Journal of Neurochemistry, 2003, **85**, 1455–1467 doi:10.1046/j.1471-4159.2003.01788.x

Microglial apolipoprotein E and astroglial apolipoprotein J expression *in vitro*: opposite effects of lipopolysaccharide

Josep Saura,*,†,[1] Valerie Petegnief,†,[1] Xin Wu,† Yanbin Liang† and Steven M. Paul*,†

*Department of Pharmacology, Toxicology and Psychiatry, Indiana School of Medicine, Indianapolis, Indiana, USA
†Neuroscience Discovery Research, Lilly Research Laboratories, Indianapolis, Indiana, USA

Abstract

Apolipoprotein E (apoE) and apoJ are lipid carriers produced in the brain primarily by glial cells. A variety of glial-activating stimuli induce a parallel upregulation of both apolipoproteins expression *in vivo* and *in vitro*. To further characterize the cell type and mechanisms by which apoE and apoJ expression are upregulated in activated glia, mixed glial cultures from neonatal rat cortex were treated with the endotoxin lipopolysaccharide (LPS). LPS induced dose-dependent increases in apoJ and decreases in apoE expression and secretion with maximum effects at 1–10 ng/mL and 0.1–1 μg/mL, respectively. Experiments with enriched astroglial and microglial cultures demonstrated that apoE and apoJ expression are predominantly microglial and astroglial, respectively. Given the pivotal role that nuclear factor-κB (NF-κB) plays in glial activation, we assessed its possible role in mediating apoE and apoJ expression by activated glia. LPS robustly increased NF-κB activation in mixed glial cultures. Two NF-κB inhibitors, aspirin (10 mM) and MG-132 (0.1 μM), blocked basal apoE and apoJ secretion as well as LPS-induced apoJ secretion. These data demonstrate that glial apoE and apoJ expression are independently regulated by LPS in microglia and astroglia, respectively, and that activated microglia are the predominant source of apoE in mixed glial cultures. The transcription factor NF-κB appears to be a critical mediator of LPS-stimulated apoJ expression from astroglia.

Keywords: apolipoprotein E, apolipoprotein J, astrocytes, glial activation, microglia, NF-κB.
J. Neurochem. (2003) **85,** 1455–1467.

Apolipoprotein E (apoE) and apolipoprotein J (apoJ; also known as clusterin among many other names) are components of plasma and cerebrospinal fluid (CSF) lipoprotein particles (Holtzman *et al*. 1999b). By binding to specific receptors they direct the utilization and clearance of lipids contained in lipoprotein particles by various cells and tissues. ApoE has recently attracted the attention of neuroscientists as it has been consistently shown to be a major susceptibility gene for late-onset Alzheimer's disease (AD; Strittmatter *et al*. 1993). While it is still unclear exactly how apoE contributes to AD pathogenesis, we have recently reported a marked reduction in amyloid-β peptide (Aβ) deposition and an almost complete absence of thioflavine-S-positive amyloid in transgenic mice over-expressing a human mutant amyloid precursor protein transgene that lack apoE (Bales *et al*. 1997, 1999b). Expression of human apoE in these mice suppressed early thioflavine-S-negative Aβ deposition (Holtzman *et al*. 1999a) but resulted in fibrillar Aβ deposits at a later age (Holtzman *et al*. 2000). These data suggest that apoE is critical for Aβ fibrillization into amyloid plaques in brain. In line with these findings, it has previously been shown that apoE is an avid Aβ binding protein (Wisniewski *et al*. 1993) and is localized to amyloid plaques in AD brain (Namba *et al*. 1991; Kida *et al*. 1994). Interestingly, apoJ is also an Aβ binding protein (Wisniewski *et al*. 1993) and reportedly the major Aβ binding protein in human CSF (Golabek *et al*. 1995). *In vitro* reports suggest an anti-amyloidogenic role for apoJ as apoJ binding to Aβ prevents Aβ aggregation and polymerization *in vitro* (Oda

Resubmitted manuscript received February 28, 2003; accepted March 4, 2003.

Address correspondence and reprint requests to Steven M. Paul, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA. E-mail: Paul_Steven_M@Lilly.com

[1]JS and VP equally contributed to this work.

Abbreviations used: Aβ, amyloid-β peptide; AD, Alzheimer's disease; apoE, apolipoprotein E; apoJ, apolipoprotein J; CSF, cerebrospinal fluid; DIV, days *in vitro*; DTT, dithiothreitol; GFAP, glial fibrillary acidic protein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PBS, phosphate-buffered saline; PMSF, phenylmethylsulphonyl fluoride.

et al. 1995; Matsubara *et al*. 1996), whereas *in vivo* data point to a proamyloidogenic role for apoJ (DeMattos *et al*. 2002). Understanding how apoE and apoJ expression and secretion are regulated in brain may therefore be critical to understanding the role these two apolipoproteins play in the pathogenesis of AD. ApoE and apoJ are highly expressed in brain (Elshourbagy *et al*. 1985; de Silva *et al*. 1990), mainly in glial cells (Boyles *et al*. 1985; Murakami *et al*. 1988; Pasinetti *et al*. 1994), and both apolipoproteins are upregulated when glia are activated by neuronal injury (Messmer-Joudrier *et al*. 1996; Page *et al*. 1998; Schauwecker *et al*. 1998; Petegnief *et al*. 2001) possibly to mediate the lipid transport required for membrane repair and recycling.

Nuclear factor-κB (NF-κB) has been shown to be a key transcription factor in glia which upon activation in the cytosol translocates to the nucleus and transactivates the transcription of proinflammatory cytokine genes (O'Neill and Kaltschmidt 1997). We and others (Bales *et al*. 1998; Akama *et al*. 1998; Akama and Van Eldik 2000; Yates *et al*. 2000) have previously demonstrated that exposure of cultured glia to Aβ robustly increases NF-κB activation and expression of proinflammatory cytokine genes. Although apoE and apoJ have also been reported to be upregulated upon glial activation induced by various stimuli, little is known about the possible involvement of NF-κB in this process.

In the current study, we have further examined the effects of NF-κB inhibitors on apoE and apoJ expression and secretion by glial cells activated *in vitro* with lipopolysaccharide (LPS), a widely used *in vitro* model of glial activation. In neurodegenerative disorders, glial activation is most often induced by neuronal injury and in the case of AD probably also by Aβ. Differences may exist in the activated state of glia depending on the inducing stimulus and therefore caution should be exercised when extrapolating the LPS effects to the pathological condition. However, LPS-induced glial activation results in typical features of glial activation induced by inflammatory stimuli such as NF-κB activation (Nishiya *et al*. 1995; Pahan *et al*. 1997; Petrova *et al*. 1999), upregulation of proinflammatory cytokines (Lieberman *et al*. 1989; Kong *et al*. 1997; Pahan *et al*. 1997), inducible nitric oxide (NO) synthase and increased NO release (Boje and Arora 1992; Pahan *et al*. 1997). We observed that LPS exposure markedly induces apoJ secretion and expression in mixed glial cultures, but unexpectedly decreased apoE expression. Using cultures highly enriched in astrocytes or microglia, we found that the effects of LPS on apoE and apoJ were primarily due to its effect on microglia and astrocytes, respectively. Finally, we observed that NF-κB inhibitors reduce basal apoE secretion from microglia and markedly suppress LPS-induced apoJ secretion from astroglia.

Materials and methods

Materials

Cell culture media, serum, antibiotics, Trizol reagent and Superscript-II kit were from Life Technologies (Gaithersburg, MD, USA). Deoxyribonuclease I and LPS (055:B5) were from Sigma (St Louis, MO, USA). DiI-acetylated low-density lipoprotein (LDL) was from Biomedical Technologies Inc. (Stoughton, MA, USA). Protease inhibitor cocktail Complete and lactate dehydrogenase (LDH) release kit were from Roche Molecular Biochemicals (Indianapolis, IN, USA). BCA Protein Assay kit was from Pierce (Rockford, IL, USA). NuPAGE 4–12% Bis Tris and 6% DNA retardation gels were from Novex (San Diego, CA, USA). Hybond-ECL nitrocellulose membranes, ECL chemiluminescence kit, Hybond-NX nylon membranes, Ready To Go random priming kit and all radiochemicals were from Amersham Pharmacia Biotech (Piscataway, NJ, USA). The 28S rRNA internal control oligonucleotide was from Ambion Inc. (Austin, TX, USA).

Goat anti-human apoE and apoJ antibodies were from Chemicon (Temecula, CA, USA), mouse anti-rat clusterin α subunit antibody was from Upstate Biotechnology (Lake Placid, NY, USA), mouse ED-1 monoclonal antibody was from Serotec (Kindlington, UK), rabbit anti-cow glial fibrillary acidic protein (GFAP) antibody was from Dako (Glostrup, Denmark), mouse monoclonal anti-MAP-2 and anti-galactocerebroside-C were from Roche Molecular Biochemicals (Indianapolis, IN). All horseradish peroxidase-conjugated antibodies and antibodies for supershift experiments were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Human apoE was obtained from PanVera (Madison, WI, USA). SYTO-16 was from Molecular Probes (Eugene, OR, USA). The gel shift assay kit containing T4 kinase and transcription factors consensus sequences and the Griess Reagent System kit for NO determination were from Promega (Madison, WI, USA).

Cell cultures

Mixed glial cultures were prepared according to the method of Giulian and Baker (1986). Cerebral cortices from 1-day-old neonatal Sprague–Dawley rats were dissected, stripped of their meninges and digested for 30 min with 0.25% trypsin at 37°C. Trypsinization was stopped by addition of an equal volume of glial culture medium (Dulbecco's modified Eagle medium : F-12 nutrient mixture : fetal bovine serum 45 : 45 : 10, penicillin 100 U/mL and streptomycin 100 μg/mL) containing 0.02% deoxyribonuclease I. The solution was pelleted, resuspended in glial culture medium and brought to a single-cell suspension by repeated pipetting followed by passage through a 105-μm pore mesh. Cells were seeded onto 6- or 48-well plates at a density of 2.5×10^5 cells/mL and cultured at 37°C in humidified 5% CO_2– 95% air. Medium was replaced by 50% after 6 days *in vitro* (DIV). Cells reached confluency on 6–8 DIV and were used between 8 and 12 DIV.

To obtain enriched astroglial cultures a 75-mL flask of rat primary mixed glial cultures on 10–12 DIV was shaken for 2 h at 200 rpm, trypsinized (0.05%) and subcultured at low density (4×10^4 cells/mL) in 6- or 48-well plates with glial culture medium (see above). One day after subculturing, plates were manually shaken for 1 min and medium was fully replaced with glial culture medium. Cells reached confluency on 5–7 DIV and were used on DIV 7–10.

Table 1 Characteristics of the glial cell cultures used in this study

	Mixed glial	Astroglial	Microglial
% Astrocytes	75%	> 99%	< 1%
% Microglia	20%	< 1%	> 98%
% Oligodendrocytes	< 5%	< 1%	< 2%
% Neurons	< 1%	ND	ND
Cell density (total cells/mm^2)	1669 ± 406	374 ± 130	424 ± 184
Astroglial density (astrocytes/mm^2)	1251 ± 305	370 ± 129	< 6
Microglial density (microglia/mm^2)	334 ± 81	< 5	416 ± 180
Other	Primary Confluent	Secondary Confluent	Secondary Non-confluent

Total cell density was estimated by SYTO-16 staining. The proportion of the various cell types was estimated by immunocytochemistry with specific antibodies as indicated in Materials. All cultures were prepared from P1 rat cerebral cortex. ND, not detected.

Microglial cultures were prepared by shaking 75-mL flasks of rat primary mixed glial cultures on 7–8 DIV for 2 h at 200 rpm. Floating cells were pelleted and subcultured at 400 000 cells/mL in 6- or 96-well plates. One day after subculturing, medium was fully replaced by macrophage serum-free medium. Cells were used on 2 DIV.

The cell density as well as the percentage of the various cell types in mixed glial, astroglial and microglial cultures are listed in Table 1. The total cell density on the day of the experiment was estimated by counting cells stained with SYTO-16. Astrocytes were identified by GFAP immunocytochemistry, microglia by DiI-acetylated-LDL uptake (in live cultures) or by ED-1 immunocytochemistry (in fixed cultures), neurons by MAP-2 immunocytochemistry and oligodendrocytes by Gal-C immunocytochemistry.

In all experiments, the medium was completely changed to serum-free medium 16–24 h prior to LPS addition. Treatment with aspirin or MG-132 was carried out 6 h prior to LPS. LDH release and NO were determined with kits according to manufacturer's instructions.

Western blot analyses

For analysis of secreted apoE and apoJ, aliquots of 50 μL of conditioned medium were collected and, after addition of Complete protease inhibitor cocktail, aliquots were either stored at 4°C for immediate assay or frozen at − 20°C for subsequent analyses. For analysis of cellular proteins, cultures were washed with cold phosphate-buffered saline (PBS) and harvested in a lysis buffer [50 mM Tris–HCl, 150 mM NaCl, 1% Nonidet P-40, 1% sodium dodecyl sulfate (SDS), 0.5% deoxycholate] containing Complete protease inhibitor cocktail. Cell extracts were kept at − 20°C until protein determination was performed with the BCA Protein Assay kit.

Samples of conditioned medium (10 μL) or cell lysates (4 μg protein except for GFAP, 0.5 μg protein) were denatured and separated on a NuPAGE 4–12% Bis Tris gel under reducing conditions and transferred to a nitrocellulose sheet. Blots were blocked overnight at 4°C in PBS containing 5% non-fat dry milk followed by a 60-min incubation at room temperature with one of the following antibodies: goat anti-human apoE 1 : 3000, mouse anti-rat clusterin α subunit 1 : 400, mouse ED-1 1 : 300 or rabbit anti-cow GFAP. After 3 × 7-min washes in PBS− 0.05% Tween, blots were incubated for 60 min at room temperature in the appropriate horseradish peroxidase-conjugated antibody, washed extensively and developed by chemiluminescence (ECL). Autoradiograms were scanned with a Molecular Dynamics laser scanner and band density was quantified with IPLab Gel (Signal Analytics Corporation, Vienna, VA, USA) image analysis software. The linear range of the relationship between protein concentration and optical density of the autoradiogram was established in pilot experiments using known amounts of human apoE.

Northern blots

The apoE cDNA was prepared by RT–PCR using total RNA from rat cortical astrocytes. RNA was reversed transcribed using a Superscript-II kit and PCR performed on a PTC-100 thermal controller (MJ Research, Waltham, MA, USA) using 30 cycles of 55°C 2 min, 72°C 3 min and 94°C 1 min. The rat apoE probe used for the northern blots was 349 bp and was amplified with the forward primer 5′-TGATGGAGGACACTATGACGG and the reverse primer 5′-TCACGGATAGCCACTCACACC. The apoJ probe was produced by PCR from rat brain cDNA using the forward primer 5′-TTCTGGATGAACGGGGACCGCATC and the reverse primer 5′CGTTTAGCTCCTGGCGCAGGTTAG. The resulting amplified fragment is 533 bp. After PCR-product resolution by agarose electrophoresis, the cDNAs were purified from agarose slices and labeled with α-[^{32}P]dCTP using a random priming labeling kit. The probe for the 28S rRNA internal control is an oligonucleotide 5′ end-labeled with γ-[^{32}P]ATP using T4 kinase.

Six to 24 h after LPS treatment, mixed glial, pure astrocyte or microglia cultures were lysed with Trizol reagent for RNA isolation according to the phenol–chloroform protocol. RNA was isolated from two 950-mm^2 wells. For northern blot analysis, 5 μg of total RNA were separated on 1.3% agarose-formaldehyde gel, transferred to a nylon membrane and UV cross-linked. The membrane was prehybridized for 2–4 h at 42°C and hybridized overnight at 42°C with apoE probe. The hybridization solution consisted of 5 × saline–sodium phosphate–EDTA (SSPE), 10 × Denhart's, 50% formamide, 0.5% SDS and 0.1 mg/mL denatured salmon sperm DNA. Blots were washed with 2 × saline–sodium citrate (SSC)/0.1% SDS at 37°C for 30 min, 1 × SSC/0.1% SDS at 37°C for 30 min and 0.1 × SSC/0.1% SDS at 45°C for 15 min. After several strippings in a 0.1% SDS solution, membranes were briefly washed in 2 × SSC and hybridized with the apoJ probe at 42°C. Confirmation of equivalent RNA loading was done after a second stripping by rehybridizing membranes with the 28S rRNA probe.

Autoradiograms were scanned in a phosphoimager and quantified using the ImageQuant computer program (Molecular Dynamics).

Electrophoretic mobility shift assay

Nuclear proteins were extracted from mixed glial cultures at various times after treatment with 1 or 100 ng/mL LPS. Cells from two 950-mm^2 wells were scrapped in cold PBS, centrifuged for 4 min at 7000 *g*. The resulting pellet was resuspended in 400 μL of buffer A: 10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride (PMSF), 1 mM dithiothreitol (DTT) and 0.1 mM EGTA and cells were swollen on ice for 15 min. After addition of 25 μL of 10% Nonidet-40 (buffer B), cells were vigorously vortexed for 10 s. After a 10-min incubation on ice, the broken cells were successively spun for 10 min at 3000 *g* and for 5 min at 14 000 *g*. The pellet was resuspended in 30–50 μL of buffer C consisting of 20 mM HEPES pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF and 1 mM DTT. Solutions A, B, C and PBS were supplemented with 1 × Complete. After a 90-min gentle shaking at 4°C, nuclei were pelleted by a 5-min spin at 2000 *g*. The supernatant containing nuclear proteins was collected and protein amount was determined by BCA assay. The gel shifts were performed using the Promega gel shift assay kit: 3 μg of nuclear proteins were incubated for 20 min at 25°C with γ-[^{32}P]NF-κB consensus sequence (25 000 cpm/reaction assay) and separated by electrophoresis on 6% polyacrilamide gels in native conditions at 4°C. The composition of protein in the DNA-protein complexes was determined by supershift in presence of anti-p65 and anti-p50 antibodies for NF-κB. Competitive binding was performed by incubation with 20x of cold specific or unrelated consensus sequence DNA. Gels were dried and scanned in a phosphoimager. Optic density of the specific bands was quantified using IPLab Gel software.

Statistics

Differences in means of multiple groups were analyzed by ANOVA and individual group differences were assessed by ANOVA contrast. All data are means + SD. p-Values < 0.05 were considered significant. Data were analyzed with JMP (SAS Institute Inc., Cary, NC, USA).

Results

LPS decreases apoE and increases apoJ release in mixed glial cultures

Mixed rat glial cultures were treated with LPS and the apoE and apoJ content was analyzed in the medium at various points in time. LPS induced a significant decrease in extracellular apoE at 48 h, with a weaker effect at 24 h and no effect at 6 or 12 h (Fig. 1a). In contrast, LPS markedly increased apoJ levels in the medium. The effect was first observed 12 h after treatment with LPS and was very robust after 24 and 48 h (Fig. 1b). We next examined the concentration–response relationship for the effects of LPS on apoE and apoJ release measured in the medium at 48 h. As shown in Fig. 1(c), the LPS-induced decrease in

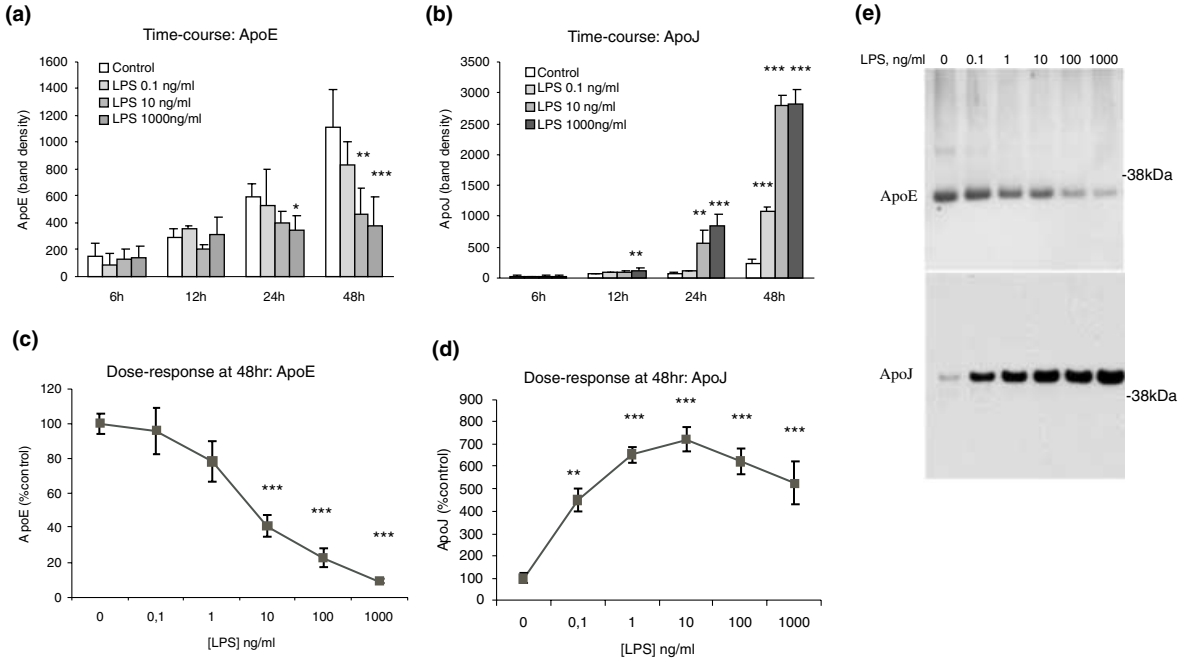

Fig. 1 Effect of LPS on apoE and apoJ secretion by mixed glial cultures. Time-course (a and b) and dose–response (c and d) of LPS-induced changes in secreted apoE (a and c) and apoJ (b and d). Samples from medium conditioned by mixed glial cultures were separated by electrophoresis and analyzed by western blot (see Materials and methods for details). Data are expressed as mean + SD of either four (a and b) or eight (c and d) independent determinations. In (a) and (b) *p < 0.05, **p < 0.01, and ***p < 0.001 indicate significant differences versus control. (e) Representative western blot of a dose–response experiment at 48 h.

apoE was concentration-dependent with a maximal effect at 1 μg/mL LPS (91% inhibition) and an IC_{50} of approximately 3 ng/mL. Interestingly, LPS was far more potent at increasing the level of apoJ than decreasing the level of apoE. The maximal effect on apoJ, a 7.2-fold increase, was obtained with 10 ng/mL and a half-maximal increase in apoJ was obtained at 0.1 ng/mL (Fig. 1d), a concentration that did not affect secreted apoE. At LPS concentrations higher than 10 ng/mL, apoJ levels typically reached a plateau, but in some experiments a moderate decrease was observed at ≥ 100 ng/mL. LPS was not toxic to mixed glial cultures at concentrations ≤ 1 μg/mL. The latter resulted in a 20% increase in LDH release (data not shown). From these experiments, the concentrations of 1 ng/mL and 100 ng/mL were selected to further characterize the LPS effects on apoJ and apoE, respectively.

The observed decrease or increase in apoE or apoJ induced by LPS in glial-conditioned medium, respectively, could be caused by a corresponding change in lipoprotein uptake or secretion. To address this question, we first analyzed intracellular apoE and apoJ levels in mixed glial cultures treated with LPS for 24 h. LPS at 1 and 100 ng/mL reduced the intracellular apoE content by 47–48% and increased the intracellular apoJ content by 5.7- to 6.2-fold (Fig. 2). These data are consistent with the effects of LPS on extracellular apoE and apoJ being caused by changes in synthesis and secretion rather than in uptake. We also analyzed the effects of LPS on two intracellular glial markers: GFAP, the prototypical astroglial marker, and ED-1, a lysosomal microglial marker. Whereas GFAP levels were unchanged by LPS, ED-1 levels were decreased by 1 ng/mL (− 28%) and 100 ng/mL (− 44%) of LPS (Fig. 2).

To confirm that the LPS-induced decrease in intracellular apoE and increase in intracellular apoJ proteins were preceded by changes in their corresponding mRNA levels, we analyzed total RNA from mixed glial cultures treated with 1 or 100 ng/mL LPS for 6, 12 or 24 h using northern blots. LPS at 100 ng/mL (but not at 1 ng/mL) decreased apoE mRNA levels by 40–55% and this effect was already apparent by 6 h (Fig. 3). In contrast, LPS induced a 2.7- and 4.5-fold increase in apoJ mRNA at 12 h and 48 h, respectively. As seen with intracellular and secreted apoJ, 1 and 100 ng/mL LPS had a similar quantitative effect on apoJ mRNA (Fig. 3).

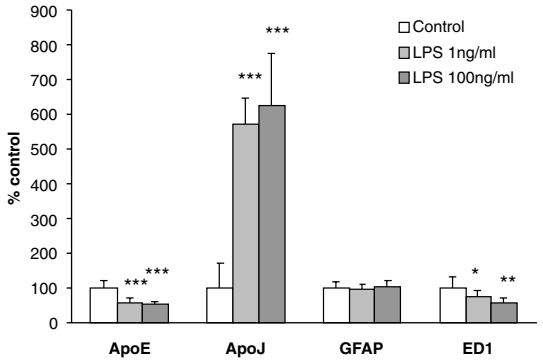

Fig. 2 Effect of LPS on intracellular apoE, apoJ, GFAP, and ED-1 in mixed glial cultures. Western blot analysis of intracellular proteins 24 h after 0, 1 or 100 ng/mL LPS (see Materials and methods for details). LPS induces a significant increase in intracellular apoJ and decreases in apoE and ED-1. Data are expressed as mean + SD of eight independent determinations. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

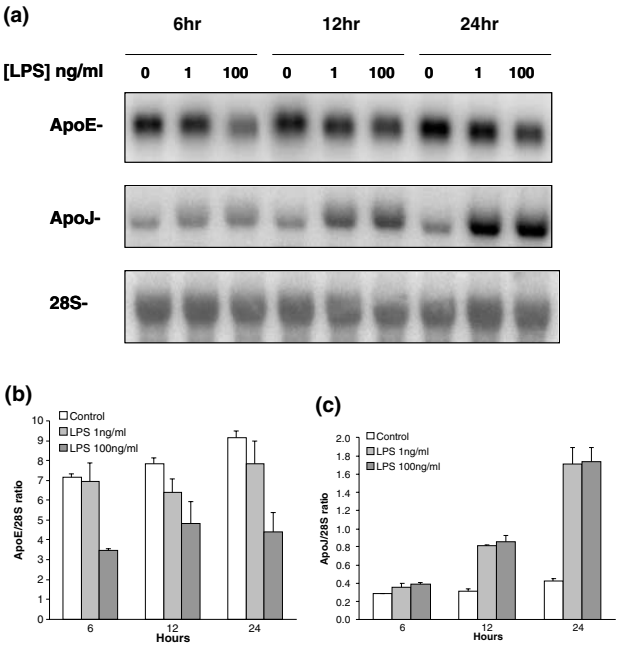

Fig. 3 Effect of LPS on apoE and apoJ mRNA expression in mixed glial cultures. (a) Northern blot analysis of apoE, apoJ, and 28S mRNA obtained from mixed glial cells treated for 6, 12 or 24 h with 0, 1 or 100 ng/mL LPS (see Materials and methods for details). (b and c) The quantitation of the northern blot bands, expressed as apoE/28S (b) and apoJ/28S (c) ratios. The values are the mean and range of two independent experiments.

LPS increases apoJ secretion and expression in astroglial cultures

The most abundant cell type in mixed glial cultures is the astrocyte, a cell type known to synthesize both apoE and apoJ. To explore whether the LPS-induced changes in apoE and apoJ secretion in mixed glial cultures could be explained solely by the actions of LPS on astrocytes, we analyzed apoE and apoJ levels in medium following LPS treatment of astroglial cultures. In control astroglial cultures, extracellular levels of apoE were very low, 0–4% of the levels found in medium conditioned by control mixed glial cultures. LPS did not induce significant changes in extracellular apoE in astroglial cultures (Fig. 4a). On the other hand, apoJ levels in

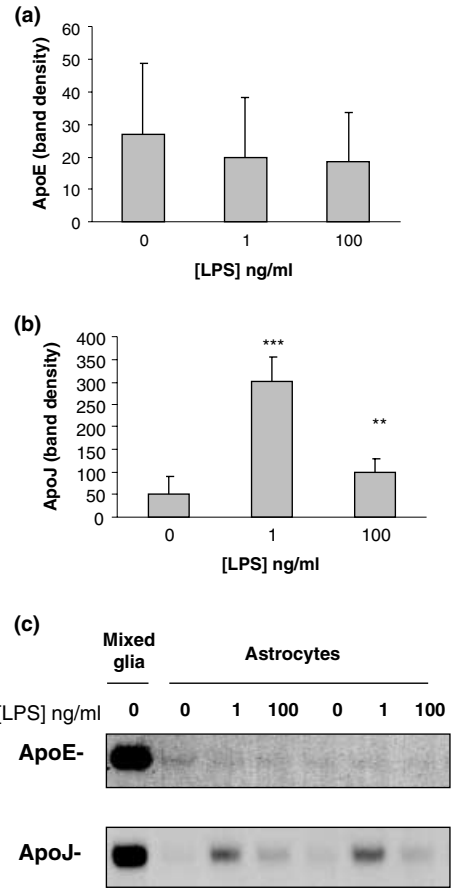

Fig. 4 Effect of LPS on apoE and apoJ secretion by astrocytes. Astroglial cultures were treated with 0, 1 or 100 ng/mL LPS and conditioned medium was sampled 48 h later and analyzed by western blot for apoE (a) and apoJ (b) content. Data in (a) and (b) are expressed as mean + SD of 12 independent determinations. $^{**}p < 0.01$ and $^{***}p < 0.001$ denote significant differences from control. (c) A representative image of apoE and apoJ levels in medium conditioned by LPS-treated astroglial cultures. For comparison a sample of medium conditioned by mixed glial cultures for 48 h was run in parallel.

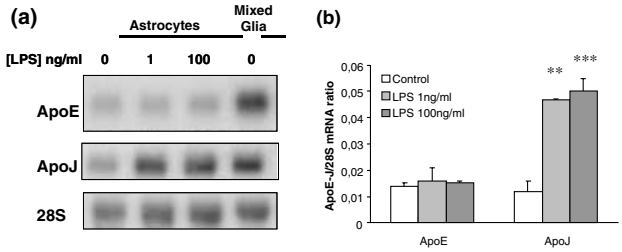

Fig. 5 ApoE and apoJ mRNA in astroglial cultures. (a) Northern blot analysis of apoE, apoJ, and 28S mRNA obtained from control mixed glial and astroglial and from astroglial cultures treated for 24 h with 1 or 100 ng/mL LPS. (b) The quantitation of the hybridization signals detected by northern blots. Data are expressed as apoE/28S and apoJ/28S ratios. The values are the mean and error bars SD ($n = 3$). $^{**}p < 0.01$ and $^{***}p > 0.001$.

control astroglia-conditioned medium were 5–10% of apoJ levels in control mixed glia-conditioned medium. However, in astroglial cultures, LPS treatment induced a marked increase in extracellular apoJ levels, especially at 1 ng/mL (5.9-fold increase) but also at 100 ng/mL (1.9-fold increase; Fig. 4b). We next analyzed apoE and apoJ mRNA levels in control and LPS-treated (24 h) astroglial cultures. ApoE mRNA levels in control astroglial cultures represented only 10.5% of apoE mRNA levels measured in control mixed glial cultures and, as seen for secreted apoE protein, they were unaffected by LPS treatment of 1 or 100 ng/mL (Fig. 5). By contrast, apoJ mRNA in astroglial cultures represented 30% of apoJ mRNA levels measured in control mixed cultures and were significantly increased 24 h after LPS treatment of 1 ng/mL (3.3-fold increase, $p < 0.01$) and 100 ng/mL (4.0-fold increase, $p < 0.001$; Fig. 5), also in agreement with the protein data. As seen in mixed glial cultures, LPS at 1 or 100 ng/mL did not induce cell death in astroglial cultures measured by LDH release (not shown).

LPS decreases apoE secretion by microglial cultures

In medium conditioned by control microglial cultures for 48 h, apoE levels were similar (80–200%) to those measured in medium conditioned by control mixed glial cultures for 48 h. Furthermore, LPS at 100 ng/mL, but not at 1 ng/mL, significantly reduced (− 63%) the apoE concentration in microglia-conditioned medium (Fig. 6a), similar to that induced by LPS in mixed glial cultures. In contrast, apoJ levels in medium conditioned by microglial cultures for 48 h were very low, representing 0–7% of apoJ levels in mixed glia-conditioned medium, and were unaffected by LPS (Fig. 6b). In microglial cultures apoE mRNA levels were similar (112%) to the levels in mixed glial cultures whereas no apoJ mRNA could be detected in microglial cultures (not shown). LPS did not induce toxicity in microglial cultures as analyzed by LDH release (not shown).

LPS induces morphological changes in microglia and stimulates NO release in mixed glial and microglial cultures

The LPS-induced decrease in ED-1 (Fig. 2) indicates a less phagocytic state of microglia that in some models, especially *in vivo*, is concomitant with a less activated state of microglia. In order to better characterize the state of activation of microglial cells after LPS we analyzed their morphology and NO release. LPS at 100 ng/mL, but not at 1 ng/mL, changed the morphology of microglial cells from round with few and short processes or no processes at all, to an elongated shape with more and longer processes. This pattern of microglial changes was observed both in isolated cultures (Fig. 7a) and in mixed glial cultures (not shown). The typical 'fried-egg' morphology and increase in size that

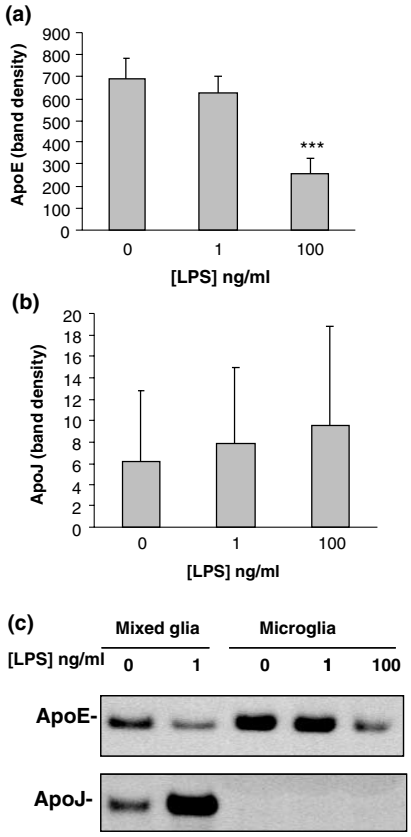

Fig. 6 The effect of LPS on apoE and apoJ secretion by microglia. Microglial cultures were treated with 0, 1 or 100 ng/mL LPS and conditioned medium was sampled 48 h later and analyzed by western blot for apoE (a) and apoJ (b) content. Data in (a) and (b) are the mean + SD of eight independent determinations. ***$p < 0.001$ denotes a significant difference with control. (c) A representative image of apoE and apoJ levels in medium conditioned by LPS-treated microglial cultures. For comparison samples of medium conditioned by mixed glial cultures for 48 h were run in parallel.

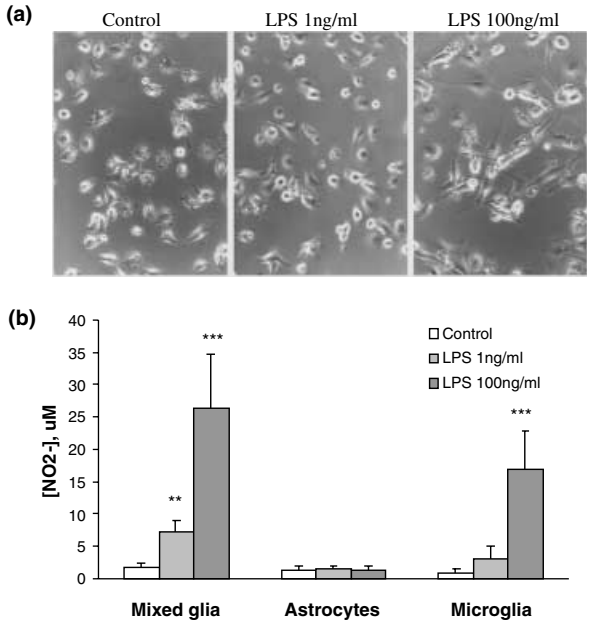

Fig. 7 LPS-induced changes in morphology and NO release. (a) Morphological changes induced by LPS in microglial cultures. Cells were treated with LPS or vehicle for 24 h. Note the absence of marked morphological changes after 1 ng/mL and the elongation and ramification of cells treated with 100 ng/mL. The same pattern of morphological changes was seen induced by LPS to microglia in mixed glial cultures (not shown). (b) LPS-induced NO release in mixed glial, astroglial and microglial cultures. NO_2^- concentrations were measured by the Griess method in medium conditioned by control or LPS-treated cultures for 48 h. Results are expressed as the mean of 4–8 experiments and error bars show SD. **$p < 0.01$ and ***$p < 0.001$ denote significant differences from control.

LPS induces in microglia at higher doses and that is associated with an increased phagocytic state was not observed.

LPS-induced NO release was measured by NO_2^- accumulation in conditioned medium for 48 h. The NO_2^- concentration in medium conditioned by control mixed glial, astroglial and microglial cultures was 1.6, 1.3 and 0.9 μM, respectively. In mixed glia LPS significantly increased NO release at 1 ng/mL (7.2 μM) and 100 ng/mL (26.2 μM). In microglial cultures, LPS induced a significant increase in NO release at 100 ng/mL (16.8 μM). In contrast, LPS did not induce changes in NO release by astroglial cultures (Fig. 7b).

LPS activates NF-κB in mixed glial cultures and NF-κB inhibitors suppress LPS-induced apoJ secretion

In mixed glial cultures, LPS induced an upregulation of two NF-κB-binding complexes that were identified by supershift experiments as p65/p50 and p50/p50 (Fig. 8). The activation of p65/p50 was maximal 2 h after LPS treatment and was sustained until at least 16 h (Fig. 8). A similar effect was observed for p50/p50 with a somewhat delayed time-course of activation (Fig. 8). LPS concentrations of 1 and 100 ng/mL were equally effective at inducing NF-κB activation. We next studied how NF-κB inhibitors affected basal and LPS (1 ng/mL)-induced changes on apoE and apoJ secretion. Aspirin (10 mM) and MG-132 (0.1 μM) significantly reduced apoE secretion in both control and LPS-treated (1 ng/mL) mixed glial cultures (−65% to −77%; Fig. 9). Basal apoJ secretion was reduced by aspirin (10 mM) but not by MG-132. LPS-induced apoJ secretion, however, was reduced by aspirin at 3 mM (−34%) and 10 mM (−95%) and by MG-132 at 0.03 μM (−31%) and 0.1 μM (−90%; Fig. 9). Gel shifts experiments showed that aspirin and MG-132 did not affect basal NF-κB activation. However, aspirin (10 mM) and MG-132 (0.1 μM) completely blocked LPS-induced activation of NF-κB (Fig. 9). Interestingly, MG-132 inhibited the LPS-induced translocation of p65/p50 and p50/p50 complexes, whereas aspirin only decreased p65/p50

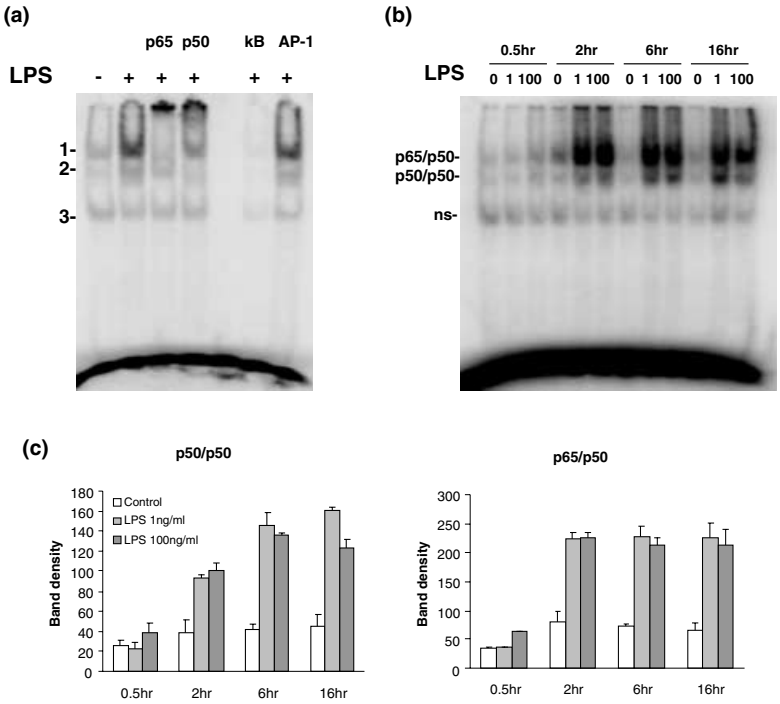

Fig. 8 LPS treatment induces NF-κB activation in mixed glial cultures. (a) Three DNA/protein retardation bands were seen in EMSA with control and LPS-treated mixed glial nuclear extracts. The anti-p65 antibody supershifted only band 1, whereas the anti-p50 antibody supershifted bands 1 and 2, indicating that band 1 corresponds to the p65/p50 complex and band 2 to the p50/p50 complex. All retardation bands were supressed by incubation with a 20-fold excess of the non-labeled NF-κB consensus-containing oligonucleotide, whereas a 20-fold excess of an oligonucleotide containing an AP-1 consensus site had no effect. (b) Nuclear extracts of mixed glial cultures treated with 0, 1 or 100 ng/mL LPS for 30 min, 2, 6 or 16 h were analyzed by NF-κB EMSA. Note that the p65/p50 and p50/p50 complexes are upregulated by LPS from 2 h on. (c) Quantitation of the p65/p50 and p50/p50 retardation bands from mixed glial cultures treated with LPS. Data show the mean and range of two independent experiments.

translocation. At the concentrations used, aspirin and MG-132 were not toxic to mixed glial cultures as assessed by LDH release (not shown).

Discussion

Our initial observation that LPS treatment upregulates apoJ but downregulates apoE in mixed glial cultures was to a certain extent unexpected. There have been reports of LPS-induced downregulation of apoE expression/release in macrophages (Werb and Chin 1983; Menju *et al*. 1989; Zuckerman and O'Neal 1994) and astrocytes (Mouchel *et al*. 1995) and of LPS-induced increases in apoJ expression and decreases in apoE expression in peripheral organs (Hardardottir *et al*. 1997). In a recent study on the effects of Aβ on glial apoE and apoJ secretion, LaDu *et al*. (2000) reported that LPS, at a concentration higher than those used in our study (10 μg/mL), stimulated apoJ secretion without affecting apoE secretion from astroglial cultures. However, in most studies apoE and apoJ levels have been shown to concomitantly increase upon glial activation, e.g. following rat striatal deafferentation by unilateral cortical ablation (Schauwecker *et al*. 1998), in excitotoxic neuronal injury induced by AMPA infusion into the rat hippocampus (Page *et al*. 1998) or in neuronal/glial spinal cord rat cultures treated with glutamate (Messmer-Joudrier *et al*. 1996). It therefore appears that in terms of apolipoprotein response, glial activation induced by LPS differs from that of most other models. Whereas apoJ expression is upregulated in all cases, suggesting that this is an intrinsic component of glial activation, apoE is upregulated in most but not all glial activation models, showing that upregulation of apoE expression is not an invariable feature of glial activation although it often accompanies it. What are the differences between glial activation induced by LPS and the other models that may explain the different apoE response? It is interesting to note that most previous reports describing upregulation of apoE expression or release by activated glia involve neuronal injury. It is possible that cell debris or factors released by injured neurons are necessary for apoE upregulation by activated glia (see Petegnief *et al*. 2001). The morphological changes and ED-1 decrease observed in microglia treated with LPS in the present study reflect a less phagocytic state and support an association between

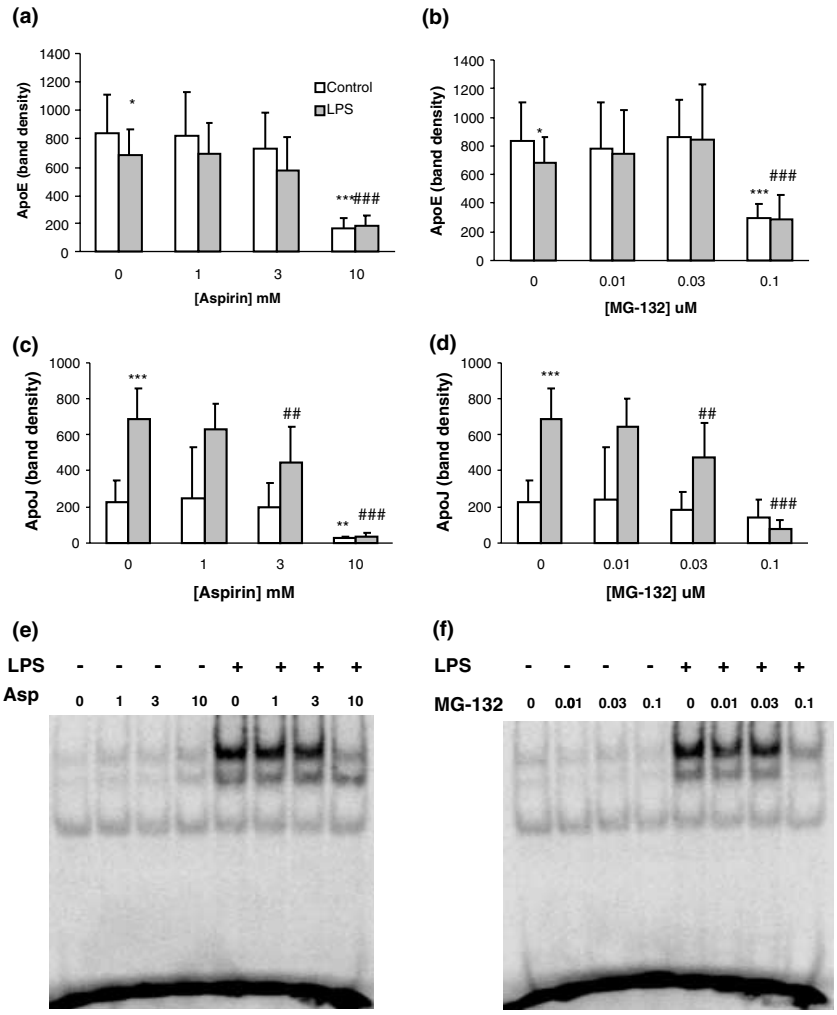

Fig. 9 Effects of NF-κB inhibitors on basal and LPS-induced glial apoE and apoJ secretion. Mixed glial cultures were treated with the NF-κB inhibitors aspirin (a and c) and MG-132 (b and d) in the absence or presence of LPS (1 ng/mL). Conditioned medium was sampled 48 h later and analyzed by western blot for apoE (a and b) and apoJ (c and d). The graphs show the mean + SD of 6–12 independent determinations. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ denote significant differences from control. $\#\#p < 0.01$ and $\#\#\#p < 0.001$ denote significant differences from LPS. (e and f) They confirm the inhibitory effects of aspirin and MG-132 on LPS-induced NF-κB activation. Nuclear extracts were obtained 12 h after addition of LPS (1 ng/mL). The images in E and F are representative of three independent experiments.

phagocytic activity and apoE expression. Another factor that may trigger apoE expression in some cases of glial activation is glial cell division, as LPS, in contrast to neuronal injury, activates glial cells without inducing cell proliferation (Suzumura *et al*. 1991; Lee *et al*. 1994; Casal *et al*. 2001).

Our finding of a completely opposite regulation of apoE versus apoJ expression and secretion by LPS-activated glial cells indicates that, at least in some cases, the physiological need for the two apolipoproteins is different. Little is known about the role of apoJ in the CNS but it is often assumed that apoE and apoJ, the two major CNS apolipoproteins, play a similar role in lipid trafficking within the CNS. Unlike apoE receptors, the only known receptor for mamalian apoJ (megalin/LRP2; Kounnas *et al*. 1995) is not expressed by neurons or glial cells, but by ependymal and endothelial cells (Kounnas *et al*. 1994). This different receptor localization suggests that brain apoJ could be involved in lipid blood–brain and brain–CSF transport, whereas brain apoE would be involved in neuron↔glia lipid transport (Zlokovic *et al*. 1996).

LPS increased apoJ secretion and expression in astroglial cultures, as in mixed glial cultures, but not in microglial cultures. On the other hand, LPS decreased apoE secretion and expression in microglial cultures, as in mixed cultures, but not in astroglial cultures. These results suggest that in mixed glial cultures apoJ expression is primarily astroglial in origin and is upregulated by LPS, whereas apoE expression is primarily microglial in origin and is downregulated by LPS. That astrocytes, and not microglia, synthesize and secrete apoJ is in agreement with previous reports (Garden *et al*. 1991; Danik *et al*. 1993; Pasinetti *et al*. 1994; Zwain *et al*. 1994; see, however, Xu *et al*. 2000). However, our finding that in mixed glial cultures microglia are the main apoE-producing cell type was unexpected. *In vivo* studies have shown that astrocytes contain apoE mRNA (Diedrich *et al*. 1991; Poirier *et al*. 1991; Lorent *et al*. 1995; Stone *et al*. 1997) and protein (Boyles *et al*. 1985; Pitas *et al*. 1987; Diedrich *et al*. 1991; Baskin *et al*. 1997), whereas only some microglial cells contain apoE mRNA (Poirier *et al*. 1991; Stone *et al*. 1997) or protein (Schmechel *et al*.

1996). Most studies conclude that astrocytes are the main apoE-producing cell in the brain (Danik *et al*. 1999 for review). However, a recent study showed that entorhinal cortex lesion induced an increase in apoE immunoreactivity in some astrocytes but the majority of apoE-positive cells were reactive microglia (White *et al*. 2001). *In vitro* it has also been shown that astrocytes produce apoE (Pitas *et al*. 1987; Baskin *et al*. 1997) and in microglia apoE mRNA has been detected (Nakai *et al*. 1996; Stone *et al*. 1997) but apoE secretion has been shown only in the BV2 cell line (Bales *et al*. 1999a; Xu *et al*. 2000). While our results confirm that astrocytes synthesize and secrete apoE, we show that in point of fact microglia produce more apoE than astrocytes in our cultures and they appear to be the main apoE-producing cell type in rat mixed glial cultures.

Microglia have been reported to be in a very activated state in pure microglial and mixed neonatal glial cultures as shown by their phagocytic activity and the expression of antigens characteristic of activation (Giulian and Baker 1986; Glenn *et al*. 1992). Clearly, this condition does not mimic their state in the healthy adult brain. It is possible therefore that astrocytes are the main source of apoE in non-diseased brain. However, our findings indicate that in pathological situations in which microglia become activated, e.g. following stroke, aging or AD, microglia may become a significant source of brain apoE. In this line, it is interesting to note that despite astrocytes express apoE in human control and diseased brain, in the close vicinity of senile plaques apoE is located in microglial cells but not in astrocytes (Shao *et al*. 1997; Uchihara *et al*. 1995), suggesting that microglial apoE may be especially relevant to AD pathogenesis.

NF-κB is a transcription factor that translocates to the nucleus in activated glial cells promoting the transcription of proinflammatory genes (O'Neill and Kaltschmidt 1997). NF-κB is thought to play a pivotal role in the inflammatory process in many cell types and, consequently, we examined the role of NF-κB in regulating apoE and apoJ expression by activated glia. We observed a strong activation of NF-κB in glial cells induced by LPS, in agreement with previous reports (Bonaiuto *et al*. 1997; Ferrari *et al*. 1997; Tanaka *et al*. 1997). This activation could be fully blocked by aspirin and MG-132, two NF-κB inhibitors with totally different mechanisms of action. Aspirin prevents the phosphorylation of IκB, an inhibitory protein that binds to NF-κB (Grilli *et al*. 1996) and MG-132 is a proteasome inhibitor (Nasuhara *et al*. 1999). Phosphorylation and subsequent degradation of IκB in the proteasome are essential steps in the mechanism of activation of NF-κB (Palombella *et al*. 1994; Bauerle 1998). In mixed glial cultures, both aspirin and MG-132 inhibited LPS-induced secretion of apoJ with the same potency they inhibited LPS-induced NF-κB activation, suggesting the involvement of NF-κB in mediating LPS-induced apoJ expression in astroglia. There are to our knowledge no previous reports of NF-κB regulating apoJ expression. By means of MatInspector 2.2 (Quandt *et al*. 1995) we have identified putative NF-κB binding sites in the apoJ gene promoter region (GenBank accession number M64733), which could mediate a direct activation of apoJ expression by NF-κB. However, the possibility that NF-κB may stimulate apoJ expression indirectly (e.g. by promoting the expression/release of glial cytokines) must be considered.

Finally, we also observed a marked reduction of glial apoE secretion induced by both NF-κB inhibitors. In line with this finding, we have previously reported that NF-κB inhibitors decrease glial apoE secretion induced by neuronal injury (Petegnief *et al*. 2001) and microglial apoE secretion induced by Aβ (Bales *et al*. 1999a). Taken together, these observations further support the involvement of NF-κB in mediating glial apoE expression. However, in microglia, LPS induced NF-κB activation but decreased apoE expression and release, indicating that factors other than NF-κB are able to suppress glial apoE expression, even in the presence of activated NF-κB. In the light of the proposed pro-amyloidogenic role for apoE in AD (Wisniewski *et al*. 1993; Bales *et al*. 1997, 1999b) elucidating the mechanisms by which LPS downregulates apoE expression in microglia may be very relevant as it could lead to the identification of novel targets for AD prevention or treatment.

Acknowledgements

We wish to thank Dr Helena Corominola and Dr Marina Strakhova for help on the Northern experiments, Dr Neil DeLapp for help on western and northern quantitation and Dr Patrick C May and Dr Steven H. Zuckerman for fruitful discussions. This work was supported by grants from Eli Lilly and Company and a postdoctoral fellowship from the Spanish Ministerio de Educacion y Ciencia to JS.

References

Akama K. T. and Van Eldik L. J. (2000) β-Amyloid stimulation of inducible nitric-oxide synthase in astrocytes is interleukin-1β-and tumor necrosis factor-α (TNFα)-dependent, and involves a TNFα receptor-associated factor- and NF-κB-inducing kinase-dependent signaling mechanism. *J. Biol. Chem.* **275**, 7918–7924.

Akama K. T., Albanese C., Pestell R. G. and Van Eldik L. J. (1998) Amyloid β-peptide stimulates nitric oxide production in astrocytes through an NF-κB-dependent mechanism. *Proc. Natl Acad. Sci. USA* **95**, 5795–5800.

Bales K. R., Verina T., Dodel R. C., Du Y., Altstiel L., Bender M., Hyslop P., Johnstone E. M., Little S. P., Cummins D. J., Piccardo P., Ghetti B. and Paul S. M. (1997) Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat. Gen.* **17**, 263–264.

Bales K. R., Du Y., Dodel R. C., Yan G. M., Hamilton-Byrd E. and Paul S. M. (1998) The NF-κB/Rel family of proteins mediates Aβ-induced neurotoxicity and glial activation. *Mol. Brain Res.* **57**, 63–72.

Bales K. R., Xu Q., Du Y., Naidu A., Cordell B. and Paul S. M. (1999a) Amyloid-β peptide stimulates apolipoprotein E synthesis and

release in a murine microglial cell line via an NF-κB-dependent mechanism. *Soc. Neurosci. Abstract* **12**, 11.

Bales K. R., Verina T., Cummins D. J., Du Y., Dodel R. C., Saura J., Fishman C. E., Delong C. A., Piccardo P., Petegnief V., Ghetti B. and Paul S. M. (1999b) Apolipoprotein E is essential for amyloid deposition in the APPV717F transgenic mouse model of Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **96**, 15233–15238.

Baskin F., Smith G. M., Fosmire J. A. and Rosenberg R. N. (1997) Altered apolipoprotein E secretion in cytokine-treated human astrocyte cultures. *J. Neurol. Sci.* **148**, 15–18.

Bauerle P. A. (1998) Proinflammatory signaling: last pieces in the NF-κB puzzle? *Current. Biol.* **8**, R19–R22.

Boje K. M. and Arora P. K. (1992) Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. *Brain Res.* **7**, 250–256.

Bonaiuto C., McDonald P. P., Rossi F. and Cassatella M. A. (1997) Activation of nuclear factor-κB by β-amyloid peptides and interferon-γ in murine microglia. *J. Neuroimmunol.* **77**, 51–56.

Boyles J. K., Pitas R. E., Wilson E., Mahley R. W. and Taylor J. M. (1985) Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J. Clin. Invest.* **76**, 1501–1513.

Casal C., Tusell J. M. and Serratosa J. (2001) Role of calmodulin in the differentiation/activation of microglial cells. *Brain Res.* **902**, 101–107.

Danik M., Chabot J. G., Hassan-Gonzalez D., Suh M. and Quirion R. (1993) Localization of sulfated glycoprotein-2/clusterin mRNA in the rat brain by *in situ* hybridization. *J. Comp. Neurol.* **334**, 209–227.

Danik M., Chabot J. G., Michel D. and Quirion R. (1999) Clusterin and apolipoprotein E gene expression in the adult brain. In: *Clusterin in Normal Brain Functions and During Neurodegeneration* (Finch, C. E., ed.), pp. 17–34. RG Landes Company, Austin.

DeMattos R. B., O'dell M. A., Parsadanian M., Taylor J. W., Harmony J. A. K., Bales K. G., Paul S. M., Aronow B. J. and Holtzman D. M. (2002) Clusterin promotes amyloid plaque formation and is critical for neuritic toxicity in a mouse model of Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **99**, 10843–10848.

Diedrich J. F., Minnigan H., Carp R. I., Whitaker J. N., Race R., Frey I. I. W. and Haase A. T. (1991) Neuropathological changes in scarpie and Alzheimer's disease are associated with increased expression of apolipoprotein E and cathepsin D in astrocytes. *J. Virol.* **65**, 4759–4768.

Elshourbagy N. A., Liao W. S., Mahley R. W. and Taylor J. M. (1985) Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proc. Natl Acad. Sci. USA* **82**, 203–207.

Ferrari D., Wesselborg S., Bauer M. K. A. and Schulze-Osthoff K. (1997) Extracellular ATP activates transcription factor NF-κB through the P2Z purinoreceptor by selectively targeting NF-κB p65 (RelA). *J. Cell Biol.* **139**, 1635–1643.

Garden G. A., Bothwell M. and Rubel E. W. (1991) Lack of correspondence between mRNA expression for a putative cell death molecule (SGP-2) and neuronal cell death in the central nervous system. *J. Neurobiol.* **22**, 590–604.

Giulian D. and Baker T. J. (1986) Characterization of ameboid microglia isolated from developing mammalian brain. *J. Neurosci.* **6**, 2163–2178.

Glenn J. A., Ward S. A., Stone C. R., Booth P. L. and Thomas W. E. (1992) Characterisation of ramified microglial cells: detailed morphology, morphological plasticity and proliferative capability. *J. Anat* **180**, 109–118.

Golabek A., Marques M. A., Lalowski M. and Wisniewski T. (1995) Amyloid β binding proteins *in vitro* and in normal human cerebrospinal fluid. *Neurosci. Lett.* **191**, 79–82.

Grilli M., Pizzi M., Memorandum M. and Spano P. (1996) Neuroprotection by aspirin and sodium salicylate through blockade of NF-κB activation. *Science* **274**, 1383–1385.

Hardardottir I., Sipe J., Moser A. H., Fielding C. J., Feingold K. R. and Grunfeld C. (1997) LPS and cytokines regulate extra hepatic mRNA levels of apolipoproteins during the acute phase response in Syrian hamsters. *Biochim. Biophys. Acta* **1344**, 210–220.

Holtzman D. M., Bales K. R., Wu S., Bhat P., Parsadanian M., Fagan A. M., Chang L. K., Sun Y. and Paul S. M. (1999a) Expression of human apolipoprotein E reduces amyloid-β deposition in a mouse model of Alzheimer's disease. *J. Clin. Invest.* **103**, R15–R21.

Holtzman D. M., LaDu M. J. and Sagan A. M. (1999b) Clusterin-apoE lipoprotein particles. In: *Clusterin in Normal Brain Functions and During Neurodegeneration* (Finch C. E., ed.), pp. 61–69. RG Landes Company, Austin.

Holtzman D. M., Bales K. R., Tenkova T., Fagan A. M., Parsadanian M., Sartorius L. J., Mackey B., Olney J., McKeel D., Wozniak D. and Paul S. M. (2000) Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **97**, 2892–2897.

Kida E., Golabek A. A., Wisniewski T. and Wisniewski K. E. (1994) Regional differences in apolipoprotein E immunoreactivity in diffuse plaques in Alzheimer's disease brain. *Neurosci. Lett.* **167**, 73–76.

Kong L. Y., Lai C., Wilson B. C., Simpson J. N. and Hong J. S. (1997) Protein tyrosine kinase inhibitors decrease lipopolysaccharide-induced proinflammatory cytokine production in mixed glia, microglia-enriched or astrocyte-enriched cultures. *Neurochem. Int.* **30**, 491–497.

Kounnas M. Z., Haudenschild C. C., Strickland D. K. and Argraves W. S. (1994) Immunological localization of glycoprotein 330, low density lipoprotein receptor related protein and 39-kDa receptor-associated protein in embryonic mouse tissues. *In vivo* **8**, 343–351.

Kounnas M. Z., Loukinova E. B., Stefansson S., Harmony J. A., Brewer B. H., Strickland D. K. and Argraves W. S. (1995) Identification of glycoprotein 330 as an endocytic receptor for apolipoprotein J/clusterin. *J. Biol. Chem.* **270**, 13070–13075.

LaDu M. J., Shah J. A., Reardon C. A., Getz G. S., Bu G., Hu J., Guo L. and van Eldik L. J. (2000) Apolipoprotein E receptors mediate the effects of β-amyloid on astrocyte cultures. *J. Biol. Chem.* **275**, 33974–33980.

Lee S. C., Liu W., Brosnan C. F. and Dickson D. W. (1994) GM-CSF promotes proliferation of human fetal and adult microglia in primary cultures. *Glia* **12**, 309–318.

Lieberman A. P., Pitha P. M., Shin H. S. and Shin M. L. (1989) Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. *Proc. Natl Acad. Sci. USA* **86**, 6348–6352.

Lorent K., Overbergh L., Moechars D., De Strooper B., Van Leuven F. and Van den Berghe H. (1995) Expression in mouse embryos and in adult mouse brain of three members of the amyloid precursor protein family, of the α-2-macroglobulin receptor/low density lipoprotein receptor-related protein and of its ligands apolipoprotein E, lipoprotein lipase, α-2-macroglobulin and the 40 000 molecular weight receptor-associated protein. *Neuroscience* **65**, 1009–1025.

Matsubara E., Soto C., Governale S., Frangione B. and Ghiso J. (1996) Apolipoprotein J and Alzheimer's amyloid β solubility. *Biochem. J.* **316**, 671–679.

Menju M., Tajima S. and Yamamoto A. (1989) Expression of apolipoprotein E gene in a human macrophage-like cell line, THP-1. *J. Biochem.* **106**, 505–510.

Messmer-Joudrier S., Sagot Y., Mattenberger L., James R. W. and Kato A. C. (1996) Injury-induced synthesis and release of apolipoprotein E and clusterin from rat neural cells. *Eur. J. Neurosci.* **8**, 2652–2661.

Mouchel Y., Lefrancois T., Fages C. and Tardy M. (1995) Apolipoprotein E gene expression in astrocytes: developmental pattern and regulation. *Neuroreport* **7**, 205–208.

Murakami M., Ushio Y., Morino Y., Ohta T. and Matsukado Y. (1988) Immunohistochemical localization of apolipoprotein E in human glial neoplasms. *J. Clin. Invest.* **82**, 177–188.

Nakai M., Kawamata T., Taniguchi T., Maeda K. and Tanaka C. (1996) Expression of apolipoprotein E mRNA in rat microglia. *Neurosci. Lett.* **211**, 41–44.

Namba Y., Tomonaga M., Kawasaki H., Otomo E. and Ikeda K. (1991) Apoliprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeld–Jakob disease. *Brain Res.* **541**, 163–166.

Nasuhara Y., Adcock I. M., Catley M., Barnes P. J. and Newton R. (1999) Differential I-κB kinase activation and I-κB-α degradation by interleukin-1β and tumor necrosis factor-α in human U937 monocytic cells. *J. Biol. Chem.* **274**, 19965–19972.

Nishiya T., Uehara T. and Nomura Y. (1995) Herbimycin A suppresses NF-κB activation and tyrosine phosphorylation of JAK2 and the subsequent induction of nitric oxide synthase in C6 glioma cells. *FEBS Lett.* **371**, 333–336.

O'Neill L. A. J. and Kaltschmidt C. (1997) NF-κB: a crucial transcription factor for glial and neuronal cell function. *Trends Neurosci.* **20**, 252–258.

Oda T., Wals P., Osterburg H. H., Johnson S. A., Pasinetti G. M., Morgan T. E., Rozovsky I., Stine W. B., Snyder S. W., Holtzman T. F., Krafft G. A. and Finch C. E. (1995) Clusterin (apoJ) alters the aggregation of amyloid β-peptide (Aβ1–42) and forms slowly sedimenting Aβ complexes that cause oxidative stress. *Exp. Neurol.* **136**, 22–31.

Page K. J., Hollister R. D. and Hyman B. T. (1998) Dissociation of apolipoprotein and apolipoprotein receptor response to lesion in the rat brain: an *in situ* hybridization study. *Neuroscience* **85**, 1161–1171.

Pahan K., Sheikh F. G., Namboodiri A. M. S. and Singh I. (1997) Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages. *J. Clin. Invest.* **100**, 2671–2679.

Palombella V. J., Rando O. J., Goldberg A. L. and Maniatis T. (1994) The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of the NF-κB. *Cell* **78**, 773–785.

Pasinetti G. M., Johnson S. A., Oda T., Rozovsky I. and Finch C. E. (1994) Clusterin (SGP-2): a multifunctional glycoprotein with regional expression in astrocytes and neurons of the adult rat brain. *J. Comp. Neurol.* **339**, 387–400.

Petegnief V., Saura J., De-Gregorio-Rocasolano N. and Paul S. M. (2001) Neuronal injury-induced expression and release of apolipoprotein E in mixed neuron/glia co-cultures: nuclear factor kappa B inhibitors reduce basal and lesion-induced secretion of apolipoprotein E. *Neuroscience* **104**, 223–234.

Petrova T. V., Akama K. T. and Van Eldik L. J. (1999) Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. *Proc. Natl Acad. Sci. USA* **96**, 4668–4673.

Pitas R. E., Boyles J. K., Lee S. H., Foss D. and Mahley R. W. (1987) Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. *Biochim. Biophys. Acta* **917**, 148–161.

Poirier J., Hess M., May P. C. and Finch C. E. (1991) Astrocytic apolipoprotein E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. *Mol. Brain Res.* **11**, 97–106.

Quandt K., Frech K., Karas H., Wingender E. and Werner T. (1995) MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. *Nucleic Acids Res.* **23**, 4878–4884.

Schauwecker P. E., Cogen J. P., Jiang T., Cheng H. W., Collier T. J. and McNeill T. H. (1998) Differential regulation of astrocytic mRNAs in the rat striatum after lesions of the cortex or substantia nigra. *Exp. Neurol.* **149**, 87–96.

Schmechel D. E., Tiller M. O., Tong P., McSwain M., Han S. H., Ange R., Burkhart D. S. and Izard M. K. (1996) Pattern of apolipoprotein E immunoreactivity during brain aging. In: *Apolipoprotein E and Alzheimer's Disease* (Roses A. D., Weisgraber K. H., Christen Y., eds), pp. 29–48. Springer-Verlag, Berlin.

Shao Y., Gearing M. and Mirra S. S. (1997) Astrocyte–apolipoprotein E associations in senile plaques in Alzheimer disease and vascular regions: a regional immunohistochemical study. *J. Neuropathol. Exp. Neurol.* **56**, 376–381.

de Silva H. V., Harmony J. A., Stuart W. D., Gil C. M. and Robbins J. (1990) Apolipoprotein J: structure and tissue distribution. *Biochemistry* **29**, 5380–5389.

Stone D. J., Rozovsky I., Morgan T. E., Anderson C. P., Hajian H. and Finch C. E. (1997) Astrocytes and microglia respond to estrogen with increased apoE mRNA *in vivo* and *in vitro*. *Exp. Neurol.* **143**, 313–318.

Strittmatter W. J., Weisgraber K. H., Huang D. Y., Dong L. M., Salvesen G. S., Pericak-Vance M., Schmechel D., Saunders A. M., Goldgaber D. and Roses A. D. (1993) Binding of human apolipoprotein E to synthetic amyloid β peptide: isoform-specific effects and implication for late-onset Alzheimer disease. *Proc. Natl Acad. Sci. USA* **90**, 8098–8102.

Suzumura A., Marunouchi T. and Yamamoto H. (1991) Morphological transformation of microglia *in vitro*. *Brain Res.* **545**, 301–306.

Tanaka C., Kamata H., Takeshita H., Yagisawa H. and Hirata H. (1997) Redox regulation of lipopolysaccharide (LPS)-induced interleukin-8 (IL-8) gene expression mediated by NF-κB and AP-1 in human astrocytoma U373 cells. *Biochem. Biophys. Res. Commun.* **232**, 568–573.

Uchihara T., Duyckaerts C., He Y., Kobayashi K., Seilhean D., Amouyel P. and Hauw J. J. (1995) ApoE immunoreactivity and microglial cells in Alzheimer's disease brain. *Neurosci. Lett.* **195**, 5–8.

Werb Z. and Chin J. R. (1983) Endotoxin suppresses expression of apolipoprotein E by mouse macrophages *in vivo* and in cultures. *J. Biol. Chem.* **258**, 10642–10648.

White F., Nicoll J. A. and Horsburgh K. (2001) Alterations in ApoE and ApoJ in relation to degeneration and regeneration in a mouse model of entorhinal cortex lesion. *Exp. Neurol.* **169**, 307–318.

Wisniewski T., Golabek A., Matsubara E., Ghiso J. and Frangione B. (1993) Apolipoprotein E: binding to soluble Alzheimer's β-amyloid. *Biochem. Biophys. Res. Commun.* **192**, 359–365.

Xu Q., Li Y., Cyras C., Sanan D. A. and Cordell B. (2000) Isolation and characterization of apolipoproteins from murine microglia. Identification of a low-density lipoprotein-like apolipoprotein J-rich but E-poor spherical particle. *J. Biol. Chem.* **275**, 31770–31777.

Yates S. L., Burgess L. H., Kocsis-Angle J., Antal J. M., Dority M. D., Embury P. B., Piotrkowski A. M. and Brunden K. R. (2000) Amyloid beta and amylin fibrils induce increases in proinflammatory cytokine and chemokine production by THP-1 cells and murine microglia. *J. Neurochem.* **74**, 1017–1025.

Zlokovic B. V., Martel C. L., Matsubara E., McComb J. G., Zheng G., McCluskey R. T., Frangione B. and Ghiso J. (1996) Glycoprotein330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer's disease amyloid β at the blood-brain and blood–cerebrospinal fluid barriers. *Proc. Natl Acad. Sci. USA* **93**, 4229–4234.

Zuckerman S. H. and O'Neal L. (1994) Endotoxin and GM-CSF-mediated downregulation of macrophage apo E secretion is inhibited by a TNF-specific monoclonal antibody. *J. Leukoc. Biol.* **55**, 743–748.

Zwain I. H., Grima J. and Cheng C. Y. (1994) Regulation of clusterin secretion and mRNA expression in astrocytes by cytokines. *Mol. Cell. Neurosci.* **5**, 229–237.