CMLS, Cell. Mol. Life Sci. 59 (2002) 1734–1743
1420-682X/02/101734-11

CMLS Cellular and Molecular Life Sciences

Research Article

Glioma cell activation by Alzheimer's peptide Aβ_{1-42}, α_1-antichymotrypsin, and their mixture

Y. X. Sun [a], H. T. Wright [b] and S. Janciauskiene [a, *]

[a] Department of Medicine, Wallenberg Laboratory, Ing. 46, UMAS, S-20502, Malmö (Sweden), Fax + 46 40 33 70 41, e-mail: sabina.janciauskiene@medforsk.mas.lu.se
[b] Department of Biochemistry and Molecular Biophysics, Virginia Commonwealth University, P.O. Box 980614 Richmond, Virginia 23298-0614 (USA)

Received 23 May 2002; received after revision 6 August 2002; accepted 13 August 2002

Abstract. We compared the effects of Alzheimer's peptide (Aβ_{1-42}), α_1-antichymotrypsin (ACT) and an ACT/Aβ_{1-42} mixture on human glioma DK-MG cells. The solution of Aβ (5 µM) formed by 2-h incubation at room temperature induced tumour necrosis factor-α (TNF-α) and interleukin (IL)-6 levels by 55 and 45%, respectively, and increased gelatinase B activity by 67%, while exposure of cells to the ACT/Aβ_{1-42} mixture (1:10 molar ratio ACT: Aβ_{1-42}) under the same experimental conditions showed no effect on IL-6 levels or gelatinase B activity, but strongly induced TNF-α (by 190%), compared to the controls. Stimulation of the cells with Aβ_{1-42} alone, but not with ACT, increased by about 20% low-density lipoprotein (LDL) uptake and mRNA levels for LDL receptor and HMG-CoA reductase, while the ACT/Aβ_{1-42} mixture significantly increased LDL uptake (by 50%), up-regulated mRNA levels for LDL receptor and HMG-CoA reductase by 48 and 63%, respectively, and increased lipid accumulation by about 20-fold. These data suggest a possible new role for Aβ in Alzheimer's disease through its interaction with the inflammatory reactant, ACT.

Key words. Alzheimer's disease; α_1-antichymotrypsin; Alzheimer's peptide Aβ_{1-42}; inflammation; cytokine; lipid metabolism.

Amyloid-beta peptide (Aβ), in particular Aβ_{1-42}, has long been implicated in the pathogenesis of Alzheimer's disease (AD) [1, 2]. However, as yet no definitive in vitro mechanism for Aβ-induced neuronal damage can be extrapolated to the in vivo state. Some studies attribute Aβ neurotoxicity to a direct interaction with neuronal cells [3], while others suggest that Aβ-induced inflammatory responses are the proximal cause of neuronal damage [4].

Aβ peptide is normally produced and secreted as a proteolytic product of amyloid precursor protein (APP) and at physiological levels it can promote neurite outgrowth [5]. Aβ can undergo an α helix to β sheet secondary structure change to a form that spontaneously polymerises to fibrillar Aβ. The occurrence of fibrillar Aβ in amyloid plaques, and its similarity to other polymerised proteins associated with pathological amyloidoses have focused attention on fibrillar Aβ as the most likely neurotoxic form of Aβ [6]. However, other observations, particularly in vivo, do not support a causal, neurotoxic role for fibrillar Aβ [7, 8] and recent evidence indicates that non-fibrillar, soluble forms of Aβ peptides possess neurotoxic properties and play an important role in AD pathogenesis [9–11]. In vitro, Aβ can form small soluble oligomers under conditions that block fibril formation, such as interaction of Aβ with other molecules, including proteins

* Corresponding author.

found in neuritic plaques [12]. These oligomers were found to be highly neurotoxic [9], although the basis for their potent toxicity is not known. Oligomeric Aβ promotes lipid release from neuronal membrane, which may lead to the disruption of neuronal lipid homeostasis and the loss of neuronal function [13]. AD patients have significantly higher low-density lipoprotein (LDL) and lower high-density lipoprotein (HDL) levels than controls [14, 15] and there is a positive correlation between total serum cholesterol levels and the amount of Aβ in AD brains that is independent of apoE genotype [16]. Aβ has been reported to modulate membrane functions [17, 18], and cholesterol metabolism in plasma membrane and cellular cholesterol status have been linked to the biosynthesis of Aβ from APP [19, 20]. We have also shown in our previous in vitro work that neurotoxicity of fibrillar Aβ_{1-42} is accompanied by perturbed intracellular lipid metabolism [21], so that lipids and lipoproteins may have multiple links to Aβ metabolism.

Neuronal damage has also been hypothesised to occur as a consequence of inflammation, to which Aβ may contribute. Aβ has been shown to induce inflammatory factors such as interleukin (IL)-1, IL-6, tumour necrosis factor-α (TNF-α) and matrix metalloproteinases (MMPs), many of which occur in senile plaques [22, 23], and the production of Aβ and synthesis of cytokines are processes that might stimulate each other [24].

Aβ also exists in vivo complexed with other proteins, including apoE and apoJ, α_1-antichymotrypsin (ACT), amyloid P component, cytokines, complement and extracellular matrix proteins, most of which bind to other amyloid-forming proteins [25–28]. These molecules may chaperone Aβ self-association and lead to the formation of toxic Aβ oligomers that are diffusible and potentially more pernicious than fibrillar Aβ [9]. The finding that apolipoprotein (apoJ) inhibits formation of fibrillar Aβ, but does not inhibit Aβ neurotoxicity, supports this hypothesis [29]. Among the molecular species associated with senile plaques, the serpin ACT, an acute-phase inflammatory protein, is consistently identified in both amorphous and classic plaques of AD [30]. Through use of monoclonal antibodies to the different forms of ACT, it has been shown that ACT in the plaques is either complexed with a target protease or proteolytically cleaved and therefore inactive [31].

We and others have shown that ACT forms complexes with Aβ_{1-42} and that ACT can inhibit or accelerate fibrillisation of Aβ_{1-42} dependent on their relative concentrations [32, 33]. The existence of both inter- and intramolecular complexes of Aβ_{1-42} with ACT may explain in part the non-linear, concentration-dependent effects of ACT on Aβ_{1-42} fibril formation, whose kinetics we have investigated over a broad range of Aβ_{1-42}:ACT ratios [34]. In agreement with other in vitro studies, we found that at a lower molar ratio (1:10 ACT:Aβ_{1-42}) at neutral pH, ACT does not stimulate Aβ fibril formation in vitro [33–35], but affects the ratio of soluble species of Aβ, some of which may represent a complex between ACT and Aβ [36]. We also showed that Aβ_{1-42} alone and in a mixture with ACT, dependent on their pre-incubation time, have different biological activities when added to neuroblastoma cells, in vitro [37].

Activated glial cells are a consistent pathological hallmark of the AD brain and have been hypothesised to contribute to neuronal damage [38]. In this study, we used a glioma cell line as an in vitro model to further explore Aβ-driven inflammatory processes in AD. We stimulated cells with (i) Aβ_{1-42} peptide incubated for 2 h at room temperature and consisting of different-size soluble Aβ polymers (referred to as Aβ_{1-42} alone), (ii) ACT incubated for 2 h at room temperature (referred to as ACT alone) and (iii) a mixture of Aβ_{1-42} and ACT in a molar ratio of 10 : 1 incubated for 2 h at room temperature (referred to as ACT/Aβ_{1-42} mixture). Data to provide a firm basis for choosing a physiologically relevant concentration of Aβ (5 µM) for our in vitro model are insufficient; however, this concentration is lower than those used in neurotoxicity studies in vitro [39, 40], and it does not affect cell viability [37]. Some studies have shown that concentrations of cerebrospinal fluid Aβ are significantly reduced in patients with AD compared with age-matched normal subjects or patients with neurological diseases, possibly reflecting deposition of Aβ [41]. In the local microenvironment, the concentrations of Aβ are unlikely to be much higher than those measured in biological fluids (about 0.1–1 nM) [42]. Since we aimed to investigate conformation-dependent, but not concentration-dependent Aβ cellular effects, we used constant concentrations of Aβ_{1-42} (5 µM) and ACT (0.5 µM) in our experiments.

We found that Aβ_{1-42} and the ACT/Aβ_{1-42} mixture at a molar ratio 1:10 have distinct effects on the expression of inflammatory cytokines and on intracellular lipid metabolism compared to the Aβ_{1-42} alone in glioma cells, in vitro.

Material and methods

Material source and sample preparation

Aβ_{1-42} peptide was synthesised by Saveen (Denmark), >95% pure, MW = 4512.9. Aβ_{1-42} solution was prepared immediately before use. Aβ_{1-42} peptide (2 mg) was first dissolved in 25 µl 0.1 N NaOH, then Tris-buffered saline (0.015 M Tris, 0.15 M NaCl, pH 7.4) was added to a final volume of 0.5 ml to obtain the desired pH 7.5–8.0. Human ACT was a gift from Prof. H. Rubin, University of Pennsylvania, and gave a single protein band of about 60 kDa on SDS/PAGE [43]. ACT was dissolved in Tris-buffered saline and its concentration determined from absorbance at 280 nm using an extinction coefficient of 6.2. The stock solutions of Aβ_{1-42} peptide (125 µM), ACT (12.5 µM) and

ACT/Aβ_{1-42} mixture at a 1:10 molar ratio were incubated for 2 h at room temperature. After incubation, identical molar amounts of Aβ_{1-42} (5 µM), ACT (0.5 µM) and their mixture were added to the glioma cells for 24 h.

Electrophoresis and Western blot analysis

Samples taken from preparations of ACT and Aβ_{1-42} alone or the ACT/Aβ_{1-42} mixture after 2 h incubation time were analysed by electrophoresis through 1% agarose at pH 8.6 as previously described [44]. Gels were stained with Coomassie blue for 8 h and kept in destaining buffer overnight at room temperature. Alternatively, electrophoretically separated proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore) in 48 mM Tris, 39 mM glycine and 20% methanol, pH 9.2, using a semi-dry blot electrophoretic transfer system (Trans-Blot SD; Bio-Rad). After protein transfer, the membranes were developed using monospecific antisera against human ACT (DAKO A/S) or Aβ_{1-42} (Chemicon) (1:500) and peroxidase-conjugated anti-rabbit IgG (1:800) as second antibodies (DAKO). 3,3-Diaminobenzidine tetrahydrochloride, DAB; Sigma) was used as a peroxidase substrate.

LDL isolation and labelling

LDL was isolated by sequential preparative ultracentrifugation using an Optima XL-80K ultracentrifuge (Beckman). A narrow density range (1.034–1.054 kg/l) was used to prepare LDL for the experiments. LDL was sterile filtered and checked on a 1% agarose gel at pH 8.6 and its concentration determined by Lowry assay with human serum albumin as a standard [45]. Native LDL was labelled with ^{125}I by the iodine monochloride method [46]. Unbound ^{125}I was removed by chromatography on Sephadex G-25 columns PD-10 (Pharmacia) followed by extensive dialysis against 0.15 M NaCl, 1 mM EDTA and 0.03 M KI, and finally against 0.15 M NaCl containing 1 mM EDTA. The specific activity of ^{125}I-LDL ranged between 1.7 – 3.1 × 10^{-4} µCi/ng LDL protein.

Cell culture

The human glioma DK-MG cell line established from the glioblastoma multiform was obtained from the German Collection of Microorganisms and Cell Cultures. The cells grow adherent as a monolayer in RPMI-1640 medium (Gibco BRL) supplemented with 10% fetal calf serum (FCS; Biological Ind) at 37 °C in an atmosphere of humidified air with 5% CO_2. Cells were used after reaching confluence at 4–5 days after culture. Prior to experiments, cells were cultured in serum-free medium for 24 h.

Assay for ^{125}I-LDL uptake and degradation

Cells seeded into 12-well plates (Nunclon), 2 × 10^6 cells/well (cell counted in a Burker chamber) were incubated in RPMI-1640 medium without FCS, without or with Aβ_{1-42}, ACT or the ACT/Aβ_{1-42} mixture, and with ^{125}I-LDL (3.4 µg of LDL protein/mg of cell protein) for 12 h at 37 °C in 5% CO_2. The medium was aspirated, cells were washed with PBS and scraped into 0.5 N NaOH for ^{125}I-LDL uptake measurement (the sum of bound and internalised ^{125}I-LDL) and for cell protein determined by Lowry assay [45]. The aspirated medium was used for LDL degradation assessment measured as the quantity of free trichloroacetic acid-soluble non-iodine ^{125}I radioactivity [47]. The radioactivity was determined in an LKB 1271 automatic gamma counter (Wallac). The results are expressed as ng LDL protein taken up or degraded/mg cell protein.

Cytokine and gelatinase B (MMP-9) assays

Cell culture supernatants from glioma cells treated with Aβ_{1-42}, ACT alone or in combination were analysed to determine IL-6, TNF-α and gelatinase B (MMP-9) levels. A quantitative sandwich enzyme immunoassay (R&D Systems Europe Ltd) technique sensitive to pg/ml assay levels was used according to the manufacturer's instructions. Optical density was determined using a microplate reader at 450 nm. The readings at 570 nm were subtracted from those at 450 nm for wavelength correction. Duplicates were used for each standard and sample. The readings were averaged and the average zero standard optical density was subtracted.

RNA isolation and RT-PCR

Total RNA from glioma cells was isolated as outlined by Chomczynski and Sacchi [48]. The quantity of RNA was estimated from absorbance at 260 and 280 nm and the quality was analysed by agarose gels. LDL receptor (LDLr) and HMG-CoA mRNA were quantified by RT-PCR as described previously [49]. The 5′ primer (5′-CAATGTCTCACCAAGCTCTG-3′) and 3′ primer (5′-TCTGTCTCGAGGGGTAGCTG-3′) oligonucleotides for LDLr, and the 5′ primer (5′-TACCATGTCAGGGGTACGTC-3′) and 3′ primer (5′-CAAGCCTAGAGACATAATCATC-3′) for HMG-CoA reductase were purchased from Pharmacia Biotech. The internal control template pAW 109 cRNA was obtained from Perkin Elmer Cetus (N808-0037). We used 700,000 copies of the control template per reaction mixture for LDLr and 1,000,000 copies for HMG-CoA reductase. Amplification was performed with a Perkin Elmer Cetus thermocycler using the following cycle profile for LDLr: denaturation at 95 °C for 1 min, primer annealing and extension at 60 °C for 1 min. The initial denaturation step was prolonged to 3 min, and after 30 cycles, the reaction mixture was incubated at 72 °C for 1 min and then cooled to 4 °C. The cycle profile for HMG-CoA reductase was: denaturation at 95 °C for 1 min, primer annealing and extension at 53 °C for 1 min. The initial denaturation step was prolonged to 3 min, and after 29 cycles, the reaction mixture was incubated at 64 °C for 7 min and then cooled to 4 °C.

Quantitative analysis of mRNA

Each PCR product (20 µl) was electrophoresed along with a DNA molecular weight marker (Pharmacia Biotech) in a 4% agarose-sieving gel (2:3 (w/w) NuSieve Agarose and 1:3 (w/w) SeaKem LE Agarose; In Vitro) in TAE (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.4) running buffer at 98 V for 2–2.5 h at room temperature. The gel was scanned in a FluorImager SI (Molecular Dynamics) using an excitation wavelength of 488 nm (argon laser). Images were analysed using ImageQuant software (Molecular Dynamics) and the signal intensity was calculated by the 'integrated volume' method. The amounts of LDLr and HMG-CoA reductase PCR fragment were normalised to that of the internal standard. The values are expressed as a percentage of levels of LDL receptor mRNA in control glioma cells.

Cholesterol synthesis assay

Cellular synthesis of cholesterol was estimated by measuring ^{14}C-acetate incorporation into sterols from cell extracts, as described elsewhere [50] and counted in a β-counter (Liquid Scintillation System TRI-CARB 300C).

Red Oil staining

Glioma cells were grown on cover slips in the presence of test reactants for 12 h. At the end of the incubation period, cells were washed with PBS and fixed with 4% PBS-buffered formaldehyde for 15 min. Cells were then rinsed with water, dipped for a few seconds in 60% isopropanol, stained in the Oil Red O for 10–15 min and rinsed again in 60% isopropanol to remove excess stain. Cell nuclei were stained for a few seconds in the haematoxylin solution, washed with water and mounted with commercially available mounting medium (DAKO). Samples were analysed microscopically (Olympus Bx60) using the PC program Olympus MicroImage. Images were taken by digital camera (Sony DKC-5000) at × 100 magnification.

[^{3}H]-thymidine incorporation assay

Cells were incubated in 12-well plates with and without added A$\beta_{1–42}$ or ACT components for 20 h. [^{3}H]-thymidine (Amersham) was then added to the cells (0.1 µCi/ml) for a further 4-h incubation at 37 °C. The [^{3}H]-thymidine incorporation assay was performed as described earlier [37].

Statistical Analysis

The differences in the means of experimental results were analysed for their statistical significance with the Student two-sample two-sided t test and/or one-way ANOVA combined with a multiple-comparisons procedure (Scheffé multiple-range test), with an overall significance level of $\alpha = 0.05$. Statistical Package (SPSS for Windows, release 11.0) was used for the statistical calculations.

Results

Interaction between Aβ_{1-42} and ACT

A$\beta_{1–42}$ peptide alone and a mixture of A$\beta_{1–42}$ and ACT at a molar ratio of 10:1 co-incubated for 2 h at room temperature were examined by native 1% agarose gel electrophoresis. Figure 1 shows the electrophoretic pattern of A$\beta_{1–42}$ peptide alone and incubated with ACT. The peptide alone loaded on the gel at a molar concentration of 125 or 62.5 µM showed three or two bands with higher electrophoretic mobility towards the anode (negatively charged) (lanes 1 and 4). The same concentrations of A$\beta_{1–42}$ peptide in the ACT/A$\beta_{1–42}$ mixture lacks the highest mobility band(s) observed for the peptide alone, and produced a few blurred, slower-migrating protein bands and closely spaced bands with a migration distance from the application point (lanes 2 and 5) similar to that produced by ACT alone, which migrates as a single band (lanes 3 and 6).

To further characterise the components of the ACT/A$\beta_{1–42}$ mixture, samples were subjected to 1% agarose gel followed by Western blot analysis using antibodies against A$\beta_{1–42}$ or ACT. A$\beta_{1–42}$ alone formed two immunoreactive bands (fig. 2A), one with increased mobility towards the anode. Co-incubation of A$\beta_{1–42}$ with ACT resulted in a loss of the fastest-mobility band of A$\beta_{1–42}$ and the appearance of slower-mobility bands. Western blot analysis using anti-ACT antibodies (fig. 2B) also showed formation of a new, faster-migrating immuno-

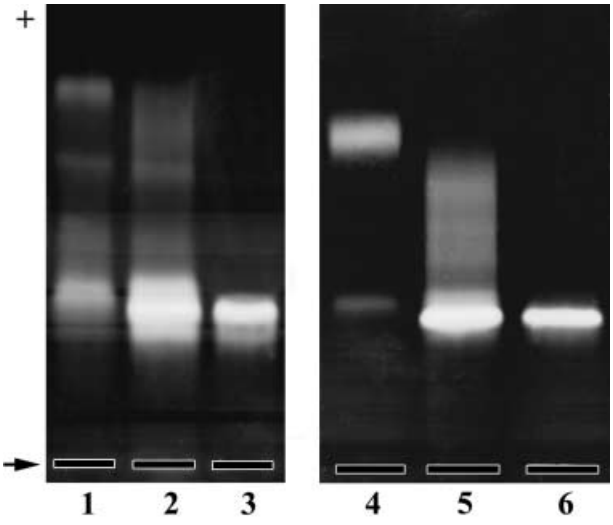

Figure 1. 1% agarose electrophoresis, at pH 8.6 of A$\beta_{1–42}$, ACT and ACT/A$\beta_{1–42}$ mixture at a molar ratio of 1:10 after 2 h incubation at room temperature. Lanes 1 and 4, A$\beta_{1–42}$ alone (125 and 62.5 µM, respectively); lanes 2 and 5, ACT/A$\beta_{1–42}$ mixture (12.5 µM ACT plus 125 µM A$\beta_{1–42}$ and 6.25 µM ACT plus 62.5 µM A$\beta_{1–42}$, respectively); lanes 3 and 6, ACT alone (12.5 and 6.25 µM, respectively). The anode is at the top. Sample application is indicated by the arrow. The constant volume of each sample applied per well was 9 µl.

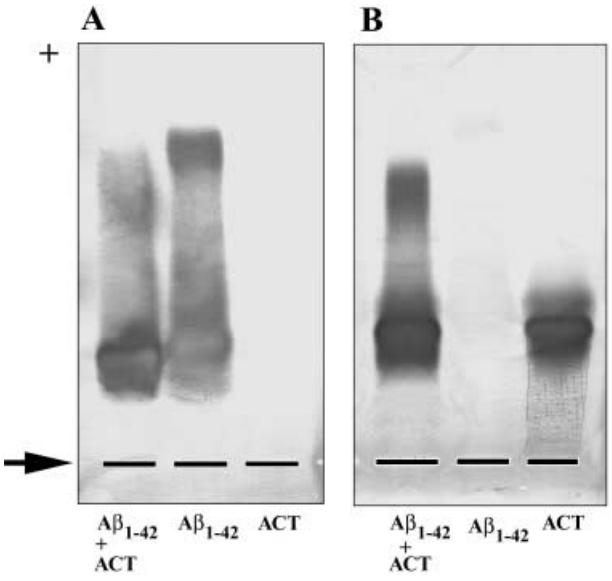

Figure 2. Interaction between Aβ_{1-42} and ACT studied by Western blot analysis. Samples were applied to a 1% agarose gel, pH 8.6 and immunoblotted with specific antibodies against Aβ_{1-42} (*A*) and ACT (*B*). The anode is at the top. Sample application is indicated by the arrow and the constant volume of each sample applied per well was 9 µl. Sample concentrations were as follows: Aβ_{1-42}, 125 µM; ACT/Aβ_{1-42} mixture, 12.5 µM ACT plus 125 µM Aβ_{1-42}; ACT, 12.5 µM.

precipitation band for ACT incubated with Aβ_{1-42} relative to that for ACT alone. Comparison of the immunoblots produced using antibodies against ACT and Aβ_{1-42} showed that the incubation of Aβ_{1-42} with ACT clearly created new molecular species with altered migration properties in an agarose gel.

Effects of Aβ_{1-42}, the ACT, and the ACT/Aβ_{1-42} mixture on cytokine and MMP production by glioma cells

We examined the levels of pro-inflammatory cytokines IL-6, TNF-α and the metalloprotease, gelatinase B (MMP-9) in medium from glioma cells cultured for 24 h with Aβ_{1-42} or ACT alone or the ACT/Aβ_{1-42} mixture. As shown in figure 3, Aβ added at a constant concentration to cells increased TNF-α secretion by 55% ± 2 ($p < 0.01$) and IL-6 secretion by 45% ± 4 ($p < 0.01$) while incubation of cells with ACT had no significant effect on TNF-α or IL-6 levels compared to controls. Similarly, secreted MMP-9 was found to be increased in cells treated with Aβ_{1-42} (67% ± 3, $p < 0.01$) compared to controls (fig. 3C). Cells treated with the ACT/Aβ_{1-42} mixture for 24 h (fig. 3) showed no changes in IL-6 or MMP-9 activity, but sharply increased TNF-α (190% ± 8.4, $p < 0.01$) compared to control cells. Treatment of cells with ACT alone resulted in a slight but significant reduction in MMP-9 levels in the medium of about 30% ± 5 ($p < 0.05$).

In these experiments, the IL-1β concentration in the supernatants was also measured, but its levels were reproducibly found to be below the detection limits of the assay (< 1 pg/ml).

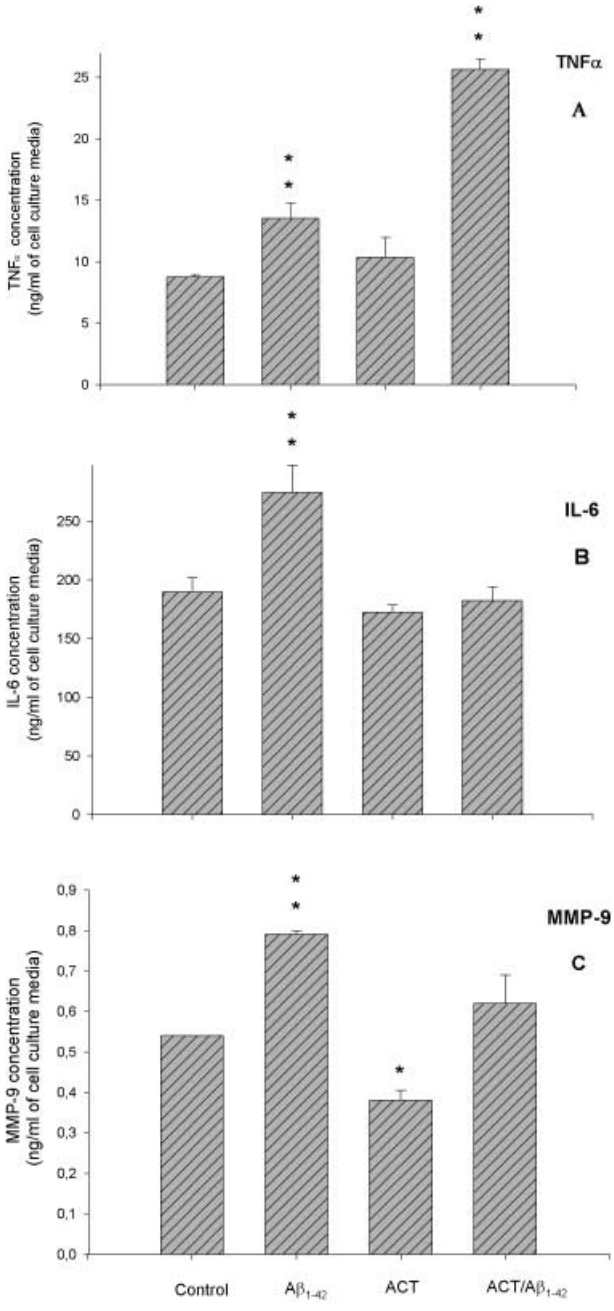

Figure 3. Effects of Aβ_{1-42} (5 µM), ACT (0.5 µM) and the ACT/Aβ_{1-42} mixture (0.5/5 µM) on pro-inflammatory cytokines TNF-α (*A*) and IL-6 (*B*), and gelatinase B (MMP-9) (*C*) generation in glioma cells. Each bar represents the mean ± SD of four to five separate experiments performed in duplicate for each sample. Asterisks indicate the probability level of a random difference between controls and stimulated cells (**$p < 0.01$, *$p < 0.05$).

Cell viability was not compromised by any of the Aβ forms studied here. In fact, both Aβ_{1-42} and the ACT/Aβ_{1-42} mixture slightly stimulated cell proliferation measured by ^{3}H-thymidine incorporation (data not shown).

Effect of Aβ_{1-42}, ACT and the ACT/Aβ_{1-42} mixture on glioma cell lipid homeostasis

An association of pro-inflammatory activation of cells with increased lipid uptake has been suggested [51]. To evaluate the effects of ACT, Aβ_{1-42} and the ACT/Aβ_{1-42} mixture on uptake and degradation of ^{125}I-LDL, cells were incubated with each of these plus ^{125}I-LDL.

Various time course studies indicated that the intracellular lipid level peak can be reached at 12 h [52, 53]. Using an incubation time of 12 h, the ACT/Aβ_{1-42} mixture significantly stimulated LDL uptake by 50% ($p < 0.01$), but had no significant effect on LDL degradation, compared to controls (fig. 4). Under the same experimental conditions, Aβ_{1-42} alone slightly, but not significantly, increased LDL uptake (by 22%), but had no effect on LDL degradation. We further examined the effects of Aβ_{1-42}, ACT and the ACT/Aβ_{1-42} mixture on intracellular lipid accumulation. Cells were treated with each in the presence of LDL (10 µg/ml) for 12 h and the intracellular lipid content was determined by Oil Red staining. As determined by the number of stained cytoplasmic cholesterol droplets (fig 5), the ACT/Aβ_{1-42} mixture promoted intracellular lipid accumulation by 20-fold ($p < 0.05$). Together, these data indicate that the ACT/Aβ_{1-42} mixture strongly induces LDL uptake and lipid accumulation in glioma cell cultures in vitro.

To determine the relative contributions to this intracellular lipid accumulation of LDL-mediated uptake and endogenous cholesterol biosynthesis, we measured mRNA levels for LDLr and HMG-CoA reductase, the rate-determining enzyme in cholesterol biosynthesis [54]. As shown in table 1, the ACT/Aβ_{1-42} mixture induced LDLr

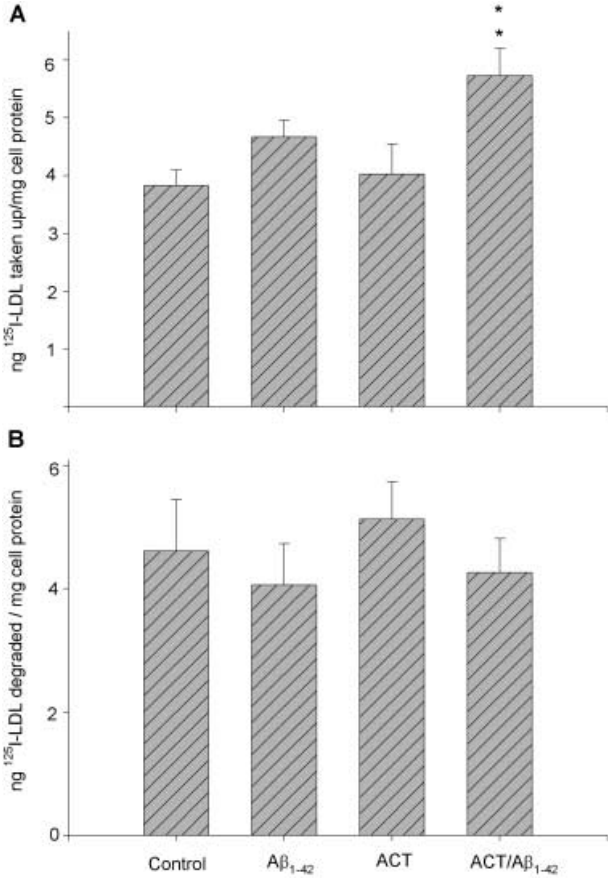

Figure 4. Combined effects of Aβ_{1-42} (5 µM), ACT (0.5 µM) and ACT/Aβ_{1-42} mixture (0.5/5 µM) on ^{125}I-LDL uptake (*A*) and degradation (*B*) by glioma cells after incubation at 37 °C for 12 h. Each bar represents the mean ± SE of three to five separate experiments tested by one-way ANOVA and the Scheffé multiple-comparison test ($\alpha = 0.05$). ACT/Aβ_{1-42} significantly induced LDL uptake while having no significant effect on LDL degradation. Asterisks indicate the probability level of a random difference between controls and stimulated cells (** $p < 0.01$).

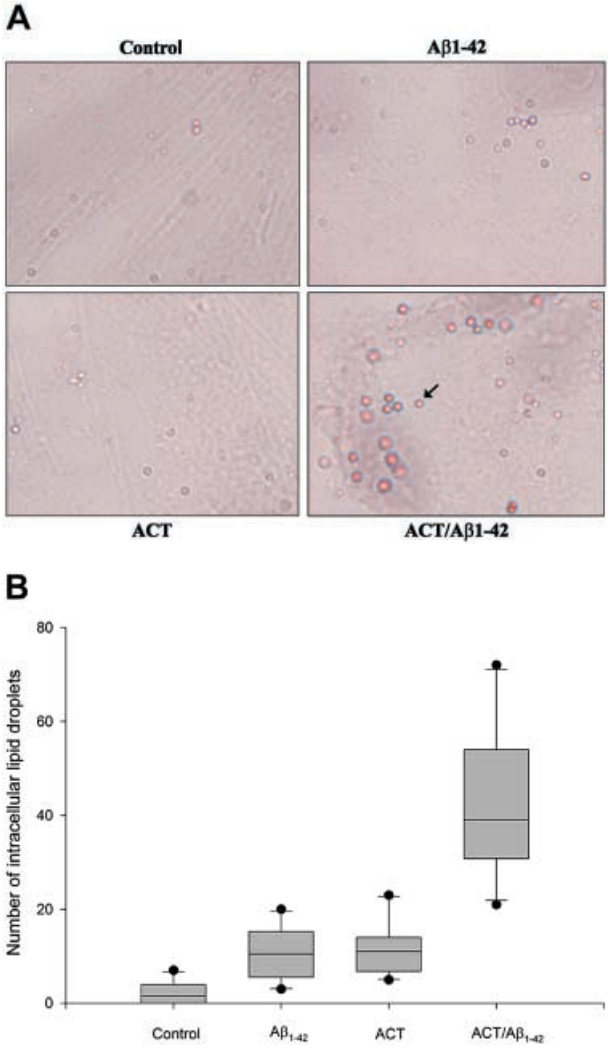

Figure 5. Accumulation of lipids in glioma cells visualised by staining with Oil Red O ×100 (*A*) and statistical calculation of intracellular lipid droplets (*B*). Glioma cells were incubated with Aβ_{1-42} (5 µM), ACT (0.5 µM) separately or together for 12 h. The cells were fixed and stained with Oil Red. The arrow indicates Oil-Red-O-stained vacuoles. The probability level of a random difference between controls and cells stimulated with ACT/Aβ_{1-42} is $p < 0.01$.

Table 1. Effects of Ab and the ACT/Aβ_{1-42} mixture on HMG-CoA reductase and LDLr mRNA levels in glioma cells.

Cell stimulation	LDL receptor mRNA		HMG-CoA reductase mRNA	
	mean	SD	mean	SD
Control	100	2	100	1.5
Pravastatin (0.1 mM)	164**	21	151**	19.5
Aβ_{1-42} (5 µM)	127	5.6	124	6.4
ACT (0.5 µM)	108	8.4	111	3
ACT/Aβ_{1-42} mixture (0.5/5 µM)	148**	6	163**	4

Mean and SD (% of control) of six independent experiments. **$p < 0.01$.

mRNA levels by 48% versus 27% for Aβ_{1-42}, while ACT alone had no effects. Pravastatin (0.1 mM), used as a positive control under the same experimental conditions, induced mRNA for LDLr by 64%. Similarly, cells treated with the ACT/Aβ_{1-42} mixture or pravastatin increased HMG-CoA reductase mRNA levels by 63% and 51%, respectively, while Aβ_{1-42} alone induced mRNA by 24% and ACT had no significant effect.

We also examined intracellular cholesterol synthesis and found that neither Aβ_{1-42} alone nor the ACT/Aβ_{1-42} mixture had any significant effect on cholesterol synthesis in glioma cells, while in the same experimental model, pravastatin suppressed cholesterol synthesis by about 90% ± 5 (data not shown). This means that the entire increase in cellular cholesterol was the result of LDL uptake, and the increase in HMG-CoA reductase mRNA made no specific contribution to cellular cholesterol levels. Our experiments show that LDLr and HMG-CoA reductase mRNA expression in human glioma cell culture were co-ordinately up-regulated on exposure to the ACT/Aβ_{1-42} mixture at levels comparable to those observed with pravastatin, a hydrophilic HMG-CoA reductase inhibitor [55]. Despite these similarities, Aβ_{1-42} alone as well as the ACT/Aβ_{1-42} mixture showed no effect on intracellular cholesterol synthesis, while pravastatin strongly inhibited cholesterol synthesis. Further studies will be necessary to adequately address these anomalies.

Discussion

Amyloid plaque and neuroinflammation, manifested as activated glial cells, are the most characteristic recurring markers of the AD brain. As amyloid-forming peptide, Aβ has been exhaustively studied in vitro, but its function is still not known nor is the in vivo mechanism by which it exerts its putative neurotoxicity. The resemblance of Aβ to other cytokines in some of its cellular effects [24] suggests that it may be an intrinsic component of the brain inflammatory response.

Aβ may play a role in AD pathology, not necessarily through direct neurotoxicity, but through interaction with other molecules to create complexes or new molecular forms that gain biological activities or lose essential regulatory activities, and thereby foster chronic inflammation. Microglia and astrocytes in brain regions with abundant amyloid plaques over-express many pro- and anti-inflammatory cytokines and other reactants, as well as Aβ [56], and some forms of Aβ can activate microgial cells to synthesise and release pro-inflammatory cytokines chemokines and reactive oxygen species, which can cause neuronal damage [57]. ACT is an anti-inflammatory reactant and a component of amyloid plaques that is known to interact with Aβ. We showed earlier that ACT forms electrophoretically distinct, soluble complexes with Aβ in vitro, changes the kinetics of Aβ polymerisation, and loses protease inhibitor activity as a consequence of complex formation with Aβ [34].

Little work has been done on the biological activities of Aβ complexes with inflammatory reactant molecules like ACT. In the work described here, we incubated Aβ_{1-42} with ACT under conditions that straddle the time- and concentration-dependent transition of some of the soluble forms of Aβ_{1-42} to complexed form(s) of Aβ_{1-42} with ACT. Product(s) of this incubation have an induction profile for the inflammatory reactants IL-6, TNF-α and MMP-9 distinct from that of Aβ_{1-42} alone. Aβ_{1-42} alone increases levels of all three, while treatment of the cells with the ACT/Aβ_{1-42} mixture under the same conditions showed no effects on IL-6 or MMP-9 levels, but elevated TNF-α much more strongly than did Aβ_{1-42} alone. Thus, the ACT/Aβ_{1-42} mixture may be more pro-inflammatory than Aβ_{1-42} through both its large enhancement of TNF-α expression as well as through ablation of ACT protease inhibitor activity by complex formation. These effects of ACT/Aβ_{1-42} mixture on cytokine expression are reminiscent of the effects of ACT complexed with target proteases on cytokine expression [58]. Since TNF-α, IL-6 and IL-1 regulate ACT expression in brain cells [59, 60], these reciprocal effects could create a self-sustaining cycle of inflammation.

An important consequence of the binding of Aβ to ACT is the loss of protease inhibitor activity of ACT [33]. In vivo, this effect would remove the protease-regulating, anti-inflammatory effect of ACT and may lead to increased protease activity and proteolytic release of Aβ which may further abolish inhibitor activity of ACT and increase the population of ACT/Aβ molecules. ACT has also been shown to be a free radical scavenger [61], acting through the NADPH oxidase (respiratory burst) pathway that is also activated by Aβ [62, 63]. Therefore, the interaction of Aβ with ACT not only eliminates ACT as an anti-inflammatory reactant, but also, if ACT is indeed a chaperone promoting formation of multiply polymeric forms of Aβ, further transforms ACT into a pro-inflam-

matory reactant, thereby making a double contribution to the inflammatory state.

Cholesterol metabolism is known to be perturbed during the inflammation [51] and APP processing and Aβ generation are sensitive to cellular cholesterol levels [64]. Increasing cellular cholesterol levels in vitro decrease secretion of soluble APP [65], while decreasing cellular cholesterol diminishes Aβ secretion [66]. Mice maintained on high-fat diets show reduced levels of secreted APP as well as reduced Aβ levels [20], and Aβ has been found to influence neuronal cholesterol esterification, synthesis and trafficking [17, 18, 67]. Thus, there may be a link between inflammation and Aβ mediated changes in cholesterol metabolism. In our earlier studies, we showed that specific molecular forms of Aβ_{1-42} may have distinct effects on cellular lipid metabolism. For example, fibrillar but not a soluble form of the Aβ_{1-42} peptide, at micromolar concentrations was found to strongly increase LDL uptake and binding by pheochromocytoma, PC12 cells [21]. These effects were found to be partially inhibited by anti-LDL receptor antibody from which we suggested that the interaction of Aβ_{1-42} fibrils with LDL receptors might contribute to fibril neurotoxicity. In addition, the ACT/Aβ_{1-42} mixture formed by 2 h incubation at a 1:10 molar ratio of ACT : Aβ increased uptake and depressed degradation of native and oxidised LDL in neuroblastoma Kelly cells. In contrast, Aβ_{1-42} alone and an ACT/Aβ_{1-42} mixture prepared from a longer incubation (24 h) showed no significant effect on LDL metabolism [37]. These observations clearly show that only certain molecular forms of Aβ may interfere with intracellular lipid metabolism.

In the present study we observed that the ACT/Aβ_{1-42} mixture prepared by 2-h incubation stimulates uptake of native LDL, but has no significant effect on LDL degradation in glioma cells, and this effect correlates with increased cellular cholesterol storage as determined by cholesterol cytoplasmic droplet staining. In addition, the ACT/Aβ_{1-42} mixture significantly induced mRNA levels for both HMG-CoA reductase and LDL receptors, but had no effect on intracellular cholesterol synthesis. Since LDLr activity is under tight metabolic control through a cholesterol-sensitive feedback system that controls both the rate of cholesterol uptake from LDL and the rate of cholesterol synthesis [68], our observations indicate that new molecular species in the ACT/Aβ_{1-42} mixture perturb intracellular lipid homeostasis. Similarly, previous studies found that fibrillar, but not soluble, Aβ peptides alters cellular cholesterol homeostasis by increasing the free cholesterol content in neuronal cells and inhibiting cellular cholesterol esterification [69]. We also found that the ACT/Aβ_{1-42} complex mixture prepared by 2-h incubation, but not Aβ alone, when added to neuroblastoma Kelly cells induces LDL uptake and expression of the transcription factors peroxisome proliferator-activated receptor-gamma and nuclear factor-κB which are known to be involved in regulation of genes linked to lipid metabolism and inflammation in AD [37].

Our present data showing that an ACT/Aβ_{1-42} mixture strongly induces TNF-α, up-regulates mRNA levels for LDLr and HMG-CoA reductase and increases lipid accumulation in glioma cells suggest that the effects of ACT/Aβ_{1-42} mixture on cellular lipid homeostasis may be mediated via induction of the expression of inflammatory cytokines such as TNF-α. At least two pro-inflammatory cytokines, TNF-α and IL-1β, have recently been shown to disrupt cholesterol-mediated LDLr regulation, resulting in formation of lipid-loaded cells [70].

These findings together indicate that the ACT/Aβ_{1-42} complex mixture is pro-inflammatory and perturbs intracellular lipid status. Several studies showing that intracellular cholesterol levels influence the generation of Aβ in brain cells [20, 63, 64] indirectly support the relevance of these observations to the in vivo case and point to a possible self-perpetuating cycle of Aβ biosynthesis. While the mechanisms by which an ACT/Aβ_{1-42} mixture induces changes in lipid metabolism remain to be elucidated, the molecular forms arising from this interaction clearly have pro-inflammatory properties and can alter cellular cholesterol homeostasis.

Aβ_{1-42} and ACT are inflammatory reactants and constituents of amyloid plaques, and almost certainly interact in vivo. In vitro co-incubation of ACT and Aβ_{1-42} results in a molecular species with activities that can be directly linked to inflammation, Aβ metabolism and thereby to AD. The complex of Aβ_{1-42} with ACT may be one of many such complexes with biological activities that contribute to the neurodegeneration characteristic of AD. While increasing the complexity of this disease, they may also offer new points for pharmacological intervention.

Acknowledgement. We are grateful to Prof. Harvey Rubin for providing purified recombinant ACT. This work was funded in part by a grant from the Thomas F. and Kate Miller Jeffress Memorial Trust (HTW), the Swedish Medical Research Council (K01-72X), the Medical Faculty, Lund University and the Tore Nilsson and Alfred Österlunds Foundation.

1 Glenner G. G. and Wong C. W. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. **120:** 885–890

2 Verbeek M. M., Ruiter D. J. and Waal R. M. de (1997) The role of amyloid in the pathogenesis of Alzheimer's disease. Biol. Chem. **378:** 937–950

3 Kawahara M. and Kuroda Y. (2000) Molecular mechanism of neurodegeneration induced by Alzheimer's beta-amyloid protein: channel formation and disruption of calcium homeostasis. Brain Res. Bull. **53:** 389–397

4 Hensley K., Carney J. M., Mattson M. P., Aksenova M., Harris M., Wu J. F. et al. (1994) A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. Proc. Natl. Acad. Sci. USA **91:** 3270–3274

5 Koo E. H., Park L. and Selkoe D. J. (1993) Amyloid beta-protein as a substrate interacts with extracellular matrix to promote neurite outgrowth. Proc. Natl. Acad. Sci. USA **90:** 4748–4752
6 Lorenzo A. and Yankner B. A. (1994) Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. Proc. Natl. Acad. Sci. USA **91:** 12243–12247
7 Irizarry M. C., Soriano F., McNamara M., Page K. J., Schenk D., Games D. et al. (1997) Abeta deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. J. Neurosci. **17:** 7053–7059
8 Martin L. J., Pardo C. A., Cork L. C. and Price D. L. (1994) Synaptic pathology and glial responses to neuronal injury precede the formation of senile plaques and amyloid deposits in the aging cerebral cortex. Am. J. Pathol. **145:** 1358–1381
9 Lambert M. P., Barlow A. K., Chromy B. A., Edwards C., Freed R., Liosatos M. et al. (1998) Diffusible, nonfibrillar ligands derived from Abeta1–42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. USA. **95:** 6448–6453
10 Hartley D. M., Walsh D. M., Ye C. P., Diehl T., Vasquez S., Vassilev P. M. et al. (1999) Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. J. Neurosci. **19:** 8876–8884
11 Zhu Y. J., Lin H. and Lal R. (2000) Fresh and nonfibrillar amyloid beta protein(1–40) induces rapid cellular degeneration in aged human fibroblasts: evidence for AbetaP-channel-mediated cellular toxicity. FASEB J. **14:** 1244–1254
12 Bohrmann B., Tjernberg L., Kuner P., Poli S., Levet-Trafit B., Naslund J. et al. (1999) Endogenous proteins controlling amyloid beta-peptide polymerization: possible implications for beta-amyloid formation in the central nervous system and in peripheral tissues. J. Biol. Chem. **274:** 15990–15995
13 Michikawa M., Gong J. S., Fan Q., W. Sawamura N. and Yanagisawa K. (2001) A novel action of Alzheimer's amyloid beta-protein (Abeta): oligomeric Abeta promotes lipid release. J. Neurosci. **21:** 7226–7235
14 Wehr H., Parnowski T., Puzynski S., Bednarska-Makaruk M., Bisko M., Kotapka-Minc S. et al. (2000) Apolipoprotein E genotype and lipid and lipoprotein levels in dementia. Dement. Geriatr. Cogn. Disord. **11:** 70–73
15 Merched A., Xia Y., Visvikis S., Serot J. M. and Siest G. (2000) Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concentrations are highly correlated with the severity of Alzheimer's disease. Neurobiol. Aging **21:** 27–30
16 Kuo Y. M., Emmerling M. R., Bisgaier C. L., Essenburg A. D., Lampert H. C., Drumm D. et al. (1998) Elevated low-density lipoprotein in Alzheimer's disease correlates with brain Abeta 1–42 levels. Biochem. Biophys. Res. Commun. **252:** 711–715
17 Rodrigues C. M, Sola S., Brito M. A., Brondino C. D., Brites D. and Moura J. J. (2001) Amyloid beta-peptide disrupts mitochondrial membrane lipid and protein structure: protective role of tauroursodeoxycholate. Biochem. Biophys. Res. Commun. **281:** 468–474
18 Strosznajder J. B., Zambrzycka A., Kacprzak M. D. and Strosznajder R. P. (1999) Amyloid beta peptide 25–35 modulates hydrolysis of phosphoinositides by membrane phospholipase(s) C of adult brain cortex. J. Mol. Neurosci. **12:** 101–109
19 Yip C. M., Elton E. A., Darabie A. A., Morrison M. R. and McLaurin J. (2001) Cholesterol, a modulator of membrane-associated Abeta-fibrillogenesis and neurotoxicity. J. Mol. Biol. **311:** 723–734
20 Howland D. S., Trusko S. P., Savage M. J., Reaume A. G., Lang D. M., Hirsch J. D. et al. (1998) Modulation of secreted beta-amyloid precursor protein and amyloid beta-peptide in brain by cholesterol. J. Biol. Chem. **273:**16576–16582
21 Janciauskiene S., Wright H. T. and Lindgren S. (1999) Fibrillar Alzheimer's amyloid peptide Abeta(1–42) stimulates low density lipoprotein binding and cell association, free radical production and cell cytotoxicity in PC12 cells. Neuropept. Res. **33:** 510–516
22 Lue L. F., Walker D. G. and Rogers J. (2001) Modeling microglial activation in Alzheimer's disease with human postmortem microglial cultures. Neurobiol. Aging **22:** 945–956
23 Deb S. and Gottschall P. E. (1996) Increased production of matrix metalloproteinases in enriched astrocyte and mixed hippocampal cultures treated with beta-amyloid peptides. J. Neurochem. **66:** 1641–1647
24 Del Bo R., Angeretti N., Lucca E., De Simoni M. G. and Forloni G. (1995) Reciprocal control of inflammatory cytokines, IL-1 and IL-6, and beta-amyloid production in cultures. Neurosci. Lett. **188:** 70–74
25 Styren S. D., Kamboh M. I. and DeKosky, S.T. (1998) Expression of differential immune factors in temporal cortex and cerebellum: the role of alpha-1-antichymotrypsin, apolipoprotein E, and reactive glia in the progression of Alzheimer's disease. J. Comp. Neurol. **396:** 511–520
26 Hamazaki H. (1995) Ca(2+)-dependent binding of human serum amyloid P component to Alzheimer's beta-amyloid peptide. J. Biol. Chem. **270:** 10392–10394
27 Veerhuis R., Janssen I., De Groot C. J., Van Muiswinkel F. L., Hack C. E. and Eikelenboom P. (1999) Cytokines associated with amyloid plaques in Alzheimer's disease brain stimulate human glial and neuronal cell cultures to secrete early complement proteins, but not C1-inhibitor. Exp. Neurol. **160:** 289–299
28 Asahina M., Yoshiyama Y. and Hattori T. (2001) Expression of matrix metalloproteinase-9 and urinary-type plasminogen activator in Alzheimer's disease brain. Clin. Neuropathol. **20:** 60–63
29 Oda T., Wals P., Osterburg H. H., Johnson S. A., Pasinetti G. M., Morgan T. E. et al. (1995) Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1–42) and forms slowly sedimenting A beta complexes that cause oxidative stress. Exp. Neurol. **136:** 22–31
30 Abraham C. R. and Potter H. (1989) The protease inhibitor, alpha 1-antichymotrypsin, is a component of the brain amyloid deposits in normal aging and Alzheimer's disease. Ann. Med. **21:** 77–81
31 Rozemuller J. M., Abbink J. J., Kamp A. M., Stam F. C., Hack C. E. and Eikelenboom P. (1991) Distribution pattern and functional state of alpha 1-antichymotrypsin in plaques and vascular amyloid in Alzheimer's disease: a immunohistochemical study with monoclonal antibodies against native and inactivated alpha 1-antichymotrypsin. Acta Neuropathol. (Berl) **82:** 200–207
32 McLaurin J., Yang D., Yip C. M. and Fraser P. E. (2000) Modulating factors in amyloid-beta fibril formation. J. Struct. Biol. **130:** 259–270
33 Eriksson S., Janciauskiene S. and Lannfelt L. (1995) Alpha 1-antichymotrypsin regulates Alzheimer beta-amyloid peptide fibril formation. Proc. Natl. Acad. Sci. USA **92:** 2313–2317
34 Janciauskiene S., Rubin H., Lukacs C. M. and Wright H. T. (1998) Alzheimer's peptide Abeta1–42 binds to two beta-sheets of alpha1-antichymotrypsin and transforms it from inhibitor to substrate. J. Biol. Chem. **273:** 28360–28364
35 Fraser P. E., Nguyen J. T., McLachlan D. R., Abraham C. R. and Kirschner D. A. (1993) Alpha 1-antichymotrypsin binding to Alzheimer A beta peptides is sequence specific and induces fibril disaggregation in vitro. J. Neurochem. **61:** 298–305
36 Yamin R., Malgeri E. G., Sloane J. A., McGraw W. T. and Abraham C. R. (1999) Metalloendopeptidase EC 3.4.24.15 is necessary for Alzheimer's amyloid-beta peptide degradation. J. Biol. Chem. **274:** 18777–18784
37 Sun Y. X., Wright H. T. and Janciauskiene S. (2002) Alpha1-antichymotrypsin/Alzheimer's peptide Abeta (1–42) complex perturbs lipid metabolism and activates transcription factors PPARgamma and NFkappaB in human neuroblastoma (Kelly) cells. J. Neurosci. Res. **67:** 511–522
38 McGeer P. L. and McGeer E. G. (2001) Inflammation, autotoxicity and Alzheimer disease. Neurobiol. Aging **22:** 799–809

39 Kajkowski E. M., Lo C. F., Ning X., Walker S., Sofia H. J., Wang W. et al. (2001) Beta-amyloid peptide-induced apoptosis regulated by a novel protein containing a G protein activation module. J. Biol. Chem. **276:** 18748–18756

40 Ishii K., Muelhauser F., Liebl U., Picard M., Kuhl S., Penke B. et al. (2000) Subacute NO generation induced by Alzheimer's beta-amyloid in the living brain: reversal by inhibition of the inducible NO synthase. FASEB J. **14:** 1485–1489

41 Riemenschneider M., Schmolke M., Lautenschlager N., Guder W. G., Vanderstichele H., Vanmechelen E. et al. (2000) Cerebrospinal beta-amyloid ((1–42)) in early Alzheimer's disease: association with apolipoprotein E genotype and cognitive decline. Neurosci. Lett. **284:** 85–88

42 Kontush A., Berndt C., Weber W., Akopyan V., Arlt S., Schippling S. et al. (2001) Amyloid-beta is an antioxidant for lipoproteins in cerebrospinal fluid and plasma. Free Radic. Biol. Med. **30:** 119–128

43 Tsuda M., Ohkubo T., Kamiguchi H., Suzuki K., Nakasaki H., Mitomi T. et al. (1982) Purification, properties and identification of a serum DNA binding protein (64DP) and its microheterogeneity. J. Exp. Chin. Med. **7:** 201–211

44 Janciauskiene S. and Eriksson S. (1993) In vitro complex formation between cholesterol and alpha 1-proteinase inhibitor. FEBS Lett. **316:** 269–272

45 Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurements with folin phenol reagents. J. Biol. Chem. **193:** 265–275

46 Shepherd J., Bedford D. K. and Morgan H. G. (1976) Radioiodination of human low density lipoprotein: a comparison of four methods. Clin. Chim. Acta. **66:** 97–109

47 Weber C., Draude G., Weber K. S., Wubert J., Lorenz R. L. and Weber P.C. (1999) Downregulation by tumor necrosis factor-alpha of monocyte CCR2 expression and monocyte chemotactic protein-1-induced transendothelial migration is antagonized by oxidized low-density lipoprotein: a potential mechanism of monocyte retention in atherosclerotic lesions. Atherosclerosis **145:** 115–123

48 Chomczynski P., and Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:** 156–159

49 Janciauskiene S., Wright H. T. and Lindgren, S. (1999) Atherogenic properties of human monocytes induced by the carboxyl terminal proteolytic fragment of alpha-1-antitrypsin. Atherosclerosis **147:** 263–275

50 Shah S. N. and Johnson R. C. (1988) Effect of serum lipoproteins on growth and sterol synthesis in cultured rat brain glial cells. J. Neurochem. **50:** 1529–1536

51 Moraga F. and Janciauskiene S. (2000) Activation of primary human monocytes by the oxidized form of alpha1-antitrypsin. J. Biol. Chem. **275:** 7693–7700

52 Tanaka T., Tatsuno I., Noguchi Y., Uchida D., Oeda T., Narumiya S. et al. (1998) Activation of cyclin-dependent kinase 2 (Cdk2) in growth-stimulated rat astrocytes: geranylgeranylated Rho small GTPase(s) are essential for the induction of cyclin E gene expression. J. Biol. Chem. **273:** 26772–26778

53 Howard B. V., Howard W. J., De la Llera M. and Kefalides N. A (1976) Triglyceride accumulation in cultured human fibroblasts: the effects of hypertriglyceridemic serum. Atherosclerosis **23:** 521–534

54 Siperstein M. D. (1984) Role of cholesterogenesis and isoprenoid synthesis in DNA replication and cell growth. J. Lipid Res. **25:** 1462–1468

55 Gebhardt A. and Niendorf A. (1995) Effects of pravastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, on two human tumour cell lines. J. Cancer Res. Clin. Oncol. **121:** 343–349

56 Meda L., Baron P. and Scarlato G. (2001) Glial activation in Alzheimer's disease: the role of Abeta and its associated proteins. Neurobiol. Aging **22:** 885–893

57 Johnstone M., Gearing A. J. and Miller K. M. (1999) A central role for astrocytes in the inflammatory response to beta-amyloid: chemokines, cytokines and reactive oxygen species are produced. J. Neuroimmunol. **93:** 182–193

58 Abraham C. R. (1992) The role of the acute-phase protein alpha 1-antichymotrypsin in brain dysfunction and injury. Res. Immunol. **143:** 631–636

59 Kordula T., Bugno M., Rydel R. E. and Travis J. (2000) Mechanism of interleukin-1- and tumor necrosis factor alpha-dependent regulation of the alpha 1-antichymotrypsin gene in human astrocytes. J. Neurosci. **20:** 7510–7516

60 Kordula T., Rydel R. E., Brigham E. F., Horn F., Heinrich P. C. and Travis J. (1998) Oncostatin M and the interleukin-6 and soluble interleukin-6 receptor complex regulate alpha1-antichymotrypsin expression in human cortical astrocytes. J. Biol. Chem. **273:** 4112–4118

61 Ferrer-Lopez P., Renesto P., Schattner M., Bassot S., Laurent P. and Chignard M. (1990) Activation of human platelets by C5a-stimulated neutrophils: a role for cathepsin G. Am. J. Physiol. **258:** C1100–1107

62 Bianca V. D., Dusi S., Bianchini E., Dal Pra I. and Rossi F. (1999) Beta-amyloid activates the O-2 forming NADPH oxidase in microglia, monocytes, and neutrophils: a possible inflammatory mechanism of neuronal damage in Alzheimer's disease. J. Biol. Chem. **274:** 15493–15499

63 Kang J., Park E. J., Jou I., Kim J. H. and Joe E. H. (2001) Reactive oxygen species mediate A beta(25–35)-induced activation of BV-2 microglia. Neuroreport **12:** 1449–1452

64 Frears E. R., Stephens D. J., Walters C. E., Davies H. and Austen B. M. (1999) The role of cholesterol in the biosynthesis of beta-amyloid. Neuroreport **10:** 1699–1705

65 Galbete J. L., Martin T. R., Peressini E., Modena P., Bianchi R. and Forloni G. (2000) Cholesterol decreases secretion of the secreted form of amyloid precursor protein by interfering with glycosylation in the protein secretory pathway. Biochem. J. **348:** 307–313

66 Simons M., Keller P., De Strooper B., Beyreuther K., Dotti C. G. and Simons K. (1998) Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. Proc. Natl. Acad. Sci. USA **95:** 6460–6464

67 Koudinov A. R., Koudinova N. V. and Berezov T. T. (1996) Alzheimer's peptides A beta 1–40 and A beta 1–28 inhibit the plasma cholesterol esterification rate. Biochem. Mol. Biol. Int. **38:** 747–752

68 Osborne T. F. and LaMorte V. J. (1998) Molecular aspects in feedback regulation of gene expression by cholesterol in mammalian cells. Methods **16:** 42–48

69 Liu Y., Peterson D. A. and Schubert D. (1998) Amyloid beta peptide alters intracellular vesicle trafficking and cholesterol homeostasis. Proc. Natl. Acad. Sci. USA **95:** 13266–13271

70 Ruan X. Z., Varghese Z., Powis S. H. and Moorhead J. F. (2001) Dysregulation of LDL receptor under the influence of inflammatory cytokines: a new pathway for foam cell formation. Kidney Int. **60:** 1716–1725