Apolipoprotein B-48 or Its Apolipoprotein B-100 Equivalent Mediates the Binding of Triglyceride-Rich Lipoproteins to Their Unique Human Monocyte-Macrophage Receptor

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Abstract—Studies in animals and humans have demonstrated uptake of plasma chylomicrons (triglyceride-rich lipoprotein [TGRLP] of Sf > 400) by accessible macrophages in vivo. One potential mechanism is via a unique receptor pathway we previously identified in human blood and THP-1 monocytes and macrophages for the lipoprotein lipase (LpL)– and apolipoprotein (apo) E–independent, high-affinity, specific binding of plasma chylomicrons and hypertriglyceridemic VLDL (HTG-VLDL) to cell-surface membrane-binding proteins (MBP 200, 235; apparent Mr 200, 235 kD on SDS-PAGE) that leads to lipid accumulation in vitro. Competitive binding studies reported here demonstrate that anti-apoB antibodies specifically block the high-affinity binding of TGRLP to this receptor on THP-1 cells and on ligand blots. LpL, which binds to an N-terminal domain of apoB, also inhibits TGRLP binding both to this site on THP-1s and to MBP 200, 235 by binding to apoB. Chylomicrons of Sf > 1100 that contain apoB-48, but not apoB-100, bind specifically to MBP 200, 235, and this binding is blocked by anti-apoB IgG. In contrast, lactoferrin and heparin do not inhibit TGRLP binding. We conclude that the receptor-binding domain is within apoB-48 (or an equivalent in apoB-100) near the LpL-binding domain, but not a heparin-binding domain. Uptake of TGRLP by this mechanism could provide essential nutrients or, in HTG, cause excess lipid accumulation and foam cell formation. (Arterioscler Thromb Vasc Biol. 1998;18:968-976.)

Key Words: foam cells ■ atherosclerosis ■ hypertriglyceridemia

The majority of chylomicron remnants are taken up by the liver (after sequestration in the space of Disse), which is enriched with apoE, the mediator of hepatic remnant uptake by the LDL receptor or LRP. Human and animal studies indicate that peripheral macrophages also take up intestinally derived plasma chylomicrons (ie, partially lipolyzed plasma TGRLP of Sf > 400), which contain apoB-48 as the only apoB species. Ross and Zilversmit demonstrated extrahepatic uptake of ≈40% of plasma chylomicrons in rabbits that was decreased by inhibition of the reticuloendothelial system, which implicated monocyte-macrophage–macrophages in this extrahepatic uptake. Studies in marmosets (a primate) and rabbits demonstrated substantial (20% to 40% of total) uptake of chylomicrons in vivo by accessible peripheral macrophages, particularly in bone marrow (both animals) and spleen (marmosets and several nonprimate animals). Studies in humans demonstrate that dietary lipoproteins deliver retinyl esters to blood cells, as well as to the liver, suggesting that plasma chylomicrons serve as a nonmodified, native source of lipid and lipid-soluble vitamins for monocyte-macrophage nutrition in the normal state. In pathological states in which chylomicrons persist in the fasting state, TGRLP (including chylomicrons and their remnants) appear to be involved in the conversion of monocyte-macrophage–macrophages to foam cells. For example, foam cells are found in the skin, spleen, atherosclerotic lesions, and bone marrow of non-diabetic subjects with fasting chylomicrons (hyperlipoproteinemia types 1, 3, and 5). Diabetic subjects with fasting chylomicronemia also develop monocyte-macrophage–derived foam cells in eruptive xanthomas that are filled with triglyceride and cholesterol ester due to uptake of chylomicron-sized lipoproteins by monocyte-macrophages, as demonstrated by chemical analysis and electron microscopy. The in vivo rate and magnitude of TG-rich chylomicron uptake by bone marrow monocyte-macrophages in rabbits and marmosets (20% to 40% of chylomicrons cleared from the plasma by 20 minutes) suggest this uptake is at least in part receptor mediated, since a bone marrow equivalent to hepatic-like sequestration of chylomicrons has not been reported. This rapid uptake of TG-rich chylomicrons in vivo by accessible bone marrow and spleen macrophages was not, however, accelerated by infusion of apoE, a surprising finding because apoE is required for the uptake of large (Sf > 60) TGRLP by members of the LDL receptor gene

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family and for hepatic uptake of chylomicron remnants and β-VLDL. Moreover, in rabbits, infused apoE diverted much of the uptake from the peripheral macrophages to the liver, suggesting that the observed preinfusion chylomicron uptake by peripheral macrophages was not mediated by apoE and that these macrophages therefore have an alternative apoE-independent uptake mechanism for these TGRLP.

We have demonstrated in human and murine macrophages a receptor that has the ligand-binding characteristics suggested by the above in vivo studies. It is an apoE- and LpL-independent TGRLP receptor pathway that differs from the LDL receptor family and the scavenger receptor family in its properties, including: (1) constitutive expression during differentiation; (2) retarded intracellular lipid degradation; (3) ligand specificity; (4) apparent mass of the candidate receptor proteins (M, 200, 235 kD); and (5) cellular distribution. Uptake of the high-affinity ligands plasma chylomicrons (S,>400), HTG-VLDL (S, 100 to 400), and tryp-VLDL (S, 100 to 400) immunochemically devoid of apoE, was prepared as previously described.12,13,20–22,30 Although tryp-VLDL is devoid of immunochromatically detectable apoE, the ligand blot was extracted into organic solvent. For competitive ligand blots, TGRLP was biotinylated32 as described and dialyzed extensively as initially published data, 1998), it retains essentially all immunochemically detectable apoB as fragments of 100 kD and less, as determined by SDS-PAGE (size) and radioimmunoassay13,27 or by SDS-PAGE and quantitative dot-blot analysis of parent VLDL and tryp-VLDL. For cell-binding studies, lipoproteins were radiolabeled by the iodine monochloride method as described11 and as we used previously.11,12,13,20,21,27 Specific activities ranged from 100 to 200 cpm/ng protein. Less than 10% of the label was extracted into organic solvent. For competitive ligand blots, TGRLP was biotinylated as described and dialyzed extensively before use.

Lipoproteins
HTG-VLDL (S, 100 to 400 and 60 to 100) was isolated from plasma of fasting subjects with type 4 lipoprotein profiles or in some cases from lipemic plasma obtained from the Red Cross. To obtain chylomicrons and remnants enriched in apoB–48, normal and hypertriglyceridemic volunteers consumed a standardized solid, high-fat meal at 1000 calories per meter squared. Blood for the isolation of lipoproteins was drawn 4 hours after the meal was consumed. Total chylomicrons S,>400 were isolated directly from plasma by flotation for 20 minutes at 20 000 RPM in an SW27 rotor at 23°C. Chylomicron subfractions (S,>3200, S, 1100 to 3200, and S, 400 to 1100) and HTG-VLDL S, 100 to 400 were isolated from total chylomicrons or the <1.006 fraction, respectively, by cumulative flotation through a discontinuous NaCl gradient from d=1.063 to 1.006 g/mL and as detailed previously for VLDL subspecies. Protein concentrations of the lipoproteins were obtained by a modified Lowry procedure.12,29 Tryp-VLDL, reisolated and devoid of immunochromatically detectable apoE, was prepared as previously described.12,13,27 Functional loss of apoE was demonstrated by lack of binding of tryp-VLDL to partially purified bovine LDL receptors on ligand blots.12,13,20–22,30 Although tryp-VLDL is devoid of immunochromatically detectable apoE12,13,22 and apoCII (W.A. Bradley, unpublished data, 1998), it retains essentially all immunochromatically detectable apoB as fragments of 100 kD and less, as determined by SDS-PAGE (size) and radioimmunoassay13,27 or by SDS-PAGE and quantitative dot-blot analysis of parent VLDL and tryp-VLDL.12 For cell-binding studies, lipoproteins were radiolabeled by the iodine monochloride method as described11 and as we used previously.11,12,13,20,21,27 Specific activities ranged from 100 to 200 cpm/ng protein. Less than 10% of the label was extracted into organic solvent. For competitive ligand blots, TGRLP was biotinylated as described and dialyzed extensively before use.

Cells and Cell Culture
THP-1 cells (a human monocytic leukemia cell line) were purchased from the American Type Culture Collection and grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mmol/L glutamine, 100 μg/mL penicillin, 100 U/mL streptomycin, and 5×10–5 mol/L 2-mercaptoethanol. Cells were maintained in tissue-culture flasks at 37°C in a humidified atmosphere of 5% CO2 and 95% air at <1.0×106/mL. For differentiation, cells (1.5×104 cells per well of 6-well plates) were seeded in complete media: phorbol 12-myristate, 13-acetate was then added (10–7 mol/L final concentration), as described. Human skin fibroblasts were early-passage cells derived from newborn foreskin and maintained as previously described.

Cell-Binding Studies
Lipoprotein binding studies were carried out essentially as initially described by Goldstein and Brown.11 THP-1 monocytes were seeded in 6-well tissue-culture plates (1.5×105 cells per well), phorbol ester (10–7 mol/L) was added to induce adherence, and then they were used for experiments after 24 hours. As controls, cultured human skin
fibroblasts were subcultured and grown to ~75% confluence (3 to 4 days after subculture at a 1-to-4 split ratio) in complete medium (DMEM containing 10% fetal bovine serum, 2 mMol/L glutamine, 100 µg streptomycin per milliliter, and 100 U penicillin per milliliter), washed with sterile saline, and preincubated in DMEM containing 5% lipoprotein-deficient serum for 36 hours to induce the LDL receptor.1,13,22 Cells were then preincubated for 30 minutes at 4°C to cool the cells. Cells were then incubated with RPMI-1640 (THP-1 cells) or DMEM (fibroblasts) containing 10 mMol/L HEPES, pH 7.4, 2 mg BSA per milliliter, and indicated amounts of 125I-HTG-VLDL or 125I-tryp-VLDL alone and in the presence of 200 µg/mL unlabeled VLDL or other potential competitors for 1.5 hours at 4°C before extensive washing with cold buffered saline containing 2 mg BSA per milliliter35 as previously described.1,13,20–22 Cells were dissolved in 0.1 N NaOH prior to the measurements of cell-associated radioactivity and cell protein. Dishes with no cells were used to correct for the amount of nonspecific binding to the plastic wells, as described.37

Antibodies
Sheep anti-human apoB IgG (1001400, Boehringer Mannheim Biochemicals) was purified by affinity chromatography using an LDL-conjugated Sepharose column, prepared as previously described.36 Immunoaffinity-purified rabbit anti-sheep IgG conjugated to alkaline phosphatase and sheep γ-globulin were purchased from Jackson Laboratories. Rabbit anti-human apoB antibodies were isolated by ammonium sulfate precipitation of serum from rabbits immunized intradermally with human LDL, isolated at d = 1.03 to 1.05 g/mL, and emulsified in adjuvant. The anti–apoB-100 antibodies generated and/or affinity purified in our laboratory were monospecific for apoB and did not recognize apoE, apoCs, or apoHDL. Anti apo-E was generated in rabbits using human apoE purified in our laboratory and was monospecific for apoE. Affinity-purified goat anti-apoCIII and anti-apoCII were generous gifts from Dr Ronald Krauss and Dr G.M. Anantharamaiah, respectively.

Preparation of Cell Extracts
THP-1 monocytes (1.5 × 10^6) were harvested and washed twice with 50 mL of buffer A (0.15 mMol/L NaCl containing 50 U aprotinin per milliliter, 5 mMol/L benzamidine, and 0.1 mMol/L PMSF) and resuspended in 2 mL of 20 mMol/L Tris, pH 8.0, 50 mMol/L NaCl, 0.1 mMol/L EDTA, containing the protease inhibitor mix of buffer A plus leupeptin and τ-phelalanyl-1-propyl-L-arginine chloromethylethylketone (PPACK) and solubilized with 1% Triton X-114 for 15 minutes on ice. Aqueous-phase extracts were prepared as previously described.36–38,21,22 by method of Bordier23 and immediately frozen in liquid nitrogen after the addition of glycerol to a final concentration of 10% (vol/vol). Protein content was estimated by the Bradford method using the Bio-Rad protein assay reagent.38

Ligand Blotting
The ligand-blotting assay was performed essentially as described earlier20–22 with minor modifications. Aliquots of the detergent extracts were electrophoresed on 5% polyacrylamide gels containing 0.1% SDS under nonreducing conditions in a Bio-Rad minigel apparatus and electrotransferred to nitrocellulose. After blocking for 1 hour with 5% Carnation nonfat dry milk in ligand buffer (50 mMol/L Tris-HCL, pH 8, 90 mMol/L NaCl, and 2 mMol/L CaCl2), the blots were rinsed with 0.5% milk in ligand buffer before incubation with lipoproteins in ligand buffer containing 0.05% milk.20–22 Biotin-labeled lipoproteins, with and without antisera, IgGs (the 50% [NH4]2 SO4 precipitate of antisera), or other potential competitors, were preincubated for 30 minutes at 4°C and then incubated with the nitrocellulose strips for 1.5 to 3 hours as indicated. After extensive washing, bound lipoprotein was detected by incubation with streptavidin linked to alkaline phosphatase, followed by the substrates BCIP and NBT (Bio-Rad). In some experiments without antibodies as potential competitors, native, unlabeled TGRLPL was used as the ligand and bound TGRLPL was detected with anti-apoB followed by alkaline phosphatase-linked secondary antibody. Ligand blots were scanned on an optical scanner (Hewlett Packard), and binding activity was quantified using the Image Quant software (Molecular Dynamics densitometer) as previously described.34,21

Results

Anti-ApoB Antibodies Inhibit the Binding of HTG-VLDL S, 100 to 400 to MBP 200, 235

Previous experiments in human blood-borne and THP-1 monocyes and macrophages1,2,22 with the surrogate ligand tryp-VLDL, which retained essentially all apoB immunoreactivity (in fragments of ~100 kDa) but was devoid of immunochromically detectable apoE20,21,22 and apoCII (S.H. Gianturco, unpublished data, 1998) and failed to bind to the LDL receptor in cells2,13 and in ligand blots,20–22,27,30 suggested that apoB may be the ligand for this monocyte-macrophage cell site and corresponding MBPs. Thus, competitive ligand-blotting experiments were done with several polyclonal anti-apoB–specific antibodies to determine whether they were capable of specifically blocking binding of HTG-VLDL to the putative TGRLPL receptor proteins MBP 200, 235. In the representative experiment shown in Figure 1, THP-1 monocyte extracts were electrophoresed and transferred to nitrocellulose, blocked, and incubated with biotinylated VLDL in the absence (lane 1) and presence of 2 levels of a monospecific rabbit anti-apoB IgG (40 µg/mL, lane 2; 400 µg/mL, lane 3), or the corresponding preimmune IgG (90 µg/mL, lane 4; 400 µg/mL, lane 5). Binding of biotinylated HTG-VLDL S, 100 to 400 was visualized with streptavidin-
linked alkaline phosphatase and the image digitized (Figure 1a) and quantified by scanning densitometry (Figure 1b). The lower level (40 μg/mL, lane 2) of anti-apoB IgG blocked ~50% of binding, and the higher level (400 μg/mL, lane 3) blocked all visibly detectable (~80% by densitometry) HTG-VLDL binding to MBP 200, 235. In contrast, the preimmune IgG at 90 μg/mL blocked none of the binding (lane 4) and at 400 μg/mL blocked ~25% (lane 5). In a separate ligand-blotting experiment, this apoB antibody did not inhibit the binding of the same biotinylated VLDL to the bovine LDL receptor (data not shown). The experiment shown in Figure 1 is representative of 12 separate experiments with 4 different anti-apoB antibodies; in all cases, anti-apoB antibodies specifically inhibited binding of HTG-VLDL, chylomicrons, or tryp-VLDL to MBP 200, 235. These experiments suggest that apoB is involved in the binding of HTG-VLDL to the MBPs. These results are consistent with studies demonstrating specific, high-affinity binding of tryg-VLDL and implicated apoB in the binding of TGRLP to MBP 200, 235. Alternatively, the anti-apoB antibodies could sterically hinder another component that is the ligand for MBP 200, 235.

Antibodies Against Other Apoproteins of HTG-VLDL Fail to Inhibit Its Binding to MBP 200, 235

ApoB is only ~30% of the total protein mass in HTG-VLDL S, 100 to 400; apoE is 6% to 8%, and apoCs are ~63%.[12,13,34] On a molar basis, HTG-VLDL S, 100 to 400 contains 1 mol apoB, approximately 3 to 6 mol apoE, and ≥150 mol apoCs (primarily apoCIII) per mole VLDL. To directly determine whether any of these other apoproteins are the ligand, sterically hindered by the anti-apoB antibodies in the experiments represented by Figure 1, or whether they contribute to the binding of HTG-VLDL to MBP 200 and 235, we did a series of competitive ligand-blotting experiments with polyclonal antibodies against the other major apoproteins of HTG-VLDL. All antibodies used recognized their antigens in native VLDL and the anti-apoCII antibody is used to isolate apoCIII-rich LDLs (Dr Ronald Krauss, personal communication, April 1996). One experiment is shown in Figure 2. In this ligand blot, as in many but not all, MBP 200, 235 activities (either or both) appear as a complex of 2 or more bands due to the existence of several permissible oxidation states and/or disulfide isomers, as previously published.[22] In the experiment shown in Figure 2A, biotinylated HTG-VLDL S, 100 to 400 incubated with buffer (lane 1) or with nonimmune IgG (lane 3) binds to MBP 200 and 235 to similar extents. Neither anti-apoE (lane 4) nor anti-apoCIII (lane 5) diminishes the binding of HTG-VLDL to MBP 200, 235 but anti-apoB again effectively blocks ~90% of the binding of HTG-VLDL (lane 2 and Figure 2B). The finding that the anti-CIII antibody failed to block binding of HTG-VLDL to MBP 200, 235, even though the total apoCIII mass is ~2 times the mass of apoB in HTG-VLDL S, 100 to 400, argues against the alternative explanation offered above that the anti-apoB antibodies blocked by sterically hindering another apoprotein’s interaction with the MBPs. In other ligand-blotting experiments, we determined that the concentration of anti-apoE IgG used here blocked all binding of HTG-VLDL to the LDL receptor. Additional competitive ligand-blotting studies with anti-apoCII IgGs demonstrate that these antibodies do not inhibit binding (data not shown). Taken together, the competitive ligand-blotting studies strongly suggest that apoB, but not apoE, apoCIII, or apoCII, mediates the binding of HTG-VLDL to MBP 200, 235.

Anti-ApoB Antibodies Inhibit the Binding of TGRLP to the TGRLP Receptor of THP-1 Monocytes, but Not to the LDL Receptor of Fibroblasts

To confirm that apoB mediates the binding of TGRLP to the LpL- and apoE-independent TGRLP cellular receptor, competitive cell-binding studies with THP-1 monocyte-macrophages were conducted under experimental conditions that minimize the expression of the LDL receptor, the LRP, LpL, and apoE (1 day after adherence was induced by phorbol myristate, 13-acetate) as previously described.[22] As a control, competitive binding studies were also done simultaneously with cultured human skin fibroblasts with upregulated LDL receptors, since HTG-VLDL S, 100 to 400 binds to the LDL receptor via apoE and not via apoB.[12–15] Consistent with the ligand-blotting studies (Figure 1) and shown in Figure 3A, the high-affinity, specific binding of [125I]–HTG-VLDL to THP-1 cells was inhibited by antibodies to apoB, but not by the equivalent level of nonimmune IgGs. In contrast, and indicating the specificity of the blocking experiments in THP-1 cells, the same anti-apoB antibody did not inhibit the LDL receptor-specific binding of [125I]–HTG-VLDL to the fibroblasts (Figure 3B), consistent with previously published studies.[12–15] This representative experiment
shows that the inhibition of $^{125}$I-HTG-VLDL binding to THP-1 by anti-apoB antibodies was not significantly different from the inhibition by homologous, unlabeled HTG-VLDL (self). This finding indicates that apoB is the component of TGRLP responsible for its high-affinity, specific binding to THP-1 cells when the LDL receptor, LRP, LpL, and apoE are suppressed.

Effects of Lactoferrin, Heparin, and LpL on Binding of HTG-VLDL to THP-1 Monocyte-Macrophages

A series of competitive ligand-blotting studies were carried out to further distinguish MBP 200 and 235 from receptors of the LDL receptor family and to further delineate the binding domains in apoB for this distinct receptor. As shown in Figure 4, neither lactoferrin nor heparin is an effective inhibitor of the binding of HTG-VLDL to MBP 200 and 235. In this representative experiment, nitrocellulose strips containing MBP 200, 235 were incubated with 0.5 μg biotinylated HTG-VLDL per milliliter in the absence (lane 1) or presence of lactoferrin at 50 μg protein per milliliter (lane 2) or 500 μg protein per milliliter (lane 3); heparin at 10 U/mL (lane 4) and 100 U/mL (lane 5); or unlabeled HTG-VLDL at 25 μg/mL (lane 6) or 5 μg/mL (lane 7). Biotinylated HTG-VLDL binding was detected with streptavidin-linked alkaline phosphatase (digitized image, A) and quantified by densitometry (B). Lacto indicates lactoferrin; Hep, heparin.

Figure 4. Effects of lactoferrin and heparin on binding of HTG-VLDL to MBP 200, 235. THP-1 monocyte aqueous-phase extracts were electrophoresed and transferred to nitrocellulose. The nitrocellulose strips were incubated for 4 hours at 4°C with 0.5 μg biotinylated HTG-VLDL per milliliter in the absence (lane 1) or presence of lactoferrin at 50 μg protein per milliliter (lane 2) or 500 μg protein per milliliter (lane 3); heparin at 10 U/mL (lane 4) and 100 U/mL (lane 5); or unlabeled HTG-VLDL at 25 μg/mL (lane 6) or 5 μg/mL (lane 7). Biotinylated HTG-VLDL binding was detected with streptavidin-linked alkaline phosphatase (digitized image, A) and quantified by densitometry (B). Lacto indicates lactoferrin; Hep, heparin.
receptors, competitive ligand-binding studies were carried out with levels of LpL reported to enhance binding of lipoproteins to LDL receptor family members or to HSPG on cells. In the representative experiment shown in Figure 5, THP-1 monocyte extracts were electrophoresed and transferred to nitrocellulose, blocked, and incubated at 4°C with biotinylated HTG-VLDL, 100 to 400, (3 μg of protein per milliliter) in the absence (lane 1) or presence of LpL (0.2 μg/mL, lane 2; 2.0 μg/mL, lane 3; 20 μg/mL, lane 4) or in the presence of bovine serum albumin (0.2 μg/mL, lane 5; 2.0 μg/mL, lane 6; 20 μg/mL, lane 7). Binding was detected by incubation with streptavidin/alkaline phosphatase and the image was digitized (A) and quantified by densitometry (B).

Figure 5. Inhibition by LpL of binding of HTG-VLDL to MBP 200, 235. THP-1 monocyte aqueous-phase extracts were electrophoresed and transferred to nitrocellulose, blocked, and incubated at 4°C with biotinylated HTG-VLDL, 100 to 400, (3 μg of protein per milliliter) in the absence (lane 1) or presence of LpL (0.2 μg/mL, lane 2; 2.0 μg/mL, lane 3; 20 μg/mL, lane 4) or in the presence of bovine serum albumin (0.2 μg/mL, lane 5; 2.0 μg/mL, lane 6; 20 μg/mL, lane 7). Binding was detected by incubation with streptavidin/alkaline phosphatase and the image was digitized (A) and quantified by densitometry (B).

The specific inhibition of HTG-VLDL and tryp-VLDL binding to cells and to MBP 200 and 235 on ligand blots implicates the N-terminal domain of apoB. Previous studies showed that HTG-VLDL, but not normal VLDL, binds with high affinity to cells, causes lipid accumulation, and binds to MBP 200 and 235. HTG-VLDL subfractions from subjects with elevated plasma triglyceride (>150 mg/dL) contain more apoB-48 than normal VLDL subfractions from subjects with normal plasma triglycerides (<150 mg/dL) after purification by cumulative flotation because of delayed chylomicron remnant clearance. Taken together, these results suggest that apoB-48 may be a preferred ligand, or at least contain a preferred conformational domain of apoB that enhances binding to this receptor. Thus, we studied chylomicron subfractions isolated 4 hours after a standardized fat load. Chylomicrons, ie, TGRPLP of Sf 1100 to 3200, were purified further by cumulative flotation into more homogeneous subfractions of Sf 1100 to 3200 (CM I), Sf 1100 to 3200 (CM II), and Sf 400 to 1100 (CM III). The largest 2 chylomicron fractions (CM I and II) contained apoB-48 as the only detectable apoB species (Figure 6, lanes 1 to 4), whereas the smallest fraction (CM III; lanes 5 and 6) contained both apoB-48 and apoB-100, as determined by immunoblotting.

Table 1. Competition of Specific TGRLP Binding to THP-1 Monocyte-Macrophages

<table>
<thead>
<tr>
<th>Additions</th>
<th>125I-Tryp-VLDL</th>
<th>125I-HTG-VLDL</th>
<th>125I-Tryp-VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (control)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>4*</td>
<td>5*</td>
<td>ND</td>
</tr>
<tr>
<td>Heparin (10 mg/mL)</td>
<td>14</td>
<td>18</td>
<td>ND</td>
</tr>
<tr>
<td>Unlabeled VLDL (20x)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*100 μg/mL (experiment 1) and 500 μg/mL (experiment 2). †1.6 μg/mL (experiment 1) and 1.0 μg/mL (experiment 3).
determined by immunochemical blotting (Figure 6), which allowed us to estimate that <0.1%, or <1 in 1000 particles, contains apoB-100 in the Sₜ 1100 subfractions. Lane 7 contains a typical fasting HTG-VLDL Sₜ 100 to 400 with apoB-48, as well as apoB-100 and apoE. All chylomicron subfractions contained immunochemically detectable apoE (Figure 6), as well as apoCs (not shown).

The 3 chylomicron subfractions were then tested for binding to MBP 200, 235 and to the partially purified bovine LDL receptor by ligand-blotting analysis; a representative experiment is shown in Figure 7. All of the chylomicron subfractions, added at equivalent concentrations, bound with high affinity to MBP 200, 235 (lanes 1, 3, and 5), as well as to the LDL receptor (lanes 2, 4, and 6). Since apoB-48 is the only apoB species immunochemically detectable in the largest 2 chylomicron subfractions (CM I and CM II; Figure 6), apoB-48 or an apoB-48 domain is strongly implicated as the primary apoprotein binding determinant in postprandial TGRLP for the distinct human apoE- and LpL-independent monocyte-macrophage receptor for TGRLP and to the cell-surface receptor candidate proteins MBP 200 and 235. 20–22

Discussion
Our earlier reported studies with tryp-VLDL, in which apoE and apoCs were removed from TGRLP by trypsinolysis and refloation, leaving apoB fragments as the only apparent apoproteins associated with the particle, suggested apoB mediates TGRLP binding to the apoE- and LpL-independent monocyte-macrophage receptor for TGRLP and to the cell-surface receptor candidate proteins MBP 200 and 235. 20–22 The tryp-VLDL bound with high affinity and specificity to this macrophage receptor and MBP 200, 235 and failed to bind to the LDL receptor. 20,21

Herein we present several additional lines of evidence that apoB is the ligand for this receptor. First, anti-apoB antibodies specifically inhibit the high-affinity, specific binding of TGRLP Sₜ 1100 both to this receptor on THP-1 cells and to the MBP on ligand blots. In contrast, the same anti-apoB antibodies failed to
inhibit the binding of HTG-VLDL $S_r$ 100 to 400 to the LDL receptor, which is known to be mediated by apoE,\textsuperscript{12–15,30} indicating that the blocking in THP-1 was a specific effect on apoB and not due to nonspecific effects on other TGRLP apoproteins. Second, antibodies that bind to the other major apoproteins that make up \approx 70% of the total protein mass of native TGRLP $S_r$ 100 to 400 (apoE, apoCII, and apoCIII) do not inhibit binding, further emphasizing the specificity of the anti-apoB inhibition. Because anti-apoB IgG alone is as effective as homologous unlabeled TGRLP in competition for this macrophage receptor (ie, anti-apoB IgG can block essentially all of the high-affinity, specific binding of TGRLP $S_r$ \textgreater 100 to this receptor, but not to the LDL receptor), apoB appears to be the essential TGRLP component directly involved in binding to this receptor. Third, LpL, which binds to an N-terminal domain of apoB,\textsuperscript{42} also inhibits the binding of TGRLP both to the cellular site and to MBP 200, 235. Inhibition by LpL is highly specific and effective, occurring at low levels (<2 \mu g/mL) and in a concentration-dependent manner. The mechanism by which LpL inhibits the TGRLP-receptor interaction appears to result from its binding to the N-terminal portion of apoB,\textsuperscript{42} since LpL preincubated with the receptor on nitrocellulose does not inhibit the binding of TGRLP subsequently added. In contrast to the effective inhibition of TGRLP binding by LpL, neither lactoferrin nor heparin, used at much higher concentrations, inhibited binding, emphasizing the specificity of inhibition by LpL and the differences of this binding site’s characteristics from those of the LDL receptor family. Fourth, plasma chylomicron subfractions that contain apoB-48 as the only apoB species ($S_r$ \textgreater 3200 and $S_r$ 1100 to 3200 isolated 4 hours postprandially) bind to MBP 200, 235, and this binding is also specifically inhibited by anti-apoB IgG. Together, these results implicate an apoB-48 domain (or an equivalent in apoB-100) as the receptor-binding determinant in TGRLP for its specific, high-affinity binding to MBP 200, 235 and to the cellular apoE- and LpL-independent monocyte-macrophage TGRLP receptor. This TGRLP/apoB 48/100 receptor-binding domain that is present in plasma chylomicrons, HTG-VLDL, and tryg-VLDL apparently is relatively inaccessible in normal VLDL and LDL, which do not exhibit high affinity for the cellular TGRLP receptor or for MBP 200, 235, as previously demonstrated by direct and competitive binding studies both in cells and on ligand blots.\textsuperscript{20,21} Our previous studies demonstrated, however, that trypsinization followed by reflation of normal VLDL $S_r$ \textgreater 60 exposes the receptor-binding domain, since trypsinized normal VLDL $S_r$ 100 to 400, but not native normal VLDL, binds with high affinity to the MBPs on ligand blots.\textsuperscript{20} In contrast, trypsinization of LDL does not induce high-affinity binding to this site on macrophages or to MBP 200, 235 on blots (data not shown), and it has little to no effect on its binding to the LDL receptor in cells or on ligand blots, as previously document- ed.\textsuperscript{12,13,27} These data parallel, but in the reverse sense, a characteristic of apoB that we and others have demonstrated in the past that the exposure of metabolically important domains of apoB are determined in part by the size of the lipoprotein particle in which they are found. For example, the binding domain in apoB in LDL, IDL, and VLDL $S_r$ 20 to 60 for the LDL receptor is not accessible in TGRLP $S_r$ \textgreater 60.\textsuperscript{12–15}

The studies reported here also add to the list of properties that distinguish this TGRLP/apoB receptor from other lipoprotein receptor families. ApoB-mediated binding to the LDL receptor\textsuperscript{22} or LpL-mediated binding to members of the LDL receptor family or to cell-surface HSPGs can be inhibited by heparin.\textsuperscript{44} In contrast, heparin has little effect on the binding of TGRLP to this distinct cellular receptor or the MBPs (present study) or on macrophage lipid accumulation under conditions in which this receptor, but not the LDL receptor, is fully expressed.\textsuperscript{44} Lactoferrin, which inhibits the uptake of chylomicron remnants by the liver,\textsuperscript{40} also fails to inhibit the interaction of TGRLP with THP-1 monocyte-macrophages and with MBP 200, 235, indicating they are not related to putative hepatic remnant receptors. The inhibition by LpL of TGRLP binding both (1) to THP-1 cells grown under conditions in which this receptor, but not the LDL receptor or LRP, is expressed and (2) to MBP 200, 235 also distinguishes this receptor from members of the LDL receptor family, because LpL can augment binding of lipoproteins to these receptors.\textsuperscript{41,43} These competitive binding studies indicate that the receptor-binding domain in TGRLP is within apoB-48 (or a corresponding domain in apoB-100) at or near the LpL-binding domain and not in a heparin-binding domain.

In addition, the specific and parallel inhibition of TGRLP binding to the cellular site and to MBP 200, 235 by anti-apoB and LpL provides further evidence that corroborates the previously published data supporting their role as the high-affinity monocyte-macrophage LpL- and apoE-independent TGRLP receptors.\textsuperscript{21,22} Finally, since chylomicrons that contain apoB-48 as the only apoB species bind to this receptor and this binding is inhibited by anti-apoB antibodies, we conclude that an apoB-48 domain is sufficient to mediate the binding of TGRLP to the receptor and suggest that this receptor functions as a TGRLP/apoB-48 monocyte-macrophage receptor.

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