Published in final edited form as:
Neurobiol Aging. 2008 May ; 29(5): 707–715.

Degradation of fibrillar forms of Alzheimer's amyloid β-peptide by macrophages

Amitabha Majumdar[#,Ξ], **Haeyong Chung**[*,#,Ξ], **Georgia Dolios**[†], **Rong Wang**[†], **Nikiya Asamoah**[#], **Peter Lobel**[Δ], and **Frederick R. Maxfield**[#&]

#*Department of Biochemistry, Weill Medical College of Cornell University, New York, NY 10021.*

**Department of Pathology, Columbia University-College of Physicians and Surgeons, New York, NY 10032.*

†*Department of Human Genetics, Mt. Sinai School of Medicine, New York, NY 10029*

Δ*Center for Advanced Biotechnology and Medicine, Robert Wood Johnson Medical School, Piscataway, N.J. 00854*

Abstract

Cultured microglia internalize fibrillar amyloid Aβ (fAβ) and deliver it to lysosomes. Degradation of fAβ by microglia is incomplete, but macrophages degrade fAβ efficiently. When mannose-6 phosphorylated lysosomal enzymes were added to the culture medium of microglia, degradation of fAβ was increased, and the increased degradation was inhibited by excess mannose-6-phosphate, which competes for binding and endocytic uptake. This suggests that low activity of one or more lysosomal enzymes in the microglia was responsible for the poor degradation of fAβ. To further characterize the degradation of fAβ in late endosomes and lysosomes, we analyzed fAβ-derived intracellular degradation products in macrophages and microglia by mass spectrometry. Fragments with truncations in the first 12 N-terminal residues were observed in extracts from both cell types. We also analyzed material released by the cells. Microglia released mainly intact $A\beta_{1-42}$, whereas macrophages released a variety of N-terminal truncated fragments. These results indicate that initial proteolysis near the N-terminus is similar in both cell types, but microglia are limited in their ability to make further cuts in the fAβ.

Keywords

Alzheimer's disease; A-beta; macrophages; clearance; mass spectrometry

1. Introduction

Microglia comprise about 10% of the cells in the CNS of an adult mouse [19]. In a normal adult brain microglia are quiescent cells that monitor the microenvironment to protect the brain from injury or infection [9,16], and during pathological conditions such as stroke or

Correspondence to: Rong Wang.

&To whom correspondence should be addressed: Weill Medical College of Cornell University, Department of Biochemistry, 1300 York Avenue, Room E215, New York, NY 10021, Tel: (212) 746-6405, Fax: (212) 746-8875, email: frmaxfie@med.cornell.edu.

ΞThese two authors contributed equally to this work.

Disclosure Statement: The authors declare that there is no actual or potential conflict of interest with any person or organization.

neurodegeneration, microglia become activated. In Alzheimer's disease (AD) microglia become activated, and it is believed that the associated inflammatory response exacerbates the disease pathology [10]. However, in mice immunized against Aβ, microglia can degrade fAβ in the CNS [3,25]. This suggests that microglia could help to limit the accumulation of fAβ under some circumstances.

Although animal model studies indicate that microglia can degrade fibrillar forms of Aβ peptide, tissue culture studies of degradation have produced apparently conflicting results. Studies on primary microglia have shown that microglia internalize fAβ via scavenger receptors, but they degrade fAβ poorly even though they have functional lysosomes and can degrade α2-macroglobulin and acetylated-LDL quite effectively [7,17,18]. In these studies it was shown by double label fluorescence microscopy that fAβ is internalized efficiently and delivered to late endosomes and lysosomes that are able to degrade these other proteins. On the other hand there are reports showing that primary microglia were able to clear out Aβ deposits from unfixed cryostat sections of AD cortex containing Aβ plaques when they were activated by passive immunization [4]. It was also reported that microglia cultures obtained from autopsy samples of AD patients could remove aggregated Aβ from tissue culture dishes via phagocytosis [22]. It was reported that rat microglial cultures and an activated human monocyte cell line could degrade Aβ42 added to the culture medium and that microglia could remove Aβ fibrils immobilized as plaque-like deposits on culture dishes [27]. In a different study, ingested, non-degraded amyloid remained within phagosomes for up to 20 days, suggesting a very limited effectiveness of microglia in degrading Aβ fibrils [11]. These discrepancies may arise from differences in the activation state of the cells or from differences in the specific properties of the Aβ (e.g., fibrillar vs. soluble). Unfortunately, in many studies these issues are not addressed clearly.

In the present study, we further investigated the fAβ degradation process in microglia in order to understand why degradation is incomplete. In parallel experiments, we compared fAβ degradation in murine microglia and macrophages. We found that macrophages could degrade fAβ particles efficiently, but the identical particles were only partially digested by microglia. Analyses of internalized fAβ in the microglia and the macrophage cells using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) showed that both cell types produced similar N-terminally cleaved fAβ fragments. Incubation of microglia with mannose-6-phosphate-tagged lysosomal enzymes increased the ability of microglia to degrade fAβ, indicating that the lack of activity of some enzymes may be limiting the ability of microglia to degrade fAβ.

2. Materials and Methods

2.1. Cells

Primary microglial cultures were isolated and maintained as described previously [17]. Primary mouse peritoneal macrophages were kindly provided by the laboratory of Dr. Carl Nathan. These macrophages were obtained from 25–35 g female mice that had been injected intraperitoneally with 2 ml of 4% thioglycollate broth (Brewer, DIFCO, Detroit, MI) 3 to 4 days prior to cell harvesting [35]. After centrifugation at 170 × *g* for 10 min, the cell pellets were suspended in RPMI 1640 (Hazelton Biologics, Lenexa, KS) containing 10% heat-inactivated fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT), 200 U/ml penicillin, and 200 μg/ml streptomycin (Hazelton Biologics). The macrophages were plated at ~80% confluence in either 24-well plates or coverslip-bottom dishes in complete RPMI 1640 medium containing 10% FBS and incubated at 37°C in a 5% CO_2 tissue culture incubator. Experiments were performed one or two days after the cells were plated. J774.A1 macrophage-like cells (American Type Culture Collection) [29] were maintained in spinner culture in DMEM (high glucose) containing 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin (GIBCO-BRL),

and 2 mM glutamine at 37°C under 5% CO_2. The medium was replaced with fresh medium daily.

2.2. Proteins and peptides

$A\beta_{1–42}$ peptides were purchased from Bachem (Torrance, CA). ^{125}I-labeled-fAβ and Cy3-fAβ were prepared using protocols described previously [18]

2.3. Degradation of ^{125}I-fAβ and Cy3-fAβ in macrophages and microglia

Degradation of ^{125}I-fAβ and Cy3-fAβ in J774 cells, peritoneal macrophages and microglia were measured following protocols described previously [7]. For Cy3- fAβ degradation, the cells were imaged using a Leica epifluorescence microscope with a cooled CCD camera (Princeton Instruments Micromax 521BFT, Roper Scientific, Trenton, NJ), and the Cy3 fluorescence power was quantified using MetaMorph Imaging System software (Molecular Devices, Downingtown, PA).

2.4. Degradation of Cy3-fAβ in microglia after lysosomal enzyme augmentation

A pool of mannose-6 phosphorylated lysosomal proteins was purified from human brain by mannose-6-phosphate receptor affinity as described previously [28]. To measure degradation of Cy3-fAβ following lysosomal enzyme augmentation, microglia were incubated with Cy3-fAβ for 1 hour, as described previously [7], and then the mixed enzyme pool was added to the chase medium at a final concentration of 30nM. The molarity of the enzyme concentration was estimated based on a molecular weight of 60,000g/mol for a Man-6 P tagged lysosomal enzyme yielding the concentration for the mixed enzyme pool as approximately 30 nM. Cells were incubated for 3 days, and then after that the Cy3 fluorescence power in cells was measured. Mannose-6 phosphate (10 mM) was included in the incubation medium of parallel cultures to inhibit mannose-6 phosphate receptor mediated endocytosis.

2.5. Mass spectrometry (MALDI-TOF-MS)

Cells were grown in 6-well plates and incubated with fAβ particles for 8–16 h. The cells were rinsed and chased in DMEM (for microglia and J774 cells) or RPMI (for peritoneal macrophages) with 1% FBS for 1 – 3 days. At the end of each chase time, the medium was collected and subjected to TCA precipitation as described previously [7]. The cells were rinsed and lysed using a lysis buffer of phosphate buffered saline with 2% CHAPS, 0.1 mM TLCK, 1 mM PMSF, 2 mM EDTA, 10 mM leupeptin, 1 mM pepstatin A, and 0.2 mM TPCK. The cells were sonicated at 4~5W for 20s followed by ultracentrifugation at 100,000 × *g* for 1 h at 4°C in order to separate the soluble vs. fibrillar Aβ. The pellet fractions from the cell lysates and the TCA precipitates from the chase medium were solubilized with 70% formic acid (100 μL) and neutralized with 2 M Tris. Aβ peptides in these preparation were immunoprecipitated with monoclonal anti-Aβ antibody 4G8 [33] and 3μl of Protein G Plus/Protein A-agarose beads (Oncogene Science, Inc., Cambridge, MA) at 4°C for 3hr. Immunoprecipitated Aβ peptides were analyzed using a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS) (Voyager-DE STR BioSpectrometry Workstation, PerSeptive Biosystems) as described previously [30].

3. Results

3.1. Degradation of internalized ^{125}I-labeled and Cy3-labeled fAβ by macrophages

In previous studies we found that microglia degraded internalized fAβ slowly and incompletely even though the fAβ is delivered to late endosomes and lysosomes [7,17]. In this study we examined whether other cell types would be able to degrade fAβ that could not be degraded by primary microglia. We examined the degradation by macrophages because these cells are

closely related developmentally to microglia and because macrophages have a biological role similar to microglia. We found that primary mouse peritoneal macrophages and J774 macrophage-like cells could degrade fAβ almost completely within a few days (Fig. 1A). Following internalization of ^{125}I-fAβ, macrophages released 80% of the ^{125}I as TCA-soluble material into the chase medium within 2–3 days. In parallel experiments, the microglia released only about 20% of the ^{125}I. Chloroform extraction of the TCA-soluble fraction of the chase medium [7] verified that the TCA-soluble material is ^{125}I associated with small peptides or amino acids.

Similar results were obtained when fAβ degradation in macrophages was monitored using Cy3-fAβ (Fig.1B). Less than 30% of the internalized Cy3-fAβ was retained in the macrophages after a 3 day chase, whereas over 80% of Cy3-fAβ was still cell-associated in the microglia after 3 days chase. These data show that both J774 cells and primary macrophages can degrade fAβ. However, even in macrophages the rate of fAβ degradation is still slower than for soluble proteins such as LDL [24].

3.2. Degradation of fAβ after mannose-6-phosphate tagged enzyme augmentation

One reason for poor degradation of fAβ by microglia might be inadequate activity of lysosomal enzymes that are needed for fAβ hydrolysis. To test this, we measured fAβ degradation after incubating cells with mannose-6-phosphate tagged lysosomal enzymes. The mannose-6-phosphate modification is responsible for the intracellular targeting of many lysosomal enzymes from the Golgi apparatus to lysosomes [8], and some of the mannose-6-phosphate receptor is also found on the cell surface [12]. Many studies have shown that the cell surface mannose-6-phosphate receptor binds extracellular lysosomal enzymes and delivers them to late endosomes and lysosomes [8]. This uptake system has even been used for enzyme replacement therapy in humans [5,26]. As shown in Figure 2, incubation with mannose-6-phosphorylated lysosomal enzymes increased the ability of microglia to degrade fAβ, as compared with parallel untreated control cells. Because the mannose phosphorylated at the 6 position is the entity recognized by the receptor, uptake of lysosomal enzymes can be inhibited by excess mannose-6 phosphate [14,15]. When the enzyme incubation was done in the presence of 10mM mannose-6 phosphate to compete for receptor binding, microglia failed to degrade fAβ. This indicates that the increased degradation of fAβ was specifically related to the uptake of lysosomal enzymes by mannose-6-phosphate receptors.

3.3. Composition of fAβ fragments in cells after uptake

Lack of specific enzymes in late endosomes and lysosomes might lead to the accumulation of a set of degradation intermediates in these organelles. To test this, we investigated the molecular identity of fAβ and its breakdown products in late endosomes and lysosomes using mass spectrometry. Cells were incubated with fAβ overnight, in order to allow enough intracellular accumulation of fAβ to be observed by mass spectrometry, and then the cells were chased for periods up to three days. The fAβ was isolated from cell lysates at each chase time, and Aβ peptides were selectively isolated by immunoprecipitation with monoclonal antibody 4G8, which recognizes residues 17–24 of Aβ [30] and analyzed by MALDI-TOF-MS [30]. We found that there was a significant amount of N-terminal trimming of the Aβ in both microglia and J774 macrophages (Figure 3). Similar fragments were observed in both cell types, although the relative abundance of specific degradation intermediates was different. Some fragments are seen at chase day 0 since cells are incubated overnight with fAβ, which would allow a significant exposure to hydrolases in late endosomes and lysosomes. Interestingly, the smallest fragment observed in either cell type was fAβ12–42. We did not observe any fragments smaller than fAβ12–42, nor any C-terminal trimming, which indicated that the N-terminal truncation is not the rate-limiting step in fAβ degradation. We know from the degradation studies that macrophages are degrading fAβ to amino acids or small peptides within 2–3 days. It is possible

that a proteolytic clip beyond residue 12 facilitates release of Aβ from the fibril, and once Aβ dissociates these degradation intermediates can be quickly further degraded into amino acids and small peptides. It should be noted that after 2 days of chase most of the fAβ internalized by J774 cells has been degraded to low molecular weight, TCA-soluble species (Fig. 1), and our mass spectrometry protocol is only designed to analyze macromolecular species containing the epitope site (residues 17–24). Thus, the signal-to-noise becomes poor in the extracts from J774 cells after 2 days chase (Fig. 3B).

3.4. Composition of fAβ fragments released into the chase medium

As reported previously, microglia release TCA-precipitable (i.e., macromolecular) Aβ from late endosomes and lysosomes during chases after uptake of fAβ [7]. MALDI-TOF-MS analysis of Aβ released into the chase medium after uptake revealed that mainly full length Aβ1–42 was released from microglia (Fig. 4A). We needed to allow the released material to collect in the medium for 3 days in order to have enough released Aβ to make a measurement.

We also analyzed the material released from macrophages. Shortly after the chase there was no detectable material released into the medium, indicating that the rinsing protocols were effective and there was little fAβ that was weakly adhering to the cells or the tissue culture dish. Within 24 hours of chase some full length peptide and a variety of N-terminally truncated fragments were released into the medium by the J774 macrophages (Fig. 4B). During the 2nd day, release of these fragments continued, but very little of the full length material was released from the cells during this period. Again, we note that this assay should detect any Aβ fragments containing the epitope site (residues 17–24). Very small fragments that lacked this site would not be detected, and most of the material released from macrophages is TCA-soluble (Fig.1), indicating that it is amino acids or very small peptides.

4. Discussion

Although fAβ plaques in the brain are extracellular, there is compelling evidence that fAβ can be internalized by microglia, and the fate of this internalized fAβ can have significant consequences in the development of AD plaques. If the fAβ is digested efficiently, as has been suggested to occur in the brains of mice immunized against Aβ [25], then microglial uptake will lead to reduced plaque growth and even removal of existing plaque. Conversely, the acidic conditions in the endosomes and lysosomes could promote assembly or growth of fAβ plaques, which could then be released from the cells into the extracellular space [31,32,34]. In that case, microglia might promote the nucleation and assembly of plaques. We have shown previously that cultured mouse microglia internalize fAβ, but they are inefficient in degrading it and actually release a large fraction of internalized fAβ back into the culture medium [7]. Like mouse microglia, microglia obtained at autopsy from Alzheimer's disease patients can also take up fAβ [22]. However it is not clear if they can completely degrade fAβ [1]. In this paper we examined whether the fAβ that we used in our studies was intrinsically indigestible by mammalian cells. We also examined the molecular identity of the fAβ that was retained in cells and the nature of the material that was released.

In the current study, we found that macrophages can degrade fAβ particles that are only partially degraded by cultured microglia. It is important to note that several replicates of these experiments were carried out using the same preparations of fAβ in parallel on the different cell types. This eliminates any variability in the fAβ properties as an explanation for the differences in degradation. Our data show clearly that primary macrophages and the J774 macrophage cell line are able to fully digest fAβ that cannot be digested fully by cultured primary microglia. This suggests that with proper augmentation of hydrolytic activity microglia might also become capable of digesting fAβ.

One way to increase the hydrolytic activity of late endosomes and lysosomes is to deliver additional enzymes using the enzyme uptake pathway that utilizes the mannose-6-phosphate receptor. Since most lysosomal enzymes carry this unusual post-translational modification, and since all cells use this receptor to deliver lysosomal enzymes to lysosomes, adding lysosomal enzymes with mannose-6-phosphate modifications to the extracellular medium is a very effective way to deliver enzymes to cells [15]. When we incubated mannose-6 phosphate tagged lysosomal enzymes with the microglia in order to deliver enzymes to the late endosomes and lysosomes, the microglia were able to degrade some of their internalized fAβ. This showed that increasing the hydrolytic capacity of microglia could lead to degradation of fAβ. When this enzyme augmentation was done in the presence of mannose-6 phosphate, which inhibits binding to and uptake by cell surface mannose 6-phosphate receptors [15], microglia failed to show any enhanced degradation of fAβ. This showed that the uptake of the enzymes via the endocytic route was responsible for enhanced degradation, and there was no inadvertent phenomenon, such as activation of the microglial cells by a contaminant in the enzyme samples, causing the enhanced fAβ degradation. We cannot formally rule out the possibility that ligand binding to the mannose 6-phosphate receptor activates a signaling process that enhances degradation.

We carried out a mass spectrometry analysis of the fAβ-derived degradation products that were either released into the chase medium or retained in the endosomes/lysosomes of macrophages and microglia. The purpose of these experiments was two-fold. First, we wanted to see if a particular degradation intermediate accumulated in the microglial cells to a greater extent than in the macrophages. This might point to a particular enzyme that was lacking in the microglia relative to the macrophages. Second, the pattern of degradation intermediates might provide information about the slow step in degradation.

We found that both microglia and macrophages had fAβ fragments that were digested from the N-terminus, and similar fragments were produced in both cell types. We did not observe C-terminal truncations in material extracted from either macrophages or microglia. The fact that microglia and macrophages produce similar fragments from fAβ indicates that microglia are not functionally deficient in the enzymes that produce the N-terminal truncations. These data are consistent with previous findings that N-terminal truncated Aβ peptides were secreted into the culture medium by primary neurons, PC12 cells and by COS cells overexpressing APP [6]. Small N-terminal truncations of Aβ peptides were reported in amyloid plaques found in the cortical sections of AD patients [23]. However, these generally show loss of only a few amino acids as compared to the larger truncations found in the fAβ isolated from microglia in this study. Since the monoclonal antibody we used, 4G8, recognizes an epitope between amino acids 17–24 of Aβ, any fragments resulting from N-terminal degradation beyond residue 17 would not have been detected in our system. Studies using antibodies with appropriate epitopes will be necessary to detect fragments smaller than Aβ17–42.

The difference between the microglia and the macrophages appears to be that the macrophages are eventually able to fully digest the fAβ, whereas it remains only partially digested in the microglia. The lack of detectable fragments in the range Aβ13–42 through Aβ16–42 suggests that if such fragments are generated in macrophages, they must be digested rapidly to smaller fragments. This suggests that an enzyme able to cut in or near this region may be a key factor in the efficient degradation by macrophages.

From structural studies it is known that the C-terminal portion, beginning around residue 11 forms β-sheet like structures [2,20], whereas the N terminal, as shown by NMR studies and protease digestion studies, is not involved in the beta sheet network and has a relatively flexible structure [13,21]. Since β-sheet like structures are resistant to enzymatic degradation, it is, perhaps, not surprising that the core of Aβ amyloid can resist degradation even in late

endosomes and lysosomes. It is possible that once degradation of an Aβ peptide proceeds past residue 12, the remaining degradation proceeds rapidly since part of the β-sheet core has been disrupted.

In a previous study, we showed that microglia release previously internalized fAβ into the chase medium as TCA-precipitable material [7]. The mass spectrometry analysis of the macromolecular material released from microglia shows that most of it is released as intact Aβ1–42 (Figure 4). These data support the idea that under some circumstances fAβ can be released from microglia and may contribute to the formation of extracellular plaque deposits. In macrophages, most of the ingested fAβ is released from the cells as low molecular weight, TCA-soluble amino acids or short peptides. A large fraction of the macromolecular material that is released from macrophages contained N-terminal deletions.

In summary we have found that the murine macrophages can degrade fAβ particles which murine microglia can only degrade incompletely. The low level of fAβ proteolysis in microglia was partially compensated by adding lysosomal enzymes from outside the cell, indicating that low hydrolytic activity of one or more enzymes in the late endosomes and lysosomes of microglia is limiting the degradation of fAβ. Further studies will be required to determine which are the key enzymatic activities for lysosomal degradation of fAβ and also whether similarly poor degradation occurs *in vivo*. Additionally, one would like to know if microglia in human brains are ineffective at degradation of fAβ and whether increased hydrolytic activity in human microglia would be beneficial. Understanding the basis for the extreme resistance to proteolysis of Aβ fibrils in microglia may lead to the development of methods to promote clearance and degradation of Aβ.

Acknowledgments

We are grateful to Drs. Carl Nathan and Aihao Ding for providing us the mouse peritoneal macrophages. We thank Dr. Ira Tabas and Cecilia Devlin for providing us with Ac-LDL. Supported by NIH grants NS 34761 and DK27083 (FRM), DK54317 (PL), and Alzheimer's Association RG1-96-070 (RW). The mass spectrometry analysis was conducted using an instrument funded by an NIH NCRR Shared Instrumentation grant.

References

1. Akiyama H, Schwab C, Kondo H, Mori H, Kametani F, Ikeda K, McGeer PL. Granules in glial cells of patients with Alzheimer's disease are immunopositive for C-terminal sequences of beta-amyloid protein. Neurosci Lett 1996;206(2–3):169–172. [PubMed: 8710178]
2. Antzutkin ON, Leapman RD, Balbach JJ, Tycko R. Supramolecular structural constraints on Alzheimer's beta-amyloid fibrils from electron microscopy and solid-state nuclear magnetic resonance. Biochemistry 2002;41(51):15436–15450. [PubMed: 12484785]
3. Bacskai BJ, Kajdasz ST, Christie RH, Carter C, Games D, Seubert P, Schenk D, Hyman BT. Imaging of amyloid-beta deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. Nat Med 2001;7(3):369–372. [PubMed: 11231639]
4. Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 2000;6(8):916–919. [PubMed: 10932230]
5. Barton NW, Furbish FS, Murray GJ, Garfield M, Brady RO. Therapeutic response to intravenous infusions of glucocerebrosidase in a patient with Gaucher disease. Proc Natl Acad Sci U S A 1990;87(5):1913–1916. [PubMed: 2308952]
6. Busciglio J, Gabuzda DH, Matsudaira P, Yankner BA. Generation of beta-amyloid in the secretory pathway in neuronal and nonneuronal cells. Proc Natl Acad Sci U S A 1993;90(5):2092–2096. [PubMed: 8446635]

7. Chung H, Brazil MI, Soe TT, Maxfield FR. Uptake, degradation, and release of fibrillar and soluble forms of Alzheimer's amyloid beta-peptide by microglial cells. J Biol Chem 1999;274(45):32301–32308. [PubMed: 10542270]
8. Dahms NM, Lobel P, Kornfeld S. Mannose 6-phosphate receptors and lysosomal enzyme targeting. J Biol Chem 1989;264(21):12115–12118. [PubMed: 2545698]
9. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 2005;8(6):752–758. [PubMed: 15895084]
10. Forman MS, Trojanowski JQ, Lee VM. Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. Nat Med 2004;10(10):1055–1063. [PubMed: 15459709]
11. Frackowiak J, Wisniewski HM, Wegiel J, Merz GS, Iqbal K, Wang KC. Ultrastructure of the microglia that phagocytose amyloid and the microglia that produce β-amyloid fibrils. Acta Neuropathol (Berl) 1992;84(3):225–233. [PubMed: 1414275]
12. Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: new twists in the tale. Nat Rev Mol Cell Biol 2003;4(3):202–212. [PubMed: 12612639]
13. Kheterpal I, Williams A, Murphy C, Bledsoe B, Wetzel R. Structural features of the Abeta amyloid fibril elucidated by limited proteolysis. Biochemistry 2001;40(39):11757–11767. [PubMed: 11570876]
14. Kyttala A, Heinonen O, Peltonen L, Jalanko A. Expression and endocytosis of lysosomal aspartylglucosaminidase in mouse primary neurons. J Neurosci 1998;18(19):7750–7756. [PubMed: 9742145]
15. Lin L, Lobel P. Production and characterization of recombinant human CLN2 protein for enzyme-replacement therapy in late infantile neuronal ceroid lipofuscinosis. Biochem J 2001;357(Pt 1):49–55. [PubMed: 11415435]
16. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005;308(5726):1314–1318. [PubMed: 15831717]
17. Paresce DM, Chung H, Maxfield FR. Slow degradation of aggregates of the Alzheimer's disease amyloid β-protein by microglial cells. J Biol Chem 1997;272(46):29390–29397. [PubMed: 9361021]
18. Paresce DM, Ghosh R, Maxfield FR. Microglial cells internalize aggregates of the Alzheimer's Disease amyloid β-protein via a scavenger receptor. Neuron 1996;17:553–565. [PubMed: 8816718]
19. Pessac B, Godin I, Alliot F. [Microglia: origin and development]. Bull Acad Natl Med 2001;185(2):337–346. [PubMed: 11474589]discussion 46-7
20. Petkova AT, Buntkowsky G, Dyda F, Leapman RD, Yau WM, Tycko R. Solid state NMR reveals a pH-dependent antiparallel beta-sheet registry in fibrils formed by a beta-amyloid peptide. J Mol Biol 2004;335(1):247–260. [PubMed: 14659754]
21. Petkova AT, Ishii Y, Balbach JJ, Antzutkin ON, Leapman RD, Delaglio F, Tycko R. A structural model for Alzheimer's beta -amyloid fibrils based on experimental constraints from solid state NMR. Proc Natl Acad Sci U S A 2002;99(26):16742–16747. [PubMed: 12481027]
22. Rogers J, Lue LF. Microglial chemotaxis, activation, and phagocytosis of amyloid beta-peptide as linked phenomena in Alzheimer's disease. Neurochem Int 2001;39(5–6):333–340. [PubMed: 11578768]
23. Saido TC, Iwatsubo T, Mann DMA, Shimada H, Ihara Y, Kawashima S. Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques. Neuron 1995;14:457–466. [PubMed: 7857653]
24. Sakr SW, Eddy RJ, Barth H, Wang F, Greenberg S, Maxfield FR, Tabas I. The uptake and degradation of matrix-bound lipoproteins by macrophages. J Biol Chem 2001;276(40):37649–37658. [PubMed: 11477084]
25. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, Walker S, Wogulls M, Yednock T, Games D, Seubert P. Immunization with amyloid-β attenuates Alzheimer disease-like pathology in the PDAPP mouse. Nature 1999;400:173–177. [PubMed: 10408445]
26. Schiffmann R, Murray GJ, Treco D, Daniel P, Sellos-Moura M, Myers M, Quirk JM, Zirzow GC, Borowski M, Loveday K, Anderson T, Gillespie F, Oliver KL, Jeffries NO, Doo E, Liang TJ, Kreps

C, Gunter K, Frei K, Crutchfield K, Selden RF, Brady RO. Infusion of alpha-galactosidase A 23 reduces tissue globotriaosylceramide storage in patients with Fabry disease. Proc Natl Acad Sci U S A 2000;97(1):365–370. [PubMed: 10618424]

27. Shaffer LM, Dority MD, Gupta-Bansal R, Frederickson RCA, Younkin SG, Brunden KR. Amyloid β protein (Aβ) removal by neuroglial cells in culture. Neurobiol Aging 1995;16(No 5):737–745. [PubMed: 8532106]
28. Sleat DE, Lackland H, Wang Y, Sohar I, Xiao G, Li H, Lobel P. The human brain mannose 6-phosphate glycoproteome: a complex mixture composed of multiple isoforms of many soluble lysosomal proteins. Proteomics 2005;5(6):1520–1532. [PubMed: 15789345]
29. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. New England J of Medicine 1989;320(14): 915–924.
30. Wang R, Sweeney D, Gandy SE, Sisodia SS. The profile of soluble amyloid beta protein in cultured cell media. Detection and quantification of amyloid beta protein and variants by immunoprecipitation-mass spectrometry. J Biol Chem 1996;271(50):31894–31902. [PubMed: 8943233]
31. Wegiel J, Imaki H, Wang KC, Wronska A, Osuchowski M, Rubenstein R. Origin and turnover of microglial cells in fibrillar plaques of APPsw transgenic mice. Acta Neuropathol (Berl) 2003;105 (4):393–402. [PubMed: 12624793]
32. Wegiel J, Wang KC, Imaki H, Rubenstein R, Wronska A, Osuchowski M, Lipinski WJ, Walker LC, LeVine H. The role of microglial cells and astrocytes in fibrillar plaque evolution in transgenic APP (SW) mice. Neurobiol Aging 2001;22(1):49–61. [PubMed: 11164276]
33. Wisniewski HM, Wen GY, Kim KS. Comparison of four staining methods on the detection of neuritic plaques. Acta Neuropathol (Berl) 1989;78(1):22–27. [PubMed: 2472039]
34. Wisniewski T, Ghiso J, Frangione B. Review: biology of Aβ amyloid in Alzheimer's disease. Neurobiology of Disease 1997;4:313–328. [PubMed: 9440120]
35. Wright SD, Weitz JI, Huang AJ, Levin SM, Silverstein SC, Loike JD. Complement receptor type three (CD11b/CD18) of human polymorphonuclear leukocytes recognizes fibrinogen. Proc Natl Acad Sci U S A 1988;85(20):7734–7738. [PubMed: 2971974]

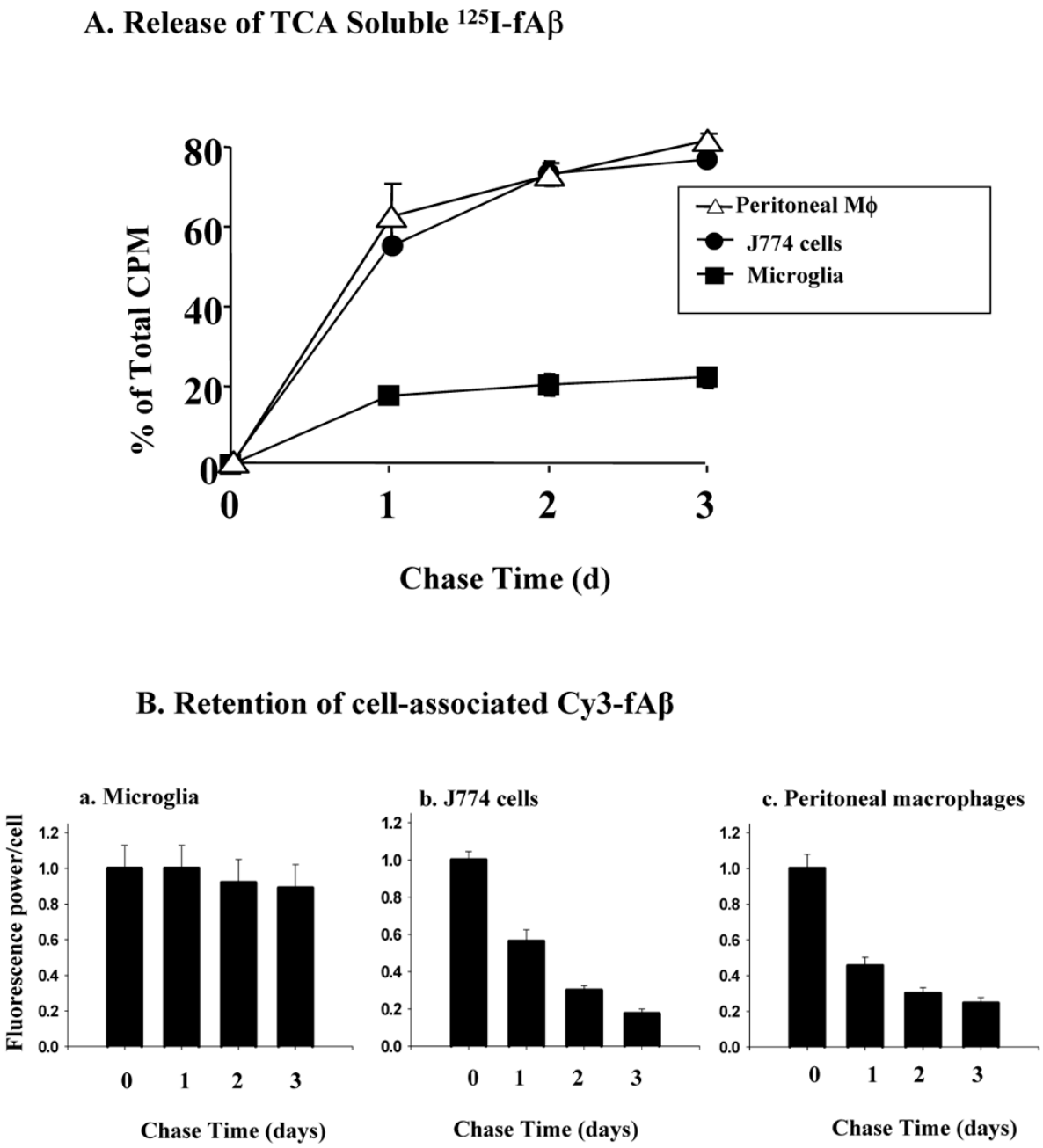


Figure 1. Degradation of labeled fAβ in macrophages and microglia

(A) ^{125}*I-labeled fAβ*. Microglia, J774 cells and primary mice peritoneal macrophages were incubated for 1 h with ^{125}I-labeled fAβ 1 μg/ml) after which the cells were washed and chased for varying times. In parallel samples, excess Ac-LDL (200 μg/ml) or fucoidan (500 μg/ml) was added to the radiolabeled fAβ to determine the extent of nonspecific cell-associated radiolabeled fAβ, for which all measurements were corrected. At each chase time, the chase medium was collected, and cells were solubilized. The chase medium was subjected to TCA precipitation to monitor degradation. The radioactivity of TCA-soluble fractions in peritoneal macrophages (Δ), J774 cells (●), and microglia (■) are shown as the % of total radioactivity.

The data presented are averages of the radioactive counts from three dishes per condition from six different experiments using fAβ42. *Error bars*, S.E.

(B) *Cy3-fAβ*. Cells were incubated for 45 min with Cy3-fAβ42 (1 μg/ml) and chased for various times. After each chase time, the cells were rinsed extensively, fixed and imaged by digital fluorescence microscopy. The Cy3 fluorescence intensity remaining in each cell over the course of the chase was quantified. The integrated cell-associated fluorescence power (arbitrary units) normalized to the fluorescence power at day 0 over the course of 3days is shown in the figure. Data for each time point shows the average of the normalized fluorescence power values from 3 different experiments done on 3 different days. *Error bars*, represent S.E.

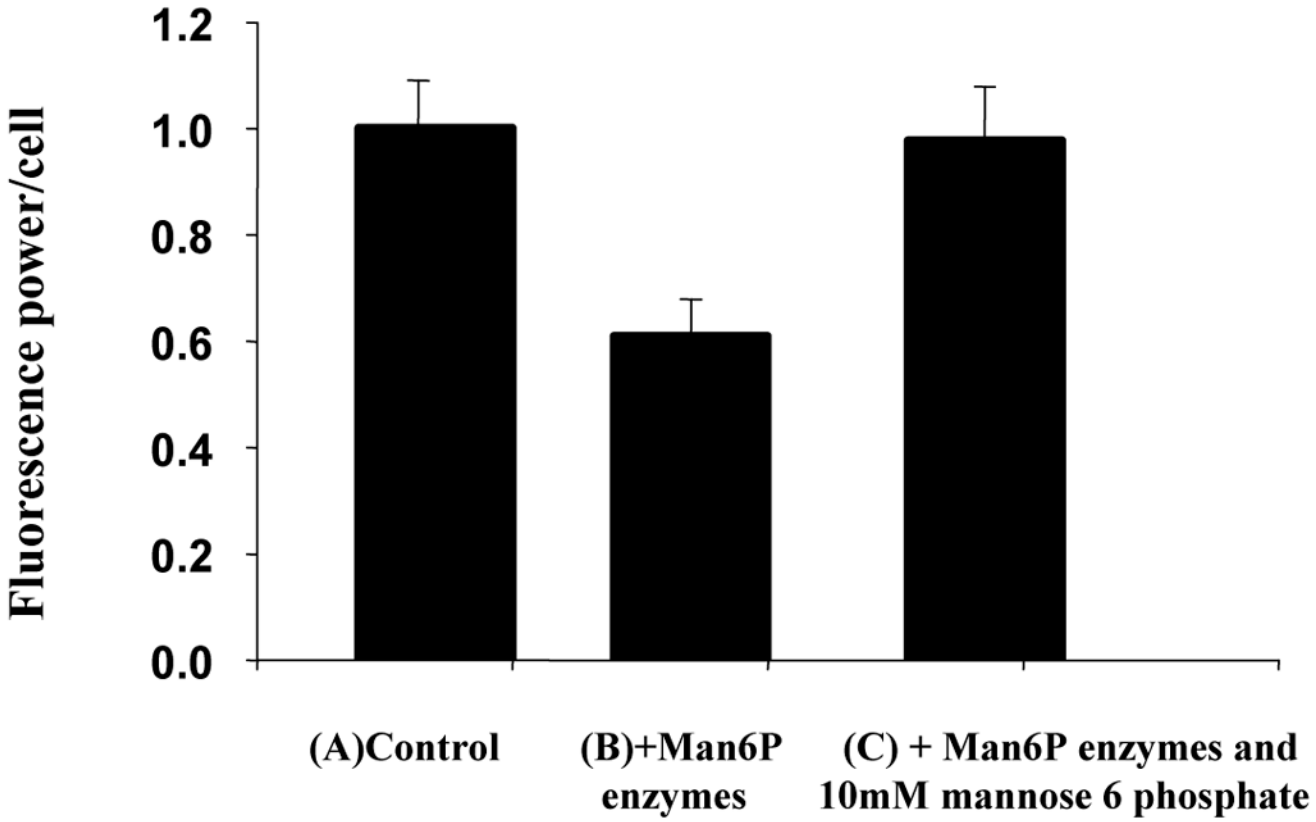
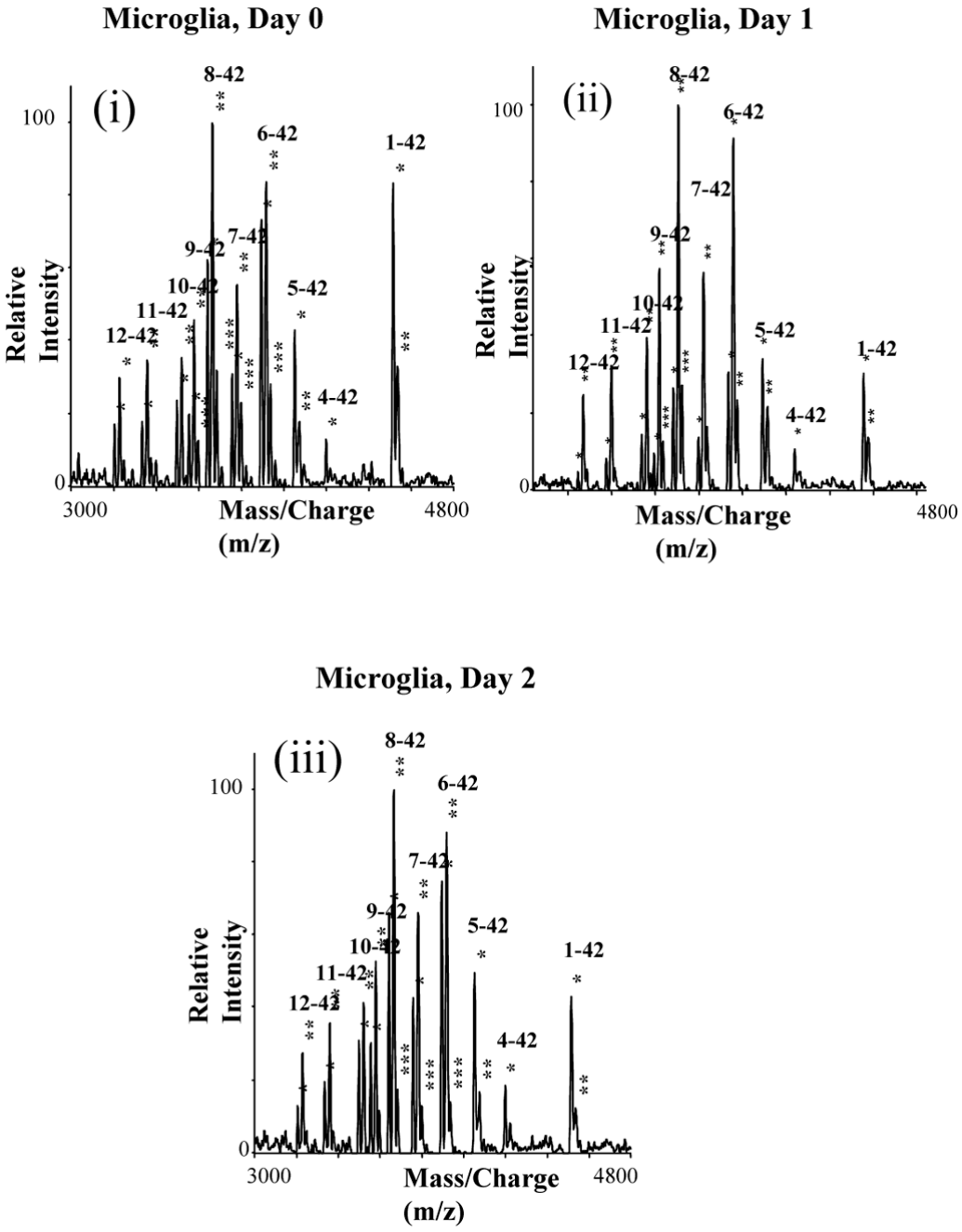


Figure 2. fAβ degradation by microglia in the presence of Man6P tagged enzymes

Cells were incubated for 45 min with Cy3-fAβ42 (1 μg/ml). After incubation the cells were rinsed extensively with complete media and were chased in (A) complete media (B) complete media+30nM mannose-6 phosphate phosphorylated lysosomal enzyme (C) complete media +30nM mannose-6 phosphate phosphorylated lysosomal enzyme + 10mM mannose-6 phosphate. After 3 days, the cells were rinsed extensively, fixed, and imaged by digital fluorescence microscopy. The integrated Cy3 fluorescence power per cell before and after the chase was quantified. The integrated cell-associated fluorescence power (arbitrary units) after 3 days normalized to the fluorescence power at day 0 is shown in the figure. The P value (unpaired Student's t test) for fAβ degradation by microglia after add-back of 30nM mannose-6 phosphate phosphorylated lysosomal enzyme was less than 0.005 compared to either control microglia or enzyme addition in the presence of 10mM mannose-6 phosphate. Data for each condition show the average of the fluorescence power values from 3 different experiments done on 3 different days. *Error bars*, represent S.E.



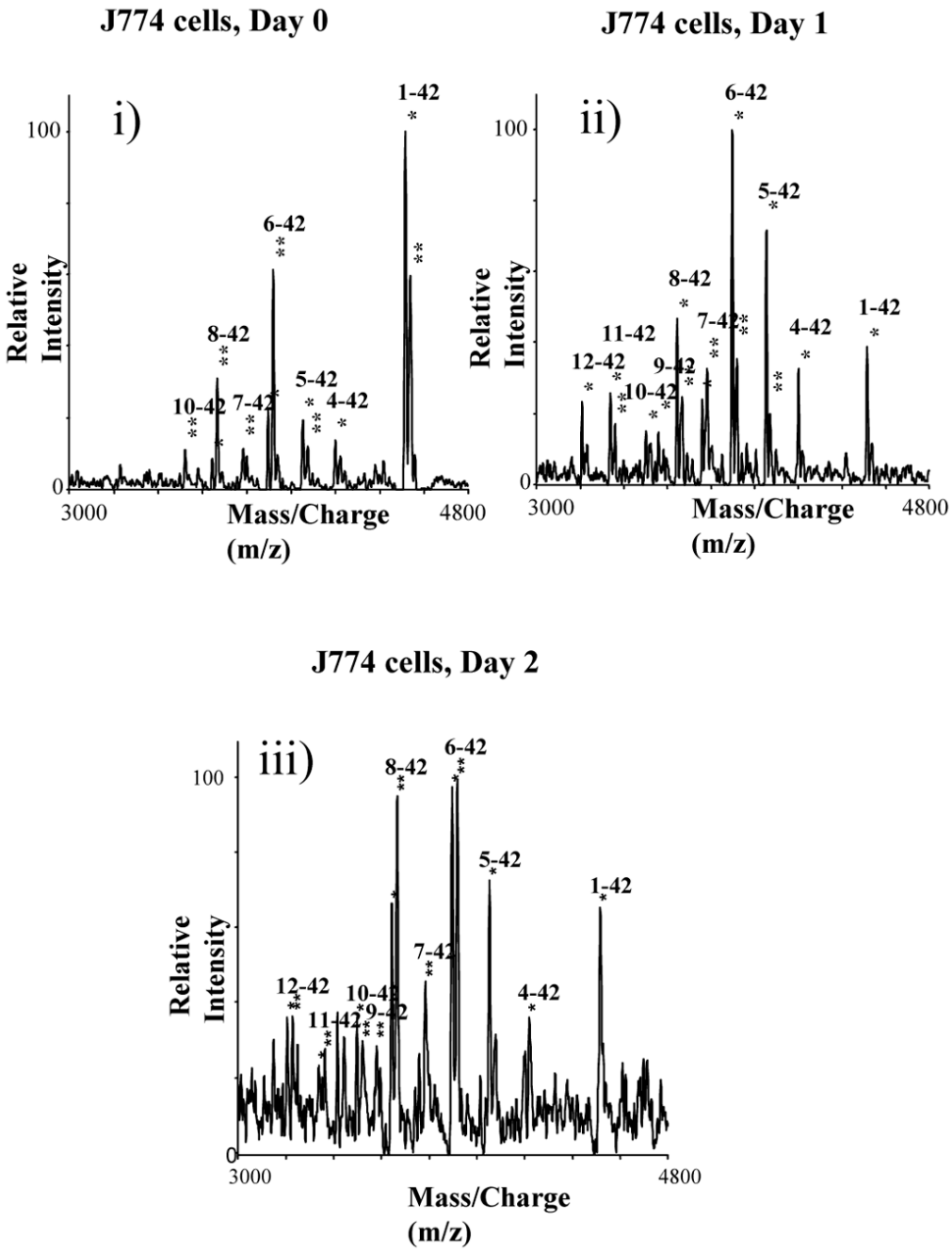


Figure 3. MALDI-TOF-MS spectrum of fAβ fragments in cells after uptake

A). Microglia [(i) chase day 0 (ii) chase day 1 and (iii) chase day 2] B) J774 macrophages [(i) chase day 0 (ii) chase day 1 and (iii) chase day 2] were pulsed with fAβ peptides overnight. The cells were then rinsed and chased for indicated times. At the end of each chase time, the cells were rinsed and lysed in lysis buffer. The cells were sonicated and centrifuged at 100,000 × *g* for 1 h at 4°C. The resulting pellet was solubilized in 70% formic acid, followed by immunoprecipitation using monoclonal Aβ antibody 4G8. The molecular masses of eluted samples were measured using mass spectrometry. The identities of the observed peaks are indicated using human Aβ sequence numbers. Representative spectra from microglia and J774

cells from ten experiments of each cell type are shown. * indicates the protonated ion peak; ** indicates the mono-sodium adduct peak; *** indicates the disodium adduct peak.

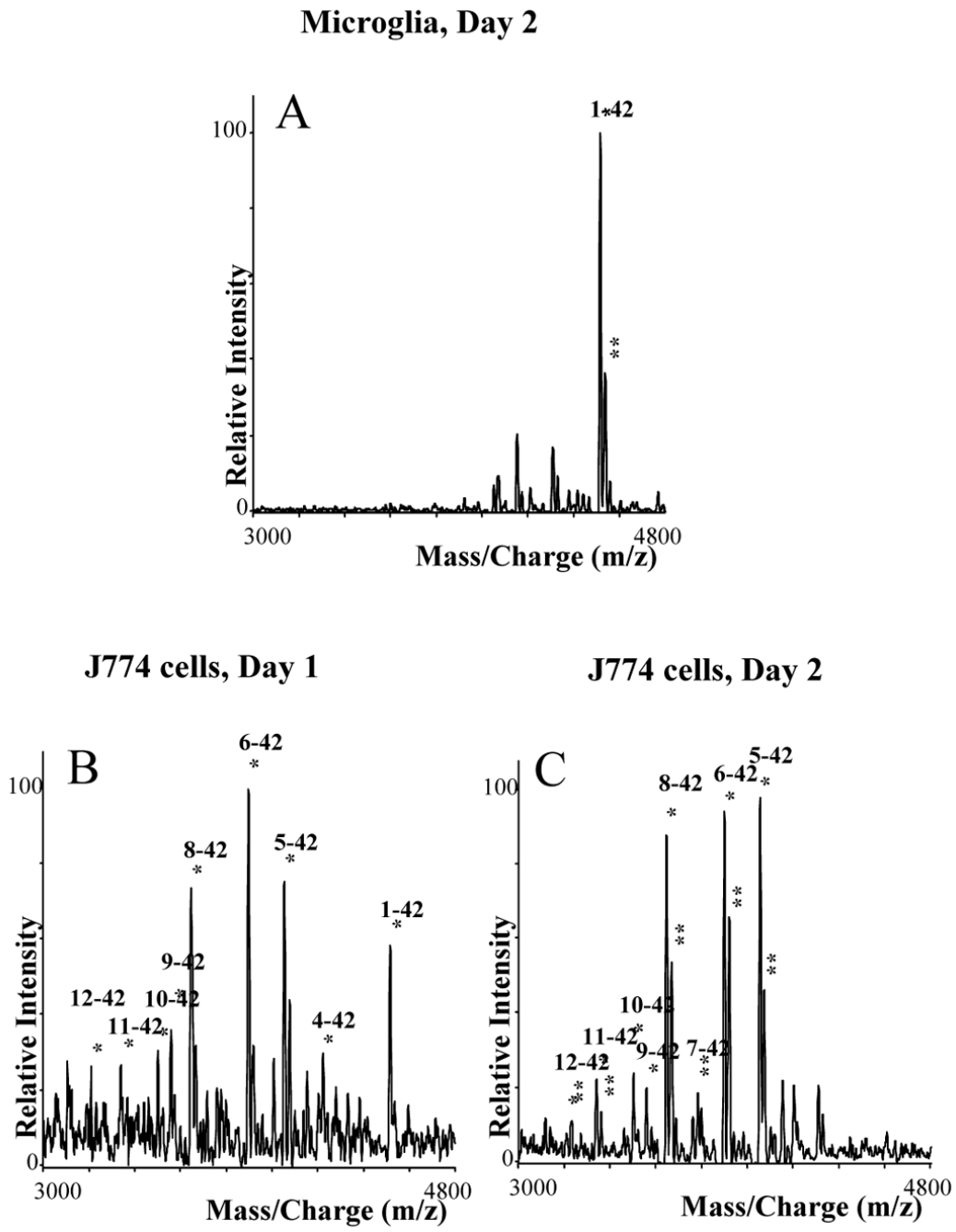


Figure 4. MALDI-TOF-MS of fAβ fragments released into the chase medium

The chase media from microglia (A; chase day 2), J774 cells (B; chase day 1), and J774 cells (C; chase day 2) that were pulsed with fAβ peptides overnight were collected and subjected to TCA precipitation. The TCA insoluble material was solubilized in 70% formic acid, and immunoprecipitated using monoclonal Aβ antibody 4G8. The eluted samples were measured using mass spectrometry. The identities of the observed peaks are indicated using human Aβ sequence numbers. Representative spectra from ten experiments are shown. * indicates the protonated ion peak; ** is the mono-sodium adduct peak.