Competition of Aβ amyloid peptide and apolipoprotein E for receptor-mediated endocytosis

Karl Winkler,[1,*] Hubert Scharnagl,* Ursula Tisljar,* Heinz Hoschützky,[†] Isolde Friedrich,* Michael M. Hoffmann,* Manfred Hüttinger,[§] Heinrich Wieland,* and Winfried März*

Department of Clinical Chemistry,* Albert Ludwigs-University, Freiburg, Germany; NanoTools Antikörpertechnik,[†] Teningen, Germany; and Department of Medical Chemistry,[§] University of Vienna, Austria

Abstract **The genetic polymorphism of apolipoprotein E (apoE) is associated with the age of onset and relative risk of Alzheimer's disease (AD). In contrast to apoE3, the wild type allele, apoE4 confers an increased risk of late-onset AD. We demonstrate that the β-amyloid peptide isoforms Aβ (1–28), Aβ (1–40), and Aβ (1–43) compete for the cellular metabolism of apoE3 and apoE4 containing β-very low density lipoproteins. An antibody raised against Aβ (1–28) cross-reacted with recombinant apoE. Epitope mapping revealed positive amino acid clusters as common epitopes of Aβ (13 through 17; HHQKL) and apoE (residues 144 through 148; LRKRL), both regions known to be heparin binding domains. Aβ in which amino acids 13 through 17 (HHQKL) were replaced by glycine (GGQGL) failed to compete with the cellular uptake of apoE enriched βVLDL. These observations indicate that Aβ and apoE are taken up into cells by a common pathway involving heparan sulfate proteoglycans.**—Winkler, K., H. Scharnagl, U. Tisljar, H. Hoschützky, I. Friedrich, M. M. Hoffman, M. Hüttinger, H. Wieland, and W. März. **Competition of Aβ amyloid peptide and apolipoprotein E for receptor-mediated endocytosis.** ***J. Lipid Res.*** **1999.** 40: **447–455.**

Supplementary key words Aβ amyloid • Alzheimer's disease • apolipoprotein E • heparin binding domain

Accumulation of the β-amyloid (Aβ) peptide in the brain is a defining feature of all forms of Alzheimer's diseases (AD), irrespective of the genetic background (1). Aβ is derived from the proteolytic processing of the β-amyloid precursor protein (APP). APP is thought to be cleaved by at least three different proteolytic activities (2). Cleavage by alpha-secretase occurs between residues 688 and 689 of mature APP. As these residues correspond to positions 16 and 17 of the Aβ peptide (3, 4), alpha-secretase prevents the formation of Aβ (5, 6). Minute amounts of Aβ are, however, generated constitutively through the action of two other putative endoproteolytic activities (7, 8): the amino terminal cleavage is mediated by beta-secretase, carboxy terminal cleavage by gamma-secretase (2). Mutations in the genes for APP, presenilin 1, and presenilin 2 have been shown to increase the formation of Aβ and may thus cause early onset forms of AD (1). Apolipoprotein E (apoE) is another constituent of amyloid plaques in the brain of patients with AD. ApoE is genetically polymorphic. There are three frequent alleles at the apoE gene locus: E2, E3, and E4 (9). This polymorphism strongly affects the risk of developing AD. In individuals with late-onset AD, the E4 allele is 2- to 3-fold more frequent than in the general population (10, 11). Recently, we demonstrated that the E4 allele not only correlates with the development of amyloid deposits, but also with the formation of neurofibrillary tangles, another major histopathological hallmark of AD (12), strongly implicating apoE in the pathogenesis of AD.

The mechanistic link between the apoE polymorphism and AD has not been unraveled so far. There are several hypotheses that may account for the association between apoE4 and AD. ApoE and Aβ form dodecyl sulfate-resistant complexes in vitro, apoE4 complexing more rapidly than apoE3 (13, 14). When Aβ and apoE are co-incubated, unique monofibrillar structures evolve, apoE4 yielding a denser matrix than apoE3 (15). According to Castaño and co-workers (16), apoE enhances both the rate and the amount at which fibrils are generated from soluble amyloid in vitro, apoE4 being more effective compared to apoE3. In contrast to apoE3, apoE4 reduces the branching and outgrowth of neurites in different types of neuronal cells (17–19). It has been suggested that apoE4, but not apoE3 promotes depolymerization of microtubuli (20, 21). ApoE may thus intimately participate in maintaining the integrity of the cytoskeleton.

Abbreviations: BCIP, 5-bromo-4-chloro-3-indolyl phosphate; DAB, diamino benzidine; EDTA, ethylenediaminetetraacetic acid; FPLC, fast protein liquid chromatography; HSPG, heparan sulfate proteoglycans; LRP, LDL receptor-related protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; VLDL, LDL, very low and low density lipoproteins, respectively; AD, Alzheimer's disease.

[1] To whom correspondence should be addressed.

In the central nervous system, apoE mRNA is found in astrocytes and glial cells, but not in neurons (22–26). As neurons contain immunoreactive apoE (27), a metabolic pathway must exist by which apoE is taken up. This pathway most likely involves endocytosis by the LDL receptor-related protein (LRP) (18, 19). Current opinion predicates that this process is initiated by binding of apoE-containing particles to heparan sulfate proteoglycans (HSPG) on the cell surface from where they are subsequently transferred to LRP (28, 29). Aβ has also been found to bind to heparan sulfate proteoglycans (30–36). Therefore, we examined the hypothesis that Aβ and apoE-containing lipoproteins are taken up into cells by a common pathway. Our results indicate that Aβ peptide and apoE compete with each other for receptor-mediated endocytosis by virtue of their heparin binding domains. These observations link the cellular metabolism of apoE and Aβ and may provide another clue to the mechanism underlying the association between the apoE polymorphism and AD.

MATERIALS AND METHODS

Materials

Polyclonal anti-Aβ(1–28) IgY raised against Aβ(1–28) peptide in chicken was from Nanotools, Denzlingen, Germany, and affinity-purified using Aβ(1–28) peptide covalently coupled to divinyl sulfone-activated agarose (37). Peroxidase-conjugated anti-chicken IgY from rabbit was from Jackson ImmunoResearch Laboratories, West Grove, PA. Alkaline phosphatase-conjugated anti-chicken IgG from goat was from Dianova, Hamburg, Germany. βVLDL were prepared from plasma of 1% cholesterol-fed rabbits (38). Recombinant human apoE (E2, E3, and E4) expressed in baculovirus (39, 40) was from PanVera, Madison, WI. Aβ(1–28) peptide, Aβ(1–40) peptide, and Aβ(1–43) peptide were from Bachem, Bubendorf, Switzerland. ^{125}I-labeled Aβ was from Immundiagnostik (Bensheim, Germany). Aβ peptides were stored freeze-dried at −20°C. In each experiment they were freshly dissolved at a concentration of 1 mg/ml at pH 8 in PBS and then used immediately.

Binding, uptake, and degradation of lipoproteins

Human skin fibroblasts were from skin biopsies of normolipidemic individuals. Binding, uptake, and degradation of lipoproteins were measured according to Goldstein, Basu, and Brown (41) with slight modifications (42). βVLDL and recombinant apoE were iodinated using the iodine monochloride method (43). Loading of βVLDL with recombinant human apoE was accomplished by incubating βVLDL at a protein concentration of 7.5 mg/ml with 2.4 mg/ml apoE in phosphate buffer at 37°C for 1 h (17). The apoE-loaded βVLDL were then used for cell culture experiments at a final concentration of 7.5 μg/ml and 2.4 μg/ml of βVLDL protein and apoE, respectively.

Fast protein liquid chromatography

Gel filtration of lipoproteins was performed with a column containing 120 ml of Superdex 200 HiLoad (Pharmacia Biotech, Uppsala, Sweden). The elution buffer contained 50 mmol/l Tris, 200 mmol/l NaCl, 0.2 g/l Na-azide, and 0.1% Brij35, pH 7.5. The flow rate was 60 ml per h and the size of the collected fractions was 2 ml.

Competitive enzyme immunoassay

Aβ(1–43) peptide (10 μg/well) was covalently bound to microtiter plates (Covalink NHR, Nunc, Roskilde, Denmark) according to the manufacturer's recommendations. Polyclonal anti-Aβ antibodies were incubated overnight at room temperature with the respective competitors: recombinant apoE or Aβ(1–43) peptide in 10 mmol/l phosphate, pH 7.4, 137 mmol/l NaCl, 2.7 mmol/l KCl (PBS), containing 10 g/l bovine serum albumin, 0.5 g/l Tween 20, and 0.05 g/l EDTA. After incubation, the antigen–antibody mixtures were loaded to the coated microtiter plates. Antibodies not neutralized by the competitors and binding to the solid phase were measured using appropriate peroxidase-conjugated secondary antibodies and *o*-phenylenediamine as substrate.

Epitope mapping of an apoE peptide library

Peptides were synthesized by Fmoc chemistry on an activated cellulose membrane using an ABIMED Auto-Spot Robot ASP 222 (Abimed, Langenfeld, Germany) according to the manufacturer's instructions and standard spot synthesis protocols (44). The cellulose-bound peptide libraries were incubated and probed essentially like immunoblots. Blocking of membranes and antibody dilutions were performed in PBS containing, in addition, 10% (v/v) fetal calf serum and 2 g/l Tween 20. Primary antibodies were used at 200 μg/l; visualization was performed with goat anti-chicken alkaline phosphatase conjugate and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as substrate.

RESULTS

We examined whether Aβ was able to compete with the uptake of apoE-containing lipoproteins in cultured human skin fibroblasts. Recombinant apoE isoforms expressed in the baculovirus system (39, 40) were loaded to ^{125}I-labeled βVLDL from cholesterol-fed rabbits. As expected, supplementation of apoE3, and apoE4, but not apoE2, enhanced cellular binding, uptake, and degradation approximately 2.5- and 3.0-fold, respectively, compared to VLDL alone (**Fig. 1 A–C**). When we added Aβ(1–43) at increasing concentrations, the increases in binding, uptake, and degradation mediated by apoE3 and apoE4 were almost completely abrogated, suggesting that Aβ and apoE competed for cellular uptake. This effect might have been non-specific as Aβ is cytotoxic. To exclude this possibility, competition experiments were done with the less toxic and aggregatable Aβ(1–28) and Aβ(1–40) (45–47). These Aβ peptides had virtually equal effects on binding, uptake, and degradation of apoE3 compared to Aβ(1–43) (**Fig. 2 A–C**). In addition, lactate dehydrogenase activities were measured in the culture medium after incubation with Aβ(1–43) peptide. There was no change of lactate dehydrogenase activity when we exposed fibroblasts for 4 and 24 h, respectively, to those Aβ(1–43) concentrations used in cell culture experiments. These data suggest that the competition of Aβ with the uptake of apoE-loaded βVLDL was not due to non-specific effects of Aβ. As Aβ (1–28) was active as a competitor, the competing domain obviously resided within the aminoterminal part of Aβ. To evaluate the possibility that the decreased uptake of apoE-containing particles was due to a displacement of apoE by Aβ from the surface of the βVLDL, we examined whether Aβ was able to liberate apoE from βVLDL under our experimental conditions. Cell culture medium was supplemented with ^{125}I-labeled apoE-loaded βVLDL and incu-

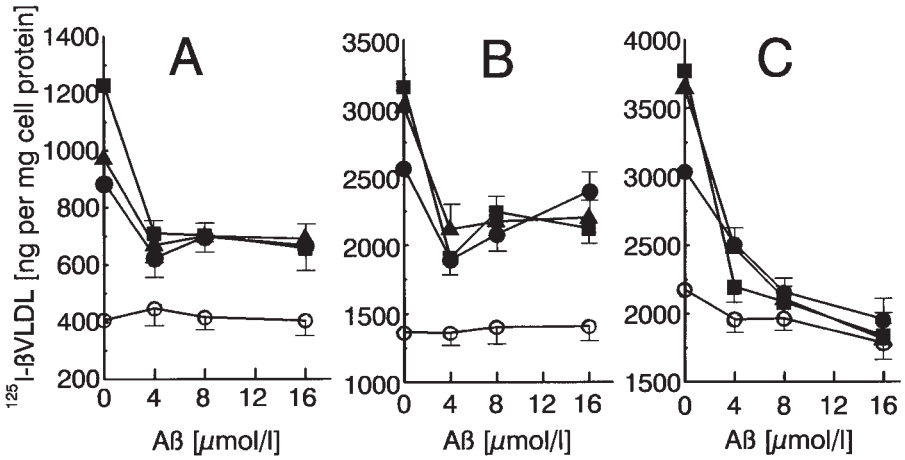

Fig. 1. Effect of Aβ on binding, uptake, and degradation of ^{125}I-labeled βVLDL in cultured human skin fibroblasts. βVLDL were prepared by ultracentrifugation from the plasma of cholesterol-fed rabbits, labeled with ^{125}I, and complexed with recombinant apoE as described (17, 38, 39). Human skin fibroblasts were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum. Cells received 7.5 μg/ml ^{125}I-labeled βVLDL protein (open circles), βVLDL complexed with 2.4 μg/ml recombinant apoE2 (closed circles), apoE3 (closed triangles), or apoE4 (closed squares), respectively. Aβ was used as unlabeled competitor at the concentrations indicated. Binding (panel A), uptake (panel B), and degradation (panel C) were determined as described (41, 42). Each data point represents the average value from triplicates, error bars represent standard deviations.

bated with human skin fibroblasts as in the binding studies. Aβ(1–43) peptide was then added to the medium (**Fig. 3**, panel A), but was omitted from the respective control (Fig. 3, panel B). After incubation for 1 h, the culture medium was subjected to gel-filtration and the radioactivity was measured in the eluted fractions. There was no difference in the amounts of ^{125}I-labeled apoE associated with the βVLDL fraction, regardless of whether or not Aβ was present in the incubation medium.

We also wished to exclude the possibility that Aβ bound to βVLDL during the cell culture experiments, thereby masking the cell surface binding domains of apoE. Cell-culture medium was supplemented with apoE/βVLDL, incubated with human skin fibroblasts, and ^{125}I-labeled

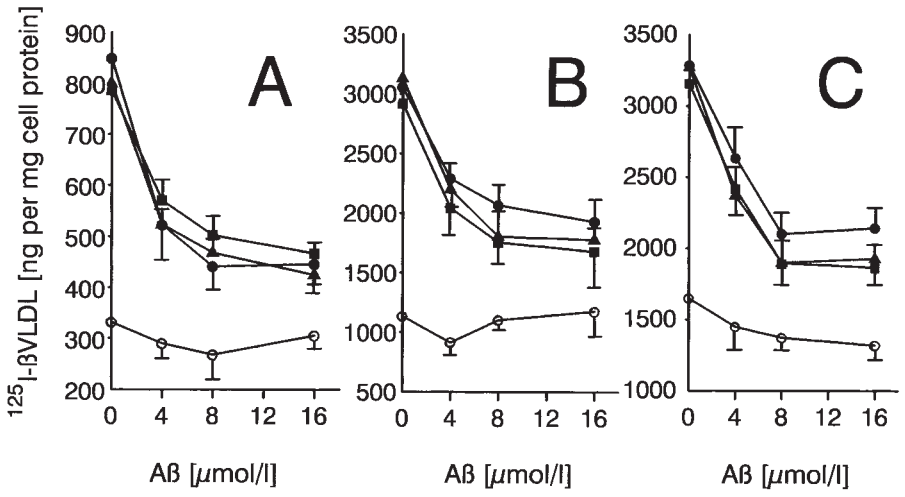

Fig. 2. Effect of various Aβ peptides on binding, uptake, and degradation of ^{125}I-labeled βVLDL in cultured human skin fibroblasts. βVLDL were prepared by ultracentrifugation from the plasma of cholesterol-fed rabbits, labeled with ^{125}I, and complexed with recombinant apoE as described (17, 38, 39). Human skin fibroblasts were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum. Cells received 7.5 μg/ml ^{125}I-labeled βVLDL protein (open circles). βVLDL complexed with 2.4 μg/ml recombinant apoE3 was used with Aβ of various lengths (Aβ(1–28) solid circle; Aβ(1–40) solid triangle and Aβ(1–43) solid square) as unlabeled competitors at the concentrations indicated. Binding (panel A), uptake (panel B), and degradation (panel C) were determined as described (41, 42). Each data point represents the average value from triplicates; error bars represent standard deviations.

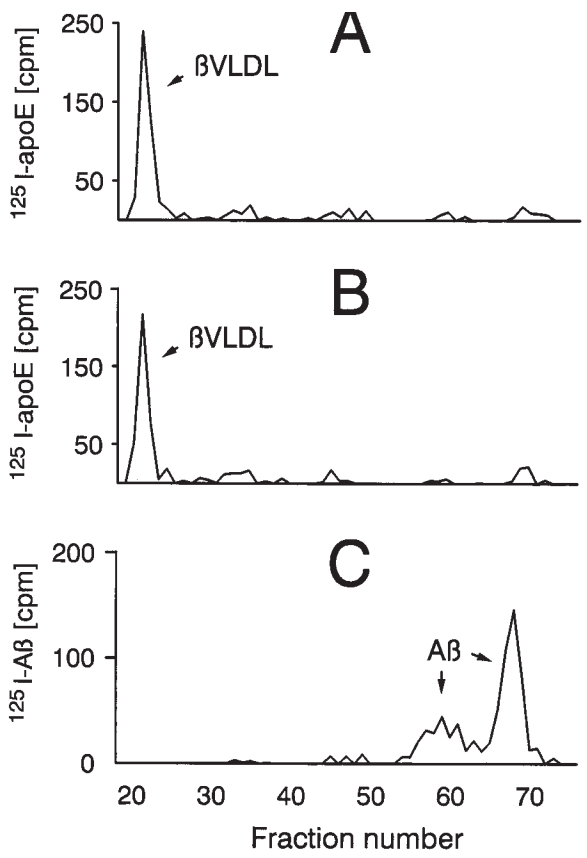

Fig. 3. Effect of Aβ on the apoE contents of ^{125}I-labeled apoE-enriched βVLDL and interaction of ^{125}I-labeled Aβ with apoE enriched βVLDL. βVLDL and ^{125}I-labeled apoE3 were incubated at room temperature for 1 h. ApoE3 loaded βVLDL (final concentrations: 2.4 μg/ml ^{125}I-labeled apoE and 7.5 μg/ml βVLDL protein) were then added to cell culture medium and incubated at 4°C with human fibroblasts. Sixteen μg/ml (4 μM) Aβ(1–43) peptide was added within 1 min after the addition of ^{125}I-labeled apoE/βVLDL to the cell culture, whereas in the control experiment Aβ was omitted. After 60 min of incubation, the cell culture medium was removed from the cells and subjected to gel filtration. The amount of radiolabeled apoE was quantified in each of the eluted fractions. Panel A: incubation with Aβ(1–43) peptide. Panel B: control experiment without Aβ(1–43) peptide. Panel C: βVLDL and unlabeled apoE3 were incubated at room temperature, then added to cell culture medium and incubated at 4°C with human fibroblasts as described above. Sixteen μg/ml (4 μM) ^{125}I-labeled Aβ(1–43) peptide was added within 1 min after the addition of apoE/βVLDL to the cell culture. After 60 min of incubation, the cell culture medium was removed from the cells and subjected to gel filtration. The amount of ^{125}I-labeled Aβ was quantified in each of the eluted fractions.

Aβ(1–43) peptide was added to the medium. After 60 min of incubation the culture medium was withdrawn and subjected to gel filtration (Fig. 3, panel C). No βVLDL-associated radioactivity could be detected. Therefore, we concluded that Aβ(1–43) neither displaced radiolabeled apoE from βVLDL nor did it bind to βVLDL to a relevant extent under the conditions used here.

To investigate whether structural similarities of Aβ and apoE exist which could explain a competition between Aβ and apoE for cell surface binding sites, we raised a polyclonal antiserum in chicken, directed against Aβ(1–28), the hydrophilic domain of Aβ. Using a solid-phase competitive enzyme immunoassay, we analyzed the binding of this antiserum to recombinant apoE. Aβ(1–43) was immobilized to microtiter plates. Recombinant apoE isoforms and Aβ peptide(1–43) were pre-incubated at increasing concentrations with the antiserum and the mixtures were then loaded to the Aβ-coated wells. In this assay, the polyclonal antiserum prepared against Aβ(1–28) cross-reacted with apoE. The affinities of the antiserum for the Aβ(1–43) peptide and apoE isoforms were all in the same order of magnitude (K_D around 0.2 μmol/l). The three major apoE isoforms (E2, E3, E4) did not differ regarding their reactivities (**Fig. 4**). We wished to identify the epitope(s) of apoE producing cross-reactivity with the Aβ antibodies. We generated a library of peptides containing hexameric sequential parts of the amino acid sequence of apoE, starting at spot number 12 with position 1 through 6 of mature apoE (44). This peptide library was probed with polyclonal anti-Aβ(1–28) from chicken which was previously affinity purified using immobilized Aβ(1–28). Immunostaining of the peptide library revealed that the antibody recognized multiple regions of the apoE molecule, all of which contain positively charged amino acids (48). As shown in **Fig. 5**, the most intense staining was seen with peptides covering positions 144 through 148 of mature apoE (48). No staining was seen in the control incubations using either crude IgY from pre-immune chicken or affinity-purified anti-myoglobin IgY. We also screened a peptide library of dodecamers derived from Aβ. Only one domain of antibody binding was detected, comprising amino acids 9 through 19 (**Fig. 6**). Amino acids 12 through 17 and 144 through 147 of Aβ and of apoE, respectively, constitute heparin binding domains (30, 32–36,

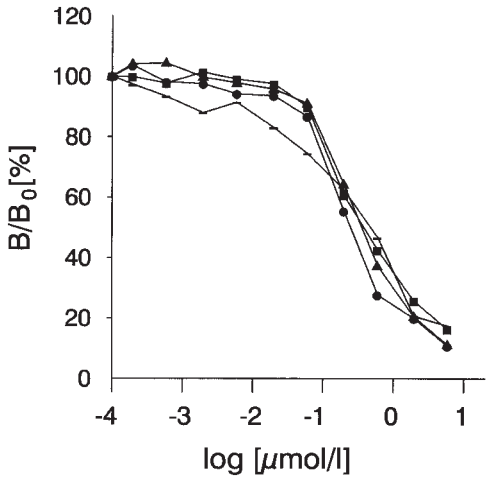

Fig. 4. Apolipoprotein E binding of a polyclonal antibody raised against Aβ(1–28). Panel A: ApoE2 (circles), apoE3 (triangles), apoE4 (squares), and Aβ(1–43) amyloid (dashes) were preincubated overnight at the concentrations indicated with polyclonal anti-Aβ(1–28) antiserum (40 μg/ml). The mixture was applied to microtiter plates coated with Aβ(1–43). Antibodies bound to the solid phase were detected with peroxidase-conjugated anti-IgY (1.6 μg/ml). B_0 (100%) is the absorbance with no competitor added and B is the absorbance in percent of B_0 at the indicated concentrations of the respective competitors.

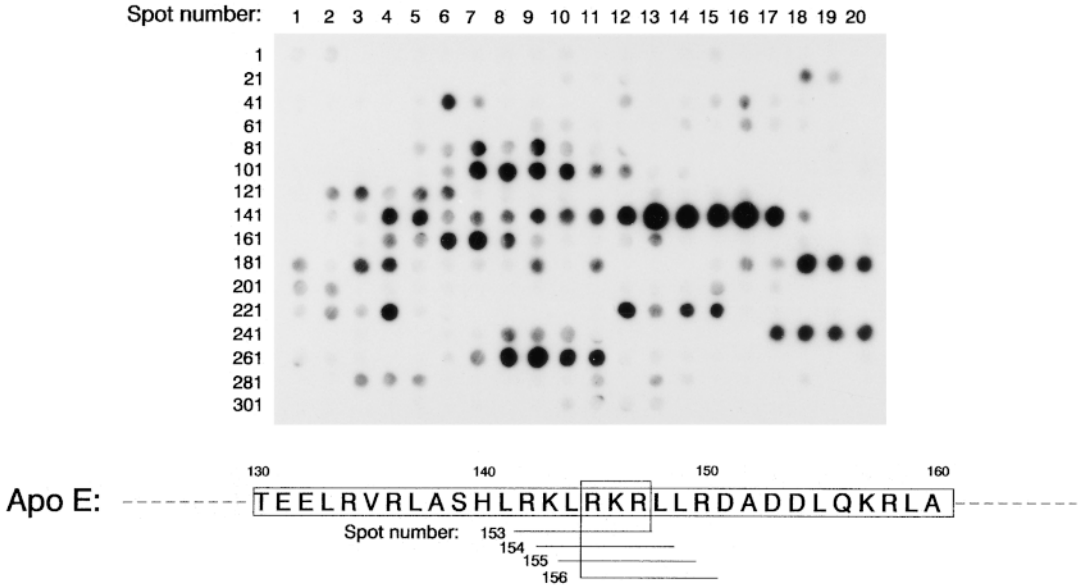

Fig. 5. Epitope mapping using a library of peptides derived from the apolipoprotein E amino acid sequence. Individual peptides were synthesized according to the following scheme: X-X-A1-A2-A3-A4-A5-A6-X-X-bA-bA-cellulose, where X = equimolar mixture of the natural amino acids with the exception of C and W, bA = β-alanine, A1 to A6 = hexapeptides consisting of six subsequent amino acids. Spot numbers 1 through 11 represent a part of the leader sequence of immature apoE. Spot number 12 consists of amino acids 1 to 6 of the mature apoE protein, spot number 13 of amino acids 2 to 7, and so on. Binding of affinity-purified anti-Aβ(1–28) antibody was visualized using goat anti-chicken antibodies conjugated to alkaline phosphatase and BCIP/MTT.

49, 50). The uptake of lipoproteins mediated by apoE involves initial binding of the ligand to cell surface HSPG (28, 29). We reasoned that the homology of the heparin binding motifs of apoE and Aβ revealed by the immunochemical studies was responsible for the competition of Aβ for the uptake of apoE-enriched βVLDL. To test this hypothesis, we generated an Aβ variant (1–43), designated Aβ*, in which residues 13 through 16 (HHQK) were replaced by GGQG. This variant peptide was earlier shown to be completely defective in binding to heparin (30). In contrast to wild-type Aβ, the Aβ* peptide was also not able to compete with apoE-supplemented βVLDL for binding, uptake, and degradation in cultured cells (**Fig. 7**), indicating that amino acids 13 through 16 of Aβ are crucial for the displacement of apoE from cell surface binding sites.

DISCUSSION

The cellular uptake of apoE-enriched lipoproteins is thought to occur in two steps. In the first step, apoE mediates binding of these particles to cell surface proteoglycans. In the second step, lipoproteins are transferred to lipoprotein receptors such as the LDL receptor-related protein (LRP) to undergo endocytosis (28, 29). So far, very little is known about the cellular catabolism of Aβ. Both Aβ and apoE possess heparin binding domains. We, therefore, examined whether Aβ was able to affect the endocytosis of apoE-containing lipoproteins. Our results demonstrate that Aβ(1–28), Aβ(1–40), and Aβ(1–43) decreased the uptake of apoE-loaded βVLDL into cells. As Aβ peptides are highly lipophilic, we wished to rule out that decreased binding and uptake of apoE-enriched βVLDL was due to displacement of apoE by βA4 directly interacting with the βVLDL. When we co-incubated apoE-enriched βVLDL and Aβ, we were not able to demonstrate that Aβ detached radiolabeled apoE from the βVLDL. In addition, there was no evidence that Aβ bound to apoE-containing βVLDL under the conditions used here, thus excluding the possibility that Aβ modulated the ability of apoE to interact with the surface of cultured cells. We conclude from this data that Aβ decreased apoE-mediated binding to the cell surface by competition of Aβ with apoE-loaded βVLDL rather than by displacement of apoE from βVLDL or by direct binding of Aβ to apoE-containing βVLDL. This assumption is further supported by the fact that Aβ(1–28), which lacks the hydrophobic carboxy-terminus most likely interacting with apoE, was effective in diminishing cellular uptake of apoE-containing βVLDL as well.

Competition of Aβ and apoE for cellular binding would require that the two polypeptides share structural similarities. As there is no homology of the amino acid sequences of apoE and Aβ, we decided to use an immunological approach to investigate whether domains of apoE might be similar to Aβ by three-dimensional structure or charge distribution. A polyclonal antibody against the Aβ(1–28) peptide, the hydrophilic region of Aβ(1–43), was raised in chicken. The anti-Aβ(1–28) antiserum was immunologically reactive with both, Aβ(1–43) amyloid and recombi-

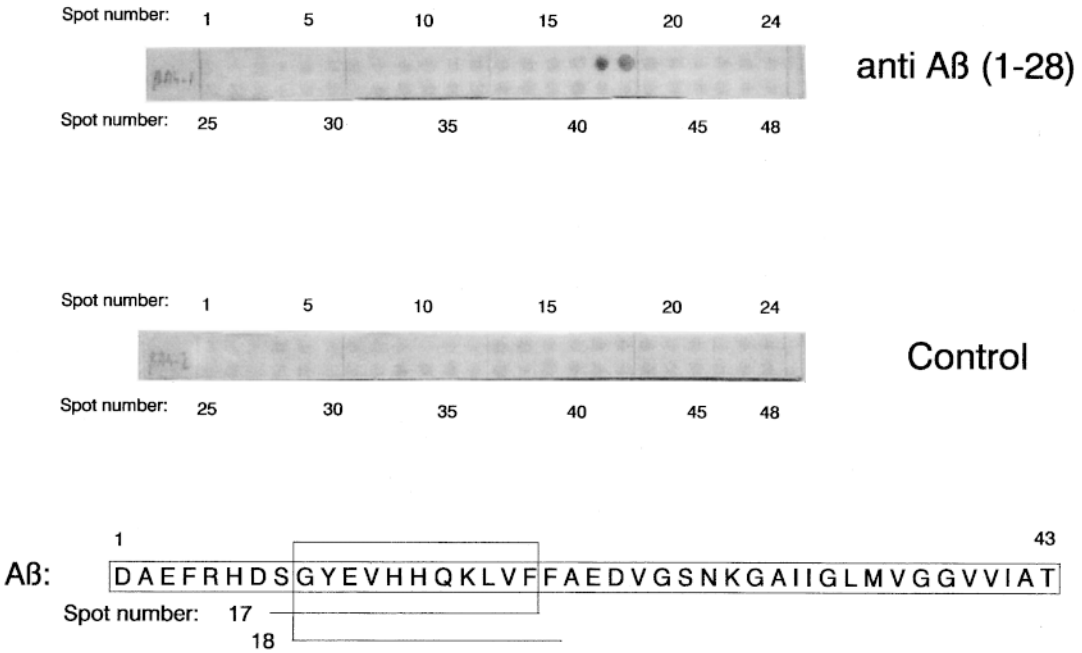

Fig. 6. Epitope mapping using a library of peptides derived from the Aβ peptide amino acid sequence. Individual peptides were synthesized according to the following scheme: X-X-A1-A2-A3-A4-A5-A6-A7-A8-A9-A10-A11-A12-X-X-bA-bA-cellulose, where X = equimolar mixture of the natural amino acids with the exception of C and W, bA = β-alanine, A1 to A12 = dodecapeptides consisting of twelve subsequent amino acids. Spot numbers 1 through 9 represent amino acids of the amyloid precursor protein (APP) preceding the Aβ peptide. Spot number 10 consists of amino acids 1 to 12 of the mature Aβ peptide, spot number 11 of amino acids 2 to 13, and so on. Binding of affinity purified anti-Aβ(1–28) antibody was visualized using goat anti-chicken antibodies conjugated to alkaline phosphatase and BCIP/MTT.

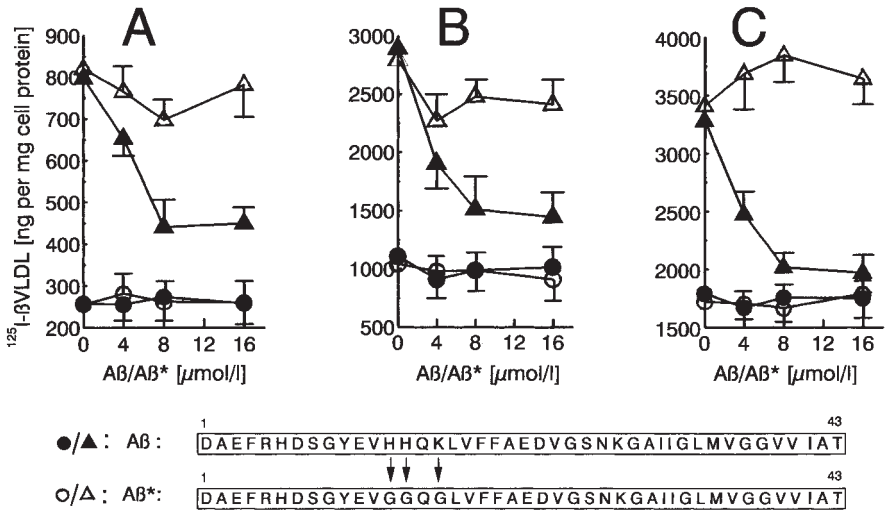

Fig. 7. Effects of Aβ and Aβ* on binding, uptake, and degradation of ^{125}I-labeled βVLDL in cultured human fibroblasts. βVLDL were prepared by ultracentrifugation from the plasma of cholesterol-fed rabbits, labeled with ^{125}I, and complexed with recombinant apoE as described (17, 38, 39). Human skin fibroblasts were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum. Cells received 7.5 μg/ml ^{125}I-labeled βVLDL protein (circles), βVLDL complexed with 2.4 μg/ml recombinant apoE3 (triangles). Aβ (positions 13 through 16 HHQK, closed symbols) and Aβ* (positions 13 through 16 GGQG, open symbols) were used as unlabeled competitors at the concentrations indicated. Binding (panel A), uptake (panel B), and degradation (panel C) were determined as described (41) with slight modifications (42). Each data point represents the average value from triplicates; error bars represent standard deviations.

nant apoE, recognizing both antigens with approximately equal affinities.

Epitope mapping suggested amino acids 9–19 of the amyloid-peptide and amino acids 144 through 148 of apoE as the common epitope. Both epitopes consist of clusters of positive amino acids. Amino acids 144 through 148 of apoE are part of the receptor binding domain of the molecule which extends between residues 136 and 150 (51) and they represent one out of at least two heparin binding sites of the apoE molecule (48–50). In the Aβ peptide, residues 12 through 16 mediate heparin binding (32–36); replacing the positive amino acids between positions 13 and 16 of Aβ (HHQK) by glycine (GGQG) completely abolishes the binding of Aβ to heparin (30). Consistently, we found that the peptide containing G at positions 13, 14, and 16 lost the ability to compete with apoE-enriched βVLDL for cellular binding, uptake, and degradation. Taken together, these data indicate that apoE and Aβ share homology of their heparin binding sites and they suggest that the heparin binding site in the aminoterminal region of Aβ is able to interact with cellular sites responsible for the endocytosis of apoE. Thus far, our findings are completely consistent with a report by Ida, Masters, and Beyreuther (51), who obtained evidence that the aminoterminus of Aβ was involved in receptor-mediated uptake of this peptide.

What is the molecular nature of the cellular site binding Aβ and apoE? ApoE is a ligand of the LDL receptor (52), the LRP (53, 54), and the VLDL receptor (55). The VLDL receptor is not expressed in fibroblasts. The uptake experiments were conducted in the presence of fetal calf serum as a source of cholesterol and LDL receptor activity was thus down-regulated. Finally, the increase in cellular uptake which is produced by supplementing βVLDL with apoE is attributable to the HSPG/LRP pathway rather than to the LDL receptor (28, 29, 56). Together, these considerations imply that in this study Aβ inhibited endocytosis of apoE by interacting with the HSPG/LRP complex.

Our observations may have implications for the mechanism underlying the association of the apoE polymorphism and the risk for AD. In the central nervous system there appears to be a surplus of about 100-fold of apoE over Aβ (100–300 nm versus 3 nm, respectively (57, 58)). ApoE might therefore modify the clearance of Aβ by pathways involving heparan sulfate proteoglycans even in situations where the generation of Aβ is increased. In this respect, it is interesting that the three common apoE isoforms differ by their binding to heparan sulfate proteoglycans. Whereas apoE4 binds more avidly (about 125% of apoE3), binding of apoE2 is approximately one-third lower compared to apoE3 (40, cf. Fig. 1, panel A; Scharnagl, J. Acar, G. Feussner, H. Wieland, and W. März, unpublished results). One might therefore speculate that the three apoE isoforms inhibit the decay of Aβ in the order apoE4 > apoE3 > apoE2. This would provide an explanation for both the positive correlation between Aβ deposition and apoE4 (10–12) and the apparent protective effect of apoE2 (59), but this hypothesis still needs to be proven.

Further, it may be of interest that amino acids 13 to 16 of Aβ, the epitope shared with apoE, are adjacent to the site at which APP is cleaved by alpha-secretase (amino acids 16/17). One might, therefore, speculate that intracellular apoE is able to modify the processing of APP by 'trapping' alpha-secretase. The possible implications of our findings are currently under further investigation.

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