Isolation and Characterization of Apolipoproteins from Murine Microglia

IDENTIFICATION OF A LOW DENSITY LIPOPROTEIN-LIKE APOLIPOPROTEIN J-RICH BUT E-POOR SPHERICAL PARTICLE

Qiang Xu, Yonghong Li, Connie Cyras, David A. Sanan‡, and Barbara Cordell§

From the Scios Inc., Sunnyvale, California 94085 and §Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, California 94141-9100

Amyloid Aβ deposition is a neuropathologic hallmark of Alzheimer’s disease. Activated microglia are intimately associated with plaques and appear to facilitate Aβ deposition, an event believed to contribute to pathogenesis. It is unclear if microglia can modulate pathogenesis of Alzheimer's disease by secreting lipoprotein particles. Here we show that cultured BV2 murine microglial cells, like astrocytes, secrete apolipoprotein E (apoE) and apolipoprotein J (apoJ) in a time-dependent manner. To isolate and identify BV2 microglial particles, gel filtration chromatography was employed to fractionate BV2-conditioned medium. Analyses by Western blot, lipid determination, electron microscopy, and native gel electrophoresis demonstrate that BV2 microglial cells release spherical low density lipoprotein (LDL)-like lipid-containing particles rich in apoJ but poor in apoE. These microglial particles are dissimilar in size, shape, and lipoprotein composition to astrocyte-derived particles. The microglial-derived particles were tested for functional activity. Under conditions of suppressed de novo cholesterol synthesis, the LDL-like particles effectively rescued primary rat cortical neurons from mevastatin-induced neurotoxicity. The particles were also shown to bind Aβ. We speculate that the LDL-like apoJ-rich apoE-poor microglial lipoproteins preferentially bind the lipoprotein receptor, recognizing apoJ, which is abundant in the choroid plexus, facilitating Aβ clearance from the brain. BV2 cells also secrete an apoE-rich lipid-poor species that binds Aβ. Consistent with the role of apoE in Aβ fibril formation and deposition, this microglial species may promote plaque formation.

Increasing evidence indicates that inflammation is involved in the pathogenesis of Alzheimer’s disease (AD) with microglia playing a central role (1–4). Microglia found in the normal adult brain are highly ramified quiescent cells, but they become reactive during brain injury. Activation of microglia is thought to induce an inflammatory response in the brain and to mediate the amyloid-associated neurodegeneration in AD. For instance, microglial response to Aβ leads to expression of important inflammatory mediators: interleukin-1, interleukin-6, tumor necrosis factor-α, and granulocyte macrophage colony-stimulating factor (5–8). Microglia themselves are one of the Aβ-generating cell types in the brain (9) and are likely to contribute to the total cerebral Aβ burden.

Emerging data support a role for microglia in plaque progression in AD (2, 10, 11). Response of quiescent microglia to Aβ appears to represent the first step of an activation cascade (12–15). Since Aβ has been demonstrated to act as a chemotactic stimulus for microglia in vitro (16), it may signal to recruit microglia to the vicinity of extracellular Aβ in vivo. This is supported by the observation that reactive microglia co-localize with diffuse nonfibrillar Aβ only in brain regions that are involved in AD pathology (17). The acquisition of a tertiary fibrillar structure by Aβ and the development of neuritic plaques can be correlated with the intimate association of activated microglia. This is in contrast to the appearance of reactive astrocytes, which encircle but are not in immediate physical proximity to neuritic plaques (2). The close physical association of reactive microglia with the different stages of plaque formation strongly suggests that activation of microglia facilitates conversion of diffuse Aβ to fibrillar Aβ. It has been proposed that once proto-fibrils have formed, endosomal compartments in microglia could serve as efficient sites for the growth of amyloid fibrils (18).

Glia-neuronal interactions also contribute to plaque progression. For example, overexpression of interleukin-1α by plaque-associated microglia may contribute to plaque development by increasing production of astrocyte-derived S100β. This neurite growth-promoting cytokine has been implicated as contributing to the formation of dystrophic neurites within plaques (2, 4, 12). Neuronal injury arising from these cytokine-induced neuronal insults can further activate microglia with increased expression of interleukin-1, thus producing feedback amplification of this cytokine cycle (2).

Although microglia have been implicated in the pathogenesis of senile plaques and apoE has been shown to co-localize with specific plaque types (19), implying a role in plaque progression, it is unclear whether microglia can modulate the progression of AD through de novo synthesis and secretion of apoE. Since apoE is a major risk factor for AD (20–22), this is an important issue. Analyses using reverse transcriptase-polymerase chain reaction and in situ hybridization indicate that microglia express apoE mRNA (23, 24). Since peripheral macrophages secrete apoE (25), we asked whether microglia synthesize and secrete apoE protein and/or apolipoprotein-containing lipoprotein particles. As an initial step to answering

*This work was supported by Eli Lilly & Co. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

‡ To whom correspondence should be addressed: Scios Inc., 820 West Maude Ave., Sunnyvale CA 94085. Tel.: 408-616-8230; Fax: 408-616-8317; E-mail: Cordell@sciosinc.com.

§ The abbreviations used are: AD, Alzheimer’s disease; apo, apolipoprotein; SEC, size exclusion chromatography; LDL, low density lipoprotein; VLDL, very LDL; HDL, high density lipoprotein; PAGE, polyacrylamide gel electrophoresis; MTS, 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium; Tricine N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine.

This paper is available online at http://www.jbc.org

31770 This paper is available on line at http://www.jbc.org
this question, we investigated the immortalized microglial cell line, BV2, which possesses many of the features of primary microglia (5, 26–28). Using size exclusion chromatography, electrophoretic separation, electron microscopy, and native gel electrophoresis, we have found that BV-2 cells secrete apoE and apoJ, as well small spherical LDL-like lipoproteins. We also demonstrate that this microglial LDL-like lipoprotein can function in delivering cholesterol to neurons and can associate with Aβ.

**EXPERIMENTAL PROCEDURES**

**Preparation of BV-2 Microglial Serum-free Media**—The BV2 immortalized murine microglial cell line was generated by Dr. Virginia Bocchini (26) and has been described previously (5, 9). Briefly, BV2 cells were maintained in Dulbecco’s modified Eagle’s medium with high glucose (Life Technologies, Inc.) supplemented with 5% heat-inactivated fetal bovine serum (HyClone Inc, Logan, UT), 4 mM L-glutamine, 0.2 mM penicillin, 0.05 mM streptomycin, and 20 mM HEPES at 37 °C in a humidified incubator under 95%/5% (v/v) mixture of air and CO2. Once confluent, typically 1 day after initial seeding at ~10^5/ml, cells were washed once and grown in serum-free medium for 12, 24, or 48 h. Conditioned medium was collected and centrifuged at 700 × g. The medium was stored under argon at 4 °C until use. For both purification of lipoprotein particles, neuronal rescue, and Aβ binding experiments, a total of 140 ml of conditioned medium was used.

**Isolation of Lipoprotein Particles Using Size Exclusion Chromatography**—To preserve the protein composition of lipoproteins during fractionation, size exclusion chromatography (SEC) was chosen over salt density centrifugation to isolate native microglial lipoprotein particles. Serum-free BV2 microglial medium conditioned for 24 h was concentrated using Centricon-10 (Amicon, Beverly, MA) before fractionation using a SuperSEC-100 column in tandem (Amersham Pharmacia Biotech), operated by a HP1050 Chemstation (Hewlett Packard, Palo Alto, CA). The columns were equilibrated in elution buffer consisting of 0.02 M sodium phosphate, pH 7.2, 0.05 M NaCl, and 0.03% EDTA at roughly 5 mg/ml were injected via a 2-ml sample loop and fractionated in elution buffer at a flow rate of 0.25 ml/min. Fractions were collected 48 h after sample injection at 0.4 ml/fraction until free proteins were eluted from the columns. The protein elution was monitored by UV absorbance at 275 nm. To characterize BV2 microglial particles, the column system was calibrated using fresh human plasma (Pacific Blood Center, San Francisco, CA) and mouse plasma (Harland Bioproducts for Science, Indianapolis, IN) to determine the elution of very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoprotein (HDL), and free protein. Positioning of the albumin elution profile determined by Coomassie Blue staining following SDS-polyacrylamide gel electrophoresis (PAGE). All experiments involving gel filtration chromatography were performed at room temperature. Fractionated samples were stored under argon at 4 °C until use.

**Analytical Gel Electrophoresis and Western Analysis**—Two gel systems, including reducing SDS-PAGE and non-denaturing PAGE, were employed. For protein analysis in reducing SDS-PAGE, proteins in conditioned media or fractions after SEC were denatured in reducing SDS-PAGE buffer consisting of 50 mM Tris-HCl, pH 6.8, 0.4% SDS, 6% sucrose, 10 mM dithiothreitol, and 0.01% bromphenol blue and resolved on a 4–20% polyacrylamide gel (Invitrogen, Carlsbad, CA) in 25 mM Tris, pH 8.3, and 192 mM glycine. To electrophorese non-denaturing 4–20% polyacrylamide gels (Invitrogen, Carlsbad, CA) in 25 mM Tris, pH 8.3, and 192 mM glycine. To determine the particle size, a mixture of protein standards of known radii was loaded (Amersham Pharmacia Biotech), including thyroglobulin (8.5 nm), ferritin (6.1 nm), catalase (4.6 nm), lactate dehydrogenase (4.1 nm), and albumin (3.55 nm) (29). After electrophoretic separation, proteins in reducing SDS-PAGE gels were electroblotted onto to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech) in 20% methanol, 20 mM Tris, and 50 mM glycine. To electroblot the proteins after native PAGE, the gel was soaked with 0.1% SDS, 10 mM Tris, pH 7.5, for 15 min. Afterward, the polyvinylidene difluoride membranes were split; a portion of the membrane was stained with Coomassie Blue to position protein standards, and the remainder of the blot was used for Western analysis. Rabbit anti-murine apoE, apoAI, apoAI-III, and apoCIII antibodies (with apoCIII as a major determinant) were purchased from Biodiesgo International (Kennebunk, ME), and sheep anti-rat apoJ was purchased from Quidel (San Diego, CA). The antigen-antibody reaction was visualized by using a secondary antibody conjugated with horse-radish peroxidase and enhanced chemiluminescence detection reagents (Amersham Pharmacia Biotech). Western blots were quantified by densitometric analysis (Molecular Dynamics, Sunnyvale, CA).

**Immunoprecipitation and N-terminal Amino Sequence Analysis**—Immunoprecipitation of apoE was performed using 5 ml of conditioned BV2 microglial medium in the presence of 50 µl of 10% protein A-Sepharose (Amersham Pharmacia Biotech) and 5 µl of rabbit anti-rat apoE serum with an overnight incubation at 4 °C. After immunoprecipitation, protein A-Sepharose pellets were washed three times with 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5 mM EDTA, and 0.5% nonidet P-40 and twice with 10 mM Tris-HCl, 5 mM EDTA, and 0.5% Nonidet P-40. Protein A-Sepharose pellets were rinsed twice with 10 mM Tris-HCl, pH 7.5. The sample was heat-denatured in SDS-PAGE buffer consisting of 50 mM Tris-HCl, pH 6.8, 0.4% SDS, 6% sucrose, 10 mM dithiothreitol,
and 0.01% bromphenol blue. Immunoprecipitated proteins were electrophoretically separated, blotted to polyvinylidine difluoride membranes, and stained with Coomassie Blue. The polyvinylidene difluoride membranes were destained with 50% methanol and rinsed with deionized water. N-terminal sequence analysis was performed on an Applied Biosystems sequencer (Perkin-Elmer, Foster City, CA).

Lipid Determination—Two hundred-microliter aliquots of fractionated plasma or BV2 microglial medium were used to determine total cholesterol and phospholipid. Total cholesterol in fractionated samples was measured after enzymatic reactions by cholesterol oxidase in the presence of cholesterol esterase using a commercially available kit (Pierce). Phospholipid levels were determined enzymatically using a commercially kit (Wako, Richmond, VA).

Analysis ofParticles by Negative Stain Electron Microscopy—For electron microscopy, fractions originating from microglial-conditioned medium was dialyzed against a volatile buffer composed of 0.125 M ammonium acetate, 2.6 mM ammonium carbonate, 0.26 mM EDTA at a pH of 7.6 overnight at 4 °C in a 10-kDa cutoff Slide-A-Lyzer cassette (Pierce). This volatile buffer causes disc-like particles to form rouleaux, a desirable alignment that allows unequivocal discrimination from spheres. Selected fractions were concentrated 8–10-fold using 10-kDa cutoff Microcon concentrator (Amicon, Beverly, MA) and then negatively stained on carbon-filmed grids using 2% neutral sodium phosphotungstate. Carbon films were freshly made and particle suspensions selectively stained on carbon-filmed grids using 2% neutral sodium phosphotungstate. Grids were immediately examined, and electron micrographs taken in a JEM 100CX transmission electron microscope (JEOL Inc., Tokyo, Japan) operated at 80 kV. Prints at a final magnification of 100,000× were prepared and captured via video camera into an Image1/AT image analyzer (Universal Imaging Corp, West Chester, PA). After thresholding the particles, size frequencies and particle counts were made by automated quantitative morphometry. Samples of 500–1000 particles were routinely analyzed.

Primary Rat Cortical Neuronal Culture and Toxicity Studies—Primary cortical neurons were prepared from embryonic day 17 Harlan Sprague-Dawley rats as described previously (32, 33). Briefly, the uteri were removed from the gravid rat under anesthesia. Cortices were dissected and diced into small pieces. Cells were dissociated and maintained in minimum essential medium (Sigma) supplemented with 10% fetal bovine serum. For mevastatin-induced neuronal toxicity assays, cells were seeded on polylysin-coated 96-well plates at 5 × 10^4 cells/well in a growth medium. One day after the initial plating, the growth medium was replaced with minimum essential medium supplemented with 3% fetal bovine serum. Triplicate wells of neuronal cells were then treated with 50 μM mevastatin (Sigma) with or without additions of isolated BV2 microglial particles for 48 h. Quantification of apoE levels in BV2 apoE-containing particles was performed by Western analysis against purified mouse apoE. Neuronal cell viability was assayed 48 h after the start of the treatments using an MTS assay kit from Promega (Madison, WI). Loss of cell viability was quantified by the decrease in the ability of cells to metabolize the dye 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS). In this system, MTS is bioreduced by living cells into soluble formazan. The quantity of formazan produced is directly proportional to the number of living cells in culture. Scios is certified with the American Association for Accreditation of Laboratory Animal Care; hence, these experiments were reviewed by an Institutional Animal Use Care Committee to ensure justification of animal use and proper handling of animals.

Aβ Binding—Aβ association with lipoproteins was assessed using previously published procedures (34, 35). Briefly, 250 μM synthetic Aβ1–40 (Bachem, Torrance, CA) was incubated with BV2-conditioned medium for 2 h at 27 °C, after which the material was fractionated by
SEC as described above. Equal volumes of fractions eluting from the column were analyzed by Western blot using a human Ab-specific monoclonal antibody (Senetek, Maryland Heights, MO). Twelve percent reducing Tricine urea SDS-PAGE and non-reducing Tricine urea SDS-PAGE (not equivalent to native PAGE) were employed to electro-phoretically separate the proteins in each fraction.

RESULTS

Secretion of ApoE and ApoJ by BV2 Microglial Cells—The BV2 cell line was established by immortalization of murine microglia by infection with the J2 retrovirus (25) and has been shown to retain properties of microglia (5, 26–28). To determine if BV2 microglia synthesize and secrete any apolipoproteins, serum-free media were harvested after conditioning for 12, 24, and 48 h and subjected to Western blot analysis using an anti-murine apoE antibody and an anti-rat apoJ antibody as well as immunological probes for other apolipoproteins. Western blot analysis revealed that there was a time-dependent accumulation of a 33-kDa apoE in the medium from BV-2 cells incubated from 12 to 24 h (Fig. 1). Prolonged conditioning up to 48 h resulted in a slight increase in 33-kDa apoE, but with prominent accumulation of apoE fragments, most notable at 21 kDa, which we presume to be degradative products of the 33-kDa protein. The size of the immunoreactive 33-kDa protein is consistent with the molecular mass of apoE. To ensure antimurine apoE antibodies recognize authentic apoE synthesized and released from BV2 cell cultures, immunoprecipitation was performed, and the protein species at 33 kDa corresponding to the apparent molecular mass of mature apoE was subjected to N-terminal amino acid sequencing. The sequence analysis revealed that the 33-kDa species had an N terminus, EGEPEVT, that matched the terminus of the mature apoE sequence. Western blotting the same amount of medium with anti-apoJ antibody detected a protein species with apparent molecular mass at 70 kDa, the size of uncleaved apoJ holoprotein (36) (also called clusterin, SP40, 40, or sulfated glycoprotein-2). Similar to apoE, there was a time-dependent accumulation of apoJ in the medium (Fig. 1). Interestingly, the majority of apoJ remained stable, in contrast to apoE, which showed degradation in the 48-h-conditioned medium. Additional Western analyses failed to detect other apolipoproteins,
including apoAI, apoAII, or apoCIII (data not shown). Thus, apoE and apoJ appear to constitute the major apolipoproteins synthesized and released by the BV2 murine microglial cells. This observation is supported and extended by results with human primary microglia from both AD and non-demented elderly individuals, which were also shown to synthesize and secrete apoE and apoJ.

Discrete Apolipoproteins and Lipid Peaks after Fractionation of BV2 Medium—To fractionate the BV2 microglial medium in a manner that would allow for the isolation of intact lipoproteins, we adapted SEC utilizing tandem Superose-6 columns. The columns were first evaluated for their ability to fractionate defined sizes of lipoproteins present in mouse and human plasma. To determine the distribution of defined sizes of lipoproteins, aliquots of isolated fractions were assayed for phospholipid and total cholesterol across the elution profile, whereas albumin, a marker for free protein elution, was monitored by Coomassie Blue staining. As seen in Fig. 2, this SEC system clearly separates the various classes of lipoproteins in both human and mouse plasma from free protein. These profiles are comparable with previous reports for the distribution of plasma lipoproteins (37).

To isolate and identify lipoprotein particles released by BV2 microglial cells, we chose to use 24-h-conditioned medium that we previously determined to have an accumulation of intact apolipoproteins. Concentrated serum-free conditioned medium was fractionated using the above-described system, and selected samples were separated on reducing SDS-PAGE for probing with apoE and apoJ antibodies across the elution profile (Fig. 3A). Elution of albumin, indicative of free protein, peaked around fraction 53 (data not shown). Although apoE was detectable as early as fraction 9, the majority of apoE immunoreactivity eluted in fractions 43–51, which corresponds to an HDL-like size particle. In contrast, apoJ eluted from fractions 25–47, with the peak located at fraction 35, which corresponds to a small LDL-like size particle. Elution of these discrete apolipoprotein peaks ahead of the free protein peak suggests that apoJ and apoE are associated with lipoprotein particles. It has been shown that apoJ is often associated with HDL-like particles in human plasma (38) and rat astrocyte-conditioned medium (30). The early elution of apoJ observed here suggests that the apoJ released from BV2 microglial cells might be associated with particles larger than HDL.

To further characterize these lipoprotein species, the fractions used for Western blot analysis were assayed for total cholesterol and phospholipid (Fig. 3B). Levels of lipid peaked around fractions 9 and 31, corresponding in size to VLDLs and small LDLs plasma particle, respectively. Since the VLDL-like lipid peak contained low levels of apolipoproteins (Fig. 3A), it was not analyzed further. The small LDL-like lipid peak (eluting ahead of the major apoJ and apoE peaks and highly enriched for apoJ relative to apoE) contained cholesterol and phospholipid (Fig. 3A). In contrast, the HDL-like material rich in apoE was found to have only modest amounts of lipid (Fig. 3A). These data suggested that BV2 microglial cells release two different particles with distinct lipid and apolipoprotein profiles.

Spherical Particles in LDL-like ApoJ-rich ApoE-poor Lipid Peak—To study the morphology of the particles secreted by BV2 microglial cells, three discrete fractions released by BV2 microglial cells and fractionated by SEC were negatively stained for analysis by electron microscopy. Abundant lipoprotein particles were observed in the LDL-like apoJ-rich apoE-poor lipid-containing peak (Fig. 4A). Since there was no evidence of disc rouleaux, these BV2 microglia-derived particles appeared to be spherical, in contrast to rat astrocyte-derived discoidal particles (30). Careful examination of electron micrographs at low magnifications from either fractionated samples or non-fractionated medium that was dialyzed against a volatile buffer revealed no disc structures. Size-frequency analysis showed that the LDL-like particles have an average diameter at 18.28 nm (S.D. = 7.38 nm) (Fig. 4B), consistent with the elution of these particles at small LDL size upon SEC (Fig. 3). A bimodal distribution of particles is suggested from the histogram in Fig. 4B. Whether this represents two distinct populations or merely a broad size distribution for the particle is unclear. There were significantly fewer particles in the HDL-like apoJ-rich apoE-poor fraction and none in the HDL-like apoE-rich fraction (data not shown), suggesting that these species may be composed largely of protein aggregates.

ApoJ as a Major Apolipoprotein Associated with LDL-like Particles—To further investigate the nature of the microglial LDL-like particles from BV2 cultures, the material was fractionated on a 4–20% native PAGE in comparison with proteins of defined radii, other BV2 fractions, mouse HDL lipoprotein, and purified mouse apoE (Fig. 5). Coomassie Blue staining of size standards demonstrated that a 4–20% gradient native gel is adequate to separate particles with radii of 3.5 to 8.5 nm. We found that the majority of the LDL-like particles migrated with a size most similar to murine HDL particles. These small LDL-like lipoproteins contained primarily apoJ immunoreactivity and small, yet significant amounts of apoE immunoreactivity. The co-migration of apoE and apoJ immunoreactivity suggests that BV2 microglial cells may secrete LDL-like particles containing both apoE and apoJ, although it is possible that two discrete particles exist in this population, one composed of apoJ and one with only apoE. The broad electrophoretic mobility suggests heterogeneity in these microglial LDL-like particles. Such heterogeneity is a typical feature for HDL-like apoJ-containing lipoprotein in plasma and astrocyte-derived particles (30, 38).

---

2 B. Cordell, Q. Xu, and J. Rogers, unpublished observation.
LDL-like BV2 Particles Rescue Primary Cortical Neurons from Mevastatin-induced Toxicity—To determine whether the isolated spherical LDL-like BV2 particles are functional, we examined the ability of these particles to rescue mevastatin-induced neuronal toxicity in embryonic primary rat cortical neurons. Mevastatin, an inhibitor of 3-hydroxyl-3-methylglutaryl-CoA dehydrogenase, suppresses de novo cholesterol synthesis and has been shown to induce neuronal cell death in a dose- and time-dependent manner (33). The addition of 50 μM mevastatin to the rat cortical neuronal cultures effectively induced ~90% loss of neuronal viability by 48 h as measured by MTS assay (Fig. 6). The addition of purified BV2 LDL-like particles (containing 0.3 μg/ml apoE) to the neuronal culture in the presence of 50 μM mevastatin restored 55% neuronal viability relative to the mevastatin treatment (Fig. 6). Under the same conditions, the addition of equal amounts of control buffer in which the LDL-like apoJ-rich apoE-poor particles were stored did not significantly modulate neuronal viability, as expected. Further analysis indicated that the HDL-like apoE-rich BV2 fraction was ineffective in blocking mevastatin-induced neuronal toxicity (data not shown). Taken together, effective rescue of mevastatin-induced neuronal toxicity by the small LDL-like BV2 particles clearly demonstrates that these native microglial particles can function in supplying cholesterol to the compromised neurons.

BV2 Lipoprotein Particles Associate with Aβ—We examined whether the lipoprotein species released by BV2 microglial cells were competent in binding Aβ using established methods (34, 35). Synthetic human Aβ was incubated with BV2-conditioned medium, after which the medium was fractionated by SEC gel filtration chromatography to separate the different lipoprotein species. Fractions eluting from the column were analyzed on reducing and non-reducing SDS-PAGE using Western blotting with the Aβ-specific monoclonal antibody, 4G8. These non-reducing conditions are such that formerly intact particles separated by the gel filtration step will be disrupted. Aβ reactivity eluted primarily in particle-containing fractions when analyzed under non-reducing conditions (Fig. 7B). The majority of the Aβ immunoreactivity was present in the high molecular mass range, indicating that Aβ is complexed with large molecular mass species. Reactivity to moderate size entities in the high molecular mass range is presumably due to binding to contaminating serum proteins. Aβ immunoreactivity also eluted in the free protein fraction of the gradient at ~60–20 kDa, suggesting protein association, as well as at ~4 kDa, reflecting unbound Aβ. Upon reduction, interestingly, the Aβ associated with the lipoprotein-containing fractions was found to be primarily dimeric, whereas the Aβ eluting with the free protein was monomeric (Fig. 7A). This suggests that a specific form of Aβ associated with the lipoprotein particles.

**DISCUSSION**

The availability of the BV2 microglial cell line provides a valuable tool with which to elucidate the role of microglia in the evolution of AD pathology. Our analysis is the first demonstration that BV2 microglial cells synthesize and secrete apoE and apoJ as well as lipoprotein particles. These murine microglial cells are capable of releasing small spherical LDL-like apoJ-rich apoE-poor lipoprotein particles. Although both apoE and apoJ bind Aβ and are colocalized to both diffuse and mature plaques within the AD parenchyma (20, 39–41), emerging data suggest distinct roles for apoE and apoJ in Aβ fibrillogenesis. Whereas apoE promotes Aβ fibrillogenesis in vitro (41, 42) and Aβ deposition in vivo (20, 43), apoJ has been shown to slow the formation of Aβ aggregates in vitro (44) and may, therefore, prevent soluble Aβ from forming pathological fibrils. In view of these findings and the key role microglia appear to play in the development of senile plaques, the lipoprotein particles released from microglia are likely to play a role in this disease. BV2 microglial LDL-like particles are dissimilar to astrocyte- and cerebrospinal fluid-derived particles in size, shape, and apolipoprotein abundance (30, 31, 45). The microglial particles are more lipid-dense, giving rise to their spherical shape, and larger than the discoidal HDL-like particles released by astrocytes. BV2 microglial LDL-like apoJ-rich apoE-poor particles have an average diameter of 18.28 nm. In comparison,
the astrocyte-derived HDL-like lipoproteins are 15.4 nm in diameter and contain apoE and apoJ as the predominant protein components (30). The lipoproteins present in the cerebrospinal fluid are also spherical; however, they contain other apolipoproteins, such as apoAI and apoAII, in addition to apoE and apoJ.

Effective rescue of primary neuronal cells from mevastatin-induced neurotoxicity by the LDL-like microglial particles demonstrates that these particles, over other types of particles released by the BV2 microglial cells, are biologically active. Mevastatin, an inhibitor of 3-hydroxyl-3-methylglutaryl-CoA reductase, induces neuronal cell death by depleting de novo cholesterol synthesis (33). Since the viability of neuronal cells, unlike nonneuronal cells, depends on intracellular cholesterol and not on the intermediate nonsterol isoprenoid products, the LDL-like microglial particles are able to function in delivering the required cholesterol, attenuating the mevastatin-induced neurotoxicity. In view of the functions of apoE and apoJ in lipid transport and recycling (38, 46), the microglial LDL-like particles, in this scenario, may simply provide a cholesterol source to support neuronal viability. Alternatively, the LDL-like particles may attenuate neuronal apoptosis that is caused by mevastatin exposure (33). ApoJ, the major apolipoprotein in these particles, has been implicated in cell death, particularly apoptotic cell death (47). Following injury, apoJ expression is upregulated at sites undergoing tissue remodeling occurring in conjunction with apoptosis (47–50). The apoJ carried on these microglial particles might serve a function to protect against neuronal injury.

Release of distinct particles varying in apolipoprotein composition and lipid abundance by BV2 microglial cells raises an interesting question regarding lipoprotein assembly. The two lipid-poor HDL-like microglial particles might result from incomplete particle assembly. It seems possible that these particles could be modified post-secretion by the addition of lipids and lipoproteins in the local environment. For example, remodeling of nascent apoJ-lipoproteins occurs in plasma and in HepG2 cell medium (51). Furthermore, apoE and cholesterol have been reported to be independently secreted from macrophages and, after release, associate to generate HDL particles (25). Hence, both of these HDL-like BV2 particles may be the recipients of exogenous lipid and/or protein.

Current data support the involvement of apoE in Aβ deposition and clearance (43), and the particles released by BV2 cells are likely to play a role in Aβ catabolism. The role may be different for the LDL-like spherical particles carrying apoJ as the major apolipoprotein from the role of the HDL-like apoE-rich particles. We have found that the microglial LDL-like particles can associate with synthetic Aβ (dimer over monomer); hence, these particles could mediate cellular clearance of Aβ through interaction with lipoprotein receptors. Clearance of these Aβ-associated microglial particles may occur via multi-functional cell surface receptors expressed in the brain, including the LDL receptor, LDL receptor-related protein, the very low density lipoprotein receptor, apoER2, and gp330. Although all of these receptors bind apoE-rich lipoproteins, gp330 is identified as the only receptor recognizing apoJ and apoJ-Aβ complexes (52, 53). Although it remains unclear whether the microglial LDL-like apoJ-rich apoE-poor particles interacts with apoE receptors, it is tempting to speculate that these apoJ-rich particles could potentially facilitate Aβ clearance and degradation via gp330. It has been suggested that gp330 can mediate internalization of Aβ-apoJ complexes, prevent intracellular Aβ aggregation, and promote Aβ lysosomal degradation (55). Moreover, since the gp330 receptor distribution is highly prominent in the choroid plexus (54), it seems possible
that these microglial particles may facilitate transport of \( \beta \) out of the brain. Since \( \beta \) can bind to this microglial particle, clearance via gp330 may represent a primary mechanism for removal of \( \beta \). In contrast, the HDL-like apoE-rich particles may contribute to the deposition of apoE in AD brain. The intimate physical association of microglia with plaque development, the required role for apoE in plaque formation (43), and our observation that this secreted apoE-rich microglial particle can bind \( \beta \) support this concept. The relative contribution of these two different microglial particles as well as those secreted by astrocytes to \( \beta \) deposition and clearance remains to be elucidated.

Acknowledgments—We thank Mary Jo LaDu and Asha Naidu for advice and Carmen M. Bryant for providing technical assistance.

REFERENCES

1. Rogers, J., Webster, S., Lue, I. F., Brachova, L., Civin, W. H., Emmerling, M., Shivers, B., Walker, D., and McGeer, P. (1996) Neurobiol. Aging 17, 681–696.
2. Griffen, W. S. T., Sheng, J. G., Royston, M. C., Gentleman, S. M., McKenzie, J. E., Graham, D. I., Roberts, G. W., and Mrak, R. E. (1998) Brain Pathol. 8, 65–72.
3. Mackenzie, I. R. A., and Munoz, D. G. (1998) J. Neuropathol. Exp. Neurol. 57, 823–835.
4. Ishida, A., Yamaguchi, H., Ogawa, A., Sugihara, S., and Nakazato, Y. (1997) J. Neurosci. Res. 48, 141–145.
5. Davis, J. B., McMurray, H. F., and Schubert, D. (1992) Biochem. Biophys. Res. Commun. 187, 712–717.
6. Overmyer, M., Helisalmi, S., Soininen, H., Laakso, M., Riekkinen, P., and Mattson, M. P. (1999) J. Biol. Chem. 274, 20967–20971.
7. Chung, H., Brazil, M. I., and Cotman, C. W. (1992) Brain Res. 582, 223–233.
8. Sheng, F. G., Mrak, R. E., and Griffen, W. S. T. (1995) Neuropharmacology 34, 269–271.
9. Rosenmuller, J., Eichenbohm, P., Stam, F. C., Beyreuther, K., and Masters, C. L. (1989) J. Neuropathol. Exp. Neurol. 48, 674–691.
10. Sasaki, A., Yamaguchi, H., Ogawa, A., Sugihara, S., and Nakazato, Y. (1997) Acta Neuropathol. 93, 30001–30007.
11. Overmyer, M., Helisalmi, S., Soininen, H., Laakso, M., Riekkinen, P., and Mattson, M. P. (1999) J. Biol. Chem. 274, 23201–23208.
12. Sheng, F. G., Mrak, R. E., and Griffen, W. S. T. (1996) Neuropharmacology 35, 316–322.
13. Schmechel, D. E., Saunders, A. M., Strittmatter, W. J., Crain, B. H., Julette, B., C., Joo, S. H., Pericak-Vance, M. A., and Roses, A. D. (1996) Lancet 348, 90–93.