). $ApoA_I$-containing liposomes had been shown to promote cholesterol efflux from Ob1771 adipose cells whereas $ApoA_{II}$-containing liposomes were uneffective, whether or not cholesterol was present in the complexes (2). Therefore, parallel experiments to those using LpA_I and LpA_I:A_{II} particles were performed in the presence of DMPC liposomes containing various proportions of $ApoA_I$ and $ApoA_{II}$. The curves of Figure 1B indicate that the higher the proportion (and the amount) of $ApoA_I$, the faster is the decrease in cellular cholesterol content ; however the presence of $ApoA_{II}$ did not prevent $ApoA_I$ to promote cholesterol efflux.

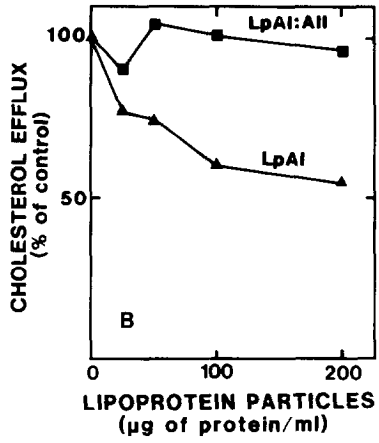

Fig.2. Dose-response curves of cholesterol efflux in differentiated Ob1771 cells
Cholesterol preloading of Ob1771 cells was performed as in Figure 1. After 5 h exposure to increasing concentrations of LpA_I or $LpA_I:A_{II}$ particles, duplicate dishes were carefully washed and assayed separately for cholesterol content (2). The mean values from duplicate dishes are reported (± 10% from the mean); they are representative of two independent experiments performed on two different series of cells from those used in Fig.1. Note that the same HDL fraction was used to prepare LpA_I and $LpA_I:A_{II}$ particles. Note also that the initial HDL fraction used in experiments of Fig.1 and Fig.2 was prepared from plasma of two different donors. 100% correspond to 36 µg cholesterol/mg cell protein.

DISCUSSION

The present study extends our previous work on the interaction of ApoE-free HDL and $ApoA_I$-containing liposomes to specific binding sites in Ob1771 cells (2). These sites are present at the cell surface of parental cells and a fraction with binding activity has been purified from crude membranes of these cells whereas, in contrast, these sites are absent from transformed cells in which the level of ApoB/E receptor sites is normal (R. Barbaras et al., unpublished work). The use of an heterologous system, i.e. human (apo)lipoproteins and mouse adipose cells, is supported by several observations i) mouse HDL (containing mainly $ApoA_I$ and $ApoA_{II}$) compete with human HDL_3 for the binding of ^{125}I-HDL_3 to receptor sites in Ob1771 cells (2), ii) mouse $ApoA_I$ and $ApoA_{II}$ resemble their human counterparts in aminoacid composition and iii) amino terminal sequence analysis demonstrates marked homology between human and mouse $ApoA_I$ (16). The plasma concentration of human LpA_I particles (300 mg/l) is actually higher than that required in vitro to observe a minimal effect (25 mg/l) or a maximal effect (200 mg/l) on cholesterol efflux. However the actual concentration of LpA_I particles is likely to be lower in the interstitial fluid bathing adipose cells than it

is in plasma, as already reported for LDL (17). Thus it is tempting to postulate that the observed effects mediated by LpA_I particles on cholesterol efflux are physiologically relevant. This point should be of major interest since patients suffering from atherosclerosis have a plasma concentration of LpA_I particles significantly lowered as compared to healthy patients, in contrast to the concentration of $LpA_I{:}A_{II}$ particles which remains unchanged (9). Clearly, the observation reported in this study that $LpA_I{:}A_{II}$ particles and LpA_I particles behave so differently for the promotion of cholesterol efflux is striking, because $LpA_I{:}A_{II}$ particles are indeed recognized by surface binding sites and thus can be considered as antagonists. This difference does not seem to be due to differences in ApoE content, since the percentage of ApoE was found to be 0.1% and 0.07% in LpA_I and $LpA_I{:}A_{II}$ particles, respectively. It is of interest to observe that $LpA_I{:}A_{II}$ particles were inactive for the promotion of cholesterol efflux (Fig.1A) in contrast to liposomes containing most likely both $ApoA_I$ and $ApoA_{II}$ (Fig.1B), although one cannot exclude that some liposomes might contain either $ApoA_I$ or $ApoA_{II}$ alone. The reasons for this opposite behaviour of native and artificial particles containing both $ApoA_I$ and $ApoA_{II}$ are unclear but might be related to the lipid composition and/or the conformation of $ApoA_I$ (or $ApoA_{II}$) in these two kinds of particles. In conclusion, the present study underlines that LpA_I particles and $LpA_I{:}A_{II}$ particles isolated from human plasma behave as distinct metabolic entities for the promotion of cholesterol efflux from cultured adipose cells.

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