DIFFERENTIAL EFFECTS OF APOLIPOPROTEIN J AND OTHER AMYLOID ASSOCIATED PROTEINS ON AMYLOID-BETA UPTAKE BY ADULT HUMAN MICROGLIA AND ASTROCYTES

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ABSTRACT

Alzheimer disease (AD) is characterized by progressive accumulation of amyloid beta (Aβ) in amyloid plaques, most likely due to a failure in Aβ clearance. Both astrocytes and microglia participate in amyloid clearance. Several amyloid associated proteins (AAPs), such as Apolipoprotein E and J (ApoE and ApoJ), α1-antichymotrypin (ACT), serum amyloid P (SAP) and complement factor C1q accumulate in amyloid plaques. These AAPs are capable of inducing aggregation and deposition of amyloid in amyloid plaques, however little is known about their effect on amyloid clearance by glial cells. Therefore, we aimed to investigate the glial cell response to Aβ in the presence or absence of AAPs in vitro. To this end, cellular uptake of Aβ oligo and Aβ fib by adult human microglia and astrocytes was determined by flow cytometry and the levels of secreted pro-inflammatory cytokines (IL6 and TNFα) were quantified by ELISA. Both microglia and astrocytes exposed to either fluorescence-labeled Aβ oligo or Aβ fib, preferably bound and internalized Aβ oligo (% Aβ positive cells, microglia: 79% versus 44%; astrocytes: 74% versus 24%; p<0.001). In addition, microglia bound and internalized more Aβ fib compared to astrocytes (p<0.05). ACT, ApoE, ApoJ and combination of SAP and C1q (SAP-C1q), reduced Aβ fib internalization by 30% in microglia, whereas only ApoJ reduced Aβ oligo uptake (60% reduction, p<0.01). Interestingly, when comparing Aβ uptake between microglia and astrocytes, ApoJ, ApoE and SAP-C1q reduced Aβ oligo but not Aβ fib uptake by adult human astrocytes. Moreover, a strong pro-inflammatory response was generated when Aβ was incubated with ApoJ, ApoE, ACT and SAP-C1q and added to the microglia, but not in astrocytes. We conclude that AAPs, especially ApoJ, prevent Aβ binding and uptake in both microglia and astrocytes differentially depending on Aβ aggregation state. Combining these results with the increased pro-inflammatory response induced by AAPs in microglia, our data suggest a detrimental role of AAPs in AD pathogenesis.
INTRODUCTION

The deposition of amyloid beta (Aβ) in extracellular plaques is one of the pathological hallmarks of Alzheimer’s disease (AD). Whereas, familial early-onset AD is primarily related to overproduction of Aβ, sporadic late onset AD may result from impaired removal of Aβ from the brain. Several pathways for Aβ clearance have been suggested: 1) extracellular degradation by Aβ-cleaving proteases 2) Aβ clearance over the blood brain barrier and 3) Aβ uptake and degradation by microglia and also astrocytes.

In non-demented controls as well as AD cases, Aβ containing astrocytes can be observed in the brain parenchyma, which suggests that astrocytes may be involved in the uptake of not (yet) aggregated Aβ from the brain parenchyma. In early stages of AD, Aβ immunoreactive microglia can be found near diffuse amyloid deposits, however clusters of microglia with an activated MHC class II positive phenotype are predominantly found associated with fibrillar Aβ plaques. Activated microglia cells and reactive astrocytes can produce and release potentially toxic products such as reactive oxygen species and pro-inflammatory cytokines which could damage surrounding neurons, implicating an important role of glia cells in disease progression. Although both astrocytes and microglia cells are closely associated with amyloid plaques in AD, the accumulation of large numbers of compact Aβ deposits suggests that there is limited success in Aβ removal.

Several proteins accumulate in amyloid plaques. Some of these amyloid associated proteins (AAPs), including α1-antichymotrypsin (ACT) and Apolipoprotein E and J (ApoE and ApoJ) are already found in diffuse Aβ plaques that are devoid of activated glial cells and tau-associated changes and thus represent an early stage in AD pathology. Further indications for the importance of these proteins in AD pathogenesis came from animal studies in which these proteins were found to enhance amyloid deposition. Recent genome wide association studies identified various factors including ApoE, ApoJ, PICALM and the complement receptor 1 (CR1) as genetic risk factors for AD. Interestingly, all these factors are potentially involved in Aβ clearance, however little is known about the exact mechanism.

Since several AAPs have shown to affect amyloid plaque pathology, we aimed to investigate how glial cells respond to Aβ in the presence or absence of AAPs in vitro. To this end, cellular uptake of oligomeric Aβ1-42 (Aβ_{oligo}) and fibrillar Aβ1-42 (Aβ_{fib}) by adult human microglia and astrocytes was determined and the levels of secreted pro-inflammatory cytokines were quantified. Similar to our findings with adult human astrocytes, microglia were found to preferentially ingest Aβ_{oligo} over Aβ_{fib}. We now extended the astrocyte study and found that ApoJ and ApoE, as well as SAP and C1q together, reduce Aβ_{oligo} but not Aβ_{fib} uptake. In contrast to the situation in astrocytes, the uptake by microglia of Aβ_{fib} is reduced in the presence of AAPs, which seems to be related to the microglial inflammatory response initiated by the presence of Aβ and AAPs.
MATERIAL AND METHODS

Isolation and cell culture

Adult primary human microglia and astrocytes were isolated from brain specimens obtained at autopsy (through the Netherlands Brain Bank) or at surgery (focal cortical temporal resection for intractable complex partial seizures). Patients gave informed consent, and the use of tissue for experiments was in compliance with the Declaration of Helsinki and approved by the local Medical Ethics Committee at the VU University Medical Center in Amsterdam. Isolated microglia cells were cultured in a mixture of DMEM and HAM-F10 (Gibco, Paisley, PA) (1:1) supplemented with 10% (v/v) fetal calf serum, L-glutamin (2mM), penicillin (100 IU/ml), streptomycin (50µg/ml) and granulocyte-monocyte colony stimulating factor (25 µg/ml), at 37°C and 5% CO₂ as described before. Approximately ten days after isolation, microglia cells were plated in 24 well plates (NuncA/S, Roskilde, Denmark) at a density of 25,000 cells per well. Cells were allowed to adhere for 24 hrs prior to stimuli exposure. Astrocytes were isolated and cultured as described before.

Aβ1-42 preparations and amyloid associated proteins

Aβ1-42 oligomers and fibrils were prepared and characterized as previously described. Prior to the cell treatment, the 100 µM Aβ stock preparations were diluted ten times in treatment medium (serum and phenol-red free DMEM-F10 1:1) and the resulting 10µM Aβ_{oligo} and 10µM Aβ_{fib} preparations were incubated at RT for 1h, either alone or in combination with AAPs. AAPs tested included ACT (Calbiochem, Darmstadt, Germany), ApoE (rPeptide, Bogart, GA), ApoJ (isolated from human plasma by affinity chromatography as described) (all in the concentration range of 1µM, 0.1µM and 0.01µM), 85 nM SAP (Calbiochem), 5nM C1q (purified in-house as described before) or SAP and C1q together (SAP-C1q). Cytochalasin B (CB) (Sigma-Aldrich, St Louis, MO) was used as a general inhibitor of endocytosis as this compound inhibits actin polymerization. The various stimuli were applied to the cells and treatment lasted over-night (18 hrs).

Cell treatment and flow cytometry

After 18 hours of treatment, cell-free microglia conditioned culture medium (centrifuged at 275g for 5 min) was collected and stored at -20°C for further analysis of lactate dehydrogenase (LDH) activity and quantification of IL-6 and TNFα release. Cells were rinsed with PBS and harvested using 0.25% trypsin, centrifuged for 5 min at 275g and washed three times in cold FACS buffer (0.25% BSA in PBS). Cellular Aβ binding and uptake of fluorescent Aβ1-42 was quantified using flow cytometry as described before. The percentage of Aβ positive cells, 5,000 counted cells per condition, was quantified using the FACS Calibur (BD Biosciences, San Jose, CA) with the CellQuest software. The same gates (fixed gates) were used throughout the experiment series and a detected event was defined as a cell gated through the front-scatter (FSC) and side-scatter (SSC) gate. An
Aβ positive cell was defined as a fluorescence (FAM)-positive microglia. Negative controls (untreated cells) were used to set the limit of background fluorescence and Staurosporin (Sigma-Aldrich; 1µM, over-night) treated microglia were used next to untreated cells to be able to distinguish dead cells and cell debris from living cells.

**Quantification of cell supernatant IL6 and TNFα**
To evaluate the baseline secretion and the pro-inflammatory response of treated microglia, the release of IL6 and TNFα in the cell supernatant of treated microglia was determined. Interleukin-6 and TNFα were both quantified using the respective Pelipair ELISA kits (Sanquin, Amsterdam, The Netherlands).

**Cytotoxicity**
In order to detect cytotoxic effects induced by the two Aβ preparations alone or in combination with AAPs, extracellular LDH activity in the conditioned medium was measured as described before. In short, cell-free conditioned medium was mixed with 100 mM potassium phosphate buffer (pH 7.4) containing 1.32 mg/ml sodium pyruvate, and 1 mg/ml NADH. The rate of decrease in absorbance at 340 nm was measured kinetically for 10 min at 37 ºC in a Microplate Spectrophotometer SPECTRAmax 250 (Molecular Devices, Sunnyvale, California). Results from all conditions were compared to the corresponding medium treated microglia.

**Statistics**
The effects of AAPs on Aβ uptake and IL6/TNFα secretion were normalized against results from Aβ-treated cells from the corresponding individual culture and expressed as means ± SD values. Comparisons between the various treatments were assessed with Mann-Whitney U tests and Bonferroni post-hoc correction on raw data and on normalized data when applicable. A p-value of <0.05 was considered to reflect statistical significance.

**RESULTS**

**Binding and uptake of Aβ oligo and Aβ fb**
Flow cytometry was used to quantify the number of Aβ-positive microglia and astrocytes after treatment with Aβ oligo or Aβ fb. Similar as seen with astrocytes, a higher percentage of microglia cells was Aβ positive upon Aβ oligo exposure (79% ± 4%; n=16), compared to Aβ fb (44%±6%; n=16; p<0.001) (Figure 1A), suggesting an Aβ-size dependent uptake mechanism. The fluorescence signal from the microglia exposed to Aβ oligo was higher that when exposed to Aβ fb, indicating that more Aβ was ingested per cell when treated with Aβ oligo (Figure 1B). Furthermore, binding and uptake of Aβ fb was significantly higher in microglia compared to astrocytes (44% vs 23% respectively; p=0.01), whereas no difference was detected for Aβ oligo (79% vs 74% respectively; p=0.42 (Figure 4A).
In this study, the effects of several AAPs on Aβ oligo and Aβ fibr binding and uptake by primary human microglia were tested and the data on astrocytes was extended. When Aβ oligo or Aβ fibr were applied to the microglia cells upon pre-incubation with various AAP (either ApoE, ApoJ or ACT in concentration from 1µM to 0.01 µM, or either SAP (85nM), C1q(5nM) or SAP-C1q (85-5nM)), varying effects of these AAPs were seen on microglial Aβ uptake. The results are depicted as normalized data, with Aβ-uptake upon Aβ treatment alone set to 100% (indicated by the horizontal line; Figures 1 and

Figure 1. Aβ binding and uptake by primary human microglia. Cells were exposed to Aβ oligo or Aβ fibr (n=16) and the Aβ positive cells were quantified (A). Mann-Whitney U test was performed, ***p<0.001. Representative histogram displaying background fluorescence of untreated microglia (black) and the distribution of cells positive for Aβ oligo (green) or Aβ fibr (red)(B). Aβ (10µM) in combination with ACT (C), ApoJ (D), ApoE (E) were tested in different concentrations (0.01µM, 0.1µM and 1µM), whereas SAP (85nM) and C1q (nM) were tested in a fixed concentration and applied either separately or in complex (F). Results from a minimum of four individual microglia cultures are presented as means with standard deviations. Mann-Whitney U test was performed with Bonferroni correction. *p<0.05, **p<0.01. See page 215 for color figure.
Figure 2. Secretion of IL6 and TNFα as determined in the cell supernatant of microglia upon treatment (18 hrs) with Aβ oligo, Aβfib and Aβ in combination with AAPs. Levels of IL6 (A) and TNFα (B) secretion upon Aβ oligo and Aβ fib stimulation were compared to those of medium treated cells. Data on fold increase in cytokine secretion upon treatment of Aβ oligo or Aβ fib in combination with ACT (C), ApoJ (D), ApoE (E) and SAP-C1q (D) compared to Aβ alone are depicted. Results from a minimum of n=4 individual microglia cultures are presented as means with standard deviations. Note: the scales (Y-axis) in C-F are logarithmic and differ in E and F from those in C and D. Mann-Whitney U test was performed with Bonferroni correction. *p<0.05, **p<0.01

4B). The largest effects of ApoJ, ApoE and ACT on microglial Aβ fib binding and uptake were seen in a 1:10 molecular ratio to Aβ fib (1µM AAP versus 10µM Aβ), although a concentration dependent effect was observed, this did not reach significance for all conditions (Figure 1C-F). The percentage of Aβ positive microglia upon Aβ fib treatment was reduced by 1µM and 0.1µM ApoJ (42% and 22% reduction, respectively; both p<0.01), 1µM ACT (36% reduction, p=0.03), 1µM, 0.1µM and 0.01µM ApoE (34% (borderline significant p=0.08), 18% (p=0.01) and 7% reduction (p<0.05), respectively) and SAP-C1q (30% reduction, p<0.01). Only 1µM ApoJ, 0.01µM ApoJ
and 0.01 μM ApoE showed a significant reduction in Aβ<sub>oligo</sub> uptake compared to Aβ<sub>oligo</sub> alone (60% (p<0.01), 11% (p<0.05), 15% (p<0.05) reduction, respectively).

Although the number of astrocyte cultures tested was increased from minimally three to minimally 5 cases per tested treatment, results did not differ from those described. Statistical differences were observed for SAP-C1q that increased Aβ<sub>fib</sub> uptake by 49%, whereas ApoJ, ApoE and SAP/C1q reduced Aβ<sub>oligo</sub> uptake in the astrocytes by 89%, 80% and 7% respectively (Figure 4B). Results between microglia and astrocytes were compared (Figure 4B-C). It was found that AAPs reduced uptake of Aβ<sub>fib</sub> in microglia, but to leave uptake of Aβ<sub>oligo</sub> largely unaffected. In contrast to microglia, uptake of Aβ<sub>oligo</sub>, but not of Aβ<sub>fib</sub>, was hampered by AAPs in astrocytes.

**Inflammatory response upon Aβ and AAP treatment**

Microglia cells are capable of generating an inflammatory response upon interaction with foreign substances in the brain. We set out to explore whether our treatments would yield a pro-inflammatory response *in vitro*. Therefore, we determined the microglial secretion of the pro-inflammatory cytokines IL-6 and TNFα into the culture medium during treatment. SECRETION of both IL-6 and TNFα seemed to be increased upon Aβ<sub>oligo</sub> and Aβ<sub>fib</sub> incubation compared to medium treated cells (Figure 2A,B), but due to large

![Figure 3](image3.png)

*Figure 3.* Possible relations between reduced Aβ uptake and pro-inflammatory secretion of IL6 and TNFα induced by AAPs were investigated by plotting the fold-change in cytokine (IL-6 and TNFα) release upon exposure of microglia to Aβ in the presence of AAPs, against the percentage reduction in Aβ<sub>oligo</sub> uptake in the presence of AAPs. No relation between increased IL6 and TNFα secretion and reduced Aβ<sub>oligo</sub> uptake was found (A and B). However, a positive correlation between the levels of secreted IL6 and TNFα and a reduction in Aβ<sub>fib</sub> uptake was found (C and D; Spearman correlation).
**Figure 4.** Differences in number of positive cells for $A\beta_{\text{oligo}}$ and $A\beta_{\text{fib}}$ between human microglia ($n=16$) and astrocytes ($n=13$) (A). The effect of several AAPs (ACT, ApoJ, ApoE and SAP-C1q) on $A\beta_{\text{oligo}}$ and $A\beta_{\text{fib}}$ uptake were compared between human adult primary microglia and astrocytes (B and C). Uptake of $A\beta$ alone was set to 100%. Uptake of $A\beta_{\text{oligo}}$ is mainly reduced by AAPs in human astrocytes, whereas uptake of $A\beta_{\text{fib}}$ is inhibited by AAPs in human microglia. Results from a minimum of five individual microglia and astrocyte cultures are presented as means with standard deviations.
variation between patients, only IL6 secretion upon Aβ\textsubscript{oligo} treatment was significantly different compared to medium treatment (1.8±0.25 fold increase, \(p=0.003, \ n=14\)). No difference in cytokine secretion was found between Aβ\textsubscript{oligo} and Aβ\textsubscript{fib} treated cells.

Incubation of Aβ\textsubscript{oligo} and Aβ\textsubscript{fib} in combination with AAPs, boosted the response of both IL6 and TNFα secretion into the cell culture medium (Figure 2C-F). The strongest inducers (>100 fold increase in cytokine expression) were Aβ\textsubscript{oligo} or Aβ\textsubscript{fib} in combination with 1μM ACT, 1μM ApoJ and 1 μM ApoE (all \(p<0.05\)). Furthermore, a positive relation between increased IL6 and TNFα secretion with reduced Aβ uptake was found for Aβ\textsubscript{fib} only (IL6: \(r=0.32, p=0.02\); TNFα: \(r=0.38, p=0.003\)) (Figure 3). Primary human astrocytes did not produce a pro-inflammatory response upon Aβ and AAP treatment, as shown before 4.

Cytotoxic effects of Aβ and AAPs

To determine if the effects of AAPs on microglial Aβ uptake resulted from cytotoxic effects of these treatments, LDH activity in the supernatants of the microglia was determined. In general no increase in LDH activity was found upon treatment with Aβ with or without AAPs, however a few treatments caused significant increases in LDH activity when compared to Aβ alone. Microglia incubated with Aβ\textsubscript{fib} in combination with C1q increased LDH release by 20% (\(p=0.008\)) and microglia incubated with Aβ\textsubscript{oligo} in combination with 0.1μM ACT, 0.01 ApoE and SAP-C1q also increased LDH release by respectively 22%, 42% and 11% (all \(p=0.008\)). This suggests that under these conditions the cytotoxicity might influence the percentage of Aβ-positive cells, however conditions where AAPs reduced Aβ uptake were not found to be cytotoxic in microglia. No cytotoxic effect were found when primary human astrocytes were exposed to Aβ and AAP treatment, as shown before 4.

**Figure 5.** Cytochalasin B (CB) is a general inhibitor of endocytosis. Here we compared the effect of CB on Aβ\textsubscript{oligo} and Aβ\textsubscript{fib} uptake by adult human microglia and astrocytes. Aβ\textsubscript{oligo} and Aβ\textsubscript{fib} in combination with CB, resulted in a reduction of Aβ positive microglia cells (A), whereas Aβ\textsubscript{oligo} and Aβ\textsubscript{fib} uptake by astrocytes was not affected by CB (B). Results of 5 individual microglia cultures and 3 individual astrocytes cultures are presented in Figure 1.
Abeta uptake mechanism

To see whether this Aβ binding and uptake is related to actin-dependent endocytosis, Cytochalasin B (CB) was added in conjunction with Aβ_{oligo} and Aβ_{fib}. CB reduced the number of Aβ positive microglia when treated with Aβ_{fib} by 90% (p<0.001) whereas Aβ_{oligo} positive cells were reduced by 50% (p<0.001) (Figure 5A). Interestingly, inhibition of Aβ_{fib} uptake was significantly greater than inhibition of Aβ_{oligo} uptake (p=0.003), suggesting different uptake mechanisms for the two Aβ species. Treatment of adult human astrocytes with CB did not affect Aβ_{oligo} or Aβ_{fib} internalization (Figure 5B).

DISCUSSION

The aim of the current study was to investigate how adult human microglia and astrocytes respond to Aβ in the presence or absence of AAPs in vitro. To this end, cellular uptake of Aβ_{oligo} and Aβ_{fib} by adult human microglia and astrocytes was determined and the levels of secreted pro-inflammatory cytokines were quantified.

Our study revealed that a larger percentage of primary human microglia and astrocytes take up Aβ_{oligo} compared to Aβ_{fib} in vitro, suggesting that smaller sized Aβ species are taken up more easily than larger fibrillar Aβ forms. Furthermore, Aβ_{fib} was taken up more efficiently in microglia compared to astrocytes. Although several studies showed that primary microglia are capable of taking up both Aβ_{oligo} and Aβ_{fib} 28-30, we are the first in comparing and quantifying both Aβ_{oligo} and Aβ_{fib} uptake by primary human microglia and astrocytes. Recently it was suggested that primary neonatal mouse microglia have higher intracellular levels of Aβ_{fib} compared to Aβ_{oligo} when treated with Aβ 28, which seem to contradict our results. Most likely, species differences and age of the brain (neonatal versus aged brain) 31, 32 are a likely cause of the observed differences, indicating the importance of primary adult human brain cells in AD research. Combining these results with post mortem findings where astrocytes contain Aβ-positive granules when no amyloid plaques are formed yet and that activated microglia are clustered around fibrillar amyloid plaques in the human brain, we could hypothesize that astrocytes play an important role in the clearance of Aβ very early in the disease and that microglia play a more important role when plaques are already formed.

Furthermore we implicate an important role of Aβ binding proteins in reducing Aβ clearance. Diffuse plaques are thought to characterize one of the earliest phases of AD pathogenesis. These plaques are decorated by several inflammatory and transport proteins, including ACT, ApoJ, ApoE and complement factors 13. Not only are these proteins locally up-regulated in AD affected brain areas and do they play an important role in the dynamic interplay between Aβ production and removal 14-17, they are also implicated as genetic risk factors for late-onset AD 19, 33 or to affect age at onset and disease duration 34, implicating a crucial role of these AAPs in amyloid plaque formation. Indeed,
in this study we described the ability of several AAPs to inhibit Aβ clearance by microglia and astrocytes. Interestingly, whereas ApoJ prevented internalization of both Aβ<sub>oligo</sub> and Aβ<sub>fib</sub> in adult microglia, ApoE, ACT and SAP-C1q reduced uptake of Aβ<sub>fib</sub> only and left the uptake of Aβ<sub>oligo</sub> unaffected, which suggests that as soon as Aβ becomes fibrillar and associated with AAPs (as seen in compact plaques), internalization is greatly inhibited. In line with our results, Yang et al showed recently that blocking the ApoE-Aβ interaction the fibrillar vascular amyloid deposition was reduced. Interestingly, the absence of ApoE was shown to reduce Aβ degradation, without influencing internalization by mouse microglia. ACT was found to inhibit Aβ-degradation both in vitro and in vivo, but did not affect Aβ uptake by human astrocytes. Here we show that ACT is capable of preventing Aβ<sub>fib</sub> internalization by microglia. Thus, both ACT and ApoE can affect Aβ uptake as well as degradation by microglia. Incubating microglia with Aβ and SAP or C1q alone did not modulate Aβ uptake in our study, whereas it was shown before that C1q is capable of inhibiting Aβ uptake. This difference is probably explained by a 125 fold higher C1q concentration used in the experimental setup of that particular study. The combination of SAP and C1q (SAP-C1q) was earlier shown to reduce Aβ endocytosis by primary human microglia. Here we confirm this finding by showing that this effect was only seen in combination with Aβ<sub>fib</sub>, implicating that some degree of fibril formation is needed for SAP in combination with C1q to inhibit Aβ uptake. In this study, ApoJ was shown to prevent binding and subsequent internalization of both Aβ<sub>oligo</sub> and Aβ<sub>fib</sub> by primary human microglia, confirming its role as a transport protein. Our results are supported by those obtained by Cole et al., who showed that ApoJ blocks binding, uptake and degradation of Aβ42 in rat microglia. On the other hand, there are reports that Aβ-ApoJ complexes can be internalized by epithelial cells via megalin-mediated endocytosis and degraded in lysomes, however there are no indications that megalin is expressed by microglia, which strengthens our finding that ApoJ inhibits rather than increases Aβ internalization in microglia. All these data together provide strong evidence for a detrimental role of amyloid binding proteins in amyloid clearance from the brain, which could explain why activated microglia that are physically associated with amyloid plaques are unable to effectively clear Aβ deposits in AD brain.
do imply a detrimental role of AAPs in AD pathology, since the AAPs are capable of reducing $\beta_{fib}$ clearance and increasing the pro-inflammatory response which will most likely lead to exacerbation of AD pathology.

Next to microglia cells, also astrocytes participate in $\beta$ clearance. Interestingly, when comparing both cell types with respect to $\beta$ uptake we found that $\beta_{fib}$ is internalized more avidly by microglia than astrocytes, suggesting a more important role of microglia in $\beta_{fib}$ clearance. Based on the effects of AAPs on $\beta$ clearance by microglia and astrocytes as shown here and in our previous study, we could speculate that in the presence of certain AAPs $\beta_{aligo}$ removal is impaired in astrocytes, whereas in microglia $\beta_{fib}$ removal is impaired in the presence of AAPs. Not only could this lead to possible $\beta$ impairment of clearance of both diffuse (oligomeric) and compact (fibrillar) plaques, but it also illustrates the importance of this combined role of astrocytes and microglia in amyloid clearance.

Several receptors have been proposed to be involved in $\beta_{fib}$ internalization, including scavenger receptors, Fc receptors, CD14, and a multiprotein complex composed of the scavenger receptor type B CD36, an $\alpha\beta$ integrin and the integrin-associated protein CD47, suggesting that several receptors, rather than only one, collaborate in endocytosis of $\beta_{fib}$. In contrast to $\beta_{fib}$, little is known about the uptake mechanism of $\beta_{aligo}$. Uptake via scavenger receptor A and fluid-phase pinocytosis has been suggested to be involved in $\beta_{aligo}$ internalization by microglia. Although all these uptake mechanisms depend on actin polymerization, we clearly find cells positive for $\beta_{aligo}$ that remain unaffected by CB treatment. This suggests that part of $\beta_{aligo}$ internalization is related to a non-actin involved mechanism, however the exact mechanism needs to be further elucidated. Interestingly, $\beta_{fib}$ and $\beta_{aligo}$ uptake by astrocytes was not sensitive to CB treatment, suggesting a different $\beta$ uptake mechanism involved in astrocytic $\beta$ clearance compared to microglia.

In conclusion, several early plaque proteins, especially ApoJ, can inhibit $\beta$ clearance in both adult human microglia and astrocytes depending on the $\beta$ aggregation state. Moreover, these proteins enhance the pro-inflammatory response in microglia. These results implicate a detrimental role of amyloid binding proteins in amyloid clearance and induction of the inflammatory response.

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