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Apolipoprotein E Genotype Regulates Amyloid-β Cytotoxicity

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The ε4 allele of apolipoprotein E (ApoE) is a risk factor for Alzheimer’s disease (AD), whereas the ε2 allele may be relatively protective. Both alleles are risk factors for cerebral amyloid angiopathy (CAA)-related hemorrhages. CAA is associated with degeneration of smooth muscle cells and pericytes. Previously, we described that synthetic amyloid-β, a dose-dependent increased risk for AD and an earlier age of onset of AD (Corder et al., 1993; Saunders et al., 1993), although a genotype-dependent regulation of γ-secretase activity. The one cell culture with the ε2/ε3 genotype was relatively resistant to Aβ-mediated toxicity compared with other cultures. Furthermore, we observed a dose-dependent protective effect of native ApoE against Aβ-mediated toxicity of cerebrovascular cells and, in addition, ApoE ε2/ε3 cells secreted more ApoE protein compared with cells with other ApoE genotypes, in particular, compared with ε4/ε4 cells. Thus, the disparity between ApoE genotype and Aβ-mediated toxicity might be related to differences in the cellular capacity to secrete ApoE. The present data suggest that one mechanism by which ApoE may alter the risk for AD is a genotype-dependent regulation of Aβ cytotoxicity, possibly via variations in its secretion levels, whereby extracellular ApoE may bind to Aβ and thereby modify Aβ-mediated cell death.

Key words: apolipoprotein E; Alzheimer’s disease; amyloid-β protein; cerebrovascular cells; CAA; cytotoxicity

Introduction

Senile plaques (SPs) and cerebral amyloid angiopathy (CAA) are two of the neuropathological hallmarks of Alzheimer’s disease (AD). The amyloid-β protein (Aβ), which consists of 40–42 amino acids, is the major constituent of both SPs and CAA. Apolipoprotein E (ApoE) has been identified as a major risk factor for the sporadic late-onset form of AD. The ApoE gene has three alleles (ε2, ε3, and ε4), the most frequent of which is the ε3 allele. Inheritance of one or two copies of the ε4 allele is associated with a dose-dependent increased risk for AD and an earlier age of onset of AD (Corder et al., 1993; Saunders et al., 1993), although individuals homozygous for ε4 may reach a high age without cognitive impairment (Henderson et al., 1995). On the contrary, the ε2 allele seems to have a protective effect against AD (Corder et al., 1994). Surprisingly, however, both the ε2 and the ε4 alleles are associated with an increased risk for CAA or CAA-related hemorrhages (Greenberg et al., 1996, 1998; Premkumar et al., 1996; Nicoll et al., 1997).

Inheritance of one or two copies of the ApoE ε4 allele is associated with a higher load of SPs and CAA (Schmechel et al., 1993; Premkumar et al., 1996). In vitro studies have shown that ApoE is able to bind to Aβ (Strittmatter et al., 1993; Pillot et al., 1999); native ApoE3 binds Aβ more efficiently than ApoE4 (LaDu et al., 1994). ApoE may also accelerate the formation of Aβ fibrils (Strittmatter et al., 1993; Sanan et al., 1994; Wisniewski et al., 1994). In mice transgenic for human amyloid precursor protein (APP) and deficient in ApoE, the amount of amyloid, but not that of Aβ immunoreactivity, was strongly reduced compared with transgenic APP/ApoE-/- mice (Bales et al., 1999), suggesting that ApoE plays a critical role in facilitating Aβ deposition. In contrast to these studies, early Aβ deposition is suppressed in mice transgenic for both APP and human ApoE on an ApoE knock-out background (Holtzman et al., 1999). These results show that the role of ApoE and its specific isoforms in Aβ fibrillogenesis, deposition, or clearance still remains unclear.

Previously, we described the toxicity of various Aβ peptides toward cultures of human brain cerebrovascular smooth muscle cells (SMCs) and human brain pericytes (HBP) (Davis-Salinas et al., 1995; Davis and Van Nostrand, 1996; Verbeek et al., 1997). We demonstrated that nonaggregated wild-type Aβ1–42 and Aβ1–40 carrying the Glu→Gln mutation at position 22 of Aβ, as...
in hereditary cerebral hemorrhage with amyloidosis-Dutch type, caused degeneration of cultured SMCs and HBPs. Either cell type secreted significant amounts of ApoE (Verbeek et al., 1999). Given the well described interactions between ApoE and Aβ, we investigated whether endogenously produced ApoE could interfere in an isoform-dependent manner with the Aβ-mediated cytotoxicity toward cultured human cerebrovascular cells. Therefore, in this study, we performed a comparative analysis of the cytotoxic effect of Aβ on cultures of HBPs and SMCs with different ApoE genotypes.

Materials and Methods

Reagents. Aβ with the “Dutch” mutation ([22 Glu→Gln; D-Aβ40,42] 89% pure) was purchased from Biosource (Etten-Leur, The Netherlands). Human recombinant ApoE produced in baculovirus in Sf insect cells was obtained from PanVera (Madison, WI). ApoE obtained from the conditioned medium of transfected human embryonic kidney 293 (HEK293) cells was generously provided by Dr. G. W. Rebeck (Georgetown University). Anti-low-density lipoprotein receptor-related protein (LRP) (β-chain) antibody was obtained from American Diagnostics (Antwerpen, Belgium). Anti-low-density lipoprotein receptor-related protein (LRP) (β-chain) antibody was obtained from American Diagnostics (Antwerpen, Belgium).

Cells. Pericytes were isolated from human brain tissue, and SMCs were isolated from leptomenigeal vessels from a number of AD patients and neurologically unaffected individuals, obtained after autopsy as described previously (Verbeek et al., 1994, 1995, 2002) (Table 1). Diagnosis and grading of AD patients was performed according to the criteria established by Braak and Braak (1991), Mirra et al. (1994), and The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer’s Disease (1997). CAA grading was established by quantification of the number of Aβ-positive vessels in one microscopic field (magnification, 2.5×). At least four microscopic fields of both the occipital cortex and the gyrus temporalis medius were analyzed and categorized as follows: 0 (−), no CAA), 0–10 (+, sparse CAA), 10–20 (++, moderate CAA), and >20 (+++, severe CAA).

Both HBPs and SMCs were maintained in Eagle’s modification of essential medium (EMEM) supplemented with 10% human serum (Sanquin Blood Bank, Nijmegen, The Netherlands), 20% newborn calf serum (Invitrogen, Paisley, UK), recombinant basic fibroblast growth factor (1 ng/ml), heparin (5 U/ml; Organon, Boxtel, The Netherlands), and antibiotics. Immunoelectron microscopic detection of Aβ accumulating at the surface of cultured cells was performed as described previously (Renssink et al., 2002, 2004). With respect to the various types of experiments, we did not observe differences between cerebrovascular cell cultures with the same ApoE genotype but derived from different types of patients (i.e., AD or control patients).

Degeneration experiments. Triplicate wells with cultured cells were preincubated with serum-free medium (EMEM with 0.1% BSA and antibiotics) for 4 h. Subsequently, cells were incubated with fresh serum-free medium supplemented with synthetic Aβ peptides at 25 μM for 6 d. Cells were routinely inspected and photographed using an Olympus (Tokyo, Japan) phase-contrast microscope. Cell viability was quantitated using a fluorescein live/dead cell assay according to the manufacturer (Molecular Probes, Leiden, The Netherlands). The cultures were examined using an Olympus fluorescence microscope, and the percentage of dead cells was determined from counting in at least five microscopic fields per well. Based on previous studies (Davis and VanNos- kendal, 1996; Verbeek et al., 1997), D-Aβ40 was used, because this peptide induced a robust degeneration of both pericytes and SMCs.

Western blot analysis. Cells were grown in serum-free medium, and both culture supernatant and cell lysates were collected to examine secretion and cell-associated expression of proteins, respectively. Culture supernatant was diluted 1:1 with reducing sample buffer. Cells were washed twice with PBS and then solubilized in the wells with lysis buffer [50 mM Tris-HCl, 150 mM NaCl, pH 7.5, 1% SDS, 5 mM EDTA, 500 μM 4-(2-aminoethyl)benzenesulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml chymostatin] for 15 min. The protein content of diluted samples of the cell lysates was determined using the bicinchoninic acid method (Pierce, Rockford, IL). Equal protein amounts were loaded, fractionated on SDS-polyacrylamide gels, and subsequently electrophoretically transferred to nitrocellulose membranes (Schleicher and Schuell, Hertogenbosch, The Netherlands) in blotting buffer (25 mM Tris-HCl, pH 8.6, 192 mM glycine, and 20% methanol). Blots were washed for 15 min in PBS containing 0.05% Tween 20 (PBST), preincubated with blocking solution (5% low-fat milk powder in PBST), washed three times with PBST, and subsequently incubated with primary antibodies and peroxidase-labeled secondary rabbit anti-mouse antibodies (Dako). Detection was performed by chemiluminescence according to the description of the manufacturer (Boehringer Mannheim, Almere, The Netherlands) and exposure to Kodak (Rochester, NY) X-OMAT-R films.

ApoE genotyping. DNA was isolated from small pieces of brain tissue, from which cell cultures were also derived, using a DNA isolation kit (Biozym, Landgraaf, The Netherlands). ApoE genotype was determined using PCR and HhaI restriction analysis according to methods described previously (Hixson and Vernier, 1990; Wenham et al., 1991).

ApoE quantification. ApoE secretion by cultured HBPs and SMCs was quantitated both by scanning of Western blots and by ELISA. The chemiluminescent signal of both ApoE standards (PanVera, Madison, WI), cell lysates, and culture supernatants obtained after Western blotting was scanned with a LabScan (Amer sham Biosciences, Aylesbury, UK). For ELISA, 5 μg/ml affinity-purified goat anti-ApoE (Biodesign International, Saco, ME) was coated overnight at 4°C on a 96-well plate. Subsequently, wells were incubated with 1% nonfat milk in PBST to block

Table 1. Overview of the source of HBP and SMC cell cultures

<table>
<thead>
<tr>
<th>Number</th>
<th>Diagnosis</th>
<th>Grade (Braak)</th>
<th>Grade (CERAD)</th>
<th>CAA grade</th>
<th>Cell type</th>
<th>ApoE</th>
<th>Age</th>
<th>Gender</th>
<th>Cause of death</th>
<th>Vascular risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>II</td>
<td>+ + +</td>
<td>−</td>
<td>HBP/SMC</td>
<td>5</td>
<td>2/3</td>
<td>F</td>
<td>Cardiac failure</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>2</td>
<td>AD VI</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP</td>
<td>4</td>
<td>3/3</td>
<td>F</td>
<td>Respiratory insufficiency</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>3</td>
<td>AD VI</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP</td>
<td>4</td>
<td>3/3</td>
<td>F</td>
<td>Cardiac failure</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>AD VI</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP/SMC</td>
<td>3</td>
<td>3/3</td>
<td>M</td>
<td>Pneumonia</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>−</td>
<td>HBP/SMC</td>
<td>4</td>
<td>3/3</td>
<td>F</td>
<td>Pneumonia</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>−</td>
<td>HBP/SMC</td>
<td>4</td>
<td>3/3</td>
<td>F</td>
<td>Ruptured aortic aneurysm</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>−</td>
<td>HBP/SMC</td>
<td>4</td>
<td>3/3</td>
<td>F</td>
<td>Pneumonia</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>8</td>
<td>AD/PD V</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP</td>
<td>5</td>
<td>3/4</td>
<td>F</td>
<td>Cardiac failure</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>9</td>
<td>AD III</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP/SMC</td>
<td>4</td>
<td>3/4</td>
<td>F</td>
<td>Pneumonia</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>III</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP/SMC</td>
<td>4</td>
<td>3/4</td>
<td>F</td>
<td>Pneumonia</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>11</td>
<td>AD VI</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP/SMC</td>
<td>3</td>
<td>3/4</td>
<td>F</td>
<td>Pneumonia</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>12</td>
<td>AD VI</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP</td>
<td>5</td>
<td>4/4</td>
<td>F</td>
<td>Sepsis</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>13</td>
<td>AD VI</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>HBP/SMC</td>
<td>3</td>
<td>4/4</td>
<td>M</td>
<td>Unknown</td>
<td>Atherosclerosis</td>
</tr>
</tbody>
</table>

F, Female; M, male; PD, Parkinson’s disease; PMI, postmortem interval in hours. Grading of AD (Braak and CERAD scores) and CAA was performed as described in Materials and Methods.
specific binding, with ApoE standards (PanVera, or from transfected HEK293 cells) or (diluted) culture supernatants, with affinity-purified biotinylated goat anti-ApoE (Biodesign International), and finally with peroxidase-labeled avidin (Dako). Tetramethylbenzidin was used as substrate, and H2SO4 was used to terminate the peroxidase reaction. No significant differences were observed with either type of standards; the use of ApoE3 as a standard only resulted in slightly higher signals than with ApoE4.

Partial purification of ApoE from culture supernatant. Purification of extracellular ApoE protein from medium of cultured HBPs was performed as described by DeMattos et al. (1999) with minor modifications. Cells were incubated with serum-free EMEM medium for 6 d. Cells remained viable during this period. Supernatant was collected and passed through a D100 weakly basic anion exchange filter (Sartorius, Goettingen, Germany) and eluted with 1 M ammonium bicarbonate. The eluted ApoE was recirculated over a HiTrap heparin column (Amersham Biosciences). Fractions containing ApoE were pooled and dialyzed against PBS. Using the ApoE sandwich ELISA described above and comparing the ApoE concentration with the total protein amount, it was demonstrated that, in the purified fractions, 25% of the total protein amount consisted of ApoE.

RNA isolation and Northern blot analysis. Total RNA (8 µg) was isolated from cultured cells by using the RNeasy kit (Qiagen, Hilden, Germany) according to the protocol of the manufacturer. Total RNA was treated with formaldehyde (Sambrook et al., 1989), separated on a 1.5% agarose gel, and blotted onto a Hybond N+ membrane (Amersham Biosciences). A 1100 bp fragment containing human ApoE3 cDNA (a kind gift from Dr. G. W. Rebeck, Georgetown University) was released from a pCMV4 expression vector by KpnI restriction cleavage. ApoE cDNA probe was radiolabeled by 32PdCTP incorporation using a random-primed DNA labeling kit (Roche Diagnostics, Penzberg, Germany). After preincubation in a hybridization mix (0.25 M sodium phosphate buffer, pH 7.2, 7% SDS, 1% BSA, 1 mM EDTA, and 0.1 mg/ml single-strand salmon sperm DNA) at 65°C, membranes were hybridized overnight with the radiolabeled probes under the same conditions. Blots were then washed at 58°C with 0.25 M sodium phosphate buffer, pH 7.2, 1% SDS, and 1 mM EDTA and autoradiographed using Kodak X-Omat-S films.

Results

HBPs and SMCs were isolated from 10 and 9 donor brains, respectively. The characterization of HBPs and SMCs was described extensively in previous studies (Verbeek et al., 1994, 2002). The cultured cells were divided into four different groups according to their ApoE genotype: ε2/ε3, ε3/ε3, ε3/ε4, and ε4/ε4 (Table 1).

After 4–6 d of treatment with 25 µM, D-Αβ1–40 cultured cells (HBPs or SMCs) lost their characteristic polygonal shape, individual cell contours became less evident, and signs of cellular atrophy were observed (data not shown). Because ApoE is codeposited with amyloid in the vessel walls in CAA, we determined whether ApoE production by perivascular cells was affected by D-Αβ1–40 treatment as well. In the absence of Αβ treatment, both cultured SMCs and HBPs secreted considerable amounts of ApoE into the culture medium, as shown by Western blot analysis (Fig. 1). In contrast, smaller amounts of ApoE were associated with the cells. In contrast, incubation with D-Αβ1–40 resulted in an inversion of ApoE distribution, with most of the ApoE remaining associated with the cells. This was confirmed by double-immunofluorescence staining of D-Αβ1–40-treated cells, showing that both Αβ and ApoE were coassociated on the cell surface (data not shown).

These experiments suggested that an interaction occurred between the endogenously produced ApoE and the exogenously administered Αβ that accumulated on the cell surface (Fig. 2). Therefore, we investigated whether the ApoE genotype of the cultured cells affected the toxicity of D-Αβ1–40. The results showed that there were striking differences in the response to D-Αβ1–40 between cell cultures with a different ApoE genotype. After treatment of HBPs cultures with the ε3/ε3 genotype with D-Αβ1–40, for 6 d, the number of viable cells was strongly reduced (Fig. 3). In contrast, cells with either the ε2/ε3 or the ε3/ε4 genotype were consistently less susceptible to D-Αβ1–40 treatment. In addition, although only a single ε2/ε3 HBP culture was available, we consistently observed (n = 9 experiments) that cell death was much lower (mean, 12 ± 10% dead cells). In addition, similar results were obtained with SMC cultures of various ApoE genotypes (data not shown). Both HBPs and SMCs expressed the ApoE receptor LRP. The effects described above, however, could not be explained by differences in LRP expression of the cells, which was similar in the cell cultures with different ApoE genotypes (data not shown). Furthermore, the effects also could not be explained by differences in the clinicopathological state of the donors [Braak or The Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) grade of AD, CAA grade] (Table 1).
These data suggested that ApoE protein modulated the association of Aβ with the cell surface and subsequent cellular degeneration. We therefore investigated whether simultaneous incubation of cells with D-Aβ1-40 and ApoE would lead to an ApoE isoform-dependent effect on HBP death. Addition of either ApoE3 or ApoE4 (up to 1.6 μg/ml), obtained from supernatants of transfected HEK293 cells, resulted in a slight but nonsignificant decrease in D-Aβ1-40-induced cell death (Fig. 4, left). The addition of any of the three ApoE isoforms (between 1.5 and 12 μg) produced by baculovirus in Sf insect cells yielded a small but significant decrease in cell death of cultured HBPs (Fig. 4, middle). Remarkably, when HBP cultures were coincubated with D-Aβ1-40 and conditioned supernatant of either e2/e3 or e4/e4 HBP cultures, a dramatic decrease in cell death was observed (Fig. 4, right). This effect was stronger when culture supernatant was used from e2/e3 cells compared with that from e4/e4 cells. Addition of ApoE, semipurified from conditioned HBP culture supernatants (up to 20 μl) containing 0.25 μg of ApoE, resulted in a dose-dependent decrease in cellular toxicity (Fig. 5).

We then studied whether the differential response of HBPs with different ApoE genotypes could be explained by differences in the ApoE production capacity of the cells. ApoE production by cells with the e4/e4, e3/e4, or e3/e3 genotype was not significantly different (p = 0.55; ANOVA), although we observed a trend toward lowest production by HBP e4/e4 cultures (Fig. 6). In two of three experiments, HBP e4/e4 cultures produced the smallest amounts of ApoE of all cultures. Interestingly, although based on only one HBP culture, cells with the ApoE e2/e3 genotype produced a mean 5.0 ± 3.1 ng of ApoE per gram of protein per day (n = 3 experiments), which is a factor threefold to 12-fold higher than the other cell cultures (p = 0.02 vs ApoE e3/e3; p = 0.21 vs ApoE e3/e4; p = 0.01 vs ApoE e4/e4). Similar results were obtained with SMC cultures of varying genotype, with the exception of one SMC e3/e3 culture that consistently secreted high ApoE levels comparable with that of the e2/e3 cell cultures. In the SMC cultures, cells with the ApoE e2/e3 genotype produced a mean 13.9 ± 5.4 ng of ApoE per microgram of protein per day (n = 3 experiments), which is a factor 4–12 higher than the other cell cultures (p = 0.01 vs ApoE e3/e3; p < 0.001 vs ApoE e3/e4; p = 0.02 vs ApoE e4/e4; data not shown). Effects on ApoE production by the cultured cells were not correlated to differences in the clinicopathological state of the donors (Braak or CERAD grade of AD, CAA grade) (Table 1).

Finally, ApoE mRNA levels in both HBP and SMC cultures were analyzed using Northern blot analysis. Although we did not observe a perfect match between protein secretion and mRNA levels, this analysis showed that the cells with the e2/e3 genotype contained the highest levels of ApoE mRNA, whereas the cells with the e4/e4 genotype contained the lowest levels (Fig. 7). There was some heterogeneity in the mRNA levels in the e3/e3 and e3/e4 cells. The same SMC culture with the e3/e3 genotype that secreted large amounts of ApoE protein also contained a high level of ApoE mRNA, comparable with that in the e2/e3 cell culture.
Discussion

Because of the initial reports of the association of the ApoE ε4 genotype with the development of AD, many research groups have tried to identify a biological explanation for this epidemiological observation. ApoE binds to Aβ and affects its fibrillogenesis, possibly in an isoform-specific manner (LaDu et al., 1994; Ma et al., 1994; Wisniewski et al., 1994). In other studies, isoform-specific neurotrophic or neurotoxic effects for ApoE have been described previously (Holtzman et al., 1995; DeMattos et al., 1998; Sun et al., 1998). In the present study, we provide a novel explanation that may, in part, account for the association of ApoE with AD development. The major findings of this investigation are as follows. First, the degree of Aβ-mediated toxicity of human cerebrovascular cells is dependent on the ApoE genotype of the cells. Cultured HBPs with an ApoE ε4/ε4 genotype were more vulnerable to Aβ-mediated cytotoxicity than cells with other ApoE genotypes. Second, ApoE protein causes a direct, concentration-dependent inhibition of Aβ-mediated toxicity of human cerebrovascular cells. Third, the production level of ApoE protein may, at least in part, depend on the ApoE genotype.

It has been suggested that in the brain, a balance exists between Aβ deposition and Aβ removal. Aβ removal from the brain may occur by several mechanisms. For example, ApoE may play a role in internalization and subsequent degradation of Aβ and in Aβ clearance via transport to the circulation. Aβ may be internalized and degraded by smooth muscle cells (Urmoneit et al., 1997) and by astrocytes (Koistinaho et al., 2004), a process that is facilitated by ApoE, because astrocytes derived from ApoE−/− mice do not degrade Aβ deposits in the brain. Alternatively, Aβ may be cleared from the brain via transport across the blood–brain barrier (BBB). LRP-1 binds free Aβ and possibly is the major receptor mediating Aβ internalization (Urmoneit et al., 1997; Koistinaho et al., 2004) and its efflux from the brain to the circulation (Shibata et al., 2000). LRP-1 most efficiently removes wild-type Aβ1–40, whereas Aβ peptides containing a higher percentage of β-sheet structures (such as wild-type Aβ42 and D-Aβ1–40) bind less efficiently, leading to accumulation of Aβ in brain microvessels (Deane et al., 2004). ApoE, which is also a ligand for LRP-1, may facilitate the clearance of Aβ from the brain, because clearance was significantly reduced in ApoE−/− mice (Shibata et al., 2000). Therefore, it is possible that at least part of the cerebral Aβ forms complexes with ApoE and that such complexes via ApoE–LRP-1 interactions may be either degraded by astrocytes or cleared from the brain by transport over the BBB. However, although the existence of such complexes in the brain has been demonstrated previously (Russo et al., 1998), their possible interaction with LRP-1 has not yet been studied.

Thus, high cerebral concentrations of ApoE may inhibit deposition of Aβ in the form of SPs and CAA. Our in vitro data suggest that, at least at the vascular level, ApoE abrogates the pathological interaction of Aβ with vascular cells and retains Aβ in a conformation that does not result in its assembly at the cell surface, possibly by a diminished interaction with LRP-1. These findings are in line with the observation that human ApoE, in an isoform-specific way, suppresses Aβ deposition in both young and aged APP-V717F transgenic mice (Holtzman et al., 1999; Fagan et al., 2002). Furthermore, human ApoE delays the shift in Aβ from soluble pools to
insoluble pools in these animals, which is in agreement with our observations of decreased accumulation of Aβ at the cell surface in the presence of elevated ApoE concentrations (Fagan et al., 2002).

Thus, relatively high concentrations of cerebral ApoE are beneficial. It can be anticipated that the levels of free cerebral ApoE are reflected by its concentration in CSF. It has been reported, however, that the CSF concentration of ApoE is similar in both control and AD groups with different ApoE genotypes (Lehtimaki et al., 1995; Yamauchi et al., 1999), although a trend toward elevated ApoE levels in patients carrying the e2 allele was observed (Lehtimaki et al., 1995). However, because the applied assays have all been designed to detect unbound ApoE, it remains possible that the levels of CSF Aβ–ApoE complexes will be different among individuals with different ApoE genotypes.

It has been suggested by Nicoll et al. (1997) and Greenberg et al. (1998) that both the e2 and e4 alleles are risk factors for CAA-related hemorrhages, the latter by increasing the vascular amyloid load and the former by increasing the susceptibility of the vessel for rupture. By incorporating these suggestions in our in vitro model, one would predict increased cell death in cells carrying the e2 allele, because this would lead to vessel weakening by (Aβ-mediated) degeneration of SMCs or HBPs. However, our in vitro data show less cytotoxicity associated with cells possessing an e2 allele. Perhaps the e2 allele promotes other changes leading to vessel rupture, such as increased extracellular proteolysis.

ApoE produced by cerebrovascular cells may play a prominent role in the pathogenesis of CAA. Second only to the liver, the brain is the organ that produces the largest amount of ApoE. Astrocytes are regarded as the major source of cerebral ApoE (Boyles et al., 1985), although neurons may be able to produce ApoE as well (Xu et al., 1999). Here, we demonstrated that both pericytes and SMCs secrete ApoE and, because both are located at the abluminal side of the blood–brain barrier, they may contribute to the pool of cerebral ApoE. This may be particularly important with regard to the etiology of CAA. An attractive model explaining the pathogenesis of vascular Aβ deposition has been proposed by Weller et al. (1998), who suggested that Aβ of neuronal origin might be transported from the brain parenchyma to the vasculature by drainage of interstitial fluid. Experimental support for this model was provided by the transgenic mice studies by Calhoun et al. (1999), who demonstrated extensive CAA formation in APP transgenic mice under the control of the neuronal Thy-1 promoter. This model of CAA formation requires the availability of local factors that enhance or otherwise stimulate Aβ deposition in the vasculature. The availability of vessel-derived ApoE, but possibly also factors such as heparan sulfate proteoglycans (van Horssen et al., 2001), may regulate Aβ clearance and/or deposition in the vasculature. Our data suggest that when ApoE is available in high-enough concentrations, Aβ may remain solubilized and be processed for clearance, whereas if ApoE is available in restricted amounts only, Aβ will be prone to deposition and cell-surface aggregation in the vessel wall.

The degree of lipidation of the ApoE molecule is crucial to its biological activities. Circulating ApoE is tightly bound to several classes of lipoproteins, such as high-density lipoprotein and very-low-density lipoprotein. It was demonstrated previously that lipitated and unlipitated ApoE differentially interact with Aβ, suggesting that the degree and type of lipidation of ApoE are of critical importance (LaDu et al., 1994). In accordance with this study, we observed differences in the reduction of Aβ-induced cellular toxicity of HBPs by ApoE, dependent on its source; ApoE, especially when secreted by HBPs, seemed very efficient. Previous studies by DeMattos et al. (1998) and Sun et al. (1998) showed that ApoE is secreted by cultured cells as specific lipid-containing particles different from those in CSF (LaDu et al., 1998). Our data suggest that between different cell types (HEK293 cells vs HBPs/SMCs), the degree of ApoE lipidation may vary. The recombinant ApoE from PanVera is posttranslationally modified, but lipidation of the molecule is probably also different in SF insect cells compared with HBPs.

Our comparative analysis of ApoE protein secretion and mRNA levels by the respective HBP and SMC cell cultures suggests that not only the ApoE genotype but also other unstudied factors may affect ApoE secretion (e.g., ApoE promoter polymorphisms) (Artiga et al., 1998) or factors involved in lipid metabolism. Furthermore, although we did not collect any evidence in this direction, we cannot completely exclude the possibility that the original cerebral environment of the cells (i.e., AD, control, or other diagnosis) contributes to the cellular phenotype in vitro. Finally, future studies on other cerebral cell types will have to reveal to what extent ApoE will affect other cellular responses to Aβ, including neuronal cell death and glial cytokine production.

References


