Lipid utilization by human lymphocytes is correlated with high-density-lipoprotein binding site activity

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The nature and physiological importance of high-density lipoprotein (HDL) binding sites on unstimulated (resting) and mitogen-activated (blast) human peripheral blood lymphocytes were investigated. Specific HDL binding on resting and blast T-lymphocytes was saturable at 50 μg of 125I-HDL/ml and of high affinity, with K_s values of 8.1 × 10⁻⁸ M and 6.5 × 10⁻⁸ M, respectively, and B_max values of 79 ng and 180 ng/mg of cell protein respectively at 4 °C. Binding of HDL double-labelled with fluorescent diodacetyldinocarbocyanine (DiI) and isotope (125I) as well as of single fluorescence- or isotope-labelled HDL was inhibited competitively by HDL apoproteins. Studies of the cholesterol flux between the cells and HDL showed that HDL, low-density lipoprotein (LDL) or BSA at a concentration of 100 μg/ml in the tissue culture medium did not result in a significant difference in exogenous [3H]cholesterol efflux from the cell membrane at 37 °C. Proliferating T-blasts incorporated more cholesterol from HDL or LDL than did resting lymphocytes. When the cells were pulsed with 125I-HDL and chased in fresh lipid-free medium, up to 80% of the radioactivity released was not precipitable with trichloroacetic acid. This percentage decreased in a competitive manner when unlabelled HDL was present in the chase incubation medium. Finally, cultivation of lymphocytes with conditioned medium from macrophages increased DiI-HDL binding/uptake, while it was decreased by mevinolin-induced inhibition of hydroxymethylglutaril-coA reductase. In conclusion, human lymphocytes possess a HDL binding site (receptor) responsible for lipid binding/uptake and concomitant internalization and degradation of apoproteins from HDL, but not for reverse cell membrane cholesterol transport. The activity of the binding site is up-regulated during cell proliferation and down-regulated during cell growth suppression.

INTRODUCTION

Low-density lipoproteins (LDL) are believed to deliver cholesterol to peripheral cells [1], while high-density lipoproteins (HDL) remove excess cholesterol from these cells and transport it to the liver for catabolism or excretion. This probably contributes to the protective effect of HDL from atherosclerosis [2]. LDL are internalized and degraded following binding to their receptor on the cell membrane [1]. For reverse cholesterol transport, the interaction of HDL with a specific cell membrane receptor may be necessary [3]. However, the pathophysiological function of this binding site (receptor) has not yet been fully elucidated at the molecular level. One controversy with regard to the HDL pathway is whether HDL bound to its surface receptor/binding site is internalized into the cells, or shows reversible binding to the plasma membrane to provide or remove cellular cholesterol without internalization. Oram et al. [4] showed that cholesterol-loaded mouse peritoneal macrophages exhibited specific binding of HDL, but cell-surface-bound HDL particles were not internalized. However, morphological analyses by Schmitz et al. [5] and Takahashi et al. [6] using the same type of cells revealed that gold-labelled HDL particles were indeed internalized and delivered to endosomes, followed by retroendocytosis. Moreover, a third type of HDL interaction with macrophages and Hep G2 cells, reported by Rinninger and co-workers [7,8], consisted of HDL particle uptake and selective utilization of HDL-associated cholesteryl esters by the cells, without parallel apoprotein degradation.

In previous studies we have applied a flow cytometric technique using diodacetyldinocarbocyanine (DiI)-labelled lipoproteins to investigate the characteristics and physiological relevance of the HDL binding site, which recognizes apoprotein (apo) A1 as a ligand, on unstimulated and mitogen-activated living human peripheral blood lymphocytes (PBL). At 37 °C, specific binding/uptake of fluorescent HDL was observed in resting T-cells, and T-blasts exhibited significantly elevated Dil-HDL uptake [9,10]. To further characterize the HDL binding site on resting lymphocytes and mitogen-stimulated T-blasts, lipoproteins labelled with DiI (labelling the lipid fraction) or 125I (labelling the apoprotein fraction) or both (fluorochrome and isotope simultaneously) were used in the present study. Furthermore, to assess the physiological function of this binding site, the flux of cholesterol between HDL and lymphocytes, as well as the relationship of cell proliferation to HDL binding site activity, were investigated using lipoproteinslabelled with [3H]cholesterol or DiI.

MATERIALS AND METHODS

Isolation and labelling of lipoproteins

EDTA plasma was pooled from normolipaemic, fasting (12–14 h) male and female donors, aged 20–35 years, prescreened to have very low levels of lipoprotein[a] (< 1 mg/dl). Lipoproteins were prepared by differential centrifugation using solid KBr to adjust the density. The following fractions were obtained: LDL, 1.020–1.050 g/ml; HDL₂, 1.125–1.230 g/ml. HDL was further purified twice on Heparin–Sepharose affinity columns (Phar-

Abbreviations used: apo, apoprotein; CM, conditioned medium; Dil, 3',3'-dioctadecylindocarbocyanine perchlorate; FACS, fluorescence-activated cell sorter; FI, fluorescence intensity; HDL, high-density lipoprotein(s); LDL, low-density lipoprotein(s); PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; PHA, phytohaemagglutinin; UdR, 2-deoxyuridine.

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macia Fine Chemicals AB, Uppsala, Sweden) to remove the apoE-containing fraction. Concentrations of all lipoproteins were determined gravimetrically by weighing an aliquot after drying, and quantities of lipoproteins are expressed in terms of total weights.

Lipoproteins were labelled with Dil (excitation 520 nm, emission 570 nm; Lambda Probes & Diagnostics, Graz, Austria) according to Pitas et al. [11] with the following slight modifications: 26 mg of HDL was incubated in 50 ml of lipoprotein-deficient plasma with 980 μg of Dil solubilized in 650 μl of dimethyl sulphoxide for 8 h at 37 °C. After adjusting the density to 1.225 g/ml with KBr, Dil-HDL was re-isolated by ultracentrifugation [10,12].

Lipoproteins were iodinated with 125I (Amersham International, Amersham, Bucks., U.K.) by the method of Sinn et al. [13], which is known to yield higher labelling efficiencies (80–90 %) and milder pH (7.6). For double labelling, lipoproteins (5 mg/ml) were iodinated with 125I following Dil labelling. More than 99 % of their radioactivity was precipitable with trichloroacetic acid, and less than 5 % was extractable into methanol/chloroform (1:2, v/v). The specific radioactivity ranged from 350 to 640 c.p.m./μg of protein.

Lipoprotein labelling with [1α,2α(n)-3H]cholesterol (Amersham) was accomplished by a modification of the procedure of Jonas et al. [14] as follows: 2 ml of HDL or LDL (5 mg/ml) in Tris buffer (0.002 m-Tris, 0.15 m-NaCl, 0.001 m-EDTA, pH 7.6) was added to [3H]cholesterol (2.5 μCi); dried down with N2 and incubated at 4 °C for 5 h with occasional mixing. The product was mixed with 20 ml of 1.240 g/ml or 1.063 g/ml KBr Tris buffer and centrifuged at 100,000 g for 24 h. The top layer of labelled HDL or LDL was collected and dialysed against Iscove’s medium at 4 °C against five changes of the Tris buffer. The specific radioactivity of the labelled HDL and LDL was 1776 and 1198 d.p.m./μg of lipoprotein respectively.

Preparation of HDL apoproteins

Apo E-free HDL was dialysed against double-distilled water containing 0.01 %, EDTA, the pH adjusted to 7.4 with 0.1 m-NaOH, lyophilized and delipidated by extractions with chloroform/methanol (2:1, v/v). ApoHDLs were solubilized in 6 m-urea and dialysed extensively against tissue culture medium before use [10].

Isolation and cultivation of PBL

Blood donors were of both sexes, aged 20–35 years. Mononuclear cells were isolated from freshly drawn anti-coagulated (heparin)-peripheral blood by density gradient centrifugation over Lympho-Paque (Nyegaard & Co., Oslo, Norway; density 1.086 g/l) as described [12]. Monocytes were depleted by plastic adherence in tissue culture flasks (Falcon, Becton Dickinson & Co., Oxnard, CA, U.S.A.). The harvested cells were referred to as PBL [9,10,12].

Serum-free tissue culture medium was Iscove’s modified Dulbecco’s medium (Gibco, Paisley, Scotland, U.K.) supplemented with 0.5 % BSA (Fraction V; Sigma), monothioglycerol (Sigma, 10 μg/ml), and human transferrin (Sigma, 1 μg/ml). Mitogen responses were performed in triplicate in round-bottomed 96-well tissue culture plates (Nunc, Roskilde, Denmark) in a final volume of 200 μl/well. PBL (2.5 × 10⁶/well) were stimulated with 12.5 μg of phytohemagglutinin (PHA)/ml (Difco, Detroit, MI, U.S.A.). Stimulation was assessed by uptake of 51[I]lodo-deoxyuridine (125I-UdR; Amersham International) at 0.1 μCi/well, added together with 10 μM-fluorodeoxyuridine (Sigma) during a 3 h pulse on day 3 or 4 of culture. Suppression of PBL proliferation by mevinolin (Sigma), a competitive inhibitor of hydroxymethylglutaryl-coA reductase, was performed as described previously [10,12]. PHA-activated T blasts were cultured under similar conditions for 4 days in 50 ml Falcon tissue culture flask in a final volume of 4 ml. If necessary, cell debris was removed by density gradient centrifugation over Lympho-Paque [10,12].

Preparation of conditioned medium (CM)

Human peripheral blood mononuclear cells were isolated as described above. Monocytes were obtained by plastic adherence after removal of the non-attached cells by washing with Iscove’s medium. Monocytes were cultured in Iscove’s medium at 37 °C for 4 days. The CM was harvested, centrifuged (1200 g, 10 min) and dialysed against Iscove’s medium.

Influx of [3H]cholesterol into lymphocytes

PBL were incubated with [3H]cholesterol-HDL or -LDL (50 μg/ml) in Iscove’s medium at 4 or 37 °C with or without PHA for various time periods. Following incubation, cells were chilled on ice, washed five times with RPMI 1640 at 4 °C and solubilized by adding 0.1 m-NaOH. The radioactivity and protein content of the lysed suspension were determined.

Determination of [3H]cholesterol efflux

To enrich plasma membranes with [3H]cholesterol from an exogenous source, PBL were incubated in 4 ml of Iscove’s medium containing [3H]cholesterol (2 μCi in 5 μl of ethanol) for 5 h at 15 °C [15]. After five washes with RPMI 1640 medium (Seromed, Berlin, Germany) without BSA, the labelled cells were again incubated with HDL, LDL or BSA in RPMI 1640 at 37 °C for different time periods. To stop possible cholesterol efflux, the suspension was chilled on ice and centrifuged at 4 °C for 10 min at 800 g. The radioactivity of an aliquot of the supernatant was determined using a scintillation counter (Beckman Instruments, Palo Alto, CA, U.S.A.).

LDL and HDL binding/uptake studies

Lipoproteins were extensively dialysed against Iscove’s medium prior to use. Binding/uptake studies were performed by incubating PBL for 2 h at 4 or 37 °C with 125I-HDL, Dil-HDL or 125I/Dil-double-labelled HDL in EDTA-containing medium (12.5 mm), with or without the indicated concentrations of unlabelled HDL or delipidated HDL (apoHDL). After five washes with phosphate-buffered saline (PBS; 0.01 m-Na2HPO4/0.003 m-KH2PO4/0.116 m-NaCl, pH 7.2), cell pellet radioactivity was determined in an LKB–Wallac 1270 Rack-gamma counter. Fluorescence measurements were performed in a fluorescence-activated cell sorter (FACS III; Becton Dickinson & Co., Englewood, NJ) equipped with an argon ion laser (model 2025; Spectra Physics, Mountain View, CA, U.S.A.) and linked to an Apple II Plus computer (Apple Computer, Inc., Cupertino, CA, U.S.A.). Details of FACS settings and methods of quantification of fluorescence intensity (FI) for uptake studies are described elsewhere [10,16,17]. FI was quantified by determining the FI of the 75th percentiles (FI = 75 %) from the cumulative frequency.

Determination of HDL degradation

PBL were preincubated with 50 μg of 125I-HDL in 2 ml of EDTA-containing medium at 4 °C for 2 h with gentle shaking (80 rev./min). The cells were washed five times with 15 ml of Iscove’s medium at 4 °C to remove unbound 125I-HDL. Further washing did not decrease cell-associated radioactivity. The cells were then distributed in 0.5 ml of Iscove’s medium per tube (2 × 10⁶ cells) and incubated at 37 °C for various time periods, referred to as chase incubation. At the time points indicated in the Figures, the cell suspension was chilled on ice and centrifuged at 4 °C for 10 min at 800 g. An aliquot of the supernatant was collected and then measured.
was precipitated by 0.125 M-trichloroacetic acid followed by centrifugation. The pellet and supernatant were counted for radioactivity separately in the y-radiation counter in terms of trichloroacetic acid-insoluble (precipitable) and trichloroacetic acid-soluble (non-precipitable) products. Finally the cells were dissolved in 0.1 M-NaOH, and the amount of cell-associated radioactivity and the protein content were determined.

After chase incubation for 4 h as described above, the incubation medium was collected and chromatographed at room temperature on a 1.2 cm x 15 cm column of Sephadex G-25 equilibrated in Tris buffer containing 0.1% BSA. The column was eluted with the same buffer and fractions of 10 drops (about 0.33 ml) were collected and counted. The column was calibrated using 125I-HDL solution containing about 1% free 125I.

Cells preincubated with 125I-HDL at 4 °C after washing were first incubated at 4 °C or 37 °C for 5 min with or without trypsin (1 mg/ml), or at 37 °C for 5 min and then for another 5 min with trypsin. These cell suspensions were then centrifuged at 800 g for 10 min at 4 °C. Radioactivity of the cells and supernatants was determined in a y-radiation counter.

T blasts were incubated with 125I-HDL at 4 °C with or without 100 μM-chloroquine (Sigma) or 10 mM-NH4Cl for 2 h. After washing, the cells were re-incubated at 37 °C in the presence or absence of chloroquine or NH4Cl for 2 h. Radioactivity in both the cells and supernatants was determined.

Other analytical procedures

Protein contents of samples were determined using the Bio-Rad protein assay (Bio-Rad Laboratories G.m.b.H., Munich, Germany). Statistical analyses were performed using an unpaired Student's t test.

RESULTS

HDL binding and competition studies

Resting and mitogen-activated PBL were incubated with increasing concentrations of 125I-HDL at 4 °C for 2 h in the presence or absence of a 50-fold excess of unlabelled HDL. Saturable binding at about 50 μg of 125I-HDL/ml occurred with high affinity, with Kd values of 8.1 x 10-9 M and 6.5 x 10-9 M and Bmax values of 79 ng and 180 ng/mg of cell protein for T-cells and T blasts respectively (Fig. 1), indicating that the binding sites on both resting T-cells and T blasts are of similar affinity but different capacity.

To further characterize the relationship between HDL lipid and apoprotein binding/uptake, competition experiments were carried out with HDL subjected to different labelling procedures. As shown in Fig. 2, binding/uptake of Dil-HDL, 125I-HDL or Dil/125I double-labelled HDL can be competed for by delipidated HDL (apoHDL). Competition of 50% was achieved with a concentration of 25 μg of apoHDL/ml. No significant difference with respect to competitive capacity emerged between fluorescent and isotope-labelled HDL. Dil and 125I label the lipid and apoprotein fractions of lipoproteins respectively. In double-labelling experiments, the radioactivity and fluorescence intensities were simultaneously detectable on T blasts during competition with apoHDL, indicating that both HDL lipids and apoproteins can be bound and taken up in parallel by the cells.

Flux of cholesterol between HDL and lymphocytes

To examine the ability of HDL to cause an efflux of plasma membrane cholesterol, resting and mitogen-activated PBL were pulsed with [3H]cholesterol and the efflux was measured during a 12 h incubation with HDL, LDL or BSA at 37 °C, over different observation periods. At a concentration of 100 μg/ml, HDL, LDL and BSA were similarly effective in removing [3H]cholesterol from the cell surface (Fig. 3).

The time course of [3H]cholesterol influx into resting lymphocytes and PHA-stimulated T blasts from labelled HDL and LDL is shown in Fig. 4. Saturation of [3H]cholesterol influx from labelled HDL and LDL was reached at 12 h of incubation in resting lymphocytes. However, in T-blasts, the degree of [3H]cholesterol influx from both labelled HDL and LDL showed a linear increase with increased incubation times, indicating the high demand of growing T cells for lipoprotein constituents.

Degradation of HDL by lymphocytes

The initial incubation with 125I-HDL was performed at 4 °C and the chase was carried out at 37 °C or 4 °C. The results show that, at 37 °C, the cells progressively released small-molecular-mass radioactive molecules into the medium, such that after 3 h only about 20–30% of the originally bound 125I-HDL remained associated with the cells. There was a rapid release rate of 125I radioactivity from cells carrying receptor-bound 125I-HDL during the initial chase incubation (Fig. 5a). At 3 h of chase incubation,

![Fig. 1. Binding of 125I-HDL to T-lymphocytes](image-url)

(a) PBL (1 x 10^7) were cultured for 4 days at 37 °C with or without PHA (25 μg/ml) in a total volume of 4 ml of Iscove's medium. After three washings, resting PBL (○, □, △; 2 x 10^⁶) and T blasts (■, ▲; 1 x 10^⁶) were incubated for 2 h at 37 °C in the absence (○, ■) or presence (▲, △) of a 50-fold excess of unlabelled HDL with the indicated concentrations of 125I-HDL in a total volume of 400 μl of EDTA (12.5 mM)-containing medium. Specific binding of 125I-HDL to the cells (□, ■) was determined by subtracting labelled HDL binding values in the presence of unlabelled HDL from those in the absence of unlabelled HDL. (b) Scatchard analyses of 125I-HDL specific binding on resting T-cells (□) and T blasts (■).
only 10–20% of the radioactivity present in the medium was precipitable with 0.125 M trichloroacetic acid (Fig. 5b), and 80–90% was not precipitable (Fig. 5c). Similar release patterns of bound 125I-HDL were observed in resting PBL and T-blasts, although the amounts of cell-bound and released radioactivity were less in the resting lymphocytes (Fig. 5). Cells incubated at 4°C released only 10–20% of the initially bound radioactivity in 3 h, compared to 80–90% by the cells incubated at 37°C, indicating that the release of cell-bound radioactivity is an energy-dependent process.

The effect of unlabelled HDL in the chase medium on the release of cell-associated radioactivity by T-blasts is shown in Fig. 6(a). The amount of trichloroacetic acid-precipitable radioactivity released was higher in the presence of unlabelled HDL (Fig. 6b). Specifically, increased precipitable radioactivity was reflected by lower cell-associated and trichloroacetic acid-soluble radioactivity (Fig. 6c). These results suggest that the presence of unlabelled HDL in the chase medium enhanced lipoprotein release, resulting in increased radioactivity in the medium.

The radioactive products released during the chase period by T-blasts preincubated with 125I-HDL were further analysed by gel filtration chromatography (Fig. 7). As a control, 125I-HDL was incubated for 3 h at 37°C in the same medium as was used for cell culture. The 125I-HDL and chase-incubated-cell media were passed over the column, and sharp high- and low-molecular-mass peaks were obtained. The former generally corresponded to the intact HDL fraction, and the latter to amino acids, i.e. metabolized products. In chase-incubated medium only a small peak was observed in the high-molecular-mass fraction. These results complement those of the trichloroacetic acid precipitation experiments (see Fig. 5).

On most cell types the HDL binding site has proven relatively resistant to trypsin treatment, but trypsinization can dissociate bound HDL from its cell surface binding site [18–20]. In the present experiments, trypsin treatment for 5 min at 37°C caused 75% release of the total bound 125I-HDL from T-blasts, while only 45% of bound 125I-HDL was released from cells preincubated at 37°C for 5 min before the 5 min trypsinization, indicating that 30% of cell-associated 125I-HDL was internalized.
Interactions of high-density lipoproteins with human lymphocytes

Fig. 5. Effect of temperature on the release of cell-associated radioactivity

Resting PBL (---) and T-blasts (-----) were incubated with 50 μg of 125I-HDL/ml for 2 h at 4 °C, washed with Iscove’s medium at 4 °C and re-incubated at 4 °C (■) or 37 °C (○) for the indicated times. Cell-associated (a) as well as trichloroacetic acid (TCA)-insoluble (b; precipitable) and TCA-soluble (c; non-precipitable) radioactivity in the medium was determined. Values are means ± S.E.M. of three experiments.

Fig. 6. Effect of unlabelled HDL in the chase medium on the release of cell-associated radioactivity

T-blasts were incubated for 2 h with 50 μg of 125I-HDL/ml at 4 °C, washed with Iscove’s medium at 4 °C and chased in the absence (■) or presence (○) of 50 μg of unlabelled HDL/ml at 37 °C for the times indicated. Cell-associated (a), trichloroacetic acid (TCA)-insoluble (b; precipitable) and TCA-soluble (c; non-precipitable) radioactivity were determined at the indicated time points. Values are means ± S.E.M. of three experiments. *Statistically significant difference, P < 0.05.

during the 5 min preincubation at 37 °C. Furthermore, 100 μM-chloroquine or 10 mM-NH4Cl did not affect HDL binding and degradation on T-blasts (Table 1).

Relationship between cell proliferation and HDL binding site activity

In the present experiments, lymphocytes were studied during two phases, i.e. resting and proliferating. Resting PBL can be stimulated to proliferate in vitro by mitogens, such as PHA, and the rate of cell proliferation correlates positively with increased PHA concentrations up to 25 μg/ml. Interestingly, Dil-HDL and Dil-LDL binding/uptake by the cells increased in parallel, and reached a plateau at PHA concentrations of 5–15 μg/ml (Fig. 8a). This increased binding was evident whether the affinity or the number of binding sites changed during proliferation. Saturation curves and Scatchard analysis on resting PBL and T-blasts demonstrated that the number of HDL binding sites increased without affecting binding affinity (Fig. 1). Furthermore, the increased HDL binding sites on T-blasts were due to increased density rather than an enlarged cell surface, since the total bound 125I-HDL on the T-blasts was much higher than that on resting PBL based on cellular protein weights (Fig. 5).

Although the composition of the CM from human macrophages is very complex, several constituent factors are known to promote cell proliferation of PHA (25 μg/ml)-stimulated lymphocytes. In this experiment, the CM only served as a source of growth factors to enhance T-cell proliferation. With increased rates of cell growth at various concentrations of CM (up to 100%, v/v), up-regulation of Dil-HDL and Dil-LDL binding/uptake was obtained (Fig. 8b). The activities of the HDL binding site and LDL receptor showed a linear increase from 100% (control, without CM) to 135%, in parallel with increased 125I-UdR incorporation by lymphocytes. Moreover, when cellular cholesterol synthesis was simultaneously inhibited by mevinolin, a competitive inhibitor of hydroxymethylglutaryl-coA reductase, without lipid supplement in the medium, LDL receptor activity increased from 100% to 200%, indicating that the suppressed cells attempt to obtain exogenous cholesterol via the LDL receptor. Surprisingly, Dil-HDL binding/uptake of cells decreased to 50%; thus the high-affinity HDL binding site
DISCUSSION

Investigations of HDL binding are complicated by the dynamic exchange of apoproteins and lipids between lipoprotein particles, liposomes and cell membranes. In our previous studies, a fluorescent label (Dil) was chosen to trace the movement of lipids from surface-bound HDL into lymphocytes. Those experiments failed to determine whether this takes place by simple lipid transfer or by internalization of the HDL particles. The requirement for an apoprotein receptor binding for lipid exchange between HDL and cells has been questioned [21,22]. In the present paper, we address this point by assessing binding/uptake of isotope- and/or fluorescence-labelled HDL on resting T-cells and T-blasts. Three types of labelled HDL, Dil-HDL, 125I-HDL and Dil/125I-double-labelled-HDL, revealed similar ligand characteristics, i.e. specificity (competed strongly by apoHDL), high affinity $[K_d (6.5-8.1) \times 10^{-8} \text{ M}]$ and saturation (50 $\mu$g of HDL/ml). These data compare favourably with those reported previously for T-cells [10,23], and support the existence of a specific HDL receptor that may be responsible for the binding/uptake of holo-HDL by lymphocytes, since the radioactivity and fluorescence intensity of the cells incubated with double-labelled Dil/125I-HDL decreased in parallel upon competition with apoHDL.

The mechanism of cholesterol exchange between cells and HDL has been the subject of much investigation and controversy, in part because the outcome (delivery/removal) varies depending on the cell types and the experimental procedures. Three mecha-
nisms have been suggested: (1) reverse cholesterol transport from the surface of extrahepatic cells [3,24], (2) establishment of a bidirectional equilibration of cholesterol between cell and HDL particle [25–27], and (3) a postulated unidirectional selective uptake of HDL cholesterol by the cells with or without degradation of the HDL particle [5–8,28,29]. We now show that human T-lymphocytes cultured at 37 °C can incorporate HDL cholesterol in parallel with a PHA-induced proliferation in serum-free medium. Resting lymphocytes incorporate significantly less cholesterol from HDL, reaching a plateau at 12 h. This may be explained in two ways: increased binding of [3H]cholesterol-labelled HDL on T-blasts by increased HDL receptors, and increased [3H]cholesterol-labelled HDL particle uptake by proliferating T-cells. LDL are able to supply proliferating cells with cholesterol via its receptor, while HDL uptake may serve to supplement either cholesterol or phospholipids. Delivery of cholesterol via HDL to T-cells is considered to be non-specific, since HDL can rescue mevinolin-suppressed cell proliferation only at high concentrations (> 250 μg/ml) in the medium [10]. In the present study with a long culture period (up to 96 h), non-specific incorporation of HDL [3H]cholesterol may also have occurred, since 30–40% of total HDL binding/uptake is independent of the high-affinity HDL binding site at an HDL concentration of 50 μg/ml during a 2 h incubation at 37 °C (Fig. 1). No evidence for the specific transport of exogenous cholesterol from the lymphocyte plasma membrane to HDL was found, since HDL, LDL and even BSA showed a similar ability to mediate cholesterol efflux from the cell membrane.

The absence of intact HDL in the chase medium of cultured luteal cells was reported by Rajan & Menon [30]. Our results indicate that retroendoysis of HDL, reported by several authors [6,28,29], may not occur in lymphocytes since human fibroblasts only degraded less than 20% of total bound [125I]-HDLS in a 2 h incubation at 37 °C (results not shown). Furthermore, chloroquine and NH4Cl, unrelated compounds which can inhibit the lysosomal pathway of HDL degradation [31], did not inhibit HDL degradation in T-lymphocytes, indicating that a lysosome-independent process of HDL degradation is involved.

Thus an HDL receptor on human lymphocytes identified by differently labelled HDL may be responsible for binding, internalization and degradation of holo-HDL particles. However, it is difficult to clarify the factors that regulate HDL receptor activity on lymphocytes. Oram et al. [32,33] showed that HDL receptor activity on fibroblasts, aortic smooth muscle cells and endothelial cells was up-regulated by increased cellular cholesterol content and inhibition of cell proliferation [34], and down-regulated by serum growth factors [35] and insulin [36], presumably to prevent loss of intracellular pools of cholesterol needed for membrane synthesis. These results are at variance with our observations on human T-lymphocytes. Mitogen (PHA) can stimulate quiescent T-cells to proliferating blasts, and CM from human monocytes/macrophages promotes PHA-activated cell proliferation in culture. HDL receptor activity is up-regulated on these proliferating T-cells, in contrast to the down-regulation reported by Oppenheimer et al. [34–36] on human fibroblasts. Obviously, proliferating T-cells require more nutrients from the environment to meet the requirement for cell organelle synthesis. It seems reasonable that HDL may provide more lipids, i.e. phospholipids or fatty acids, by increased activity of the HDL receptor, rather than mediating reverse cholesterol transport from the cells.

LDL receptor activity can obviously be up-regulated by a low cellular cholesterol content. In proliferating T-cells stimulated by PHA or CM, LDL receptor activity up-regulation is dependent on the greater requirement for cholesterol, as further substantiated by the suppression of the mitogen response after inhibition of hydroxymethylglutaryl-coA reductase, the rate-limiting enzyme for intracellular cholesterol synthesis. In this case, LDL receptor activity is increased by more than 100%, due to the decreased intracellular cholesterol content.

The main function of LDL consists of providing cholesterol to proliferating T-cells via the apoB,E receptor, while HDL may also supply lipids to these cells via its receptor. HDL was not considered to be a cholesterol donor for the cells because HDL receptor activity was down-regulated by decreased intracellular cholesterol content, e.g. when synthesis of endogenous cholesterol was inhibited by mevinolin in mitogen stimulation tests under serum-free conditions. Exogenous fatty acids or phospholipids may be provided by HDL in lipid-free medium, since cultured lymphocytes required an exogenous source of fatty acids or phospholipids for optimal proliferation in response to mitogens [16,37–40]. When cell proliferation was suppressed by inhibition of hydroxymethylglutaryl-coA reductase, the activity of the HDL receptor was decreased, possibly because less fatty acids or phospholipids were required under these unfavourable conditions.

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