Cellular binding site and membrane binding proteins for triglyceride-rich lipoproteins in human monocyte-macrophages and THP-1 monocytic cells

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Abstract Triglyceride- and cholesterol-rich foam cells derived from monocyte-macrophages are commonly associated with some forms of hypertriglyceridemia. In this report, direct binding studies at 4°C demonstrate that human monocyte-macrophages (HMM) 1–6 days after isolation from blood and human THP-1 monocytic cells, before and up to 7 days after differentiation with phorbol ester, exhibit a high affinity (K_d 3–6 nM), saturable, specific, and apolipoprotein (apo) E-independent binding site for the uptake and degradation of certain triglyceride-rich lipoproteins (TGRLP). Ligand blotting analysis identified two membrane binding proteins (MBP) of apparent molecular weights of 200 and 235 kDa (MBP 200 and MBP 235) in both cell types that share the same ligand specificity as the cellular site and bind hypertriglyceridemic (HTG) VLDL, trypsinized VLDL devoid of apoE (tryp-VLDL), and dietary plasma chylomicrons from normal subjects but not LDL, acetyl LDL, or normal VLDL with high affinity. Neither lipoprotein lipase nor apoE are required for TGRLP binding to the cells or the isolated MBPs. The cellular binding site and the MBPs are expressed at similar levels at all stages of differentiation, unlike the LDL or the acetyl LDL receptor. TGRLP that bind to the MBPs induce rapid, saturable, cellular triglyceride accumulation in monocytes as well as macrophages; normal VLDL does not. In addition, the cellular high affinity binding site and MBP 200 and 235 are not affected by the media sterol content, unlike the LDL receptor. Taken together, these data indicate that human monocyte-macrophages exhibit a high affinity, saturable, specific, apoE- and lipoprotein lipase-independent binding site and membrane binding proteins for TGRLP that differ in expression, specificity, and molecular size from receptors of the LDL receptor gene family or the acetyl LDL receptor. The shared characteristics of the cellular binding site with MBP 200 and MBP 235 suggest that they are candidates for the receptor-mediated, apoE-independent uptake of HTG-VLDL and chylomicrons by monocytes and macrophages and therefore may be involved in foam cell formation.—**Gianturco, S. H., M. P. Ramprasad, A. H-Y. Lin, R. Song, and W. A. Bradley.** Cellular binding site and membrane binding proteins for triglyceride-rich lipoproteins in human monocyte-macrophages and THP-1 monocytic cells. *J. Lipid Res.* 1994. **35:** 1674–1687.

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Hypertriglyceridemia is a common, heterogeneous disorder (1). When chylomicrons persist in the fasting state, lipid-filled, macrophage-derived foam cells can accumulate in the spleen, liver, bone marrow, atherosclerotic lesions, and skin (1). Foam cells containing triglyceride and cholesteryl ester are found in eruptive xanthomas in untreated hypertriglyceridemic (HTG), Type 5 diabetics (2); these foam cells are resolved after treatment with insulin reduces plasma triglyceride levels and eliminates fasting chylomicrons, indicating the triglyceride-rich lipoproteins (TGRLP) had induced foam cell formation.

A number of studies (3–8) also indicate that hypertriglyceridemia is a risk factor for coronary heart disease and myocardial infarction, sequelae of atherosclerosis, although the independence of this relationship is not supported by all studies (4, 7, 9). Monocyte-macrophages play a key role in atherogenesis, accounting for many of the foam cells characteristic of atherosclerotic lesions (10–13). The occurrence of foam cells in hypertriglyceridemic subjects described above (1, 2) suggests that TGRLP induce foam cell formation in vivo.

Studies in vitro have suggested a number of potential mechanisms for these in vivo observations. The first demonstration that very low density lipoproteins (VLDL) from HTG subjects were abnormal and potentially atherogenic came from studies that showed that HTG-VLDL,

Abbreviations: apoE, apolipoprotein E; β-VLDL, cholesteryl ester-enriched β migrating VLDL from cholesterol-fed rabbits; C, cholesterol; HMM, human monocyte-macrophages; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HTG, hypertriglyceridemic; LPDS, lipoprotein-deficient serum; LRP, LDL receptor-related protein/α_2-macroglobulin receptor; MBP, membrane binding protein; PMA, phorbol 12-myristate, 13-acetate; TGRLP, triglyceride-rich lipoprotein; tryp-VLDL, trypsinized VLDL; VLDL, very low density lipoprotein; LDL, low density lipoprotein.

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but not normal VLDL, deliver cholesterol to cultured fibroblasts via the LDL receptor (14). The abnormality in HTG-VLDL is primarily in the $S_f > 60$ subfraction which, in contrast to normal VLDL $S_f > 60$, specifically binds to the LDL receptor via apolipoprotein (apo)E (15–20). Other studies demonstrated that lipolysis of normal VLDL $S_f > 60$ permits binding of the lipolytic remnant to the LDL receptor (21, 22). Lipoprotein lipase secreted by macrophages (23, 24) has been shown to hydrolyze VLDL and enhance its cellular uptake (25) and may facilitate uptake through localization of TGRLP to membrane heparin sulfate proteoglycan (26) and/or through binding to the LDL receptor-related protein/α_2-macroglobulin receptor (LRP) (27). In addition to these mechanisms which enhance uptake by the LDL receptor and LRP, VLDL and chylomicrons from certain HTG subjects, but again not VLDL from normal subjects, bind to a distinct, apoE- and lipoprotein lipase-independent, high affinity binding site on murine macrophages (28–30). Macrophage uptake of HTG-VLDL and chylomicrons by this receptor also leads to rapid (≤ 4 h) mass accumulation of triglyceride (28–30) and cholesteryl ester (29). The lipids are visible as cytoplasmic droplets by phase-contrast microscopy and by oil red-O staining, giving the macrophages a foam cell morphology (28, 29).

Because of the potential importance of an apoE-independent, receptor-mediated pathway for TGRLP in the formation of foam cells in human pathology, particularly in HTG subjects, the studies reported here were undertaken to determine whether human monocyte-macrophages (HMM) express a high affinity cellular binding site and membrane binding proteins specific for HTG-VLDL and chylomicrons. In addition to HMM, the human monocytic cell line THP-1 was studied as a plentiful and reproducible potential human source for characterization of the pathway and purification of the receptor, as THP-1 cells share many relevant characteristics with normal HMM. For example, THP-1 monocytes in suspension express the LDL receptor but not the acetyl LDL receptor; phorbol ester-induced differentiation reduces LDL receptor activity and induces the acetyl LDL receptor (31, 32), similar to changes in receptor activities in HMM during differentiation (33). The present studies indicate that HMM express a high affinity, specific, apoE-independent cellular pathway and membrane binding proteins for TGRLP that differ from the known lipoprotein receptors in a number of properties including *1)* expression during differentiation, *2)* intracellular ligand degradation, *3)* ligand specificity, *4)* apparent molecular weights of the candidate receptor proteins, and *5)* cellular distribution. As neither the cellular binding site nor the candidate receptor proteins require apoE (or lipoprotein lipase) for TGRLP binding and as they are not regulated by state of differentiation or by sterol, they could participate in TGRLP uptake and foam cell formation independent of apoE while the LDL receptor is suppressed.

METHODS

Cell growth

The human monocytic line THP-1 was obtained from ATCC and grown in suspension in RPMI 1640 with 10% fetal bovine serum, 2 mM glutamine, 100 μg streptomycin and 100 units of penicillin/ml, and 5×10^{-5} M mercaptoethanol. Cells were maintained at $\leq 1.0 \times 10^6$ cells/ml in a humidified incubator (5% CO_2) at 37°C, with a complete media change at least once a week. For differentiation of THP-1 monocytes into adherent macrophages, cells were seeded (1.5×10^6 cells/35-mm dish or per well of 6-well plate) in complete media; phorbol 12-myristate, 13-acetate (PMA) was then added (10^{-7} M final concentration). Adherent cells were used for experiments between 1 and 7 days, as indicated. Cells were washed twice with saline, then incubated in 2 ml of serum-free RPMI 1640 medium containing the indicated concentration of lipoprotein. For ligand blotting studies, cells were seeded in 10 ml of complete medium in 100 × 15 mm dishes prior to PMA addition and harvested between 1 and 7 days, as specified. Human mononuclear cells were isolated from the buffy coat or from whole blood of healthy normal subjects by the method of Boyum (34) as described previously (30) and placed in tissue culture dishes for 1 h in DME or RPMI 1640 medium containing 10% autologous serum or 10% fetal bovine serum to allow monocytes to adhere (34). After non-adherent cells were removed, the monocytes were washed twice and incubated in complete medium containing 10% autologous serum for 1–6 days in a CO_2 incubator before study, as indicated.

For binding, uptake, and degradation studies, duplicate dishes of cells and empty dishes were washed twice and incubated with ^{125}I-labeled lipoproteins alone and in the presence of indicated quantities of unlabeled lipoprotein at 4° or 37°C for up to 4 h as indicated and as previously reported (30). Experiments were conducted in the absence of serum components other than lipoproteins and 2 mg bovine serum albumin/ml. Total cell-associated radioactivity, representing surface-bound (4°C studies) and bound plus internalized lipoprotein (37°C studies), was determined after the cells were washed five times with chilled buffer containing 2 mg albumin/ml and once with albumin-free buffer (35). The amount of non-iodide, non-lipid TCA-soluble radioactivity in the medium was used as a measure of iodinated lipoprotein degradation (35). Each value was corrected by subtracting the amount "bound" or degraded in control dishes that contained no cells. Specific binding curves were calculated by subtracting the curve generated by plotting the amount of cell-associated ^{125}I-labeled lipoprotein in the presence of ex-

cess unlabeled homologous lipoprotein (linear plots) from the curve representing the amount bound in the absence of unlabeled lipoproteins (curvilinear). For studies of cells in suspension, incubations were done in 6-well plates; cells were harvested, washed, and processed as described by Ho et al. (36).

For lipid accumulation studies, duplicate or triplicate dishes of cells were grown in 6-well plates, and washed twice with sterile saline prior to incubation with lipoproteins and RPMI-1640 in the absence of other serum components for 4 h at 37°C. Cells were washed with chilled buffered saline containing 2 mg albumin/ml and once with albumin-free buffer prior to extraction with 2 ml of hexanes–isopropanol 3:2 (v/v). An aliquot of solvent was evaporated under nitrogen and lipids were resolubilized in kit reagent containing additional Triton X-100 (10 μl/ml reagent) as previously reported (28–30). Triglyceride mass was determined with an enzymatic kit (Boehringer Mannheim).

Lipoproteins

Plasma was obtained either from fasting subjects with normal lipid values for isolation of normal VLDL, LDL, and lipoprotein-deficient serum (LPDS) or from fasting patients with types 4 and 5 lipoprotein profiles for HTG-VLDL after informed consent was obtained. The diagnoses were based on commonly used criteria (1) as previously described (14, 17, 30). In some cases, VLDL were isolated from lipemic plasma obtained from the Red Cross. For chylomicrons, normal fasting subjects consumed a solid test meal (37) 4 h prior to blood donation. Lipoproteins for cell studies were isolated (38) from fresh plasma containing 1 mM EDTA, 1 mM NaN_3, 10 μM PMSF (Sigma Chemical Co., St. Louis, MO), 10 μM PPACK (Calbiochem Corp., La Jolla, CA), and 50 U Trasylol/ml. TGRLP were subfractionated through a discontinuous NaCl gradient from d 1.063 to 1.006 g/ml by cumulative flotation methods of Lindgren et al. (39), as previously detailed (38). VLDL used were S_f 100–400 and S_f 60–100; chylomicrons were $S_f > 400$. Characteristics of VLDL subclasses so isolated are detailed elsewhere (15, 18, 38). LDL were isolated at d 1.03–1.05 g/ml and washed at each density. Acetyl LDL was prepared as described (40). β-VLDL were isolated from cholesterol-fed rabbits as previously described (28, 29). Total protein contents of the lipoproteins were obtained by a modified Lowry method (41, 42).

To prepare TGRLP devoid of apoE, HTG-VLDL were incubated with trypsin (100:1, wt/wt) for 2 h at 37°C in 0.15 M NaCl, 20 mM Tris, pH 7.4, (buffer), or with buffer alone (control), as described previously (17, 18, 29, 30, 38). Trypsin-treated VLDL were chromatographed on immobilized *p*-aminobenzamidine linked to Sepharose to remove active trypsin (30) before reisolation by gradient flotation as described above. Each preparation of native HTG-VLDL or of protease-modified HTG-VLDL used in the macrophage studies was tested for its ability to cause LDL receptor-mediated suppression of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase activity in cultured normal human skin fibroblasts, and/or for its ability to bind to partially purified bovine LDL receptors after electrophoresis and transfer to nitrocellulose paper, two sensitive measures of LDL receptor binding determinants present on the lipoprotein (30, 38, 43). ApoE and its fragments were not detected in trypsinized VLDL by immunochemical (Western) blotting or by RIA using polyclonal apoE antibodies (17, 18, 38). In contrast to HTG-VLDL, trypsin-treated HTG-VLDL used in these studies no longer suppressed HMG-CoA reductase activity in normal human fibroblasts (17, 18, 38) nor did they bind to partially purified bovine adrenal LDL receptors in ligand blots (44) due to total loss of apoE epitopes.

Lipoproteins were iodinated by a modification of the iodine monochloride method (45). Free iodine was removed by gel filtration and extensive dialysis. Samples were filtered (0.45 μm Millex;Millipore Corporation, Bedford, MA) immediately before use; specific activities ranged from 100 to 200 cpm/ng protein. Less than 10% of the label was extractable into organic solvent.

Bovine LDL receptor preparation

The LDL receptor from bovine adrenal cortical membranes was partially purified after solubilization in Triton X-100 by chromatography on DEAE-cellulose, for use in ligand blots, as described (46).

Detergent solubilization of monocytes and macrophages for ligand blotting

For adherent macrophages, 10^{-7} M PMA was added to THP-1 monocytes in 100-mm dishes in complete medium, and the cells were grown for 1–7 days as indicated. Cells (10–20 dishes) were placed on ice, washed twice, and scraped with a rubber policeman into chilled buffer 1 (0.15 M NaCl, 50 mM Tris-HCl, pH 8.0, 50 U aprotinin/ml, 10 mM leupeptin, and 10 μm PMSF), 3 ml per dish. The cells were pelleted (1,000 rpm, 4°C, 5 min) and resuspended in buffer 1. Monocytes in suspension were harvested by low-speed centrifugation and washed with buffer 1 prior to extraction. Cell membranes were prepared using a Dounce homogenizer (10 strokes by hand), pelleting debris (800 *g* for 10 min at 4°C) and precipitating cell membranes from the supernatant (100,000 *g* for 1 h at 4°C), as previously detailed (30). Whole cell pellets or membranes were solubilized as previously reported (30): Triton X-114, which forms two phases above 25°C (47), was added (1% final concentration, vol/vol); the preparation was vortexed and placed on ice for 15 min. The suspension was centrifuged (1 h, 35,000 rpm in a 50.4 Ti rotor at 4°C), the supernatant was incubated at 37°C for 15 min, and the cloudy suspen-

sion was overlaid on a cushion composed of 6% sucrose, 50 mM Tris, pH 8.0, 0.6% Triton X-114 at 37°C (1 ml cushion/3 ml suspension). Centrifugation for 5 min at 1,000 rpm caused phase separation into a detergent phase at the bottom of the tube and an upper aqueous phase, which contained TGRLP binding activity.

Ligand blotting

Electrophoresis was performed on 6% polyacrylamide slab gels containing 0.1% SDS using the buffer system of Laemmli (48). A 40-μl sample was applied per well (40–80 μg protein), and electrophoresed in a Minigel apparatus (Bio-Rad Laboratories, Richmond, CA) at room temperature for 10 min at 100 V and 35 min at 200 V. Proteins were electrotransferred to nitrocellulose for 3 h at 100 V and 10°C. Complete transfer was confirmed by Coomassie Blue staining of the gel after transfer. For ligand blotting (49), the nitrocellulose strips were then incubated with blocking buffer [50 mM Tris-HCl, 2 mM $CaCl_2$, 5% Carnation nonfat dry milk (Carnation Co., Los Angeles, CA), and 90 mM NaCl at pH 8.0] for 1 h with shaking at room temperature, then in 0.5% milk in the same buffer containing the indicated amount of lipoproteins for 3 h, then washed with buffer without milk (three times, 5 min each), dried and visualized by autoradiography (30, 44). Alternatively, as specified, the binding activities were detected using sheep anti-human apoB IgG followed by alkaline phosphatase-conjugated second antibody and substrates BCIP and NBT. Lipoprotein particle concentrations were calculated using the following molecular masses and percentages of protein: HTG-VLDL, 30×10^6 kDa, 5% protein; tryp-VLDL, 30×10^6 kDa, 4% protein; LDL and acetyl LDL, 2.2×10^6 kDa, 25% protein. Prestained standards (Gibco BRL, Grand Island, NY) were used for estimation of apparent molecular mass: myosin (200–220 kDa, depending on the lot), phosphorylase B (97.4 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa). Autoradiograms of the ligand blots were analyzed using a 2-dimensional laser densitometer (Molecular Dynamics, Sunnyvale, CA) and quantified with the ImageQuant® software provided by the manufacturer.

Data

All data presented are representative of multiple independent experiments: 19 direct binding or uptake and degradation curves (±unlabeled VLDL) in THP-1 monocytes and macrophages and 4 in HMM; 30 ligand blotting experiments of HMM from 11 subjects and ~2,000 of THP-1 cells at different stages of differentiation; 3 sterol regulation experiments; 18 competitive cellular binding and 30 competitive ligand blotting experiments; 47 triglyceride accumulation studies.

RESULTS

Expression of a high affinity binding site and membrane binding proteins for TGRLP in THP-1 and human monocyte-macrophages

To determine whether HMM and THP-1 macrophages express a specific, high affinity, apoE-independent binding site for TGRLP, THP-1 macrophages were tested 1–7 days after treatment of monocytes with phorbol ester to induce differentiation and human monocytes were tested 1–7 days after isolation from blood and adherence for the high affinity, specific binding of ^{125}I-labeled trypsinized (tryp)-VLDL. Tryp-VLDL was used as a ligand to test for the TGRLP binding site because tryp-VLDL binds to the murine macrophage specific binding site and membrane binding proteins for HTG-VLDL (30) but does not bind to LDL receptors either in cells (17, 18) or in ligand blots (44), due to the absence of apoE or apoE fragments in tryp-VLDL (17, 18). Use of ligands with mutually exclusive receptor specificities simplifies the interpretation of comparative and competitive binding studies by reducing the ambiguity seen when HTG-VLDL is used, since HTG-VLDL binds to the LDL receptor via apoE (17) and to the murine macrophage high affinity binding site and membrane binding proteins for TGRLP independent of apoE (30). Moreover, the absence of apoE in tryp-VLDL would reduce the potential binding to other members of the LDL receptor gene family that bind via apoE, such as LRP (50, 51) or the putative rabbit muscle VLDL receptor (52) (if its equivalent is expressed in HMM). Studies were conducted at 4°C in prechilled, washed cells to preclude secretion of apoE or other components such as lipoprotein lipase which are known to influence lipoprotein binding to the LDL receptor or to LRP and the rabbit muscle VLDL receptor. The binding studies were also conducted in medium that contained no serum components other than tryp-VLDL and bovine serum albumin to avoid adding any apoE that could be in LPDS. The macrophages were incubated with iodinated tryp-VLDL in the absence and presence of a 10-fold excess of unlabeled homologous lipoprotein for 2 h at 4°C to measure the total and nonspecific binding, respectively. Representative binding experiments in THP-1 macrophages 1 day after adherence and in normal HMM 1 day after isolation from blood are shown in **Fig. 1.** The upper curve of each panel represents total binding (specific plus nonspecific) of tryp-VLDL and is curvilinear. The lower curve represents nonspecific binding that occurs in the presence of excess unlabeled lipoprotein and is linear; the calculated difference between the two curves represents specific binding (middle curve). Both THP-1 (Fig. 1A) and HMM (Fig. 1B) macrophages possess a high affinity, saturable, and specific binding site for ^{125}I-labeled tryp-

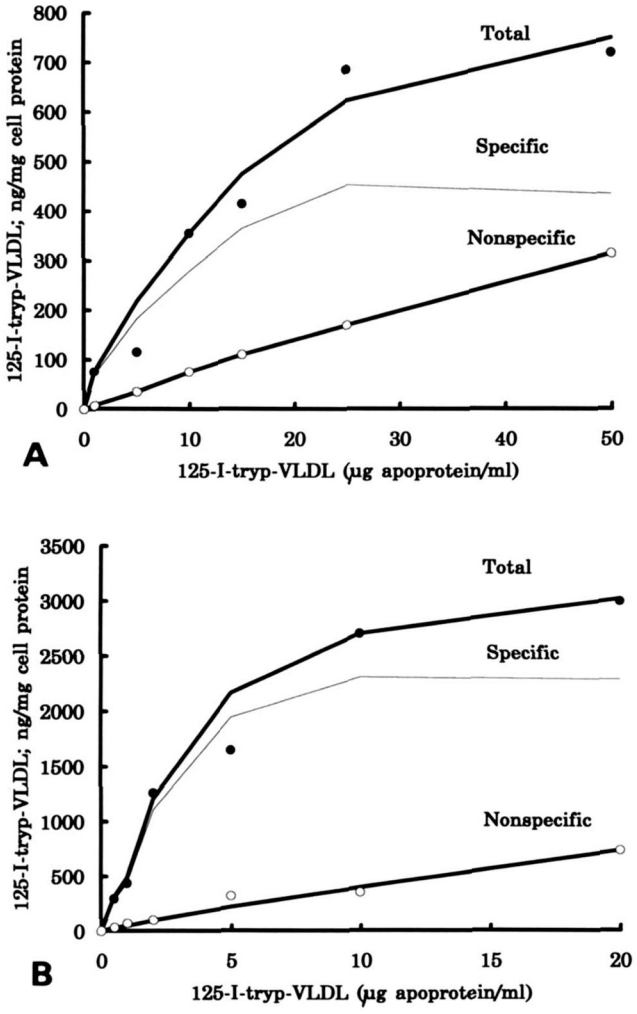
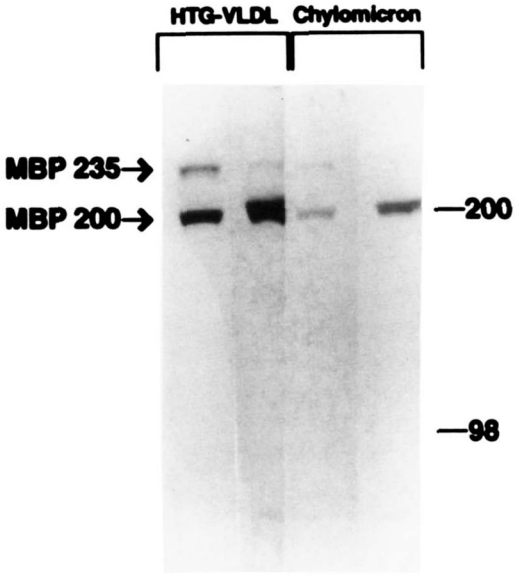


Fig. 1. Direct 4°C binding of ^{125}I-labeled tryp-VLDL to THP-1 macrophages and to human monocyte-macrophages 1 day after adherence. THP-1 monocytes were seeded in 6-well dishes, treated with PMA (10^{-7} M) and allowed to adhere for 1 day as described in Methods (panel A). Human mononuclear cells were isolated from whole blood of a healthy normal subject and placed in 6-well tissue culture dishes for 1 h to allow monocyte adherence; nonadherent cells were removed; and adherent monocyte-macrophages were cultured 1 day as described in Methods (panel B). Duplicate wells of washed cells (and no cells for background) were incubated with media containing indicated quantities of ^{125}I-labeled tryp-VLDL in the absence (total binding, closed circles) and in the presence of a 10-fold excess of the homologous unlabeled VLDL (nonspecific binding, open circles) for 2 h at 4°C. Each data point is the average of values from duplicate dishes, which differed by ≤5%. The specific binding curves were calculated by subtraction of the nonspecific binding curve from the total binding, as described in Methods.

VLDL at early stages of differentiation (1 day after adherence). As is evident from the specific binding curves in Fig. 1, saturation is typically achieved between 10 and 20 μg apolipoprotein/ml (8–16 nM) and half saturation (K_d) between 2 and 4 μg apolipoprotein/ml (3–6 nM) for both THP-1 and normal human macrophages; Scatchard analysis of the data confirm the K_ds are in the 3–6 nM range in both cell types. Similar results were obtained with tryp-VLDL in THP-1 monocytes in suspension and THP-1 macrophages 4–7 days after adherence, described below, and in HMM 4–6 days after adherence (data not shown). Thus the high affinity, saturable binding of tryp-VLDL is similar throughout differentiation and is not influenced by cellular changes known to accompany differentiation that might affect TGRLP binding, notably the induction of apoE (53–56), lipoprotein lipase (24, 55, 56) or LRP (57) or the suppression of the LDL receptor (31–33).

Ligand blotting analyses were used to identify membrane binding proteins for TGRLP in extracts of THP-1 monocytes (**Fig. 2,** lanes 1 and 3) and normal HMM 1 day after isolation (Fig. 2, lanes 2 and 4). Both cell types have two distinct membrane binding proteins of apparent molecular mass of 200 and 235 kDa (MBP 200 and MBP



Lane	1	2	3	4
THP-1	+	-	+	-
HMM	-	+	-	+

Fig. 2. Ligand blot detection of MBP 200 and MBP 235 from THP-1 monocytes and HMM. THP-1 monocytes and normal HMM 1 day after isolation were grown as described in Methods. Triton X-114 aqueous-phase extracts were prepared and 50-microliter aliquots of THP-1 extracts diluted 3-fold with extraction buffer containing protease inhibitors (lanes 1, 3) and undiluted HMM extracts (lanes 2, 4) were treated with 2% SDS at room temperature for 20 min prior to SDS-PAGE on 5% polyacrylamide minigels. After electrotransfer, the nitrocellulose strips were washed, blocked, and then incubated with either HTG-VLDL (20 μg/ml; lanes 1, 2) or with dietary plasma chylomicrons (S_f >400) isolated from a normal subject 4 h after a high-fat meal (20 μg/ml; lanes 3, 4), as described in Methods. Binding of the lipoproteins to MBPs was visualized by identification of apolipoprotein B using a monospecific, LDL affinity-purified sheep anti-human apoB IgG detected with a rabbit anti-sheep IgG conjugated to alkaline phosphatase using substrates BCIP and NBT (Bio-Rad). The ligand binding activities are identified with arrows. Molecular weight markers are indicated on the right.

235) that bind HTG-VLDL (lanes 1 and 2) and plasma chylomicrons $S_f > 400$, isolated 4 h postprandially from a normal subject (lanes 3 and 4). In the HMM extract shown in Fig. 2, the MBP 235 activity band is much less intense than MBP 200 and was detected with HTG-VLDL but not with chylomicrons under the conditions used to develop this blot. MBP 200 and 235 consistently were detected in HMM 1–7 days after isolation from 11 different subjects (both normal and HTG) and in THP-1 monocytes in suspension and in THP-1 macrophages 1–7 days after adherence. MBP 200 and 235 from both sources bind tryp-VLDL as well as HTG-VLDL and chylomicrons in the absence of other serum components. The relative intensities of MBP 200 and 235 binding activities are variable in both THP-1 and HMM extracts. Predominance of MBP 200 or MBP 235 activity, however, did not appear to correlate with state of differentiation or culture conditions. Evidence that MBP 200 and 235 are cell surface proteins comes from cell surface proteolysis and iodination studies described elsewhere (submitted for publication).

Expression of the high affinity cellular pathway and membrane binding proteins for uptake of TGRLP during differentiation of THP-1 monocytes into macrophages differs from that of the LDL receptor and the acetyl LDL receptor pathway

To distinguish the high affinity, specific binding site for TGRLP from the LDL receptor and the acetyl LDL receptor, THP-1 cells were tested for the specific uptake and degradation of LDL, tryp-VLDL, and acetyl-LDL after treatment with phorbol ester to induce differentiation. The cells were incubated with iodinated lipoproteins in the absence and presence of a 10-fold excess of unlabeled homologous lipoprotein to measure total and nonspecific cell-associated radioactivity respectively. These studies were conducted at 37°C to amplify the low level of LDL binding seen at 4°C and represent surface bound and internalized lipoprotein. There was no high affinity, curvilinear, specific, competable cell association or degradation of ^{125}I-labeled LDL with the differentiated (7-day plated) macrophages (**Fig. 3,** left panel), as reported (31, 32). In contrast, there was significant curvilinear association and degradation of ^{125}I-labeled tryp-VLDL with the differentiated macrophages that was specifically competed by a 10-fold excess of unlabeled tryp-VLDL (Fig. 3, middle panel). There was also specific cell association and degradation of acetyl-LDL that was competed by unlabeled acetyl-LDL (Fig. 3, right panel) as reported (31, 32). The amount of ^{125}I-labeled tryp-VLDL that was specifically degraded in the 4 h incubation was approximately one-fourth of the amount specifically associated with cells. In contrast, the amount of ^{125}I-labeled acetyl LDL that was degraded was nearly equal to the amount associated with the cells in the same incubation period. The ratio of degradation to cell association of TGRLP in 4 h incubations was generally diminished relative to that of acetyl LDL in differentiated cells, suggesting a different intracellular pathway for TGRLP from that for acetyl LDL.

Consistent with the cell studies (Fig. 3), ligand blotting analysis of THP-1 macrophage membrane extracts prepared 7 days after PMA-induced differentiation demonstrated an active acetyl-LDL receptor and TGRLP binding activities (**Fig. 4**). Lanes 1–3 were incubated with ^{125}I-labeled acetyl-LDL in the absence (lane 1) or in the presence of a 10-fold and 20-fold excess of unlabeled tryp-

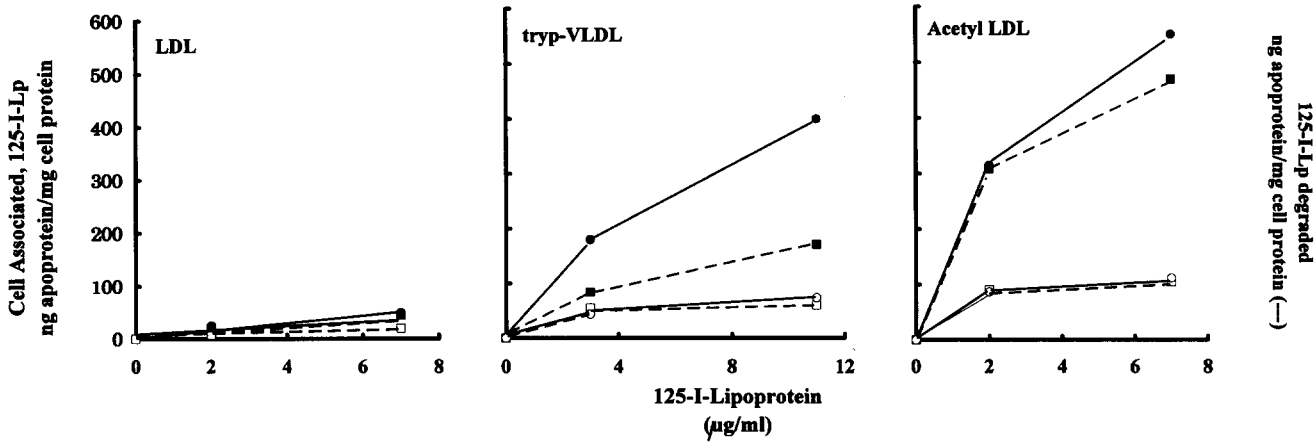


Fig. 3. Specific cell association and degradation of tryp-VLDL and acetyl LDL, but not LDL, with THP-1 macrophages 7 days after PMA-induced differentiation. THP-1 monocytes were treated with PMA (10^{-7} M) to induce adherence and allowed to differentiate for 7 days. Cells were washed and incubated for 4 h at 37°C with the indicated concentrations of ^{125}I-labeled LDL (left panel), ^{125}I-labeled tryp-VLDL (middle panel), or ^{125}I-labeled acetyl LDL (right panel), respectively, to measure the total (closed symbols) and the nonspecific (open symbols) cell-associated or degraded radioactivity in the presence of unlabeled homologous lipoprotein. Cell-associated (●, ○) and degraded (■, □) radioactivity was determined as described in Methods and expressed as ng apoprotein/mg cell protein. Each data point is the average of values from duplicate dishes, which differed by ≤5%.

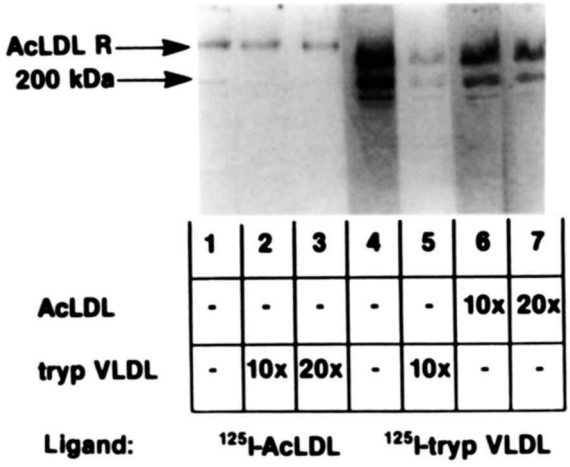


Fig. 4. Competitive ligand blotting analysis of THP-1 macrophages 7 days after PMA-induced differentiation. THP-1 cells were treated with PMA (10^{-7} M) and allowed to differentiate for 7 days, as described in the legend to Fig. 3. Triton X-114 extracts were prepared, electrophoresed, and electrotransferred, as described in Methods. The blocked nitrocellulose strips were incubated with ^{125}I-labeled acetyl LDL (AcLDL) (6 pmol/ml; lanes 1–3) either alone (lane 1) or with a 10-fold (lane 2) or a 20-fold (lane 3) molar excess of unlabeled tryp-VLDL. Lanes 4–7 were incubated with ^{125}I-labeled tryp-VLDL (6 pmol/ml) either alone (lane 4) or with a 10-fold excess of tryp-VLDL (lane 5), a 10-fold (lane 6) or 20-fold (lane 7) excess of unlabeled acetyl LDL. The nitrocellulose strips were washed, dried, and exposed to X-OMAT AR film for 16 h at room temperature. The acetyl LDL receptor and the 200 kDa molecular mass markers are indicated with arrows.

VLDL (lanes 2, 3). The acetyl-LDL receptor migrates with an apparent molecular mass of 260 kDa, in agreement with Via et al. (58). Acetyl LDL binding to its receptor was not effectively competed by a 10- or 20-fold molar excess of tryp-VLDL (Fig. 4, lanes 2, 3). Iodinated tryp-VLDL was used to probe lanes 4 through 7 alone (lane 4) or in the presence of a 10-fold excess of unlabeled tryp-VLDL (lane 5) or a 10- or 20-fold excess of unlabeled acetyl-LDL (lanes 6 and 7). ^{125}I-labeled tryp-VLDL binds primarily to MBP 200 and 235 (lane 4). In this, as in some but not all blots of THP-1 extracts, there are additional narrow bands just below MBP 200, which show little lipoprotein specificity and bind both acetyl LDL and VLDL. When these binding activities are present in extracts (< 7% of total binding activity, as quantified by densitometry), they bind any lipoprotein to which the blot is exposed, in contrast to the more specific binding of MBP 200 and 235 or the acetyl LDL receptor. A 10-fold excess of unlabeled tryp-VLDL competes 70% of the binding of iodinated tryp-VLDL (lane 5), as quantified by densitometry to correct for background differences, but acetyl-LDL has little effect on the binding of tryp-VLDL to MBP 200 and 235 at a 10-fold (lane 6) or 20-fold excess (lane 7). Conversely, a 20-fold excess of unlabeled acetyl LDL competes ~80% of the binding of labeled acetyl LDL to its receptor but neither LDL nor tryp-VLDL were effective competitors (data not shown). These data demonstrate, first, that THP-1 macrophages, 7 days after treatment with phorbol ester, express both the acetyl-LDL receptor and MBP 200 and 235; second, that the ligands for these membrane proteins are distinct and exhibit little cross competition; and, third, the membrane binding proteins for TGRLP have apparent molecular masses (200 and 235 kDa) different from the acetyl LDL receptor (260 kDa).

To determine whether the expression of the cellular binding site for TGRLP was increased or decreased upon differentiation of THP-1 monocytes into macrophages as are the acetyl-LDL receptor and the LDL receptor, re-

TABLE 1. Cell association of lipoproteins with THP-1 monocytes and macrophages during differentiation

Lipoprotein	Cells	Cell-Associated Lipoprotein (3 h 37°C) Total	Nonspecific	Specific	Relative
		ng/mg cell protein			
^{125}I-labeled tryp-VLDL	Monocytes	87.8 ± 0.7[a]	26.7 ± 1.4[a]	61.1	1
	1 d plated	62.2 ± 1.1	2.8 ± 2.8	59.4	0.97
	7 d plated	52.4 ± 1.0	6.7 ± 6.7	45.7	0.75
^{125}I-labeled LDL	Monocytes	22.6 ± 0.8	12.6 ± 0.8	10.0	1
	1 d plated	31.4 ± 4.2	19.9 ± 4.4	11.5	1.15
	7 d plated	38.9 ± 4.9	49.4 ± 6.8	0	0
^{125}I-labeled acetyl LDL	Monocytes	92.8 ± 0.7	65.9 ± 3.0	26.9	1
	1 d plated	61.9 ± 0.1	28.0 ± 5.0	33.9	1.26
	7 d plated	309.0 ± 22	50.5 ± 7.6	258	9.59

THP-1 monocytes (suspension) or macrophages 1 and 7 days (d) after treatment with 10^{-7} M phorbol ester were incubated with ^{125}I-labeled tryp-VLDL (10.5 μg protein/ml), ^{125}I-labeled LDL (7 μg/ml) or ^{125}I-labeled acetyl LDL (7 μg/ml) alone (for total cell associated) or in the presence of a 10-fold excess of unlabeled HTG-VLDL, LDL, or acetyl LDL, respectively (for nonspecific cell associated lipoprotein), for 3 h at 37°C before the cells were washed and cell-associated radioactivity was determined as described in methods.

[a]Each value is the average from duplicate dishes of cells; variation between duplicates is indicated. Specific (i.e., competable) cell association was calculated as the difference between total and nonspecifically associated lipoproteins. The last column is the relative specific cell association for each lipoprotein at different stages of differentiation relative to that observed in monocytes.

spectively (31, 32), THP-1 monocytes were divided into three groups and assayed before and at day 1 and 7 after treatment with 10^{-7} M phorbol ester for specific high affinity uptake of ^{125}I-labeled LDL, ^{125}I-labeled acetyl LDL, and ^{125}I-labeled tryp-VLDL. The specific uptake of tryp-VLDL was similar in monocytes and in THP-1 macrophages 1 day after treatment with 10^{-7} M PMA and retained 75% activity at 7 days (**Table 1**). By contrast, the specific uptake of acetyl LDL was low in THP-1 monocytes before and 1 day after treatment with PMA but was induced 9.6-fold 7 days after phorbol ester treatment (Table 1), in agreement with Via et al. (32). The specific uptake of LDL in THP-1 monocytes and 1 day after PMA treatment was low, but measurable (the cells were not preincubated in LPDS to induce LDL receptor activity in these experiments); specific LDL uptake dropped to undetectable levels 7 days after PMA treatment, in agreement with Via et al. (32). In contrast, specific, high affinity cell association of tryp-VLDL was similar throughout differentiation (Table 1) and has consistently been detected in THP-1 monocytes in suspension and adherent macrophages assayed 1–7 days after PMA treatment. These experiments indicate that the cellular binding site for high affinity uptake of TGRLP is not appreciably affected by differentiation of monocytes into macrophages, in contrast to the decreased expression of the LDL receptor pathway and the increased expression of the acetyl LDL receptor pathway upon differentiation. This also distinguishes the TGRLP binding site from LRP, which is also induced 10- to 15-fold upon differentiation (57). That specific uptake of tryp-VLDL is unchanged upon differentiation further indicates that macrophage apoE or lipoprotein lipase, which are known to be induced many fold upon differentiation of THP-1 monocytes into macrophages (55, 56), do not affect lipoprotein interaction with this site under our experimental conditions.

Expression of MBP 200 and 235 before and after PMA-induced differentiation of THP-1 cells

Ligand blots demonstrate that MBP 200 and 235 are expressed in both monocytes (Fig. 2) and in macrophages 7 days after PMA treatment (Fig. 4). As a corollary to the experiment in Table 1, total binding activities of MBP 200 and 235 were similar throughout differentiation, as determined by densitometry of MBP 200 and 235 activities on ligand blots (data not shown). By contrast, the acetyl LDL receptor was not detected by ligand blotting in extracts of THP-1 monocytes or in macrophages 1 day after PMA, but was detected at 4 (data not shown) to 7 days after PMA treatment (Fig. 4), consistent with the cell uptake studies (Table 1) and the studies of Via et al. (32).

Specific cell association of TGRLP with THP-1 monocytes is not suppressed by medium sterol content, unlike the LDL receptor pathway

To determine whether or not the high affinity binding site for the uptake of TGRLP in THP-1 monocytes is distinct from the LDL receptor in terms of regulation by sterols, THP-1 monocytes were divided into three groups and preincubated in medium containing serum in the absence and presence of cholesterol plus 25-hydroxycholesterol or in LPDS for 24 h prior to incubation with ^{125}I-labeled LDL and ^{125}I-labeled tryp-VLDL alone and with excess unlabeled ligand to assess specific cell association (**Table 2**). These studies were conducted at 37°C to allow uptake and thereby amplify the low level of LDL binding seen at 4°C. Monocytes, in contrast to differentiated macrophages, secrete little if any apoE or lipoprotein lipase (55, 56), so these are not confounding factors. As seen in

TABLE 2. Effects of media sterol content on specific cell association of ^{125}I-labeled LDL and ^{125}I-labeled tryp-VLDL with THP-1 monocytes

		Cell Associated Lipoprotein		
Lipoprotein	Preincubation	Total	Nonspecific	Specific
		ng/mg cell protein		
^{125}I-labeled tryp-VLDL	FBS	56.5 ± 0.6[a]	16.2 ± 0.8[a]	40.3
	LPDS	84.2 ± 5.0	20.3 ± 3.1	63.9
	FBS + sterols	88.1 ± 6.0	21.5 ± 0.5	66.6
^{125}I-labeled LDL	FBS	66.5 ± 6.8	23.8 ± 4.0	42.7
	LPDS	168 ± 5.0	38.1 ± 0.8	130
	FBS + sterols	59.9 ± 0.2	60.0 ± 5.5	0

THP-1 monocytes were divided into three groups and preincubated for 24 h in 6-well plates, with medium containing 10% fetal bovine serum (FBS), medium containing LPDS, or medium containing 10% FBS plus cholesterol, 16 μg/ml, and 25-hydroxycholesterol, 1 μg/ml, prior to incubation in duplicate with ^{125}I-labeled LDL, 13 μg/ml, or ^{125}I-labeled tryp-VLDL, 11 μg/ml alone (for total binding) or with a 10-fold excess of unlabeled LDL or homologous VLDL, respectively (for nonspecific binding), for 2 h at 37°C before the cells were washed, harvested, and counted as described in methods.

[a]The average value from duplicate dishes is expressed as ng lipoprotein protein per mg cell protein ± range for total and nonspecific values. Specific cell-associated lipoprotein is calculated as the difference between total and nonspecific values.

Table 2, the specific cell-associated radioactivity of THP-1 monocytes in medium containing serum (FBS), LPDS, or serum plus cholesterol and 25-hydroxycholesterol (FBS + sterols) prior to incubations with ^{125}I-labeled tryp-VLDL were similar and were effectively competed by unlabeled lipoprotein. In two out of three experiments, there was a modest increase in uptake of tryp-VLDL by cells in LPDS and in FBS plus sterols, relative to cells in FBS; in no case was binding decreased in cells treated with FBS plus sterols relative to LPDS, indicating there is no "down regulation" by sterol, similar to studies in P388D$_1$ macrophages (30). These data indicate that media sterol content has little to no effect on the specific, competable uptake of ^{125}I-labeled tryp-VLDL by THP-1 monocytes. By contrast, the specific uptake of ^{125}I-labeled LDL by THP-1 monocytes was increased 3-fold by preincubation in LPDS and was abolished by preincubation in medium containing serum plus cholesterol and 25-hydroxycholesterol (Table 2). These results indicate that THP-1 monocytes express a high affinity binding site for TGRLP which, unlike the LDL receptor (43), is not appreciably regulated by media sterol content. Moreover, there was little to no degradation of tryp-VLDL in this short (2 h) incubation yet there was substantial degradation of ^{125}I-labeled LDL, especially in cells preincubated in LPDS

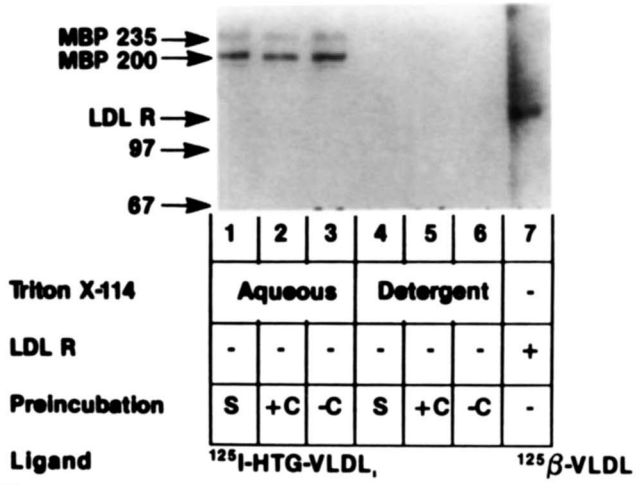


Fig. 5. Ligand blotting analysis of the binding of ^{125}I-labeled HTG-VLDL to MBP 200 and 235 from THP-1 monocytes preincubated with media containing serum, serum plus cholesterol and 25-hydroxycholesterol, or LPDS. After exposure to test media containing differing sterol levels as described in the legend of Table 2, THP-1 monocytes were harvested and extracted with Triton X-114. After phase separation of the detergent extracts, aliquots of the aqueous phase at equal protein concentrations (lanes 1–3) and the detergent phase (lanes 4–6) were electrophoresed, transferred, blocked, and incubated with ^{125}I-labeled HTG-VLDL (20 μg/ml). Preincubation conditions are indicated as serum alone (S; lanes 1, 4), serum + cholesterol and 25-hydroxycholesterol (+C; lanes 2, 5), and lipoprotein-deficient serum (−C; lanes 3, 6). Lane 7 contains a partially purified bovine adrenal LDL receptor used as a marker and visualized with ^{125}I-labeled βVLDL. Autoradiography was done as described in the legend of Fig. 4. Molecular mass markers and the LDL receptor are indicated by arrows. MBP 200 and MBP 235 relative mobilities are also indicated.

where the amount of ^{125}I-labeled LDL specifically degraded was 313 ng/mg cell protein, or 2.4-fold higher than the amount associated with the cells. In other experiments using longer (4 h) incubations of ^{125}I-labeled tryp-VLDL with THP-1 monocytes or 1-day plated cells, the specific degradation of ^{125}I-labeled tryp-VLDL ranged from approximately 15 to 50% of the amount of tryp-VLDL specifically associated with the cells. Thus, the specific degradation of tryp-VLDL by THP-1 monocytes and macrophages occurs, but it is delayed and less efficient in comparison to the specific degradation of LDL via the LDL receptor pathway. This difference indicates that the pathway for TGRLP uptake and degradation is different from the LDL receptor pathway.

As in the cell uptake studies presented in Table 2, the binding activities of MBP 200 and 235 in THP-1 monocytes were not affected by medium sterol content, as indicated by ligand blotting studies (**Fig. 5**). In the same experiment shown in Table 2, cells grown on the three test media for 24 h were harvested and extracted with Triton X-114. After phase separation, aliquots of the aqueous phase (Fig. 5, lanes 1–3) and the detergent phase (lanes 4–6) were analyzed by ligand blotting with ^{125}I-labeled HTG-VLDL; aqueous phase extracts of THP-1 monocytes incubated with serum (S, lane 1), serum plus cholesterol and 25-hydroxycholesterol (+C, lane 2), or LPDS (−C, lane 3) were electrophoresed at equal protein concentrations. ^{125}I-labeled HTG-VLDL bound to MBP 200 and 235 with similar intensities regardless of preincubation conditions, indicating that, like the cellular binding site (Table 2), expression of these binding proteins is not affected by sterol content of the medium. MBP 200 and 235 were not apparent in the detergent phase extracts of monocytes regardless of preincubation conditions (lanes 4–6).

In the same experiment illustrated in Fig. 5, identical gels were transferred and probed with iodinated tryp-VLDL. The results with tryp-VLDL confirmed those with hypertriglyceridemic VLDL; quantitation of binding of ^{125}I-labeled tryp-VLDL to MBP 200 and 235 was done by densitometry of autoradiograms and demonstrated unchanged binding of ^{125}I-labeled tryp-VLDL after the three preincubations (data not shown).

Ligand specificity of the THP-1 cellular binding site for TGRLP and MBP 200 and 235

Competitive binding studies indicate that the cellular uptake of ^{125}I-labeled tryp-VLDL is effectively competed by a 10- to 20-fold excess of HTG-VLDL but a 40-fold molar excess of LDL or acetyl-LDL shows little to no competition in THP-1 monocytes (**Fig. 6**) or in THP-1 macrophages at 1–7 days after treatment with 10^{-7} M PMA (not shown). Conversely, the cellular uptake of ^{125}I-labeled acetyl LDL by THP-1 macrophages 7 days after

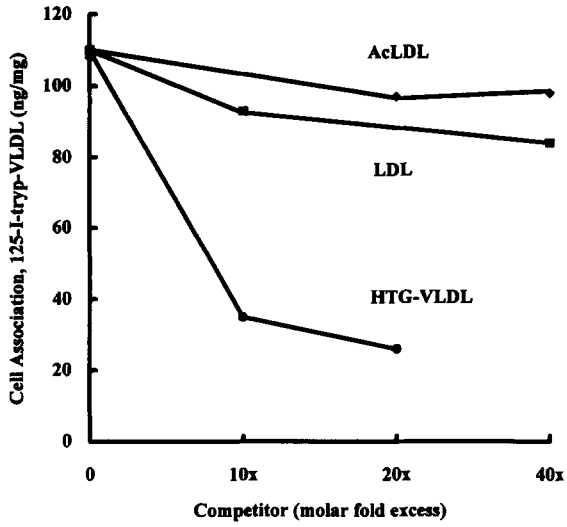


Fig. 6. Ligand specificity of the apoE-independent site for TGRLP on THP-1 monocytes. THP-1 monocytes were grown in complete medium for 24 h, washed and then incubated for 4 h at 37°C with ^{125}I-labeled tryp-VLDL in the absence and presence of the indicated concentrations of unlabeled homologous HTG-VLDL (●), LDL (■), or acetyl LDL (♦). The amount of cell-associated radioactivity was determined and expressed as ng apoprotein/mg cell protein as described in Methods. Each data point represents the average of values from duplicate dishes, which differed by ≤5%.

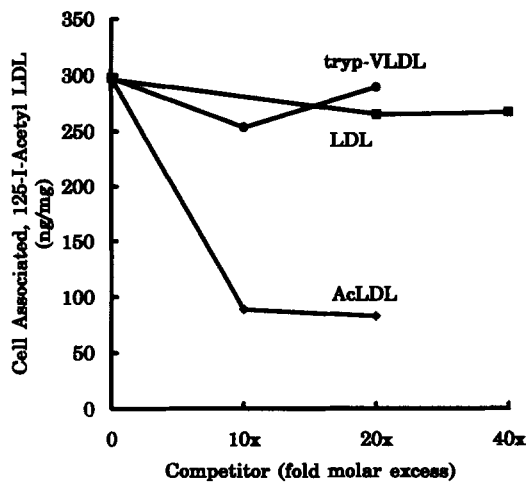


Fig. 7. Specificity of the acetyl LDL receptor pathway of THP-1 macrophages 7 days after PMA-induced differentiation. THP-1 monocytes were treated with PMA (10^{-7} M) and maintained in culture for 7 days. Subsequently, these cells were washed and incubated for 4 h at 37°C with ^{125}I-labeled acetyl LDL (7 μg/ml) in the absence and presence of indicated quantities of unlabeled acetyl LDL (♦), tryp-VLDL (●), and LDL (■). The amount of cell-associated radioactivity was determined and expressed as ng apoprotein/mg cell protein. Each data point represents the average of values from duplicate dishes, which differed by ≤5%.

PMA treatment is effectively competed by a 10-fold excess of acetyl LDL but not by a 40-fold excess of LDL or 20-fold excess of tryp-VLDL (**Fig. 7**). These studies indicate that the cellular binding site for uptake of TGRLP is distinct from the acetyl-LDL receptor and the LDL receptor in ligand specificity.

Similarly, competitive ligand blots indicate that the binding of ^{125}I-labeled tryp-VLDL to MBP 200 and 235 from THP-1 monocytes is effectively competed by a 10-fold particle excess of HTG-VLDL but shows little to no competition by a 20-fold (**Fig. 8A**) or 40-fold (Fig. 8B) excess of acetyl LDL or LDL. Some but not all LDL partially compete at high levels (40 ×), as shown in Fig. 8B, but not at low levels (10 ×). While a 10-fold excess of HTG-VLDL is as effective as a 20-fold excess (Fig. 8A), causing ≥75% competition of tryp-VLDL binding, a 10-fold excess of β-VLDL from cholesterol-fed rabbits competes less efficiently than HTG-VLDL, inhibiting binding by approximately 40% (Fig. 8B). β-VLDL from four different cholesterol-fed rabbits gave similar results. As described above (Fig. 4), binding of ^{125}I-labeled acetyl LDL to its receptor in ligand blots of THP-1 macrophage extracts was effectively competed by acetyl LDL but not by tryp-VLDL or LDL.

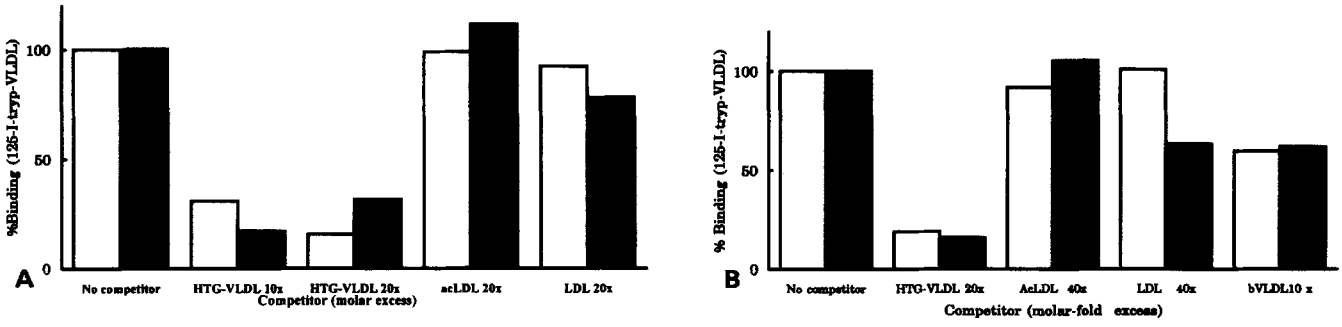


Fig. 8. Densitometric analysis of the specificity of binding of ^{125}I-labeled tryp-VLDL to MBP 200 and 235 from THP-1 monocytes. Extracts of THP-1 monocyte membranes were prepared as described in Methods and ligand blot analyses were performed as described in the legend of Fig. 4. Two-dimensional densitometry of the autoradiograms from two experiments was used to determine the amount of ^{125}I-labeled tryp-VLDL bound to MBP 200 and 235. The binding of ^{125}I-labeled tryp-VLDL in the presence of the indicated concentrations of competitors is expressed as percent of binding in the absence of competitor of ^{125}I-labeled tryp-VLDL to MBP 200 (solid bars) and MBP 235 (open bars). A: Competition by HTG-VLDL at 10- and 20-fold and acetyl LDL and LDL at 20-fold molar excess; B: competition by HTG-VLDL at 20-fold, acetyl LDL and LDL at 40-fold, and β-VLDL at 10-fold molar excess.

Thus MBP 200 and 235 have the same ligand specificities as the specific cellular binding site for TGRLP on intact monocytes and macrophages and different from the ligand specificities of the LDL receptor and members of its gene family or the acetyl LDL receptor.

Triglyceride accumulation in THP-1 monocytes and macrophages

As the specific cellular binding and uptake of tryp-VLDL and the intensities of MBP 200 and 235 on ligand blots are similar in monocytes and in macrophages, this raises the question whether tryp-VLDL can induce rapid, receptor-mediated triglyceride accumulation in monocytes as well as in macrophages. Washed THP-1 monocytes and 1-day-plated macrophages were incubated with tryp-VLDL for 4 h at 37°C in the absence of serum components, conditions previously shown in P388D$_1$ macrophages to induce receptor-mediated, lipoprotein lipase-independent and apoE-independent triglyceride accumulation (30). There was significant accumulation of triglyceride by monocytes, which secrete little to no apoE or lipoprotein lipase (55, 56), and by macrophages exposed to tryp-VLDL and HTG-VLDL for 4 h relative to buffer control (**Table 3**), indicating secretion of apoE or lipoprotein lipase is not required for triglyceride accumulation. Both baseline and TGRLP-induced triglyceride accumulation were greater in macrophages, however, suggesting increased endogenous synthesis of triglyceride. Accumulation was similar whether cells were exposed to HTG-VLDL or apoE-free tryp-VLDL. Direct comparison of HTG-VLDL and normal VLDL at equal triglyceride levels in 4-h incubations demonstrated a 4- to 7-fold greater response to HTG-VLDL than to normal VLDL, which induced a minimal and more linear triglyceride response (**Fig. 9**), similar to results in murine macrophages (30).

TABLE 3. Triglyceride accumulation in THP-1 monocytes and macrophages

Lipoprotein	Monocytes	Macrophages
	μg triglyceride/mg cell protein	
None	22.8 ± 3.94[a]	34.6 ± 6.10[a]
HTG-VLDL	59.5 ± 4.95	112.7 ± 11.3
Tryp-VLDL	64.0 ± 7.79	104.8 ± 4.4

THP-1 monocytes before and 1 day after treatment with PMA were grown in 6-well plates, washed, and incubated with HTG-VLDL or tryp-VLDL (300 μg triglyceride/ml medium) in the absence of other serum components for 4 h at 37°C prior to extensive washing, lipid extraction, and triglyceride analysis as described in Methods.

[a]Values are the mean (± SD) of triplicate dishes of cells, expressed as μg triglyceride/mg of cell protein.

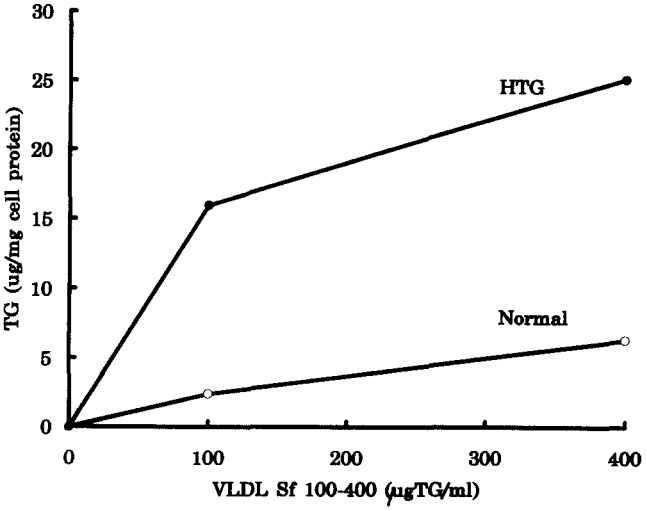


Fig. 9. Triglyceride accumulation induced by HTG-VLDL$_1$ versus normal VLDL$_1$. Cells were grown in 6-well plates and treated with phorbol ester 1 day prior to incubation with the indicated concentrations of HTG-VLDL$_1$ (●) or normal VLDL$_1$ (○) for 4 h at 37°C in the absence of other serum components, as described in Table 3 and Methods. Cells were extensively washed, extracted, and triglyceride mass was determined as described in Methods. Values represent the average from duplicate dishes.

DISCUSSION

The current studies were undertaken to determine whether or not monocyte-macrophages of human origin express a high affinity, specific, apoE-independent cellular binding site and membrane binding proteins for TGRLP. Studies were carried out both in HMM isolated from blood and in the human monocytic cell line THP-1. Several lines of evidence indicate that both cell types express high affinity cellular binding sites for TGRLP that are distinct from the LDL receptor and members of its gene family and the acetyl LDL receptor. First, 4°C binding studies in both cell types indicate the presence of a high affinity, saturable, apoE-independent, and competable site for TGRLP. Modification of HTG-VLDL, which abolishes its high affinity binding to the LDL receptor by completely degrading apoE (trypsin treatment of HTG-VLDL), was used to distinguish the TGRLP binding site from the LDL receptor and related receptors. The saturable, specific binding curves suggest that both cell types have similar high affinities for tryp-VLDL, with half-maximal binding at 3–6 nM. Second, the TGRLP binding site differs from the LDL receptor, LRP, and the acetyl LDL receptor in expression during differentiation, being present in THP-1 monocytes in suspension and in HMM and THP-1 macrophages 1 day after adherence and for at least 1 week in culture during differentiation. In contrast, the LDL receptor is diminished and the acetyl LDL receptor (this paper and refs.

31, 32) and LRP (57) are induced after differentiation of HMM and THP-1 monocytes. Third, the binding site for TGRLP on HMM differs from the LDL receptor gene family in ligand specificity in that apoE is not required for binding of the TGRLP to the site, as indicated by studies using tryp-VLDL devoid of apoE. ApoE is essential for the binding of large (S_f >60) TGRLP to the LDL receptor (17–20). Moreover, exogenous apoE must be added to apoE-rich β-VLDL to induce binding to LRP (51), yet β-VLDL without exogenous apoE binds to MBP 200 and 235 (Fig. 8 and data not shown). ApoE also mediates binding to the LDL receptor-related rabbit VLDL receptor expressed in heart and muscle (52). However, tryp-VLDL devoid of apoE bound with at least as high affinity to monocytes, to macrophages and to MBP 200 and 235 as did unmodified HTG-VLDL, indicating that apoE is not necessary for the specific binding of TGRLP particles to the human monocyte-macrophage binding site or to MBP 200 and 235. The similar high affinity 4°C binding of tryp-VLDL to washed THP-1 monocytes and HMM and THP-1 macrophages 1 day after adherence as well as at later stages of differentiation, i.e., before and after induction of apoE and lipoprotein lipase is known to occur (24, 54–56), indicates that macrophage-derived apoE or lipoprotein lipase does not influence binding under these conditions. Competitive binding studies also demonstrate that the THP-1 binding site for TGRLP is distinct from the binding sites for acetyl LDL and for LDL. Fourth, the binding site for TGRLP is not sterol-regulated, thus differing from the LDL receptor. Fifth, tryp-VLDL does not bind with high affinity to normal human skin fibroblasts (17, 18), indicating a more restricted cellular distribution for the TGRLP binding site than for the LDL receptor (43), LRP (59), or the rabbit VLDL receptor (52). Finally, the amount of tryp-VLDL specifically degraded by THP-1 cells was diminished relative to the specific degradation of LDL by undifferentiated THP-1 monocytes or of acetyl-LDL by differentiated THP-1 macrophages.

Ligand blotting studies identified two membrane binding proteins with apparent molecular masses of 200 and 235 kDa that share all the above-mentioned characteristics of the cellular binding site in *1)* expression during differentiation, *2)* ligand specificity, *3)* lack of regulation by sterols in the medium, and *4)* cellular distribution. Ligand blotting studies demonstrate the presence of MBP 235 and/or 200 only in cells of reticuloendothelial origin; for example, HMM, THP-1 monocyte-macrophages (this report), bovine and porcine aortic endothelial cells, and U937 cells, but not in human hepatoma cell lines, CHO cells, or NIH 3T3 fibroblasts (unpublished data) or in human skin fibroblasts (30, 44). The murine cell lines $P388D_1$ (30) and J774.1 macrophages (unpublished observations) express an MBP with identical ligand specificities but of slightly different apparent molecular mass (190 kDa). The apparent molecular masses of the likely candidates for the human macrophage membrane proteins responsible for cellular binding of TGRLP (M_r 200 and 235 kDa) are different from those of the LDL receptor (M_r 130 kDa) (43), the acetyl LDL receptor (M_r 260 kDa) (58), and LRP/α_2-macroglobulin receptor (M_r ~600; 515, 85 kDa) (60). Ligand blotting of the rabbit VLDL receptor has not been reported, but its cDNA length and extensive homology suggests it would be similar to the LDL receptor in molecular size (52).

Cellular uptake and degradation studies demonstrated apoE-independent specific degradation of tryp-VLDL by monocytes as well as macrophages. Further, both monocytes and macrophages rapidly accumulate triglyceride when exposed to tryp-VLDL, HTG-VLDL and plasma chylomicrons, but not normal VLDL under conditions where lipoprotein lipase is not expressed.

The cellular binding site and MBPs for chylomicrons and HTG-VLDL in human monocyte-macrophages are similar to the murine macrophage receptor pathway and MBP 190 previously described (30) in several respects (ligand specificity, lack of regulation by sterols, and detergent solubility), but not identical. Murine macrophages expressed only one receptor candidate, MBP 190, whereas two MBPs were identified in both human blood-borne and THP-1 monocyte-macrophages. The two human receptor candidates have greater apparent molecular masses than the murine macrophage MBP (200 and 235 versus 190 kDa). Evidence that MBP 200 and MBP 235 are cell surface proteins that, upon reduction, are converted into a single active binding species with intermediate apparent M_r is presented elsewhere (submitted for publication).

The purpose of this study was not to detail the binding determinants in lipoproteins for the binding site and MBPs or to exhaustively study all possible ligands. However, the specific binding of HTG-VLDL and plasma chylomicrons to human monocyte-macrophages suggests a potential role of this pathway. Studies in rabbits and marmosets demonstrate that bone marrow macrophages actively take up chylomicrons in vivo (61). The monocyte-macrophage binding site and MBP 200 and 235, described in this report, could be responsible for initial recognition of chylomicrons by monocytes and accessible macrophages leading to uptake of the lipoprotein in humans. Hence, the binding site and MBP 200 and 235 may play a role in the nutrition of circulating monocytes and accessible macrophages, such as in bone marrow, in the postprandial state.

As the binding sites on monocyte-macrophages and MBP 200 and 235, unlike the LDL receptor, are not down-regulated by exposure to sterols, this site could mediate binding of HTG-VLDL or chylomicrons even when LDL receptor activity is suppressed. This property could allow its ligands, when present for prolonged times or in large

quantities, to induce lipid accumulation both in monocytes and in macrophages (foam cell formation).

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