Regulation of the Concentration of Preβ High-Density Lipoprotein in Normal Plasma by Cell Membranes and Lecithin–Cholesterol Acyltransferase Activity†

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ABSTRACT: A minor fraction of plasma high-density lipoprotein (preβ-1 HDL) has been shown to promote cholesterol efflux from peripheral cell membranes [Castro, G. R., & Fielding, C. J. (1988) *Biochemistry 27*, 25–29]. When isolated native plasma is incubated at 37 °C, this fraction is specifically decreased. On the other hand, the level of plasma preβ-1 HDL is fully protected in the presence of even very low levels of fibroblasts, vascular smooth muscle cells, or macrophages. Blood cells were completely inactive in maintaining plasma preβ-1 HDL levels in the absence of peripheral cells, even at the relatively high levels present in whole blood. The loss of preβ-1 observed in isolated plasma was dependent upon lecithin–cholesterol acyltransferase (LCAT) activity. These data suggest that reverse cholesterol transport catalyzed by preβ-1 HDL, and subsequent LCAT-mediated cholesterol esterification, is directly dependent upon the interaction between this HDL species and competent peripheral cells.

The major protein of human high-density lipoprotein (HDL) is apolipoprotein A-I (apo A-I). All HDL particles contain this protein, usually in combination with other apolipoproteins (Fielding & Fielding, 1991). HDL includes several species with an anomalous preβ electrophoretic migration that distinguishes them from the major (α-migrating) component of total HDL. One of these (preβ-1 HDL) has low molecular weight (about 74K) and contains apo A-I as the only protein component (Castro & Fielding, 1988). A similar particle has been identified in the plasma of other primates (Melchior & Castle, 1989), and is also present in lymph (Lefevre et al., 1988). Preβ-1 HDL is a major initial acceptor among lipoproteins of free cholesterol transferred from cell membranes to plasma (Castro & Fielding, 1988; Francone et al., 1989). Several other preβ HDL fractions of larger apparent molecular weight (preβ-2 HDL, preβ-3 HDL) are also found in plasma (Francone et al., 1989). Preβ-2 and preβ-3 HDL appear to be important intermediates in the later processing of cell-derived cholesterol as part of the reverse cholesterol transport pathway (Francone et al., 1989; Fielding et al., 1991).

The plasma of normal individuals shows a considerable variation in the proportion of total HDL present in the preβ fraction (Castro & Fielding, 1988). However, the factors controlling preβ HDL levels are little known. In this study, the regulation of preβ HDL levels has been investigated, and in particular that of preβ-1 HDL, to determine whether the proportions of these HDL species are actively regulated in plasma.

EXPERIMENTAL PROCEDURES

Blood was taken from normolipemic volunteers who had fasted overnight. The blood was drawn into plastic tubes cooled in ice water and containing streptokinase (Calbiochem, San Diego, CA; final concentration 150 units/mL) as anticoagulant (Miida et al., 1990). Plasma was separated by centrifugation (2000*g*, 30 min, 0 °C), rapidly brought to 37 °C, and then used immediately (<1 min) in the individual experiments described. In some experiments, whole blood was incubated, and plasma was then separated after incubation. Streptokinase is without effect on plasma lipoprotein pattern (Miida et al., 1990) and, unlike calcium-chelating anticoagulants such as citrate or EDTA, allows the incubation of whole blood or blood plasma with adherent cell monolayers, which would otherwise be dissociated.

Cell Culture. Normal human skin fibroblasts, human vascular smooth muscle cells (gift of Dr. Robert Wall, Stanford University), and human monocytic cells (HL-60 line, American Type Tissue Culture, Rockville, MD) were cultured in 3.5-cm plastic dishes. Fibroblasts were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, smooth muscle cells in Waymouth's medium with 10% fetal calf serum, and HL-60 cells in RPMI-1640 medium supplemented with 20% fetal calf serum. HL-60 cells were differentiated by the addition of 1.5×10^{-7} M tetramyristoyl phorbol acetate (TPA) for 48 h to induce attachment and macrophage transformation (Rovera et al., 1979) before use in the studies described below.

In experiments with whole blood, screw-capped glass vials (45 × 25 mm) containing 1 mL of blood were gently shaken (1 cycle s^{-1}) on an orbital shaker during the 37 °C incubation to ensure maximal contact between plasma and the cells. Samples were taken, chilled to 0 °C, and then centrifuged 10 min to remove the cells. The plasma was then analyzed by two-dimensional electrophoresis as described below. Control vials contained plasma alone obtained from the same blood samples.

Measurement of Changes in Preβ HDL Concentration. Plasma was incubated for periods of up to 90 min at 37 °C. Under these conditions, rates of plasma cholesterol esterification were linear from zero time (Fielding & Fielding, 1981). The distribution of preβ- and α-migrating HDL species of native and incubated plasma was determined by nondenaturing two-dimensional electrophoresis as described below (Miida et al., 1990). In some experiments, the synthesis of cholesteryl

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esters in plasma by lecithin–cholesterol acyltransferase (LCAT) activity was inhibited prior to incubation by 2.0 mM dithiobis(2-nitrobenzoic acid) (DTNB) (Stokke & Norum, 1972). This has been previously shown to be without effect on the viability of cultured cell monolayers under the conditions described (Fielding & Fielding, 1981). In other experiments, LCAT was inhibited immunologically with goat anti-human LCAT antibody, as described below.

Isolation and Identification of HDL Subfractions. Nondenaturing two-dimensional electrophoresis of plasma was carried out as previously described (Miida et al., 1990). Briefly, electrophoresis was first carried out at 0 °C in 0.75% agarose on GelBond (FMC, Rockland, ME) in 50 mM barbital buffer, pH 8.6. Second-dimensional electrophoresis of the agarose strip was then carried out at the same temperature in a gradient (2–15% w/v) of polyacrylamide in 0.025 M Tris–0.2 M glycine buffer (pH 8.3). Separated proteins were transferred to a nitrocellulose membrane (Sartorius, 0.45 μm; West Coast Scientific, Inc., Emeryville, CA) using a Hoefer Transblot apparatus (Hoefer, San Francisco, CA). Individual HDL species on the membrane were then identified by immunoblotting, using purified ^{125}I-labeled goat or rabbit anti-human apo A-I or LCAT antibody (see below), followed by autoradiography (Miida et al., 1990). To quantitate the apo A-I within individual HDL species, the autoradiograph was used as a template to identify HDL fractions on the nitrocellulose sheet. These areas were cut out and ^{125}I radioactivity was measured by γ spectrometry. The reactivity of preβ- and α-migrating species of HDL was earlier shown to be equivalent with this procedure (Ishida et al., 1987).

Antibody Purification. Anti-human apo A-I polyclonal antiserum was raised in rabbits, and goat anti-human LCAT polyclonal antiserum was obtained from Alpha Biomedical (Belleview, WA). Antiserum was purified by affinity chromatography on protein G–agarose (Pierce, Rockford, IL) in 50 mM acetate (pH 5.0). The retained IgG fraction was eluted with 0.1 M glycine buffer (pH 2.8). The eluate was immediately neutralized with 0.5 M phosphate buffer (pH 7.0) and dialyzed against phosphate-buffered saline overnight at 4 °C before use. In some experiments, purified IgG was used directly for immunoprecipitation, as described below. For immunoblots, the anti-LCAT or anti-apo A-I IgG was labeled with ^{125}I as described by Markwell (1982). The nitrocellulose membranes obtained after electrotransfer were treated with labeled antibody in 5% bovine serum albumin for 90 min at room temperature. Radioactivity was detected by autoradiography at −70 °C using X-OMAT AR Kodak film with intensifier screens for 1–3 days (Miida et al., 1990).

Plasma total apo A-I was determined as previously described (Fielding & Fielding, 1980) using a solid-phase immunoassay with rabbit anti-human apo A-I antiserum. Mean plasma total A-I concentration in this study was 1.15 ± 0.21 mg mL^{-1}.

Differences in plasma HDL distribution before and after modification were compared by paired *t*-test. A *p* value of <0.05 was considered significant. Intraassay variation of multiple analyses of the same plasma samples was 5–13% of the mean for individual preβ HDL fractions.

RESULTS

Modification of Preβ HDL Levels in Plasma. The concentration of apo A-I in preβ HDL was 7.0 ± 3.1% of total apo A-I ($n = 8$) as determined by agarose gel electrophoresis, equivalent to a mean total preβ HDL concentration of 80 μg mL^{-1}. When plasma was incubated at 37 °C for 90 min, this fraction decreased to 4.7 ± 2.6%, representing a 33% decrease (Figure 1) ($p < 0.001$). There was no change in the migration rate of either preβ HDL or the predominant α-migrating band. When the same incubation was carried out in the presence of a cultured fibroblast monolayer (2–3 μg of cell cholesterol per dish), there was no detectable loss of preβ HDL (Figure 1). This finding was confirmed by quantitation of bound ^{125}I-labeled antibody to human apo A-I (Table I). While there was a significant decrease in preβ HDL during incubation in the absence of cells, in the presence of cells there was no such change. Assay of total plasma apo A-I before and after incubation (90 min, 37 °C) in the presence or absence of cells indicated no significant change (<3%). This indicates that the changes found in the proportions of HDL subspecies represent a redistribution of apo A-I within plasma.

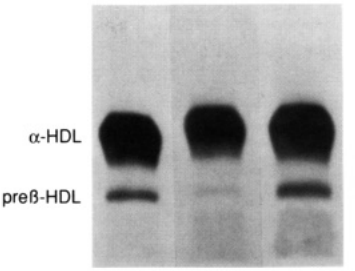

FIGURE 1: Agarose gel electrophoresis of native and preincubated plasma. Plasma was incubated at 37 °C for 90 min in the presence and absence of fibroblasts. Total preβ HDL and α-migrating HDL were then separated by agarose gel electrophoresis, and apo A-I distribution was determined as described under Experimental Procedures. Lanes left to right: original unincubated plasma; plasma incubated in the absence of cells; plasma incubated in the presence of cells.

Table I: Plasma Total Preβ HDL Determined by Agarose Gel Electrophoresis[a]

incubation	% preβ of total apo A-I at 0 min	% preβ of total apo A-I at 90 min
plasma	7.0 ± 3.1	4.7 ± 2.6[b]
plasma + fibroblasts	8.8 ± 4.2	9.2 ± 2.5

[a] Native plasma in the presence or absence of fibroblasts was incubated for 90 min at 37 °C. The distribution of apo A-I protein between preβ- and α-migrating fractions was determined following electrophoresis by electrotransfer and immunoblotting with ^{125}I-labeled rabbit anti-human apo A-I antibody as described under Experimental Procedures. Values shown are means ± 1 SD for seven experiments. [b] $p < 0.001$ compared to zero time value.

Preβ HDL contains several lipoprotein species of different apparent molecular weight separable by two-dimensional electrophoresis (Castro & Fielding, 1988; Francone et al., 1989). As shown in Figure 2, in the presence of cells, there was no significant change in the proportions of any of the different species. However, in the absence of cells, there was a striking decrease in the preβ-1 HDL species. This result was also confirmed by quantitation of bound labeled antibody (Table II). A large decrease in HDL preβ-1 (about 70% compared to 30–40% for total preβ HDL) was observed, corresponding to a mean decrease of from 38 to 14 μg mL^{-1} plasma. This was accompanied by smaller changes in the other preβ HDL species, although in the case of both preβ-2 ($p = 0.05$) and preβ-3 ($0.1 < p < 0.2$) the mean concentration did decrease somewhat with time in the absence of cells. This decrease did not occur when fibroblasts were present, when there was no significant loss in any preβ HDL species over the 90-min incubation period.

The time course of this change is shown in Figure 3. The rate of decrease in preβ-1 HDL was approximately exponential,

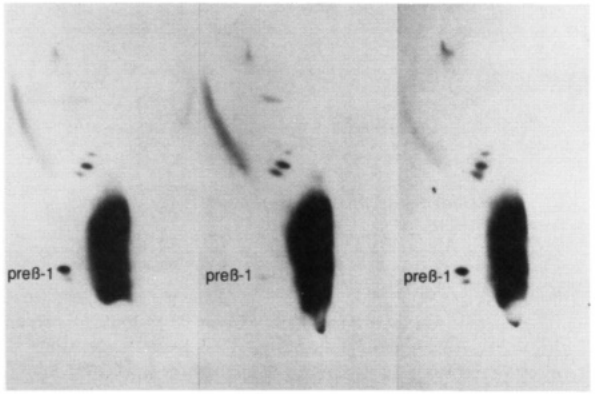

FIGURE 2: Two-dimensional electrophoretic analysis of plasma HDL subfractions after incubation in the presence and absence of cells. Plasma was incubated in the presence and absence of a monolayer of fibroblasts for 90 min at 37 °C. HDL subspecies were then separated by two-dimensional electrophoresis (agarose gel in the first dimension and nondenaturing gradient gel electrophoresis in the second dimension), and the apo A-I content of individual fractions was quantitated as described under Experimental *Procedures*. Panels: left, original unincubated plasma; center, plasma incubated in the absence of cells; right, plasma incubated in the presence of cells.

Table II: Distribution of Preβ HDL Subspecies Analyzed by Nondenaturing Two-Dimensional Electrophoresis[a]

incubation	% total apo A-I		
	preβ-1 HDL	preβ-2 HDL	preβ-3 HDL
plasma			
0 min	3.3 ± 1.4	2.7 ± 1.4	0.7 ± 0.4
90 min	1.1 ± 0.7[b]	2.3 ± 1.1	0.6 ± 0.4
plasma + fibroblasts			
0 min	3.7 ± 0.7	2.5 ± 0.7	1.2 ± 0.2
90 min	4.2 ± 1.1	2.9 ± 1.0	1.3 ± 0.5

[a] Quantitation of apo A-I was carried out as described in the legend to Figure 1. Values shown are means ± 1 SD for eight experiments.[b] $p < 0.001$ vs zero time value. Other differences did not reach significance.

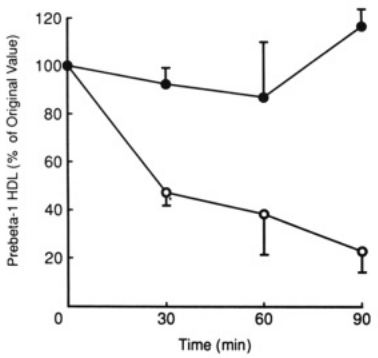

FIGURE 3: Time course of the level of plasma preβ-1 HDL in the presence and absence of cells. During incubation of native plasma at 37 °C with and without fibroblasts, samples were taken at the intervals indicated and analyzed by two-dimensional electrophoresis as described under Experimental Procedures. Values are the mean ± SD of three separate experiments. Closed circles, plasma incubated in the presence of fibroblasts; open circles, plasma incubated in the absence of fibroblasts.

with a $t_{1/2}$ of about 45 min. Figure 3 also confirms that in the presence of fibroblasts, preβ-1 HDL remains unchanged.

The major (α-migrating) HDL species consists of three major subfractions (Miida et al., 1990), corresponding to the HDL_3, HDL_{2b}, and HDL_{2a} flotational species (Anderson et al., 1977). In the absence of cells, there was a slight but significant increase in the HDL_{2a} and HDL_{2b} fractions ($p < 0.025$) (Table III). When fibroblasts were present, and preβ HDL was unchanged, there was also no change in the distribution of apo A-I in HDL_{2a} or HDL_{2b}. These results indicate that the apo A-I lost from preβ-1 HDL is transferred eventually to the larger fractions of α-HDL, possibly via an equilibrium governing the concentrations of preβ-1, -2, and -3.

Table III: Distribution of Apo A-I among α HDL Fractions and LDL[a]

	plasma only		plasma + fibroblasts	
	0 min	90 min	0 min	90 min
LDL	1.0 ± 0.7	0.8 ± 0.5	1.0 ± 0.4	1.1 ± 0.7
HDL_{2b}	21.7 ± 3.3	25.0 ± 3.2[b]	19.9 ± 3.1	21.1 ± 2.9
HDL_{2a}	41.9 ± 5.3	44.4 ± 3.4	44.4 ± 5.0	43.5 ± 4.8
HDL_3	27.6 ± 2.2	25.7 ± 3.3	27.7 ± 1.8	26.8 ± 3.2

[a] Values are percent distribution of apo A-I radioactivity before and after incubation (90 min, 37 °C) and represent means ± 1 SD of 11 experiments (–cells) and 6 experiments (+cells). Initial total preβ HDL was 7.4 ± 0.3% of total apo A-I, and final total preβ HDL in the absence of fibroblasts was 4.2%. Initial preβ-1 HDL in these experiments was 3.82 ± 1.35% and after 90-min incubation in the absence of cells was 1.09 ± 0.62% ($p < 0.001$). There was no significant change in preβ-1 HDL in the presence of cells, or in the other HDL fractions. [b] $p < 0.025$ compared to the initial value.

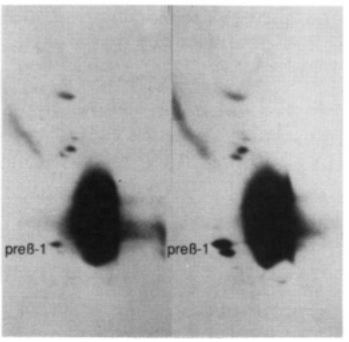

FIGURE 4: Comparison of effects of incubation of plasma with blood cells or cultured fibroblasts on the level of preβ-1 HDL. Plasma was incubated for 90 min at 37 °C with a monolayer of fibroblasts or with blood cells. Samples from each incubation were analysed by two-dimensional electrophoresis as described under Experimental Procedures. Panels: left, incubation with blood cells; right, incubation with fibroblasts.

The modulation of preβ HDL levels is therefore specific, involving changes mainly in the small preβ-1 HDL species, which is the initial acceptor of cell-derived cholesterol, and the presence of fibroblasts, at a low ratio (in terms of cell protein or cholesterol) compared to plasma, is sufficient to prevent any loss of this HDL species.

Cell Specificity in Regulating Preβ-1 HDL. The specificity with which the presence of fibroblasts modulated preβ-1 HDL levels was investigated by comparing the effects of other cell types.

Because of the large mass of blood cell membranes circulating with plasma in vivo, whole blood was used to determine whether these cells could also prevent the loss of preβ HDL. As shown in Figure 4, plasma incubated in the presence of blood cells lost most of preβ-1 HDL after a 90-min incubation. The decrease of 72% seen in the presence of blood cells can be compared with the decrease of 80% observed with plasma alone in the same experiment. Blood cells were without significant effect on the level of preβ-1 in plasma (Table IV). On the other hand, the same plasma sample incubated with about 200-fold less concentration of fibroblast membranes retained a prominent preβ-1 fraction (Figure 4). Analysis of other preβ and α-species following incubation in whole blood revealed only small changes that did not reach significance.

Table IV: Preβ-1 HDL Levels in the Presence of Vascular Smooth Muscle Cells, Macrophages, and Blood Cells[a]

cell type	% total apo A-I as preβ-1		
	0 min	90 min	% change
blood cells	3.1 ± 1.2	0.9 ± 0.4	−72
HL-60 macrophages	2.1 ± 1.2	2.7 ± 0.8	+27
smooth muscle cells	2.2 ± 0.5	2.4 ± 0.3	+10

[a] Values are means ± 1 SD for three experiments, and were obtained before and after incubation at 37 °C for 90 min.

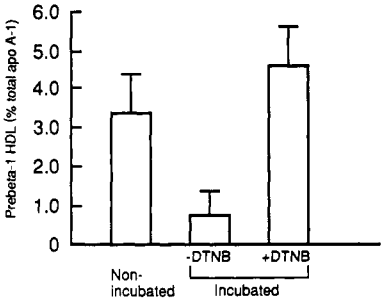

FIGURE 5: Effect of DTNB on the level of preβ-1 HDL during incubation of plasma. Plasma was incubated at 37 °C for 90 min in the absence or presence of 2.0 mM DTNB. Samples of the original plasma and the incubated material were then analyzed by two-dimensional electrophoresis in order to quantitate the preβ-1 HDL. Values shown are the mean ± SD of three experiments.

HL-60 is a human promyelocytic leukemic cell line which has been shown in previous studies to differentiate into cells with macrocytic properties in response to various agonists including TPA (Rovera et al., 1979). Following incubation with HL-60 cells (90 min, 37 °C), plasma was fractionated by two-dimensional electrophoresis as described for fibroblasts and blood cells. As shown in Table IV, there was complete retention of preβ-1 HDL under these conditions.

Similar studies were carried out with human smooth muscle cells. As shown in Table IV, these cells also prevented any loss of preβ-1 from plasma during incubation.

Together these data indicate that while several lines of cultured peripheral cells maintain or even slightly increase plasma preβ-1 levels, the high physiological level of blood cell membranes has little or no protective effect.

Role of LCAT in Determining Preβ HDL Levels. DTNB reacts nonspecifically with protein free sulfhydryl groups. DTNB blocks cholesterol esterification in plasma by reacting with LCAT free sulfhydryl groups vicinal to the enzyme active-site serine residue (Francone & Fielding, 1991a,b). At 0 °C, DTNB was without effect on the distribution of HDL species in plasma. When LCAT activity was inhibited by DTNB in cell-free plasma incubated at 37 °C for 90 min, the predicted decrease in preβ-1 HDL levels was completely blocked (Figure 5). Analysis following two-dimensional electrophoresis showed little or no effect on the proportions of other HDL species. Preβ-2 HDL was 2.0 ± 0.7% of HDL in the original plasma, 1.8 ± 0.5% of HDL in the absence of cells, and 2.1 ± 1.0% of HDL following incubation with DTNB. Preβ-3 HDL was 0.8 ± 0.4% of total HDL in the original plasma, 0.7 ± 0.4% in the absence of DTNB, and 0.8 ± 0.5% in the presence of DTNB.

To confirm that the action of DTNB was mediated by its effect on LCAT, native plasma was incubated overnight at 0 °C with purified goat anti-human LCAT IgG. In control incubations, nonimmune IgG replaced LCAT antibody. After centrifugation to remove precipitated antibody–antigen complex, plasma samples were incubated at 37 °C for 90 min, and then both experimental and control samples were analyzed by two-dimensional electrophoresis. As shown in Figure 6, there was a significant drop in the level of preβ-1 in the plasma preincubated with nonimmune IgG. On the other hand, after treatment with anti-LCAT antibody, the level of preβ-1 rose above base line to a mean of 131% of initial values, comparable to the effect observed when LCAT was inhibited with DTNB (Figure 5).

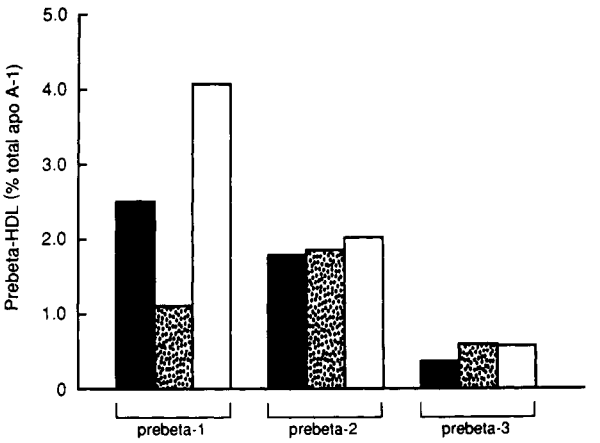

FIGURE 6: Effect of anti-LCAT antibody on the level of preβ-1 HDL. An aliquot of plasma was incubated for 20 h at 4 °C with purified anti-LCAT IgG, or with an equivalent mass of IgG from nonimmune serum, or with an equal volume of saline. The samples were then centrifuged for 15 min at 8800*g*, and the supernatant was removed. After incubation at 37 °C for 90 min, aliquots were taken for two-dimensional electrophoresis and quantitation of the preβ HDL subspecies, as described under Experimental Procedures. Closed bars, control incubations; stipplied bars, +nonimmune IgG; open bars, +anti-LCAT IgG. The data shown are the mean of two separate experiments.

Table V: Distribution of LCAT Protein among Plasma Lipoproteins[a]

	% total LCAT protein		
	preβ-3 HDL	α HDL	LDL
nonincubated plasma	31.5 ± 6.3	52.0 ± 7.2	16.5 ± 8.3
−fibroblasts	37.4 ± 8.9[b]	44.8 ± 7.5	17.7 ± 6.3
+fibroblasts	28.8 ± 7.3	47.7 ± 5.1	23.5 ± 6.1

[a] Values are means ± 1 SD for seven experiments. [b] $p < 0.02$ compared to +fibroblast value. Incubation in the presence or absence of fibroblasts was carried out for 90 min at 37 °C.

The distribution of LCAT antigen among preβ- and α-migrating HDL species was determined by immunoblotting with ^{125}I-labeled goat anti-human LCAT IgG. LCAT in preβ HDL (specifically, the high molecular weight preβ-3 fraction) preferentially esterifies cell-derived cholesterol (Francone et al., 1989), while LCAT in α-HDL is mainly active with cholesterol derived from plasma lipoproteins, particularly LDL (Fielding et al., 1991). As shown in Table V, the loss of preβ-I HDL was accompanied by a significant redistribution of LCAT within HDL, as enzyme moved into the preβ fraction from α-migrating HDL. Thus, a decrease in the plasma concentration of the proposed HDL catalyst of cell-to-plasma cholesterol transfer (Castro & Fielding, 1988) was associated with a compensatory increase in the concentration of enzyme catalyzing cell-derived cholesterol esterification.

DISCUSSION

Considerable data now indicate that preβ HDL plays an important role in delivering cholesterol from cultured peripheral cells for LCAT-mediated esterification in plasma (Castro & Fielding, 1988; Francone et al., 1989). Comparison

of esterification rates for cell- and LDL-derived cholesterol showed a considerably greater efficiency in the former case. This finding implies a mechanism in plasma that promotes a preferential utilization of cholesterol from peripheral cell membranes. The molecular basis for this selectivity has been incompletely understood, because plasma lipoproteins, particularly LDL, contain large amounts of free cholesterol that is readily utilized by LCAT in assays of native plasma in vitro (Fielding & Fielding, 1981; Park et al., 1987). Blood cells, mainly erythrocytes, provide another large pool of available substrate (Murphy, 1962; Glomset, 1968; Fielding et al., 1989).

The present research provides new information on the relationship between preβ-1 HDL and LCAT and in particular on the regulation of preβ-1 HDL levels. It was previously shown that total preβ-HDL, measured from one-dimensional agarose gel electrophoresis, decreases during incubation (Ishida et al., 1989; Neary et al., 1990). In both human and mouse plasma, total preβ HDL decreased over a 90-min incubation period, as described in the present results. However, as has been previously demonstrated (Castro & Fielding, 1988; Francone et al., 1989; Miida et al., 1990) using two-dimensional electrophoresis, preβ HDL, at least in human plasma, is heterogeneous, consisting not only of the low molecular weight, initial acceptor of cell-derived cholesterol (preβ-1 HDL) but also of later intermediates of the reverse cholesterol transport pathway, including one (preβ-3 HDL) where active esterification by LCAT is initiated (Francone et al., 1989). The present research demonstrates that only the level of preβ-1 HDL is significantly modified by incubation. The apo A-I contained in this fraction is evidently transferred to the larger α-migrating HDL fractions (HDL_{2a} and HDL_{2b}). These results indicate that the level of preβ-1 HDL is under active regulation in plasma.

This research also demonstrates that the presence of a small mass of fibroblasts is sufficient to completely protect plasma preβ-1 HDL against the effects of incubation. The same result was obtained with vascular smooth muscle cells and transformed macrophages. As extracellular fluid and lymph contain preβ HDL levels at least as high as those in plasma (Smith et al., 1984; Lefevre et al., 1988), this finding is likely to reflect HDL–cellular interactions occurring in vivo. However, blood cells were completely unable to regulate preβ-1 HDL concentration, despite the much greater number present for each milliliter of plasma (an approximately 200-fold greater concentration, in terms of cell cholesterol, compared to fibroblasts).

It has been unclear why under physiological conditions this excess of blood cells did not competitively inhibit reverse cholesterol transport from other cells, such as those of the vascular bed, through the pathway mediated by preβ-1 HDL. The present study demonstrates that blood cells do not contribute to the regulation of preβ-1 HDL concentration, and may therefore be unable to direct cholesterol into the preferred pathway of esterification utilized by other cells. It may also be relevant that erythrocytes, the major blood cell, synthesize no cholesterol (London & Schwarz, 1953) and have no LDL receptors (Gisinger et al., 1991). These cells in any case probably serve only as a buffer against transient changes in lipoprotein cholesterol concentration, for example, during postprandial lipemia (Fielding et al., 1989). The same is the case for platelets (Derksen & Cohen, 1973). Leukocytes contribute little to cholesterol synthesis (Fogelman et al., 1977), and in the circulation contain very few LDL receptors (Reichl et al., 1976). Thus, movement of cholesterol in or out of blood cells would not contribute to reverse cholesterol transport overall, that is, the net transport of peripheral cell cholesterol to the liver. The present findings suggest that the maintainance of preβ-1 HDL involves interaction with the peripheral cell surface and that blood cells are nonfunctional in this reaction.

The only function of preβ HDL identified so far is as an acceptor of cell-derived cholesterol. This lipoprotein forms part of a reaction sequence delivering cholesterol for esterification by LCAT (Francone et al., 1989). Three observations in this research link preβ HDL levels to free cellular cholesterol transport to plasma. Incubation in the absence of peripheral cells supplying free cholesterol reduces preβ HDL concentrations. Inhibition of the esterification reaction driving cholesterol transport prevents this decrease, and may even increase preβ HDL levels. Finally, this process shows cell specificity; it is effective with several lines of peripheral cells, but blood cells are incompetent. Data obtained in the present research therefore strongly suggest that interaction of preβ-1 HDL with competent cells such as fibroblasts, smooth muscle cells or macrophages is closely linked to the ability of these cells to supply cholesterol to the LCAT reaction and that this interaction is lacking with blood cells. Several laboratories have suggested that HDL interacts with cells via a receptor or binding site (Biesbroeck et al., 1983; Fidge & Nestle, 1985). The present data are most consistent with this type of interaction. While red cells can supply cholesterol to plasma lipoproteins, present evidence suggests that this is the result of thermodynamic rather than receptor-mediated transfers (Lange et al., 1983). In this case, the absence of an effect of red cells on preβ-1 HDL levels would reflect the fact that these lipoproteins do not react with the cell surface.

HDL receptors as so far described react rather nonspecifically with native HDL of different density fractions, or with synthetic HDL prepared from the recombination of phospholipid with several different HDL apolipoproteins (Graham & Oram, 1987; Fidge & Nestel, 1985; Barbaras et al., 1986). The presence of a small amount of preβ HDL in total HDL preparations used was not excluded in the earlier studies, and so it is possible that a preβ form of HDL was the active factor in the binding studies reported. Alternatively, the cell-surface factor whose presence is implied by these studies may represent an as yet undescribed HDL receptor. Further research will be required to distinguish these alternatives.

In summary, the present findings provide strong further support for a central role for preβ-1 HDL in cell-to-plasma cholesterol transport. Such specific transfer is no doubt supplemented by simple diffusion, which has been described between red cells and synthetic vesicles of various kinds (Bruckdorfer et al., 1968; Claret et al., 1978), and also between red cells and plasma lipoproteins (Quarfordt et al., 1970; Lange et al., 1983; Gottlieb, 1980). The proportions of preβ HDL-dependent and diffusion-dependent transfer of cholesterol probably depend on cell type, and possibly other local conditions.

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