Cell-surface binding sites for high density lipoproteins do not mediate efflux of cholesterol from human fibroblasts in tissue culture[1]

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Abstract The present investigation was designed to test the hypothesis that binding sites for high density lipoproteins (HDL_3) on cell surfaces of peripheral tissues mediate cholesterol efflux from these cells. This hypothesis had been formulated to explain two observations: *1*) HDL_3 binding to peripheral cells and HDL_3-mediated cholesterol efflux from these cells had both been found to saturate at similar unbound (free) HDL_3 concentrations; and *2*) both of these processes had been found to be similarly "up-regulated" by loading the cells with cholesterol. In the present study, however, we found that the "specific" binding of HDL_3 to cholesterol-loaded human fibroblasts was saturated at a free HDL_3 concentration of approximately 20 μg protein/ml, whereas efflux of cholesterol from these cells to HDL_3 did not "saturate" even at a free HDL_3 concentration of 2000 μg protein/ml. In addition, we found that the increase in cholesterol efflux caused by loading the fibroblasts with cholesterol was no greater when the acceptor particles were HDL_3 than when albumin or phospholipid vesicles served as acceptors, despite a marked increase in HDL_3 binding to these cells. Because HDL_3 binding to these cells and HDL_3-mediated cholesterol efflux from these cells do not saturate at similar free HDL_3 concentrations, and because the cholesterol-induced increase in HDL_3 binding is not accompanied by a similar increase in cholesterol efflux that is specific for HDL_3, we conclude that the described HDL_3 binding sites on human fibroblasts do not mediate cholesterol efflux. — **Mendel, C. M., and S. T. Kunitake.** Cell-surface binding sites for high density lipoproteins do not mediate efflux of cholesterol from human fibroblasts in tissue culture. *J. Lipid Res.* 1988. **29:** 1171–1178.

Supplementary key words receptors • transport • atherosclerosis

Cell-surface binding sites for high density lipoproteins (HDL) have been identified in a variety of tissues. Because HDL are thought to be involved in reverse cholesterol transport, it has been suggested that these binding sites may function in peripheral tissues to remove cholesterol from cells (1, 2). Although the aqueous solubility of cholesterol is sufficient to account for its movement between cells and HDL (3, 4), evidence has been obtained that suggests that HDL binding sites on human fibroblasts mediate cholesterol removal from these cells. This evidence includes two observations in particular: *1*) HDL binding to these cells and HDL-mediated cholesterol efflux from these cells both appear to saturate at similar unbound (free) HDL concentrations; and *2*) both of these processes appear to be "up-regulated" by loading the cells with cholesterol (2).

Under most circumstances, studies showing that the binding of a ligand to a defined site is regulated in parallel with the proposed biological function of that site would stand as strong evidence that the binding site, in fact, performs the proposed function (and is therefore a receptor). In the case of the apparent parallel regulation of HDL binding and HDL-mediated cholesterol efflux, however, an alternative explanation is possible since the mechanism of regulation (loading the cells with cholesterol) is relatively nonspecific and might be expected to have a wide variety of effects. In particular, loading cells with cholesterol would be expected to increase the chemical potential of cholesterol in the plasma membranes. This increased chemical potential might, in turn, be expected to increase the diffusional rate of cholesterol leaving the membranes to any acceptor particle, not just to HDL. If so, the increase in cholesterol efflux observed in cholesterol-loaded cells might be a nonspecific effect, unrelated to the cholesterol-induced increase in HDL binding. We have addressed this issue in the present investigation.

MATERIALS AND METHODS

Materials

Cholesterol (chromatography grade), egg phosphatidylcholine, and bovine serum albumin (BSA) (fraction V)

Abbreviations: HDL, high density lipoproteins; HSA, human serum albumin; PBS, phosphate-buffered saline; LPDS, lipoprotein-deficient serum; TNM, tetranitromethane; TLC, thin-layer chromatography.

[1]Previously published in part as an abstract (1987. *Clin. Res.* **35:** 511a).

were from Sigma Chemical Co. (St. Louis, MO). Na-^{125}I (13–17 mCi/μg I) was from Amersham Corp. (Arlington Heights, IL), and heparin linked to agarose was from Bio-Rad Laboratories (Richmond, CA). [7-^{3}H(N)]cholesterol (10–20 Ci/mmol, New England Nuclear Research Products, Boston, MA) was purified by thin-layer chromatography (TLC) on Silica Gel G in cyclohexane–ethyl acetate 3:2 (5) and stored at 8°C in benzene for up to 1 month. Sandoz compound 58-035 (3-[decyldimethylsilyl]-N-[2-(4-methylphenyl)-1-phenylethyl]propanamide) was a generous gift from Dr. John Heider of Sandoz, Inc. (East Hanover, NJ). Human serum albumin (HSA, 250 g/l, Cutter Laboratories, Emeryville, CA) was dialyzed against Dulbecco's phosphate-buffered saline (PBS) before use. It was shown to be free of apolipoproteins (apo) A-I and B by immunonephelometry (6, 7) (sensitivity 400 ng/ml in 5 mg/ml HSA), and free of apoE by radioimmunoassay (8) (sensitivity 2 ng/ml in 5 mg/ml HSA). In addition, no lipoproteins could be detected by electron microscopy in its d < 1.21 g/ml fraction. It was, however, found to contain a small amount of phospholipid (0.5 μg/ml in 5 mg/ml HSA) (9).

Methods

Lipoproteins. HDL_3 (d 1.125–1.210 g/ml) were isolated from sera of fasting (12–14 hr) normal humans by ultracentrifugation (10), which was repeated at each density. The solvent densities were adjusted with KBr. Lipoprotein-deficient serum (LPDS) was prepared as the d > 1.25 g/ml fraction of serum and stored at 4°C for up to 6 weeks as a 60 mg/ml solution in 0.9% NaCl. ApoE-free HDL_3 were prepared by heparin-agarose chromatography (11) as previously described (12). Lipoproteins were stored for up to 6 weeks at 4°C in 100 mM NaCl, 50 mM Tris, pH 7.4 at 37°C, 0.04% EDTA, and 0.05% sodium azide.

ApoE-free HDL_3 were iodinated with ^{125}I by the iodine monochloride method, as modified for lipoproteins by Bilheimer, Eisenberg, and Levy (13). The apoE-free ^{125}I-labeled HDL_3 were passed over a Sephadex G-25 column and then dialyzed; 99% of the radioactivity was precipitable with trichloroacetic acid, and <5% was extractable into ethanol–ether 3:1. The specific activity generally ranged from 300 to 350 cpm/ng protein.

Tissue culture. Human newborn foreskin fibroblasts in their 10th to 15th passages were grown under 8% CO_2 in 6-cm tissue culture dishes (Costar, Cambridge, MA) in Dulbecco's modified Eagle medium H21 (DME), supplemented with 2 mM glutamine and containing 50 μg/ml gentamicin and 10% fetal calf serum. When the cells reached confluence, the medium was changed to DME containing 10% LPDS (cholesterol-depleted cells) or DME containing 10% LPDS + cholesterol (50 μg/ml, added from a solution of 15 mg/ml in ethanol) (cholesterol-loaded cells). Twenty-four hr later the cells were washed three times with PBS containing 0.5% BSA and three times with PBS alone.

The medium was then changed to DME containing 0.2% BSA, 5 μg/ml Sandoz compound 58-035 (an inhibitor of acyl CoA:cholesterol acyltransferase activity (14) added to prevent esterification of both the labeled and the unlabeled cholesterol) and 1 μCi/dish of [^{3}H]cholesterol (the [^{3}H]cholesterol was omitted when equilibrium binding studies were to be performed). To add the [^{3}H]cholesterol to the medium, a solution of (prepurified) [^{3}H]cholesterol in benzene was evaporated to dryness in a test tube and redissolved in 20–50 μl of ethanol (final concentration in medium <0.1%). A solution of 2% BSA was then added to the [^{3}H]cholesterol solution and vortexed vigorously; the resulting solution was added to the medium (10% v/v). Twenty-four hr later the cells were washed five times with PBS containing 0.5% BSA and then five times with PBS alone. The cells were then used for binding studies or for cholesterol-efflux studies.

Binding of apoE-free ^{125}I-labeled HDL_3. The equilibrium binding of apoE-free ^{125}I-labeled HDL_3 to human fibroblasts in tissue culture was measured after incubation of the cells with the HDL for 2 hr at 37°C in PBS containing 0.2% HSA. Equilibrium was attained by 2 hr, as demonstrated by the observations that the binding was maximal by this time even at the lowest concentration of unbound (free) apoE-free ^{125}I-labeled HDL_3 examined (0.5 μg protein/ml), and that the binding was completely (>90%) reversible upon addition of a 100-fold excess of unlabeled HDL_3 after the 2-hr incubation. After the incubation, a portion of the medium was taken for measurement of the free apoE-free ^{125}I-labeled HDL_3 concentration and the rest of the medium was removed by aspiration. The cells were then washed four times with 2 ml of ice-cold PBS containing 1% BSA and two times with PBS alone. The cell monolayer and the apoE-free ^{125}I-labeled HDL_3 bound to it were then dissolved in 0.1 N NaOH. One portion of this solution was taken for measurement of the concentration of cell protein and another was taken for measurement of the ^{125}I by gamma scintillation spectrometry.

Binding analysis. Nonspecific binding was not measured directly, but instead was estimated by nonlinear least-squares curve fitting of the total binding data, as described previously (12). The advantages of this method of estimation of "specific" and "nonspecific" binding have been discussed in detail (15, 16). The function describing the binding of a ligand to a single class of noninteracting saturable sites and to an unsaturable site(s), $B = nL/(K_D + L) + aL$ (where B = concentration of bound ligand, n = concentration of saturable sites, L = concentration of free ligand, K_D = equilibrium dissociation constant of the ligand for the saturable site, and a = constant), was fitted

to the total binding data by the method of nonlinear least squares, and the best fit estimates of n and K_D were computed. In no case did consideration of a second saturable site improve the fit to the data, as assessed by the F test (95th percentile).

Measurement of cholesterol efflux. The appearance of cellular unesterified (free) cholesterol in the medium was determined from the radioactivity in the medium and the specific activity of [^{3}H]cholesterol in the cells (determined from the free cholesterol concentration and the amount of radioactivity in aliquots of hexane–isopropanol extracts of the cells: see below). For all experiments the cells were incubated for 1 or 2 hr at 37°C with PBS containing HDL_3, HSA, phosphatidylcholine vesicles, or no additives, as specified, and the radioactivity in the medium at the end of the incubation was determined by liquid scintillation spectrometry. Under all conditions cholesterol efflux from the cells was linear with respect to time for at least 2 hr. Background cholesterol efflux—efflux to PBS alone (see Results)—was subtracted from total cholesterol efflux at each point.

Miscellaneous. Unilamellar phosphatidylcholine vesicles were prepared as described by Batzri and Korn (17). When both the cholesterol and protein concentrations of the cells in tissue culture dishes were to be measured, cellular lipid was first extracted with hexane isopropanol by the method of Brown, Ho, and Goldstein (18) and the residual cellular material (including protein) was dissolved in 0.1 N NaOH. Esterified and free cholesterol concentrations in the hexane–isopropanol solution were measured by the method of Heider and Boyett (19), and the protein concentration was measured by the method of Lowry et al. (20). Protein concentrations of the lipoproteins were converted to molarity as described previously (12).

RESULTS

As described by others (2), we found that cholesterol efflux from fibroblasts to PBS alone (background efflux), although variable in magnitude, was often a significant fraction of its efflux to acceptor particles. We also found that the concentration of cholesterol in the PBS at the end of the incubation often exceeded its aqueous solubility of 10–30 nM (5), sometimes reaching micromolar concentrations. Experiments were therefore undertaken to determine the nature of the cholesterol in the medium containing PBS alone. More than 90% of the radioactivity in the medium migrated as free cholesterol as judged by TLC. This [^{3}H]cholesterol sedimented (>90%) during centrifugation at 100,000 *g* for 1 hr, but not (<20%) during centrifugation at 800 *g* for 5 min. This result raised the possibility that the [^{3}H]cholesterol in the PBS was associated with membrane fragments. We therefore examined the sedimented material by electron microscopy using negative stains. The material was very heterogeneous, but distinct structures that appeared to be cellular fragments could be identified (**Fig. 1**). This material was present in much greater quantity in medium from cholesterol-loaded cells than in medium from cholesterol-depleted cells,[2] but was similar in appearance in both. It was present when all steps in the experiment (up to the electron microscopy) were performed under sterile conditions, indicating that it was not the result of bacterial contamination. In addition, no bacteria were seen. Rectangular crystals, presumably of cholesterol monohydrate (21), could also be seen in medium from the cholesterol-loaded cells.

As reported previously (2), the "specific" binding of apoE-free ^{125}I-labeled HDL_3 to human fibroblasts in tissue culture was greatly increased by loading the cells with cholesterol (**Fig. 2** and **Fig. 3**). The number of binding sites increased without a change in their affinity for HDL (Fig. 3).

Efflux of cholesterol from fibroblasts to HDL_3 did not "saturate" over the concentration range of HDL_3 examined (up to 2000 μg protein/ml; **Fig. 4**). This contrasts with the saturation observed in the HDL binding studies (at approximately 20 μg protein/ml) and indicates a dissociation between HDL-mediated cholesterol efflux and specific HDL binding. Cholesterol efflux to albumin also failed to saturate over the concentration range of albumin studied (**Fig. 5**).

Cholesterol efflux to various acceptor particles (HDL_3, 20 μg protein/ml; HSA, 5 mg/ml; and phosphatidylcholine vesicles, 100 μg/ml) was compared in cholesterol-depleted and in cholesterol-loaded fibroblasts. With cholesterol-loading of the fibroblasts, cholesterol efflux to HDL_3 increased, but cholesterol efflux to albumin and to phospholipid vesicles increased to a similar extent (**Table 1**). In each of the experiments shown, loading the cells with cholesterol resulted in at least twofold increases in cellular free cholesterol content, cholesterol efflux, and "specific" HDL binding.[3]

[2] In this regard it should be noted that the cholesterol-loaded cells generally appeared less healthy than the cholesterol-depleted cells. The cholesterol-loaded cells were usually less firmly attached to the tissue culture dishes and often did not remain viable past the 48 hr required for the experiment.

[3] In these studies the amount of bound apoE-free ^{125}I-labeled HDL_3 was determined only at a single concentration of free apoE-free ^{125}I-labeled HDL_3 (2 μg protein/mg). Specific binding was defined as the binding that could be displaced by a 50-fold excess of unlabeled HDL_3, and was usually ~90% of the total binding. This operational definition of specific binding is justified under our present experimental conditions for reasons discussed previously (30).

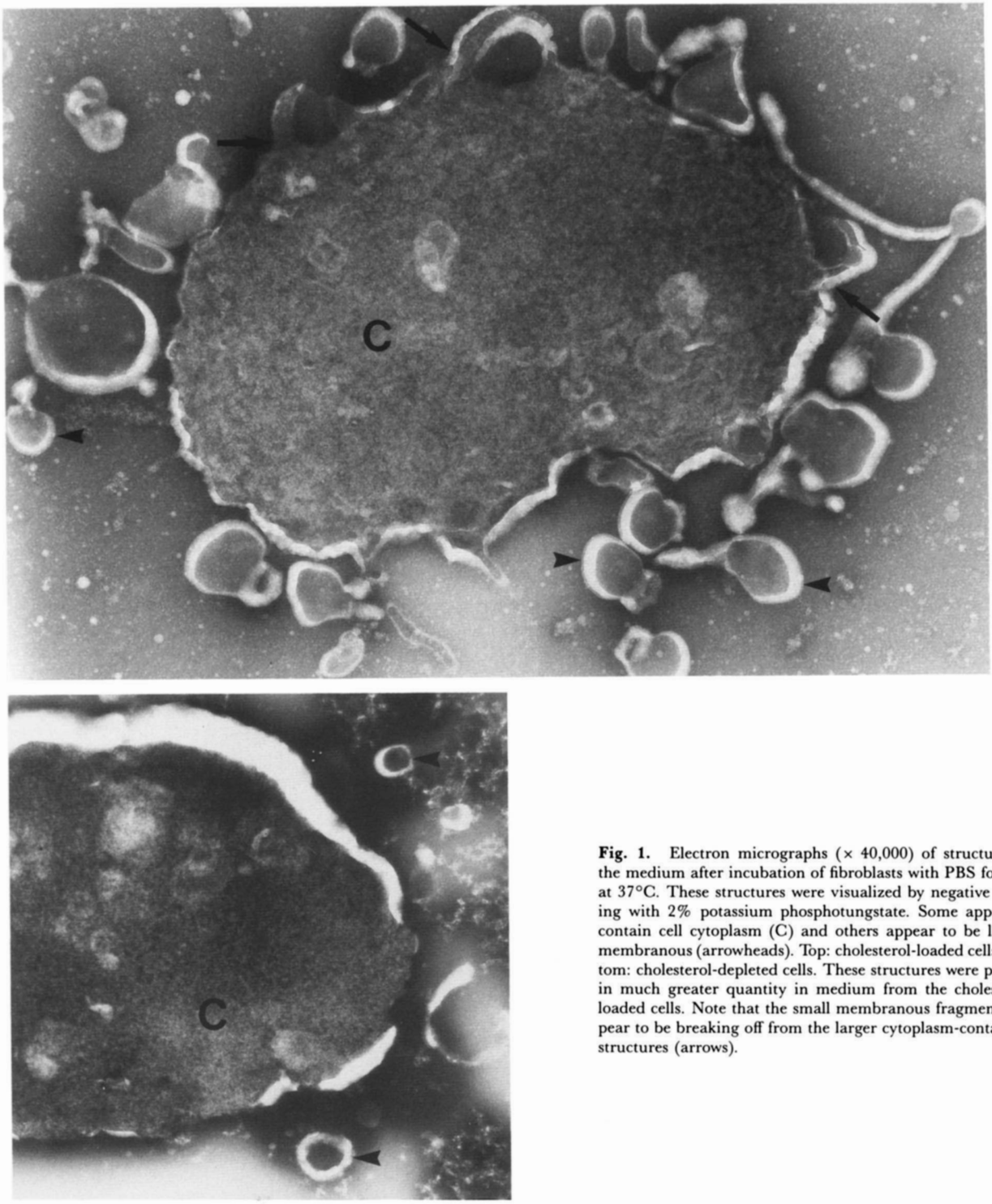

Fig. 1. Electron micrographs (× 40,000) of structures in the medium after incubation of fibroblasts with PBS for 2 hr at 37°C. These structures were visualized by negative staining with 2% potassium phosphotungstate. Some appear to contain cell cytoplasm (C) and others appear to be largely membranous (arrowheads). Top: cholesterol-loaded cells; bottom: cholesterol-depleted cells. These structures were present in much greater quantity in medium from the cholesterol-loaded cells. Note that the small membranous fragments appear to be breaking off from the larger cytoplasm-containing structures (arrows).

DISCUSSION

The current findings are inconsistent with the hypothesis that specific HDL binding sites on human fibroblasts mediate cholesterol efflux from these cells. This conclusion is based on two types of observation. First, specific HDL binding saturates at a free HDL_3 concentration of approximately 20 μg protein/ml (Figs. 2 and 3), but HDL-mediated cholesterol efflux does not "saturate" until much higher concentrations of free HDL_3 (>2000 μg protein/ml; Fig. 4). Second, the increase in HDL-mediated cholesterol efflux caused by loading the cells with

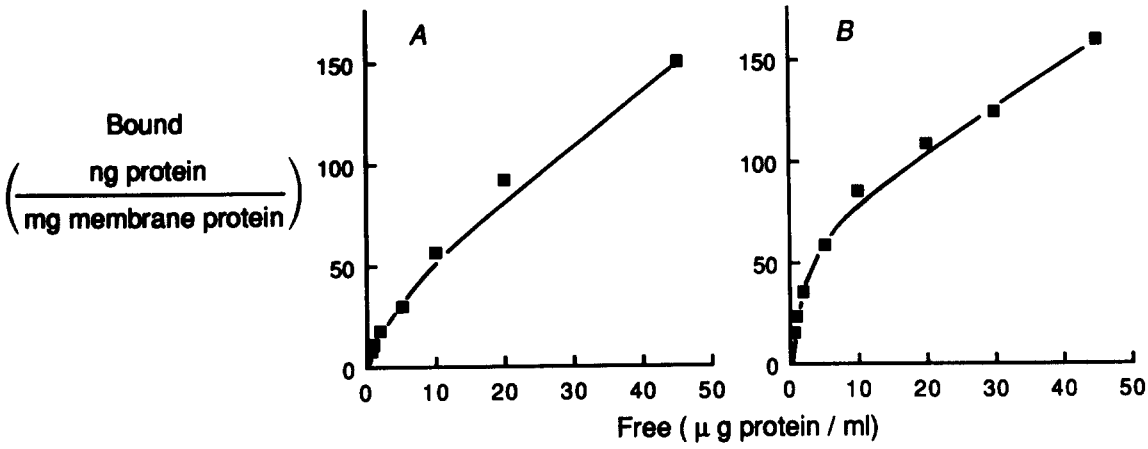

Fig. 2. Equilibrium binding at 37°C of apoE-free ^{125}I-labeled HDL_3 to human fibroblasts that were cholesterol-depleted (A) or cholesterol-loaded (B) as described in Methods. Total binding data are shown. Each point represents the mean of duplicate determinations. The experiment shown is representative of three such experiments performed.

cholesterol is no greater than the increase in efflux to other acceptor particles, despite the fact that HDL binding to these cells is greatly increased. Thus, the increase in cholesterol efflux is apparently the result of increased chemical potential of cholesterol in the cell membranes and is not related to the increase in HDL binding. Consistent with this interpretation was our finding that the fractional release of cholesterol from the cell membranes to HDL was similar in the cholesterol-loaded and the cholesterol-depleted cells (Table 1). Because HDL binding to these cells and HDL-mediated cholesterol efflux from these cells do not saturate at similar free HDL concentrations, and because the increase in HDL binding is not accompanied by a similar increase in cholesterol efflux that is specific for HDL, we conclude that the described HDL binding sites on these cells do not mediate cholesterol efflux.

Our finding that HDL binding to fibroblasts and HDL-mediated cholesterol efflux from fibroblasts do not saturate at similar concentrations of free HDL contrasts with the conclusions of Oram, Brinton, and Bierman (2) and Brinton et al. (22). It should be pointed out, however, that our data in this respect are in fact similar to those of Oram et al. and Brinton et al. The difference in the conclusions drawn stems from the fact that the latter authors divided their observed HDL-mediated cholesterol efflux into "high-affinity" and "low-affinity" components, noting that the profile of the "high-affinity" component paralleled that of HDL binding (2, 22). This mathematical manipulation of the data is difficult for us to accept. Whereas separation of such components is justified in equilibrium binding studies, it is not clearly justified in studies of cholesterol efflux. In binding studies it can be justified for two reasons. First, because binding sites being studied are usually not purified, a (lower-affinity) component of the binding representing binding to impurities is anticipated. Second, the mathematical function describing the binding of a ligand to one or more sites is precisely known, thus providing the means to separate high-affinity and low-affinity components. In contrast, in studies of cholesterol

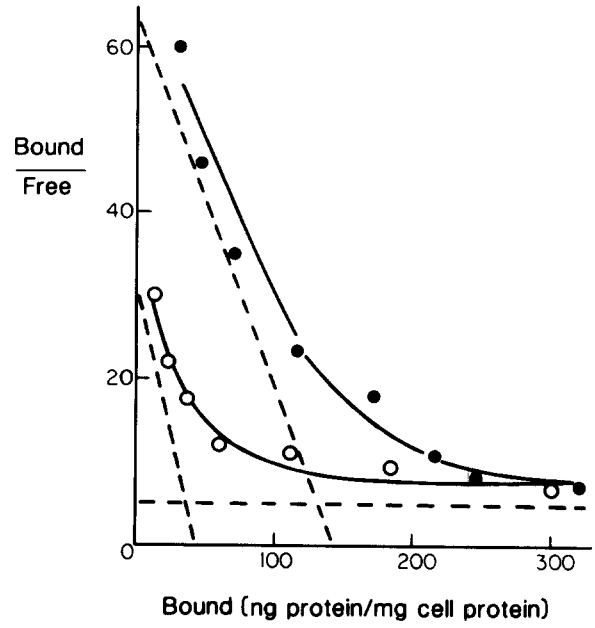

Fig. 3. Scatchard plots of the equilibrium binding data shown in Fig. 2. (●), Cholesterol-loaded cells; (○), cholesterol-depleted cells. Total binding data (——), separated into "specific" and "nonspecific" components (---) by computer-assisted analysis, are shown. In the experiment shown, the K_D of the cholesterol-loaded cells was 2.1 ± 0.4 μg protein/ml (± SEM; 21 nM) and that of the cholesterol-depleted cells was 1.4 ± 0.6 μg protein/ml. The capacity of the binding sites increased with cholesterol-loading from 40 ± 10 ng HDL_3 protein/mg cell protein to 140 ± 20 ng/HDL_3 protein/mg cell protein. The "nonspecific" component was unchanged by cholesterol-loading.

efflux, it is not clear what "high-affinity" and "low-affinity" components of efflux would signify. In addition, the mathematical function describing cholesterol efflux from cells to acceptor particles is not precisely known and is probably not straightforward. Different steps could be rate-limiting to the overall process of cholesterol efflux under different conditions, and the rate-limiting step would be expected to change as the concentration of acceptor particles changes, thus yielding an extremely complex function (3).[4] Arbitrarily dividing such a function into "high-affinity" and "low-affinity" components would seem, therefore, to require a great deal more justification than has been provided. This consideration is underscored by our present data, where the curve of HDL-mediated cholesterol efflux could no doubt be divided into "high-affinity" and "low-affinity" components, but so could the curve of albumin-mediated cholesterol efflux.

Other previous studies that could be interpreted as indicating that cell-surface receptors are involved in cholesterol efflux from cells can also be explained by known physicochemical determinants of cholesterol flux without invoking receptors. Thus, the addition of apoA-IV to liposomes (23) would be expected to increase the ability of the liposomes to remove cholesterol from cells in the absence of apoA-IV receptors by increasing the number of acceptor particles in the medium, as explained in detail by DeLamatre et al. (24). Similarly, protease treatment of cells (25) might be expected to affect cholesterol efflux even in the absence of protease-sensitive receptors by altering the surface properties of the cell membranes (3, 25).

More recently, Slotte, Oram, and Bierman (26) have also concluded that HDL binding sites do not mediate

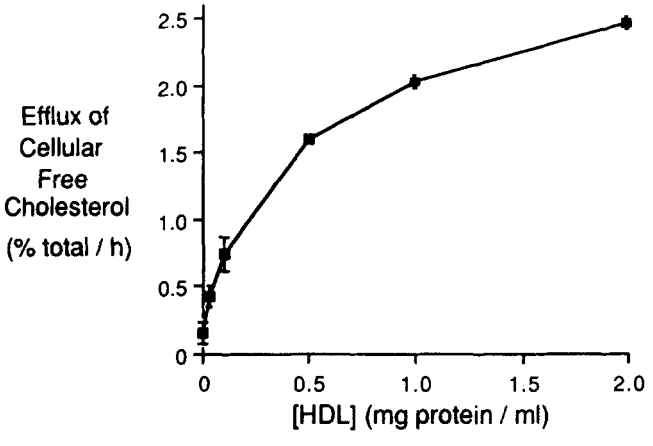

Fig. 4. Efflux of free cholesterol from cholesterol-loaded fibroblasts to medium containing HDL_3 as a function of the HDL_3 concentration. Points shown are mean ± SD of values obtained in three separate experiments. Background efflux is subtracted from each point and was 0.6 ± 0.3% of the total cellular free cholesterol/hr in these experiments. The lowest point shown is at an HDL_3 concentration of 5 μg protein/ml.

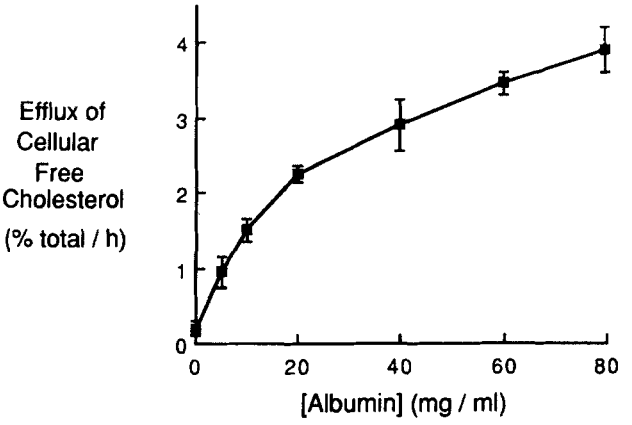

Fig. 5. Efflux of free cholesterol from cholesterol-loaded fibroblasts to medium containing human serum albumin (HSA) as a function of the HSA concentration. Points shown are mean ± SD of values obtained in three separate experiments. Background efflux is subtracted from each point and was 1.4 ± 1.0% of the total cellular free cholesterol/hr in these experiments. The lowest point shown is at an albumin concentration of 1 mg/ml.

cholesterol efflux from human fibroblasts in tissue culture. This conclusion was based on the observation that treatment of HDL with tetranitromethane (TNM) did not affect their ability to promote cholesterol efflux despite rendering them unable to bind to the putative HDL receptor, in agreement with the findings of Karlin et al. (27). While rejecting a role for the putative HDL receptor in cholesterol efflux from fibroblasts, however, these authors formulated a substitute role for HDL binding sites: that of promoting the translocation of free cholesterol from intracellular membranes to the cell-surface membrane (26). We do not believe that their data support such a role. The described profile of HDL-mediated translocation of free cholesterol does not parallel that of specific HDL binding (26). (It is only when this described profile is divided into "high-affinity" and "low-affinity" components (26) that the "high-affinity" component appears to parallel HDL binding. The lack of justification of arbitrarily dividing such a complex activity described by an unknown mathematical function into such components has been discussed above.) This apparent action of HDL, therefore, cannot presently be attributed to an

[4] It is also important to note that cholesterol efflux would be expected to appear "saturable" at high enough acceptor concentrations even when it is not mediated by a specific binding site for acceptor particles (3). As the concentration of acceptor particles in the medium is increased, it will eventually become high enough such that it is no longer at all rate-limiting to the overall process of cholesterol efflux. When cholesterol efflux thus becomes zero order with respect to acceptor particle concentration, saturation is mimicked. Many biological processes not mediated by receptors can similarly appear "saturable" (31).

TABLE 1. Relative increase in efflux of cellular free cholesterol to various acceptor particles on cholesterol-loading of the cells

Experiment #	HDL/HSA	HDL/PC
1	0.94	1.51
2	0.94	0.43
3	0.89	0.45
4	1.68	0.51
5	0.62	1.00
Mean ± SD	1.01 ± 0.40	0.78 ± 0.47

Results are expressed as the ratios of the percent increase in efflux to HDL_3 (20 μg protein/ml) and that to other acceptor particles. Background cholesterol efflux, which was <50% of the total cholesterol efflux in each of the experiments shown, was subtracted from each measurement before calculation of the ratios. HSA, human serum albumin (5 mg/ml); PC, phosphatidylcholine vesicles (100 μg/ml). The free cholesterol content of the cholesterol-depleted cells was 30.5 ± 3.4 μg/mg cell protein (± SD), and that of the cholesterol-loaded cells was 83.1 ± 19.8 μg/mg cell protein. The fractional efflux of cellular free cholesterol to HDL was 0.62 ± 0.10%/hr for the cholesterol-depleted cells, and 0.70 ± 0.14%/hr for the cholesterol-loaded cells. The fractional efflux to HSA was 0.61 ± 0.14%/hr for the cholesterol-depleted cells and 0.82 ± 0.45%/hr for the cholesterol-loaded cells. The fractional efflux to PC was 0.53 ± 0.17%/hr for the cholesterol-depleted cells and 1.03 ± 0.56%/hr for the cholesterol-loaded cells.

HDL receptor.[5] Thus, in the absence of a well-defined function, HDL binding sites on human fibroblasts cannot yet be considered receptors, regardless of whether or not they are proteins (2, 28–30).

Certain methodological aspects of this study deserve comment. Most of the experiments reported here were performed in cells that were cholesterol-loaded by adding large concentrations of nonlipoprotein cholesterol to the medium. This manipulation is clearly not physiologic. It is, however, the manipulation most commonly employed to describe putative HDL receptors in fibroblasts (2, 22, 26, 29). Thus, in a study addressing the function of the putative HDL receptor, this nonphysiologic manipulation was appropriate. In a recent study of fibroblasts that were not cholesterol-loaded, Karlin et al. (27) also concluded that HDL binding sites on these cells do not mediate cholesterol efflux. This conclusion was based largely on their finding that HDL binding and HDL-mediated cholesterol efflux were characterized by different saturation profiles.

It should also be emphasized that our present measurements of cholesterol movement between cells and medium were measurements of one-way movement (efflux) only, and not of net (mass) movement. Although it is the net movement of cholesterol between plasma and tissues in vivo that is of prime importance (and this net movement cannot be predicted from measurements of one-way movement), in this study we were examining the mechanism of cholesterol efflux from cells. Under these circumstances measurements of cholesterol efflux alone are appropriate. In fact, it should be noted that studies of the mass movement of cholesterol between cholesterol-loaded cells in tissue culture and ultracentrifugally isolated lipoproteins would not in and of themselves be any more relevant to in vivo physiology than studies of cholesterol efflux alone, since an artificial gradient of cholesterol chemical potential is created by such experimental conditions and determines the direction and amount of mass movement of cholesterol.

Another methodological aspect of this study is of particular interest. Background cholesterol efflux, although ubiquitous in these types of studies, has to our knowledge never before been characterized. Our present findings suggest that it results mainly from release of membrane-rich fragments of cells into the medium. It remains unclear whether this release of cell fragments into the medium is a normal process or whether it is a sign of impaired viability of some of the cells on the tissue culture dishes.

We thank Dr. Robert L. Hamilton for performing the electron microscopy and helping us interpret the observed structures, Judith Tweedie for expert technical assistance, Dr. John Heider of Sandoz, Inc., for providing compound 58-035, and Drs. Richard J. Havel and Robert L. Hamilton for critical review of the manuscript. This work was supported by research grants HL-14237 (Arteriosclerosis SCOR), HL-01546 (Clinical Investigator Award), and HL-31210 from the National Institutes of Health.

Manuscript received 28 December 1987 and in revised form 4 April 1988.

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[5]These authors also argued that HDL-mediated translocation of free cholesterol is receptor-mediated because TNM-treated HDL, which do not bind to the putative HDL receptor, do not perform this function. This argument seems difficult to sustain. Modification of a ligand known to interact with a receptor can provide many types of useful information. When, however, the question is still whether or not a receptor exists, modification of a putative ligand provides only very limited information. Thus, considering functions performed by HDL but not by TNM-treated HDL as being receptor-mediated seems presently little more justified than so considering functions performed by HDL but not by albumin (which also does not bind to the putative HDL receptor).

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