Low Density Lipoprotein Receptor-related Protein Mediates Endocytosis of Monoclonal Antibodies in Cultured Cells and Rabbit Liver

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Monoclonal antibodies that bound to the external domain of the rabbit low density lipoprotein receptor-related protein (LRP) were taken into rabbit fibroblasts by receptor-mediated endocytosis. Uptake occurred in fibroblasts from Watanabe-heritable hyperlipidemic rabbits, which lack low density lipoprotein receptors, as well as in normal rabbit fibroblasts. The fate of the internalized antibodies differed, depending on the domain of LRP that was recognized. LRP is synthesized as a single polypeptide chain that is cleaved to form a heterodimer of two noncovalently bound proteins, 1) a 515-kDa subunit that contains the binding domain, and 2) an 85-kDa subunit that contains the membrane-spanning region and cytoplasmic tail. A monoclonal antibody directed against the 515-kDa subunit (anti-LRP 515) rapidly dissociated from LRP at pH 5.2. After uptake by cells this antibody dissociated from the receptor and was degraded in lysosomes. A second antibody directed against the external portion of the 85-kDa subunit (anti-LRP 85) failed to dissociate at acid pH. After uptake by cells this antibody was not degraded, but instead was released from the cells in an acid-precipitable form. When administered intravenously to rabbits, both 125I-labeled antibodies were rapidly cleared from the circulation, 75-95% of the uptake occurring in the liver. The anti-LRP 515 antibody was degraded and acid-soluble products appeared in the plasma. No significant acid-soluble products appeared when the anti-LRP-85 antibody was infused. We conclude that LRP can carry receptor-mediated endocytosis and that its ligand-binding domain, like the similar domain of the low density lipoprotein receptor, undergoes an acid-dependent conformational change that ejects ligands within the endosome. We also conclude that in the body this endocytotic function is expressed primarily in the liver. Both of these conclusions lend support to the hypothesis that LRP may function in humans and animals as a receptor for apolipoprotein E-enriched lipoproteins, such as chylomicron remnants.

The low density lipoprotein receptor-related protein

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1 The abbreviations used are: LRP, LDL receptor-related protein; apo, apoprotein; β-VLDL, β-migrating very low density lipoproteins; DMEM, Dulbecco’s modified Eagle medium; LDL, low density lipoprotein; SDS, sodium dodecyl sulfate; WHHL rabbit, Watanabe-heritable hyperlipidemic rabbit; Hepes, 4-(2-hydroxyethyl)-1-piperazinediethanesulfonic acid.

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of LRP contains two copies of the sequence Asn-Pro-X-Tyr (1). In the LDL receptor, a single copy of this sequence mediates entry into coated pits, a prerequisite to efficient endocytosis (10). ApoE-dependent binding of β-VLDL to LRP has been demonstrated on nitrocellulose blots (5). This binding is abolished by incubating the β-VLDL with C apoproteins, and this correlates with the ability of apoS to prevent the β-VLDL stimulation of cholesteryl[^14C]oleate synthesis in cultured cells (6).

The above data are consistent with a role for LRP in receptor-mediated endocytosis, but this phenomenon has not yet been demonstrated directly in cultured cells. Studies with 125I-labeled apoE-enriched β-VLDL have not demonstrated conclusive LRP-dependent uptake or degradation of the protein component of the lipoprotein. This has been attributed to two problems, 1) the relatively low ratio of protein/cholesteryl ester in the β-VLDL, leading to a low expected uptake and degradation of 125I radioactivity, and 2) a relatively large amount of nonspecific binding to the cell surface and the culture dish when 125I-β-VLDL is enriched with apoE, which creates a high LRP-independent blank in the assays (2).

A second gap in our knowledge of LRP relates to its postulated function in the liver. Hepatic clearance of chylomicron remnants and artificial lipid emulsions is increased by supplementation with apoE (11–13), a property that matches the requirement for apoE in binding to LRP. However, whereas the majority of remnants are cleared by the liver (7, 14), LRP is expressed in relatively large amounts in a wide variety of organisms (1). Is it possible that the endocytosis-mediating function of LRP is expressed more efficiently in the liver than in other tissues? Finally, LRP is found in large amounts in endosomes from rat liver (3), implying that the protein may have an intracellular transport role as well as a role in endocytosis.

One way to circumvent the technical problems associated with measurements of endocytosis of β-VLDL is to use a monoclonal antibody to tag the receptor during endocytosis. This approach has been exploited previously with the LDL receptor. Monoclonal antibodies that bind to the ligand-binding domain of the LDL receptor (IgG-C7 or IgG-10C8) enter the cells with the receptor in coated pits, dissociate in endosomes, and are degraded, allowing the receptor to recycle (15–17). The release of such antibodies in endosomes is mediated by a fall in pH, the same reaction that triggers the release of 125I-LDL from the ligand-binding domain (16, 17). We have interpreted these data to indicate that the ligand-binding domain of the LDL receptor undergoes an acid-dependent conformational change that releases ligands and antibody in the acidic endosome, allowing the receptor to return unoccupied to the cell surface.

In the current experiments we have attempted to confirm that LRP functions as an endocytosis-mediating receptor by studying the binding, internalization, and degradation of monoclonal antibodies directed against various domains of LRP. The results support the hypothesis that LRP can mediate endocytosis in cultured fibroblasts. Clearance experiments with these antibodies in rabbits suggest that in the body LRP also functions as an endocytic receptor and that this function is expressed predominantly in the liver.

**EXPERIMENTAL PROCEDURES**

**Materials**—LRP was purified from rabbit liver by sequential DEAE-cellulose and immunoaffinity chromatography as previously described for rat LRP (2). The antibody used for the immunoaffinity column was IgG-11H4, a mouse monoclonal antibody directed against a synthetic peptide corresponding to the 13 COOH-terminal amino acids of human LRP (2). Trypsin (0.05%), EDTA (0.5 mM) solution was obtained from Gibco (Catalog No. 619-5800). Human protein-deficient serum (d > 1.215 g/ml) was prepared as previously described (18). Monoclonal antibodies and affinity-purified rabbit IgG directed against mouse IgG (Organon Teknika Corp., Duluth, NC) were radiolabeled with 125I by the iodogen method (19).

**Cultured Cells**—Cultured fibroblasts from normal and WHHL rabbits were derived from explants of skin and grown in monolayer culture in a 5% CO2 incubator (20). On day 0, 2.5 x 10^6 cells were seeded into each Petri dish (60- x 15-mm) containing 3 ml of Dulbecco's modified Eagle medium (DMEM) supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% (v/v) fetal calf serum. On days 3 and 5, the cells were refed with identical medium. On day 6 or 7, the cells were used for experiments.

**Monoclonal Antibodies**—Monoclonal antibodies were prepared by fusion of Sp2/O-Ag14 mouse myeloma cells (21) with splenic B-lymphocytes derived from fibroblasts for 2 days. After three injections of 20 μg each of purified rabbit LRP, hybridoma culture supernatants were screened using a solid-phase enzyme-linked immunosorbant assay procedure. Two positive hybridomas, IgG-5D7 (directed against LRP 515 and designated anti-LRP 515) and IgG-1B3 (directed against LRP 85 and designated anti-LRP 85), were obtained. Both monoclonals belong to IgG subclass 1. The hybridoma cells were subcloned three times by limiting dilution and then injected into the peritoneal cavity of athymic nude mice (22) and LRP was purified from the ascites fluid by affinity chromatography on Protein G-Sepharose 4 Fast Flow columns (Pharmacia LKB Biotechnology Inc.). Mouse monoclonal antibody IgG-11H4 (directed against the COOH-terminal 13 amino acids of human LRP and designated anti-COOH tail) (2), and mouse monoclonal antibody IgG-9D9 (directed against rabbit LDL receptor) (23) have been described previously.

**Assays of Binding, Uptake, and Degradation of 125I-Labeled Monoclonal Antibodies**—The total cellular binding of monoclonal 125I-IgG to fibroblast monolayers at 4°C and the total cellular uptake (surface + internalized) was determined exactly as described for 125I-LDL binding (18) except that the cells were washed at 4°C, four times rapidly in the standard albumin-containing buffer, then incubated for 4 min in albumin buffer, followed by two rapid washes in standard buffer without albumin. The proteolytic degradation of monoclonal 125I-IgG by fibroblast monolayers at 37°C was determined by measuring the amount of 125I-labeled trichloroacetic acid-soluble (noniodide) material formed by the cells and excreted into the culture medium (18).

**Intravenous Injection of Radiolabeled Monoclonal Antibodies and Measurement of Tissue Content of Radioactivity**—New Zealand White rabbits (male) were purchased from Myrtle Rabbitry (Thompson Station, TN). Animals were maintained on Teklad Rabbit Chow for at least 1 week before the experiment and were 1.9-2.9 kg of body weight. The rabbits were fasted for 12 h before the experiment. The injection of antibody was given as a bolus into the marginal ear vein. In some studies a single labeled antibody was injected, and in other studies two different antibodies (125I- and 131I-labeled) were injected together in 1 ml of NaCl/albumin for double-label analysis. Blood samples (1 ml) were collected from the central artery of the opposite ear at the indicated time into tubes containing EDTA and centrifuged. The plasma fractions were precipitated with 10–12% (v/v) trichloroacetic acid, and the resulting supernatants and precipitates were counted in a GammaTrac 1193 (TM Analytic) counter. In one experiment, 20 min after 125I-IgG injection the rabbits were injected intravenously with a lethal dose of pentobarbital and various organs were removed and weighed. The amount of 125I radioactivity was determined by counting either the entire organ (adrenals, spleen, and bile) or 1-2-g pieces of tissue (liver, kidneys, heart, bone marrow of femur, lungs, testes, and brain).

**RESULTS**

**Preparation of Monoclonal Antibodies against the Two Sub-units of LRP**—Fig. 1 shows the pathway for the proteolytic processing of LRP as recently defined (4). The protein is
synthesized as a single-chain molecule with an apparent molecular mass of approximately 600 kDa on SDS gels. After it is transported to the Golgi complex, LRP is proteolytically cleaved at the COOH-terminal side of a tetrasaccharide amino acid sequence. The cleavage produces a large fragment with an apparent molecular mass of 515 kDa that contains all of the ligand binding repeats but lacks a membrane-spanning region. The cleavage is blocked by incubation of cells with brefeldin A, which prevents transport to the cis-Golgi, or with monensin, which prevents transport from the cis- to the trans-Golgi (4). These findings suggest that the proteolytic clipping of LRP occurs in the cis-Golgi complex, the site where many secretory proteins undergo cleavage.

To prepare monoclonal antibodies against rabbit LRP, we purified the protein from rabbit liver by methods previously described (2) and inoculated it into mice. We isolated two hybridoma cell lines that produced monoclonal IgG antibodies against rabbit LRP (Fig. 2). One antibody, designated anti-LRP 515, reacted specifically with the 515-kDa subunit of rabbit liver LRP on nitrocellulose blots (lanes 1 and 3). The second antibody, designated anti-LRP 85, reacted only with the 85-kDa subunit of rabbit liver LRP and not with the 515-kDa subunit (lane 2). The anti-LRP 515 antibody reacted weakly with the rat 85-kDa LRP fragment (lane 4), but the anti-LRP 515 antibody showed no cross-reactivity with this species (lane 4). A previously described monoclonal antibody directed against a peptide corresponding to the extreme COOH terminus of human LRP (2) reacted strongly with the 85-kDa fragment of rabbit and rat LRP (lanes 5 and 6). None of these antibodies cross-reacted with the LDL receptor, which was present on these nitrocellulose blots.

Binding, Uptake, and Degradation of Anti-LRP Antibodies in Cultured Cells—To study the binding and metabolism of these antibodies in cultured cells, we used fibroblasts derived from WHHL rabbits, which are homozygous for a defect in low-density lipoprotein receptor activity on the cell surface (4). Equal amounts of the anti-LRP 85 antibody and the anti-LRP 515 antibody bound to the surface of the WHHL fibroblasts at 4 °C (Fig. 3), a finding consistent with the previous demonstration that the 515- and 85-kDa subunits are present in equimolar amounts on the cell surface (4). These data also demonstrate that the epitope for the anti-LRP 85 antibody is external to the plasma membrane. As expected, the anti-COOH-terminal tail antibody did not bind to LRP (Fig. 3), presumably because the epitope is inside the cell.

At 37 °C both antibodies bound to the WHHL fibroblasts with high affinity and saturability (Fig. 4A). At this temperature the affinity for the anti-LRP 85 antibody was somewhat higher than that for the anti-LRP 515 antibody. The total binding at 37 °C for both antibodies was about 5-fold higher at 37 °C than at 4 °C (compare Figs. 3 and 4A), suggesting that at the higher temperature most of the receptor-bound material had entered the cell. There was negligible uptake of the anti-COOH tail antibody (Fig. 4A). Virtually identical results were obtained in normal rabbit fibroblasts that were

![Diagram of LRP processing](image-url)
to the cells at 37 °C, only the anti-LRP 515 was degraded more than the amount bound to the cells (compare Figs. 4 and 5A). After incubation at 37 °C for 5 h, cells were chilled to 4 °C, and the total cellular content of 125I-IgG (panel A) and the amount of 125I-IgG degradation products excreted into the medium (panel B) were determined. The specific radioactivity of the 125I-labeled monoclonal antibodies was 313-482 cpm/ng. Each value represents a single incubation.

![Image](http://www.jbc.org/)  
**FIG. 4.** Saturation curves for total cellular uptake and degradation of 125I-anti-LRP monoclonal antibodies by WHHL rabbit fibroblasts. On day 7 of cell growth, monolayers of cells received 2 ml of DMEM (without glutamine) containing 5% human lipoprotein-deficient serum and the indicated concentration of the indicated 125I-anti-LRP monoclonal IgG. After incubation at 37 °C for 24 h, cells were washed and treated with trypsin to release surface-bound anti-LRP 85 IgG (panel A) or unlabeled anti-LRP 515 IgG (panel B). After incubation for the indicated time at 37 °C, the medium was removed and saved, and each monolayer was washed with 2.5 ml of either unlabeled anti-LRP 515 IgG (panel A) or unlabeled anti-LRP 515 IgG (panel B). After incubation at 37 °C for 1 h, the cell monolayers were washed at 4 °C as described under “Experimental Procedures.” Each washed monolayer then received 2 ml of chase-medium containing prewarmed (37 °C) DMEM, 2 mg/ml of bovine serum albumin, and 10 μg/ml of either unlabeled anti-LRP 85 IgG (panel A) or unlabeled anti-LRP 515 IgG (panel B). After incubation at 37 °C for 1 h, the cell monolayers were washed at 4 °C as described under “Experimental Procedures.” Each washed monolayer then received 2 ml of chase-medium containing prewarmed (37 °C) DMEM, 2 mg/ml of bovine serum albumin, and 10 μg/ml of either unlabeled anti-LRP 85 IgG (panel A) or unlabeled anti-LRP 515 IgG (panel B). After incubation at 37 °C for 1 h, the cell monolayers were washed at 4 °C as described under “Experimental Procedures.” Each washed monolayer then received 2 ml of chase-medium containing prewarmed (37 °C) DMEM, 2 mg/ml of bovine serum albumin, and 10 μg/ml of either unlabeled anti-LRP 85 IgG (panel A) or unlabeled anti-LRP 515 IgG (panel B). After incubation for the indicated time at 37 °C, the medium was removed and saved, and each monolayer was washed with 2.5 ml of ice-cold phosphate-buffered saline. The original medium and the phosphate buffered saline wash were combined, trichloroacetic acid was added to final concentration of 10% (v/v), and the resulting pellet (A) and an aliquot of the soluble fraction (B) were counted. The cells were dissolved in 0.2 N NaOH, and an aliquot were counted (O). The zero time values were obtained 10 s after the chase-medium was added. The specific radioactivity of the 125I-labeled monoclonal antibodies was 427 cpm/ng (panel A) and 489 cpm/ng (panel B). Each value is the mean of triplicate determinations.
occupied to the cell surface. To determine whether the anti-LRP 515 antibody or the anti-LRP 85 antibody could be dissociated at acid pH, we incubated the \(^{125}\)I-labeled antibodies with WHHL fibroblasts at 4°C, then washed the cells, and eluted the antibodies at either pH 7.5 (Fig. 7A) or pH 5.2 (Fig. 7B). At pH 7.5 only a small amount of either antibody was eluted. When the pH was lowered to 5.2, a large amount of the anti-LRP 515 was eluted, but there was no increased release of anti-LRP 85.

The data of Figs. 5–7 suggest that the anti-LRP 515 antibody binds and is taken up by the cells, dissociates in the acidic endosome, and is delivered to lysosomes for degradation. The LRP recycles to the cell surface and binds a new molecule of anti-LRP 515. The anti-LRP 85 antibody also binds and is taken up, but it fails to dissociate in the endosome, and thereby recycles to the cell surface while bound to the receptor. Some of the anti-LRP antibody dissociates intact from the receptor at the cell surface, and this is presumably replaced by new molecules of \(^{125}\)I-anti-LRP 85 to maintain the steady state.

To rule out the possibility that the anti-LRP 85 antibody influences the routing of the LRP within the cell, we performed an assay to determine whether unlabeled anti-LRP 85 would influence the behavior of \(^{125}\)I-labeled anti-LRP 515 (Table I). WHHL fibroblasts were incubated with \(^{125}\)I-anti-LRP 515 (5 \(\mu\)g/ml) either alone or with an excess (50 \(\mu\)g/ml) of an unlabeled monoclonal IgG. As expected, the inclusion of the control anti-COOH-terminal antibody had no effect on the uptake or degradation of the anti-LRP 515 antibody. Unlabeled anti-LRP 515 antibody competitively inhibited the uptake of the \(^{125}\)I-anti-LRP 515 as expected. The anti-LRP

![Fig. 7. Effect of pH on the release of \(^{125}\)I-anti-LRP monoclonal antibodies from the surface of WHHL rabbit fibroblasts. On day 7 of cell culture, each monolayer received 2 ml of ice-cold medium A containing 5% human lipoprotein-deficient serum and 2 \(\mu\)g/ml of either \(^{125}\)I-anti-LRP 515 IgG (357 cpm/ng) (C) or \(^{125}\)I-anti-LRP 85 IgG (497 cpm/ng) (O). After incubation at 4°C for 3 h, cells were washed at 4°C rapidly four times with the standard albumin-containing wash buffer (15), then incubated twice for 4 min in the same buffer, followed by two rapid washes in 50 mM Tris-maleate at pH 7.5. The washed monolayers were then incubated at 4°C with ice-cold buffer containing 50 mM Tris-maleate, 50 mM sodium chloride, and 5% human lipoprotein-deficient serum at either pH 7.5 (panel A) or pH 5.2 (panel B). After incubation for the indicated time, the buffer was removed and saved, and each monolayer was washed with 2 ml of ice-cold phosphate-buffered saline. The original buffer and the phosphate-buffered saline wash were combined, and trichloroacetic acid was added to a final concentration of 9% (v/v). The cells were dissolved in 0.1 N NaOH, and an aliquot of the total cellular content of \(^{125}\)I-IgG and the amount of \(^{125}\)I-IgG degradation products excreted into the culture medium were determined. Each value is the mean of triplicate incubations.](http://www.jbc.org/)

![Fig. 8. Plasma clearance curves after intravenous injection of \(^{125}\)I-labeled monoclonal antibodies into normal rabbits. Each animal was injected with the indicated \(^{125}\)I-monoclonal antibody as described under "Experimental Procedures." Blood samples were drawn at the indicated time. Aliquots were precipitated with trichloroacetic acid and processed for gamma counting. The "100% of injected dose" value for plasma radioactivity represents the trichloroacetic acid-precipitable radioactivity in plasma 1 min after injection. Each value represents the mean and range of the data obtained from three animals. Where no bars are shown, the range of values was so small that it is encompassed by the size of the points.](http://www.jbc.org/)

### Table I

| Addition to medium | Metabolism of monoclonal \(^{125}\)I-IgG |
|--------------------|--------------------------------------|
|                    | \(^{125}\)I-Labeled monoclonal IgG | Unlabeled monoclonal IgG | Cell-bound \(^{125}\)I-IgG | Degraded \(^{125}\)I-IgG |
| 5 \(\mu\)g/ml       | 50 \(\mu\)g/ml                     | ng/\(\mu\)g protein      |                         |                         |
| Anti-COOH tail     | None                                | 19                        | 26                       |
| Anti-LRP 515       | None                                | 236                       | 1550                     |
| Anti-LRP 85        | Anti-COOH tail                      | 241                       | 1016                     |
| Anti-LRP 515       | Anti-LRP 85                         | 268                       | 1693                     |
| Anti-LRP 515       | Anti-LRP 515                        | 92                        | 419                      |

85 antibody had no effect on the uptake of the \(^{125}\)I-anti-LRP 515. In other experiments we showed that the anti-LRP 85 antibody bound to LRP even in the presence of the anti-LRP 515 antibody. These findings indicate that the failure of degradation of the anti-LRP 85 antibody is not attributable to the failure of the LRP to enter the degradative pathway, but rather it is presumably due to the failure of the anti-LRP 85 antibody to dissociate from the LRP in the endosome.

### In Vivo Clearance Studies with Anti-LRP Antibodies—Rabbits were injected with various \(^{125}\)I-labeled monoclonal antibodies, plasma was sampled at intervals, and the amount of trichloroacetic acid-precipitable radioactivity remaining in plasma was measured (Fig. 8). As a negative control for the experiments, we injected \(^{125}\)I-labeled anti-COOH terminal antibody (Fig. 8D). This antibody was cleared slowly with a 50% removal time greater than 6 h. As a positive control, we injected a monoclonal antibody against the LDL receptor that was previously shown to be removed rapidly, owing to LDL receptor-mediated uptake (20). In the current experiments this antibody was rapidly cleared from the circulation with 50% removal occurring at 40 min (Fig. 8C). The anti-LRP
515 antibody was cleared from the circulation more rapidly than the anti-LDL receptor antibody, 50% removal occurring at ~8 min (Fig. 8A). The clearance curve appeared to be biphasic, with a rapid phase within the first 15 min, and a phase of slower clearance occurring over the next 6 h. The anti-LRP 85 antibody was cleared in a somewhat different pattern. Approximately 90% of the antibody was rapidly cleared within the first 15 min, but the clearance rate thereafter was much slower than that of the anti-LRP 515 antibody (Fig. 8B).

If LRP were removing the two antibodies by receptor-mediated endocytosis in vivo as it does in cultured fibroblasts, then the anti-LRP 515 antibody, but not the anti-LRP 85 antibody, should be degraded to trichloroacetic acid-soluble products. To test this hypothesis, we injected either 125I-anti-LRP 85 or 131I-anti-LRP 515 intravenously into rabbits together with 125I-anti-LRP COOH tail, which served as a control for nonspecific events. As expected, the 125I-anti-LRP 515 and 85 antibodies were both removed from plasma much more rapidly than the 131I-labeled control antibody (Fig. 9, A and C). After a lag of 10 min, acid-soluble degradation products of the 125I-anti-LRP 515 antibody began to appear in the plasma (Fig. 9B), reaching a steady-state level at 40 min and persisting for as long as 180 min. In contrast, only trace amounts of acid-soluble material was formed from the 125I-anti-LRP 85 antibody (Fig. 9D).

To determine the tissues responsible for the rapid uptake of the 125I-anti-LRP antibodies, we injected rabbits with the 131I-labeled antibodies and studied them 20 min later when the first traces of acid-soluble radioactivity appeared in the plasma. Portions of various organs were removed and assayed for their content of 125I-radioactivity (Fig. 10). Results were expressed either as the content of 131I radioactivity/g of tissue (upper panels) or per total organ (lower panels). Low amounts of radioactivity from the control anti-COOH antibody accumulated in a variety of tissues, without evidence for selectivity (Fig. 10A). In contrast, the anti-LRP 85 and 515 antibodies showed specific uptake into the liver and spleen, reaching to levels that were much higher than those seen with the control antibody (Fig. 10, B and C). Significant uptake into the kidneys was observed with one animal injected with the anti-LRP 515 antibody, but this was not observed with the other two animals injected with this antibody, nor with the animals injected with the anti-LRP 515.

When the data were expressed on a per organ basis, the liver accounted for 75-95% of the total 125I anti-LRP 85 (Fig. 10E) and the total 125I-anti-LRP 515 that was cleared (Fig. 10F). This estimation is based on the total amount of radioactivity recovered in the 10 tissues examined. We did not observe significant uptake of either antibody into the portion of bone marrow (i.e., the femur) that was sampled.

**DISCUSSION**

In the current experiments we have used monoclonal antibodies as ligands to demonstrate that LRP can mediate binding, endocytosis, and degradation of a protein in cultured rabbit fibroblasts and in rabbit livers in vivo. We observed a striking difference between the metabolism of two different monoclonal antibodies. The antibody directed against the COOH-terminal tail IgG (0) (panels A and B) or with 15 pg of 125I-anti-LRP COOH-terminal tail IgG (O) (panels C and D) as described under "Experimental Procedures." Blood samples were drawn at the indicated time. Trichloroacetic acid-precipitable (panels A and C) and soluble radioactivity (panels B and D) were determined and plotted as follows. An aliquot of plasma (0.4 ml) was precipitated with trichloroacetic acid, and the soluble phase and the precipitate were counted separately. Radioactivity specific for 125I and 131I was determined by correction for spillover from the 125I channel (17% of 131I counts) into the 125I channel. In panels A and C, solid lines represent clearance of the labeled monoclonal from plasma normalized to the acid-precipitable radioactivity recovered from plasma at 1 min after injection (100% of control). Dotted lines represent clearance of monoclonals directed against LRP 515 (panel A) and LRP 85 (panel C) normalized to the clearance of anti-LRP COOH-terminal tail monoclonal. The 125I/131I ratio of acid-precipitable radioactivity of the injected material was set at 100%, and the ratios recovered at the indicated times were plotted accordingly. In panels B and D, acid-soluble radioactivity recovered from plasma is plotted as the percentage of plasma radioactivity at zero time. This zero time value was based on the 125I/131I ratio of the injected material and on the assumption that the clearance of the 125I-labeled control monoclonal antibody between 0 and 1 min is negligible. The measured value for % acid-soluble radioactivity at 1 min after injection was set as a blank value of zero and subtracted from the values for % acid-soluble radioactivity at the later time points. Each value represents the mean and range of the data obtained from three animals. Where no bars are shown, the range of values was so small that it is encompassed by the size of the points.  

**FIG. 9.** Plasma clearance curves after intravenous double-label injection of 125I- and 131I-labeled anti-LRP monoclonal antibodies into normal rabbits. Each animal was injected either with 15 pg of 125I-anti-LRP 515 IgG (0) plus 15 pg of 125I-anti-LRP COOH-terminal tail IgG (O) (panels A and B) or with 15 pg of 125I-anti-LRP 50 IgG (0) plus 10 pg of 125I-anti-LRP COOH-terminal tail IgG (O) (panels C and D) as described under "Experimental Procedures." Blood samples were drawn at the indicated time. Trichloroacetic acid-precipitable (panels A and C) and soluble radioactivity (panels B and D) were determined and plotted as follows. An aliquot of plasma (0.4 ml) was precipitated with trichloroacetic acid, and the soluble phase and the precipitate were counted separately. Radioactivity specific for 125I and 131I was determined by correction for spillover from the 125I channel (17% of 131I counts) into the 125I channel. In panels A and C, solid lines represent clearance of the labeled monoclonal from plasma normalized to the acid-precipitable radioactivity recovered from plasma at 1 min after injection (100% of control). Dotted lines represent clearance of monoclonals directed against LRP 515 (panel A) and LRP 85 (panel C) normalized to the clearance of anti-LRP COOH-terminal tail monoclonal. The 125I/131I ratio of acid-precipitable radioactivity of the injected material was set at 100%, and the ratios recovered at the indicated times were plotted accordingly. In panels B and D, acid-soluble radioactivity recovered from plasma is plotted as the percentage of plasma radioactivity at zero time. This zero time value was based on the 125I/131I ratio of the injected material and on the assumption that the clearance of the 125I-labeled control monoclonal antibody between 0 and 1 min is negligible. The measured value for % acid-soluble radioactivity at 1 min after injection was set as a blank value of zero and subtracted from the values for % acid-soluble radioactivity at the later time points. Each value represents the mean and range of the data obtained from three animals. Where no bars are shown, the range of values was so small that it is encompassed by the size of the points.

**FIG. 10.** Tissue distribution of 125I radioactivity following intravenous injection of 125I-labeled monoclonal antibodies into normal rabbits. Rabbits were injected with the indicated 125I-labeled monoclonal antibody as described under "Experimental Procedures." After 20 min, the animals were killed, organs were obtained, and their content of 125I radioactivity was determined as described under "Experimental Procedures." Panels A-C, uptake/g of tissue was determined by the following calculation: uptake/g of tissue = (cpm/g of organ)/(cpm/ml of plasma 1 min after injection of 125I-labeled antibody) x 100. The total mass of bone marrow was not determined; the values shown were obtained from one femur. Each bar represents the mean ± S.E. of data obtained from three rabbits. In panels B and E, the mean value for the kidneys may be falsely elevated, owing to one excessively high data point denoted by the asterisk (*).
515-kDa subunit of LRP, which contains the ligand binding repeats, behaved like monoclonal antibodies directed against the ligand-binding domain of the human LDL receptor. This antibody bound to the surface with high affinity at 4 ℃ (Fig. 3) and was internalized and degraded at 37 ℃ (Fig. 4). In the steady state at 37 ℃, the cellular content of antibody was approximately two times greater than the amount of surface binding as estimated by trypsin release (Fig. 5B). After a lag phase of ~1 h, the cells degraded an amount of antibody equal to the intracellular content each hour (Fig. 5B). When the cells were allowed to take up the antibody at 37 ℃ and then switched to medium lacking antibody, most of the intracellular material was degraded in 1 h (Fig. 6B), a finding that was consistent with the results of the steady state experiments (Fig. 5B). These rates are all similar to those that were previously observed with a monoclonal antibody against the ligand-binding domain of the LDL receptor (125I-IgG-C7) in human fibroblasts (15). These findings suggest that the anti-LRP 515 is continuously binding, being internalized, and dissociating from the receptor in the endosome, thereby allowing LRP to return empty to the cell surface.

A striking difference was observed in the behavior of the anti-LRP 85 antibody. At 4 ℃ this antibody bound to the surface in amounts that were stoichiometrically equal to the binding of the anti-LRP 515 antibody (Fig. 3). At 37 ℃ the intracellular (trypsin-resistant) radioactivity was 5–7-fold higher than the surface-bound radioactivity (Fig. 5A), indicating that the WHHL fibroblasts were internalizing this antibody at least as efficiently as they internalized the anti-LRP 515 antibody. However, the anti-LRP 85 antibody did not undergo degradation. Instead, as shown by the pulse-chase experiment (Fig. 6A), the anti-LRP 85 antibody was released intact from the cell. Thus, the steady-state cellular level of this antibody was apparently maintained by continuous binding, uptake, and recycling to the cell surface.

An explanation for these findings emerged when we compared the ability of the two antibodies to dissociate from LRP at acid pH. Both antibodies bound stably to the receptor at 4 ℃. When the pH was dropped to 5.2, the anti-LRP 515, but not the anti-LRP 85, rapidly dissociated from LRP. The properties of the anti-LRP 515 thus match the properties of IgG C7, the monoclonal antibody against the ligand binding domain of the LDL receptor (15).

Considered together, these data suggest that the ligand-binding domain of LRP functionally resembles that of the ligand-binding domain of the LDL receptor (15). This is consistent with the results of the steady state experiments (Fig. 5B). These rates are all similar to those that were previously observed with a monoclonal antibody against the ligand-binding domain of the LDL receptor (125I-IgG-C7) in human fibroblasts (15). These findings suggest that the anti-LRP 515 is continuously binding, being internalized, and dissociating from the receptor in the endosome, thereby allowing LRP to return empty to the cell surface.

A striking difference was observed in the behavior of the anti-LRP 85 antibody. At 4 ℃ this antibody bound to the surface in amounts that were stoichiometrically equal to the binding of the anti-LRP 515 antibody (Fig. 3). At 37 ℃ the intracellular (trypsin-resistant) radioactivity was 5–7-fold higher than the surface-bound radioactivity (Fig. 5A), indicating that the WHHL fibroblasts were internalizing this antibody at least as efficiently as they internalized the anti-LRP 515 antibody. However, the anti-LRP 85 antibody did not undergo degradation. Instead, as shown by the pulse-chase experiment (Fig. 6A), the anti-LRP 85 antibody was released intact from the cell. Thus, the steady-state cellular level of this antibody was apparently maintained by continuous binding, uptake, and recycling to the cell surface.

An explanation for these findings emerged when we compared the ability of the two antibodies to dissociate from LRP at acid pH. Both antibodies bound stably to the receptor at 4 ℃. When the pH was dropped to 5.2, the anti-LRP 515, but not the anti-LRP 85, rapidly dissociated from LRP. The properties of the anti-LRP 515 thus match the properties of IgG C7, the monoclonal antibody against the ligand binding domain of the LDL receptor (15).

Considered together, these data suggest that the ligand-binding domain of LRP functionally resembles that of the LDL receptor in that it can rapidly dissociate ligands at acid pH. This dissociation appears to be necessary in order for the antibody to separate from the receptor, presumably in an acidic compartment such as the endosome, thereby allowing the antibody to be carried forward to the lysosome and allowing the LRP to recycle to the surface. Inasmuch as the acid-dependent dissociation of the LDL receptor has been suggested to be caused by a conformational change occurring in the epidermal growth factor precursor homology region (17), the current data raise the possibility that the epidermal growth factor homology units in LRP also undergo such an acid-dependent conformational change, which may be necessary in order to permit the receptor to recycle.

The clearance experiments with the two monoclonal antibodies in the intact rabbits suggest that LRP in the liver behaves similarly to its behavior in cultured fibroblasts. Thus, both antibodies showed a rapid phase of plasma clearance that was attributable mainly to uptake into the liver (Fig. 10). The anti-LRP 515 antibody underwent rapid degradation, and the trichloroacetic acid-soluble products appeared in the plasma, but there was no detectable rapid degradation of the anti-LRP 85 antibody (Fig. 9).

The in vivo clearance data indicate that the vast bulk of the endocytotic function of LRP is performed in the liver even though the protein can be found in a variety of other tissues (1). We have not yet performed careful quantitative comparisons to determine whether this selective hepatic function is entirely explained by a higher number of LRP molecules in the liver. It may be that a higher fraction of receptors are located on the cell surface in the liver. Alternatively, the rapid hepatic clearance may be attributable to the high rate of penetration of the antibody through the fenestrated endothelium of the sinusoidal space, allowing rapid access to hepatocytes. This rate is expected to be much faster than the rate of penetration through the nonfenestrated capillaries that are found in most organs.

If lipoproteins such as apoE-enriched chylomicron remnants were to use LRP for clearance from plasma, then they should have an even greater selectivity for hepatic uptake than did the monoclonal antibodies. These huge lipoprotein particles are essentially restricted from all tissues except those with fenestrated endothelia (14). Moreover, when these remnant particles penetrate into the hepatic sinusoidal space, they are likely to be exposed to high concentrations of newly secreted apoE which would allow them to attach to LRP. We therefore believe that the current data add further support to the hypothesis that LRP may function as a receptor for apoE-enriched chylomicron remnants and other lipoproteins in the liver.

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