Interaction of Apolipoprotein J-Amyloid β-Peptide Complex with Low Density Lipoprotein Receptor-related Protein-2/Megalin

A MECHANISM TO PREVENT PATHOLOGICAL ACCUMULATION OF AMYLOID β-PEPTIDE*

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Samar M. Hammad, Sripriya Ranganathan, Elena Loukinova‡, Waleed O. Twal, and W. Scott Argraves§

From the Cell Biology and Anatomy Department, Medical University of South Carolina, Charleston, South Carolina 29425-2204

Apolipoprotein J (apoJ) has been shown to be the predominant amyloid β-peptide (Aβ)-binding protein in cerebrospinal fluid. We have previously demonstrated that the endocytic receptor low density lipoprotein receptor-related protein-2/megalin (LRP-2), which is expressed by choroid plexus epithelium and ependymal cells lining the brain ventricles and neural tube, binds and mediates cellular uptake of apoJ (Kounnas, M. Z., Loukinova, E. B., Stefansson, S., Harmony, J. A., Brewer, B., Strickland, D. K., and Argraves, W. S. (1995) J. Biol. Chem. 270, 13070–13075). In the present study, we evaluated the ability of apoJ to mediate binding of Aβ1–40-apoJ complex to LRP-2 in vitro. Immunoblot analysis showed that incubation of apoJ with Aβ1–40 resulted in the formation of Aβ1–40-apoJ complex and the inhibition of the formation of Aβ1–40 aggregates. Using an enzyme-linked immunosorbent assay, an estimated dissociation constant (Kd) of 4.8 nm was derived for the interaction between Aβ1–40 and apoJ. Enzyme-linked immunosorbent assay was also used to study the interaction of the Aβ1–40-apoJ complex with LRP-2. The results showed that Aβ alone did not bind directly to LRP-2; however, when Aβ1–40 was combined with apoJ to form a complex, binding to LRP-2 took place. The binding interaction could be blocked by inclusion of the receptor-associated protein, an antagonist of apoJ binding to LRP-2. When LRP-2-expressing cells were given 125I-Aβ1–40, cellular uptake of the radiolabeled peptide was promoted by co-incubation with apoJ. When the cells were provided purified 125I-Aβ1–40-apoJ complex, the complex was internalized and degraded, and both processes were inhibited with polyclonal LRP-2 antibodies. Furthermore, chloroquine treatment inhibited the cellular degradation of the complex. The data indicate that apoJ facilitates Aβ1–40 binding to LRP-2 and that the receptor mediates cellular clearance of Aβ1–40 complex leading to lysosomal degradation of Aβ1–40. The findings support the possibility that LRP-2 can act in vivo to mediate clearance of the complex from biological fluids such as cerebrospinal fluid and thereby play a role in the regulation of Aβ accumulation.

A hallmark feature of Alzheimer’s disease is the accelerated cerebral accumulation of amyloid β-protein (Aβ), a small 39–42-residue proteolytically derived fragment of amyloid β-precursor protein (1, 2). The accumulation takes the form of spherical extracellular deposits of Aβ fibrils in the vicinity of morphologically abnormal axons and dendrites. Associated with these so-called plaques are microglia and astrocytes. The mechanisms that lead to accumulation of Aβ are still obscure but represent an area of intense investigation. Whereas much emphasis is currently placed on trying to determine the mechanism(s) of Aβ biosynthesis from amyloid β-precursor protein processing (3, 4), little is being done on determining possible mechanisms that mediate the catabolism of Aβ. Catabolic processes may prevent the extracellular accumulation of Aβ that is expressed under normal physiological conditions yet does not accumulate to the extent seen in Alzheimer’s disease or Down’s syndrome.

Aβ can be found in cerebrospinal fluid and blood in complex with apolipoprotein J (apoJ) or apolipoprotein E (apoE) (5, 6). Whereas apoE has been reported to promote Aβ fibrillogenesis (7–9), apoJ has been shown to slow the formation of Aβ aggregates and may therefore act to maintain Aβ in a soluble form and prevent it from forming pathological fibrils (10). Our discoveries that LRP-2 is an endocytic receptor for apoJ (11) and LRP-2 is expressed by cells that are in contact with cerebrospinal fluid (choroid plexus and ependymal cells) (12) prompted us to hypothesize that LRP-2 may mediate clearance of Aβ complexed with apoJ, thereby controlling the accumulation of Aβ. In the present study, we used in vitro solid phase binding assays as well as cellular internalization and degradation assays to evaluate the roles of apoJ and LRP-2 in mediating cellular clearance of Aβ.

EXPERIMENTAL PROCEDURES

Proteins—Human apoJ was purchased from Quidel (San Diego, CA). Synthetic Aβ fragment 1–40 and ovalbumin were obtained from Sigma. Bovine serum albumin was purchased from U. S. Biochemical Corp. Human RAP was expressed as a glutathione S-transferase fusion protein in bacteria and prepared free of glutathione S-transferase as described by Williams et al. (13). LRP-2 was purified from extracts of human placenta by protein A-Sepharose chromatography.

‡ Present address: Head and Neck Surgery Branch, Tumor Biology Section, NIDCD, NIH, 10 Center Dr., Bldg. 10, Rm. 5D55, MSC1419, Bethesda, MD 20892-1419.
§ To whom correspondence should be addressed: Medical University of South Carolina, Cell Biology and Anatomy Dept., 171 Ashley Ave., Charleston, SC 29425-2204. Tel.: 803-792-5482; Fax: 803-792-0664; E-mail: argraves@musc.edu.

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1 The abbreviations used are: Aβ, amyloid β-peptide; apoJ, apolipoprotein J; clustein; LRP-2, low density lipoprotein receptor-related protein-2/megalin; RAP, receptor-associated protein; BSA, bovine serum albumin; TBS, Tris-buffered saline; mAb, monoclonal antibody; PBS, phosphate-buffered saline; ρDBS, Dulbecco’s PBS; PAGE, polyacrylamide gel electrophoresis; OG, N-octyl-β-D-glucopyranoside; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)methyl]glycine; RA, retinoic acid; Bt,cAMP, dibutyryl cyclic AMP; DMEM, Dulbecco’s modified Eagle’s medium; ELISA, enzyme-linked immunosorbent assays; SS, serum substitute.

2 LRP-2 is synonymous with glycoprotein 330 (gp330), brushin, and megalin.
porcine kidney by affinity chromatography using a column of RAP coupled to Sepharose as described previously (36).

**Antibodies**—The mouse monoclonal antibody to LRP-2 designated 1H2 was provided by Dr. Robert McCluskey (Massachusetts General Hospital, Boston, MA). Mouse monoclonal antibody to human apoJ (mAb 4G8) was obtained from Dr. Judith Harmony (University of Cincinnati College of Medicine, Cincinnati, OH). Mouse monoclonal antibody to human Aβ (mAb 4G8) was purchased from Senetek (Maryland Heights, MO). Rabbit anti-LRP-2 IgG (rabbit 6286) were isolated by immunofinity chromatography on a column of porcine LRP-2 coupled to CNBr-activated Sepharose (Pharmacia Biotech Inc.) with minor modification to a previously described procedure (11). IgG was sequentially eluted using 100 mM glycine, pH 2.3, followed by 100 mM triethylammonium, pH 11.5, and the combined eluates were dialyzed against 50 mM Tris, pH 7.4, 150 mM NaCl. The polyclonal anti-LRP-2 IgG preparation was absorbed on a column of RAP-Sepharose followed by selection on a column of protein G-Sepharose. Control rabbit IgG was isolated from the preimmune serum of rabbit 6286 by protein G-Sepharose chromatography.

**Formation of Aβ-ApoJ Complex**—ApoJ was combined with synthetic Aβ1-40 at a 1:15 molar ratio in PBS as described previously (14) and incubated for 24 h at 37°C. Typically, a 1:15 molar ratio was maintained for complexes of unlabeled Aβ1-40 and apoJ, although the total protein concentration varied according to the assay. For SDS-PAGE immunoblot analysis of the concentration of Aβ1-40 and apoJ, 80.5 μM and Aβ1-40 was 15 μM, whereas for solid phase binding assays, apoJ was 0.095 μM and Aβ1-40 was 1.5 μM. As a control, ovalbumin was substituted for apoJ.

**Preparation and Purification of 125I-labeled Aβ-ApoJ Complex**—ApoJ (300 μg in Dulbecco’s PBS (dPBS)) was radiiodinated by the IODO-GEN (Pierce) method using 2 μCi of Na125I (Amersham Life Science, Inc.). Unincorporated 125I was removed by Sepharose G-15 chromatography using a 0.7 × 18-cm column equilibrated with dPBS. Radiiodinated Aβ1-40 peptide (100 μg) was combined with apoJ (50 μg in dPBS) at a 30:1 molar ratio and incubated for 36 h at 37°C. Following the incubation, radiolabeled Aβ apoJ complex was separated from free 125I-labeled Aβ by gel filtration chromatography using a model 650E Waters protein purification system (Waters, Milford, MA) and a Superdex-200HR column (Pharmacia) equilibrated with dPBS. The integrity of the complex was evaluated by electrophoresis under non-denaturating conditions on 4–12% polyacrylamide, Tris/glycine-containing gels (Novex, San Diego, CA) followed by autoradiography.

**Enzyme-linked Immunosorbent Assays (ELISA)**—Microrot wells were coated with LRP-2, Aβ, or BSA (each at 3 μg/ml) in 150 mM NaCl, 50 mM Tris, pH 8.0 (TBS) containing 5 mM CaCl2 for 18 h at 4°C. Unoccupied sites were either blocked with PBS containing 3% nonfat milk or treated with PBS containing 0.1% N-octyl-β-D-glucopyranoside (OG) (Calbiochem). For those wells that were blocked with PBS containing 3% nonfat milk, all subsequent incubations were carried out in TBS containing 0.1% OG. For wells treated with PBS containing 0.1% OG, all subsequent incubations were carried out in TBS containing 0.01% OG. Bound proteins were detected using monoclonal antibodies, sheep anti-mouse IgG-horseshad peroxidase (Amerham International, Buckinghamshire, UK), and the chromogenic substrate o-phenylenediamine (Sigma) (1 mg/ml in 12 mM citric acid monohydrate, 25 mM dicycic acid sodium phosphate, pH 5.0, 0.0014% hydrogen peroxide). Binding data from ELISA were analyzed using a form of the binding isotherm as described by Ashcom et al. (18).

**Immunoblotting**—To analyze Aβ apoJ complexes by SDS-PAGE, samples were electrophoresed on 10–20% acrylamide gradient, Tricine-containing gels (Novex) in the presence of SDS. The separated proteins were electrophoretically transferred to ProtranTM nitrocellulose membranes (Schleicher & Schuell) in Tris-glycine-methanol buffer for 2 h at 70 V. After transfer, the membranes were incubated with 5% nonfat dry milk in TBS (pH 7.4). The membranes were then incubated with monoclonal Aβ or apoJ antibodies followed by sheep anti-mouse IgG-horse radish peroxidase (Amerham) diluted in 5% nonfat milk, TBS, 0.1% Tween 20. To detect bound antibodies, the membranes were incubated with ECL(TM) Western blotting chemiluminescent reagent (Amerham) and exposed to Biomax™ MR film (Eastman Kodak Co.).

**Internalization and Degradation of 125I-Aβ-ApoJ Complex**—To evaluate the effect of apoJ on the internalization of 125I-Aβ, mouse teratocarcinoma F9 cells (ATCC CRL 1720) were treated for 6 days with retinoic acid (RA) and dibutyryl cyclic AMP (Bt2cAMP) as described previously (30), released by trypsin-EDTA, and reseeded onto gelatin-coated 383-mm2 wells of 12-well plates (1.5 × 106 cells/well) in Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies, Inc.) containing 10% bovine calf serum (Hyclone Laboratories, Inc.) 20 mm HEPES, pH 7.4, 100 units/ml penicillin, 100 μg/ml streptomycin (Life Technologies, Inc.) and no RA/Bt2cAMP. The cells were cultured for 18 h at 37°C, 5% CO2 and then washed with serum-free DMEM containing penicillin and streptomycin. From solution-phase apoJ binding to immobilized Aβ1-40 (70 nM) in DMEM, 1.5% BSA, 1% Nutridoma serum substitute (Boehringer Mannheim) (DMEM/BSA/SS) containing various amounts of apoJ (1–40 nM) was added to the cells and incubated for 5 h at 37°C, 5% CO2. The amount of radiolabeled Aβ that was internalized by cells was defined as the amount of radioactivity that remained associated with the cell pellets following trypsin/protease K/EDTA treatment (11, 30).

To evaluate the role of LRP-2 in the cellular internalization and degradation of 125I-Aβ-apoJ complex, RA/Bt2cAMP-treated mouse teratocarcinoma F9 cells were cultured as described above. 60 min prior to the addition of 125I-Aβ-apoJ complex, the medium was removed and the cells were treated with DMEM/BSA/SS containing either anti-LRP-2 IgG (200 μg/ml), control rabbit IgG (200 μg/ml), or chloroquine (0.1 mM). 125I-Aβ-apoJ complex (10 nM) in DMEM/BSA/SS or in DMEM/BSA/SS containing anti-LRP-2 IgG (200 μg/ml), rabbit IgG (200 μg/ml), or chloroquine (0.1 mM) was then added and incubated with the cells for 5 or 18 h at 37°C, 5% CO2. The amount of radiolabeled complex that was internalized was measured as described above. Radioactivity released into the conditioned culture medium that was soluble in 10% trichloroacetic acid was taken to represent degraded ligand. Total ligand degradation values were corrected for non-cellular mediated degradation by subtracting the amount of degradation that occurred when the radiolabeled complex was incubated in wells lacking cells.

**RESULTS**

**Amyloid β-Protein Bonds to ApoJ but Not to LRP-2**—ELISAs were used to determine whether synthetic Aβ1–40 peptide was capable of binding directly to purified LRP-2. As shown in Fig. 1A, LRP-2 did not bind to microtiter wells coated with Aβ1–40. Likewise, Aβ1–40 (at concentrations up to 125 nM) did not bind to LRP-2-coated wells (Fig. 1B). In parallel assays, Aβ1–40 was shown to bind to apoJ in a dose-dependent manner, either when Aβ1–40 was coated onto microtiter wells and apoJ was introduced in solution phase (Fig. 1A) or when apoJ was coated and Aβ1–40 was introduced in solution phase (Fig. 1B). The data for solution-phase apoJ binding to immobilized Aβ1–40 (Fig. 1A) were fit using a hyperbolic function (16), and the half-saturating level of binding (estimated Kd) was determined to be 4.8 mM. This value is in good agreement with the value of 2.0 mM reported by Matsubara and colleagues (6). By contrast, the binding of solution-phase Aβ1–40 to immobilized apoJ was not saturable (Fig. 1B). Such non-saturable binding can be expected given that Aβ1–40 has the ability to self-associate (17). The results indicate that Aβ1–40 does not bind to LRP-2 but does

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**Fig. 1.** Aβ1–40 binds with high affinity to apoJ but not to LRP-2.

In panel A, microtiter wells coated with Aβ1–40 (3 μg/ml, plus a milk block) were incubated with varying concentrations of either apoJ (0.18–400 nM) or LRP-2 (0.25–500 nM) in PBS containing 0.1% N-octyl-β-D-glucopyranoside. Bound protein was detected by ELISA using monoclonal antibodies to apoJ or LRP-2. In panel B, microtiter wells coated with either apoJ, LRP-2, or BSA (each at 3 μg/ml, no blocking with milk) were incubated with varying concentrations of Aβ1–40 (1.37–125 nM) in PBS containing 0.1% N-octyl-β-D-glucopyranoside; bound peptide was detected by ELISA using monoclonal Aβ antibody 4G8. The plotted values are means of duplicate determinations with the range indicated by bars and are representative of three experiments.
Amyloid β-Protein-ApoJ Complex Binds to LRP-2—To generate Aβ-apoJ complex, Aβ1–40 was incubated with apoJ for 24 h at 37 °C, and complex formation was evaluated by SDS-PAGE and immunoblot analysis. As shown in Fig. 2, this incubation resulted in the formation of an approximately 70-kDa band, immunoreactive with monoclonal Aβ antibody (Fig. 2B, lane 4). The anti-Aβ-reactive 70-kDa band displayed a similar electrophoretic mobility to the 70-kDa apoJ (Fig. 1C, compare lanes 4 and 6). Incubation of Aβ with ovalbumin did not produce a band having a similar molecular mass (Fig. 2B, lane 5). Coomassie Blue staining showed that in the lane containing Aβ, which had been incubated alone for 24 h at 37 °C, there was a single band having a mobility corresponding to ~4 kDa (Fig. 2A, lane 3), consistent with M, of 4392. However, immunoblot analysis of this lane (Fig. 2B, lane 3) using monoclonal Aβ antibody revealed several immunoreactive species having M, values of ~8000 and ~12,000 and a high molecular mass band that just entered the gel. These species likely correspond to Aβ dimer, trimer, and aggregate, respectively. Although the dimer, trimer, and aggregated species were not detectable by Coomassie Blue staining, they were immunoreactive with Aβ antibody, indicative perhaps of its preference for multimerized peptide versus the monomeric form. Each of these immunoreactive species was also present in the profile of the Aβ incubated with ovalbumin. However, the Aβ aggregate was missing in the lane containing Aβ incubated with apoJ (Fig. 2B, lane 4). The data indicated that incubation of apoJ with Aβ under the conditions that we described resulted in the formation of a complex of Aβ and apoJ that is stable in SDS. The conditions of SDS-PAGE were insufficient to permit resolution of the Aβ-apoJ complex as a discrete species having a M, ~4000 greater than apoJ. The results also showed that apoJ inhibited the formation of aggregated Aβ while not perturbing the formation of Aβ dimer and trimer.

To evaluate the ability of Aβ-apoJ complex to interact with LRP-2, microtiter wells coated with LRP-2 were incubated with mixtures of Aβ and apoJ or Aβ and ovalbumin that had been preincubated for 24 h at 37 °C. As shown in Fig. 3A, Aβ binding to immobilized LRP-2, as detected by Aβ monoclonal antibody, occurred when Aβ was preincubated with apoJ but not with ovalbumin. Experimental controls showed that neither of the mixtures, Aβ and apoJ or Aβ and ovalbumin, bound to wells coated with BSA (Fig. 3B). When monoclonal antibody to apoJ was used to detect apoJ binding to LRP-2, the half-saturating level of binding of apoJ to LRP-2 was not significantly modified by the inclusion of Aβ (Fig. 3C). The results indicate that apoJ mediates binding of Aβ to LRP-2 and that the affinity of the Aβ-apoJ complex for LRP-2 does not appear to be different from that of apoJ alone.

The Antagonist of ApoJ Binding to LRP-2 Blocks Binding of Aβ-ApoJ Complex to LRP-2—RAP has been shown to inhibit the binding of apoJ to LRP-2 (11). As shown in Fig. 4, incubation of Aβ-apoJ complex with RAP completely blocked the binding of the complex to immobilized LRP-2. This, taken together with the above data, indicates that apoJ can function to bridge the interaction of Aβ with LRP-2.

Cellular Endocytosis of Aβ Is Facilitated by ApoJ—To determine whether apoJ might facilitate cellular internalization of Aβ, 125I-Aβ was administered to cultured LRP-2-expressing cells in the presence of varying concentrations of apoJ. As shown in Fig. 5, exogenously added apoJ promoted the internalization of 125I-Aβ in a dose-dependent manner.

LRP-2 Mediates Cellular Endocytosis and Degradation of Aβ-ApoJ Complex—We next examined the cellular clearance of Aβ-apoJ complex and evaluated the role of LRP-2 in the process. Radiiodinated Aβ was combined with unlabeled apoJ, and the resulting complex was purified by gel filtration chromatography. Fig. 6A shows the chromatographic profiles of the individual components and the complex-containing mixture. Native gel electrophoretic analysis of the complex-containing fraction is shown in the inset of Fig. 6A. The results indicated that the chromatography procedure permitted isolation of the 125I-labeled complex from the bulk of the unincorporated Aβ; however, free radiolabeled peptide did copurify with the complex.

Equimolar amounts of 125I-Aβ or 125I-Aβ-apoJ were administered to LRP-2-expressing cells, and the level of internalization of each was measured. As shown in Fig. 6B, there was a 2-fold higher level of 125I-Aβ-apoJ complex internalized as compared with 125I-Aβ alone. In separate experiments, the amount of 125I-Aβ internalized was unchanged by the inclusion of a 1000-fold molar excess of unlabeled peptide (data not shown). Complex internalization was also measured in the presence of function blocking LRP-2 antibodies or control IgGs. As shown in Fig. 6B (and Fig. 7A), anti-LRP-2 IgG inhibited 125I-Aβ-apoJ complex internalization by 59% as compared with treatment with control rabbit IgGs. The results indicate that Aβ-apoJ complex is internalized by LRP-2-expressing cells to a greater extent than Aβ alone and that the internalization of the complex can be inhibited by LRP-2 antibodies, implicating LRP-2 in the clearance process.

We also evaluated whether the internalized complex was lysosomally degraded as is the case for other LRP-2 ligands including apoJ (11). As shown in Fig. 7, administration of 125I-Aβ-apoJ complex to LRP-2-expressing cells resulted in the internalization and degradation of the complex. Degradation was evidenced by the appearance of trichloroacetic acid-soluble radioactivity in the conditioned culture medium that could be blocked by treatment with chloroquine, an inhibitor of lysoso-
mal proteinase activity (Fig. 7B). Both internalization and degradation of the complex were also inhibited with LRP-2-antibodies. Taken together, the findings indicate that LRP-2 mediates endocytosis of Aβ-apoJ complex leading to its degradation in lysosomes.

DISCUSSION

In this study, we document the ability of the Aβ-apoJ complex to bind to the endocytic receptor LRP-2 in both cell-free and cultured cell assays. Although the physiological relevance of this interaction remains to be established, we have previously hypothesized that it is part of a mechanism in which LRP-2-expressing epithelial cells, such as those of the choroid plexus and ependyma, can clear the Aβ-apoJ complex from the cerebrospinal fluid (11). In support of such a hypothesis is the fact that both apoJ and LRP-2 are expressed at high levels in the choroid plexus epithelium as well as ependymal cells that line the ventricles of the brain and neural tube (12, 18, 19) and are therefore in direct contact with cerebrospinal fluid. In addition to LRP-2 having a possible role in clearance of Aβ-apoJ complex from the cerebrospinal fluid, there is evidence that LRP-2 may have a role in uptake of the complex from the blood by cells of the cerebral vascular endothelium (14, 20). Following our previous report on the identification of LRP-2 as the receptor for apoJ, Zlokovic et al. (14) introduced radiolabeled Aβ-apoJ complex into rat brain vasculature and observed that RAP or monoclonal antibody to LRP-2 decreased the brain uptake of the radiolabeled complex. Whereas these findings indirectly implicate LRP-2 as being responsible for the observed vascular clearance, evidence is needed to show that LRP-2 is indeed expressed by brain vascular endothelial cells. Nevertheless, the results presented in the present study provide direct evidence that LRP-2 is indeed expressed by brain vascular endothelial cells.

Our results also indicate that apoJ inhibits the formation of high molecular weight aggregates of Aβ1–40 (Fig. 2). This activity is consistent with observations made using a sedimentation assay (10) and with the hypothesis that apoJ serves to maintain Aβ in a soluble form, preventing it from forming insoluble amyloid filaments. Such filaments are the hallmark component of the senile plaque found in brain parenchyma and deposited in the cerebrovasculature of patients with Alzhei-
triplicate values and are representative of duplicate experiments. Complex (10 nM) in the presence of affinity-purified anti-LRP-2 IgG (200 μg/ml), or chloroquine (0.1 mM) for 5 h at 37 °C, 5% CO2. Shown are the amounts of 125I-Aβ1–40 or 125I-Aβ1–40-apoJ internalized by RA/Bt2cAMP-differentiated F9 cells. Cultures were incubated with the respective probe at a concentration of 10 nM in the absence of any competitor or in the presence of anti-LRP-2 IgG or control IgG (each at 200 μg/ml) 18 h at 37 °C, 5% CO2. Plotted values are means ± S.D. of triplicate values and are representative of duplicate experiments.

In this regard, the Aβ-apoJ complex may make it possible that under normal physiological conditions, a number of studies indicate that it can be created in lysosomes as a result of proteolytic processing of amyloid β-precursor protein (33, 34). It is possible that the lysosomal presentation of Aβ in the form of a complex with apoJ may make it more susceptible to proteinase degradation. It is important to note that LRP-2 is apparently not the only means by which extracellular Aβ can be internalized. For example, fibroblasts presumably lacking LRP-2 can internalize exogenously added 125I-Aβ1–42 (35). This internalization pathway was shown to lead to intracellular accumulation of Aβ in the form of aggregates that are resistant to lysosomal proteinase degradation. LRP-2-mediated internalization may avoid this outcome by both preventing intracellular aggregate formation and promoting lysosomal degradation of Aβ.

Herein, evidence is presented indicating that one consequence of LRP-2-mediated endocytosis of Aβ-apoJ is that Aβ is targeted for lysosomal degradation. This is the end result for other LRP-2 ligands following their endocytosis (e.g., urokinase and plasminogen activator inhibitor-1 complex, low density lipoprotein, and apoJ (11, 30–32)). It may, however, seem paradoxical that Aβ could be degraded in lysosomes, considering that a number of studies indicate that it can be created in lysosomes as a result of proteolytic processing of amyloid β-precursor protein (33, 34). It is possible that the lysosomal presentation of Aβ in the form of a complex with apoJ may make it more susceptible to proteinase degradation. It is important to note that LRP-2 is apparently not the only means by which extracellular Aβ can be internalized. For example, fibroblasts presumably lacking LRP-2 can internalize exogenously added 125I-Aβ1–42 (35). This internalization pathway was shown to lead to intracellular accumulation of Aβ in the form of aggregates that are resistant to lysosomal proteinase degradation. LRP-2-mediated internalization may avoid this outcome by both preventing intracellular aggregate formation and promoting lysosomal degradation of Aβ.

REFERENCES

1. Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Greschuk, K. H., Multthap, G., Beyreuther, K., and Muller-Hill, B. (1987) Nature 325, 733–736
2. Selkoe, D. J. (1994) Annu. Rev. Cell Biol. 10, 373–403
3. Haass, C., Huang, A. Y., Schlossmacher, M. G., Teplow, D. B., and Selkoe, D. J. (1993) J. Biol. Chem. 268, 3021–3024
4. Haass, C., Koo, E. H., Teplow, D. B., and Selkoe, D. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1564–1568
5. Ghiso, J., Matusabara, E., Koudinov, A., Choi-Miura, N. H., Tomita, M., Wisniewski, T., and Frangione, B. (1993) Biochem. J. 293, 27–30
6. Matusabara, E., Frangione, B., and Ghio, J. (1995) J. Biol. Chem. 270, 7563–7567
7. Ma, J., Yee, A., Brewer, H. B., Jr., Das, S., and Potter, H. (1994) Nature 372, 92–94
8. Sohn, C., Castano, E. M., Prelli, F., Kumar, R. A., and Baumann, M. (1995) FEBS Lett. 371, 110–114
9. Wisniewski, T., Castano, E. M., Golabek, A., Vogel, T., and Frangione, B. (1994) Am. J. Pathol. 145, 1030–1035
10. Oda, T., Pusenetti, G. M., Osterburg, H. H., Anderson, C., Johnson, S. A., and Finch, C. E. (1994) Biochem. Biophys. Res. Commun. 204, 1131–1136
11. Kounnas, M. Z., Loukinova, E. B., Stefansson, S., Harmony, J. A., Brewer, B. H., Strickland, D. K., and Argraves, W. S. (1995) J. Biol. Chem. 270, 13070–13075
12. Kounnas, M. Z., Haudenschild, C. C., Strickland, D. K., and Argraves, W. S. (1994) In Vivo (Athens) 8, 343–351
13. Williams, S. E., Ashcom, J. D., Argraves, W. S., and Strickland, D. K. (1992) J. Biol. Chem. 267, 9035–9040
14. Zlokovic, B. V., Martel, C. L., Matusabara, E., McComb, J. G., Zheng, G., McChuskey, R. T., Frangione, B., and Ghio, J. (1996) Proc. Natl. Acad. Sci.
15. Ashcom, J. D., Tiller, S. E., Dickerson, K., Cravens, J. L., Argraves, W. S., and Strickland, D. K. (1990) J. Cell Biol. 110, 1041–1048
16. Balbona, K., Tran, H., Godyna, S., Ingham, K. C., Strickland, D. K., and Argraves, W. S. (1992) J. Biol. Chem. 267, 20120–20125
17. Terzi, E., Holzemmann, G., and Seelig, J. (1995) J. Mol. Biol. 252, 633–642
18. Ahoja, H. S., Tenniesen, M., Lockshin, R., and Zakari, Z. F. (1999) Biochem. Cell Biol. 72, 523–530
19. Arowow, B. J., Lund, S. D., Brown, T. L., Harmony, J. A., and Witte, D. P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 725–729
20. Zlokovic, B. V., Martel, C. L., Mackie, J. B., Matsubara, E., Wisniewski, T., McComb, J. G., Frangione, B., and Ghiso, J. (1993) Biochem. Biophys. Res. Commun. 205, 1431–1437
21. Glenner, G. G., and Wong, C. W. (1984) Biochem. Biophys. Res. Commun. 120, 885–890
22. Glenner, G. G., and Wong, C. W. (1984) Biochem. Biophys. Res. Commun. 122, 1131–1135
23. Pike, C. J., Burdick, D., Walencewicz, A. J., Glabe, C. G., and Cotman, C. W. (1993) J. Neurosci. 13, 1676–1687
24. Kim, D. H., Iijima, H., Goto, K., Sakai, J., Ishii, H., Kim, H. J., Suzuki, H., Kondo, H., Saecki, S., and Yamamoto, T. (1996) J. Biol. Chem. 271, 8373–8380
25. Rebuck, G. W., Reiter, J. S., Strickland, D. K., and Hyman, B. T. (1993) Neuron 11, 579–580
26. Bu, G., Maksymovitch, E. A., Nogronne, J. M., and Schwartz, A. L. (1994) J. Biol. Chem. 269, 18521–18525
27. Rebeck, G. W., Harr, S. D., Strickland, D. K., and Hyman, B. T. (1995) Ann. Neurol. 37, 211–217
28. Zheng, G., Bachinsky, D. R., Stamenkovic, I., Strickland, D. K., Brown, D., Andres, G., and McCluskey, R. T. (1994) J. Histochem. Cytochem. 42, 531–542
29. Assmann, K. J. M., Lange, W. P. H., Tangelder, M. M., and Keene, R. A. P. (1996) Virchows Arch. 408, 541–553
30. Stefansson, S., Chappell, D. A., Argraves, K. M., Strickland, D. K., and Argraves, W. S. (1995) J. Biol. Chem. 270, 19417–19421
31. Stefansson, S., Kounnas, M. Z., Henkin, J., Mallampalli, R. K., Chappell, D. A., Strickland, D. K., and Argraves, W. S. (1995) J. Cell Sci. 108, 2361–2368
32. Stefansson, S., Lawrence, D. A., and Argraves, W. S. (1996) J. Biol. Chem. 271, 8215–8220
33. Golde, T. E., Estus, S., Younkin, L. H., Selkoe, D. J., and Younkin, S. G. (1992) Science 255, 728–730
34. Tsuzuki, K., Fukatsu, R., Takamaru, Y., Fujii, N., and Takahata, N. (1994) Brain Res. 659, 213–220
35. Knauer, M. F., Soreghan, B., Burdick, D., Kosmoski, J., and Glabe, C. G. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7437–7441
36. Kounnas, M. Z., Stefansson, S., Loukinova, E., Argraves, K. M., Strickland, D. K., and Argraves, W. S. (1994) Ann. N. Y. Acad. Sci. 737, 114–123