**TITLE:**

Cell-Based Assay to Study Antibody-Mediated Tau Clearance by Microglia

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**KEYWORDS**:

Alzheimer’s, tauopathy, tau, aggregation, microglia, BV-2, uptake, clearance.

**SHORT ABSTRACT:**

Here we describe a cell-based assay to quantitatively assess tau uptake by microglia with the aim of creating an investigational tool to better characterize the mechanisms of action of anti-tau antibodies.

**LONG ABSTRACT**:

Alzheimer’s disease (AD) is a progressive neurodegenerative condition in which aggregated tau and amyloid proteins accumulate in the brain causing neuronal dysfunction which eventually leads to cognitive decline. Hyperphosphorylated tau aggregates in the neuron are believed to cause most of the pathology associated with AD. These aggregates are assumed to be released into the extracellular compartment and taken up by adjacent healthy neurons where they induce further tau aggregation. This “prion-like” spreading can be interrupted by antibodies capable of binding and “neutralizing” extracellular tau aggregates as shown in preclinical mouse models of AD. One of the proposed mechanisms by which therapeutic antibodies reduce pathology is antibody-mediated uptake and clearance of pathological aggregated forms of tau by microglia. Here, we describe a quantitative cell-based assay to assess tau uptake by microglia. This assay uses the mouse microglial cell line BV-2, allows for high specificity, low variability and medium throughput. Data generated with this assay can contribute to a better characterization of anti-tau antibody effector functions.

**INTRODUCTION**:

Alzheimer’s disease (AD) is a progressive neurodegenerative condition characterized by the conformational change and self-assembly of amyloid β peptide and tau protein into pathological aggregates. The normal soluble amyloid β peptide is converted into oligomeric and fibrillar amyloid β, while abnormally phosphorylated tau accumulates as oligomers and neurofibrillary tangles1, 2. These protein aggregates cause neuronal death leading to memory loss and subsequent progressive cognitive decline. Other factors, including non-productive neuroinflammation and a reduced ability to clear misfolded proteins, may exacerbate and accelerate disease. Currently, intervention strategies against AD provide largely symptomatic relief, but there is no disease-modifying cure or prevention.

Increasing evidence suggests a key role of hyperphosphorylated tau aggregates in the pathology of AD. In its non-pathological state, tau is a natively unfolded protein that binds to microtubules and promotes their assembly into the neuronal cytoskeleton. When tau becomes hyperphosphorylated, it detaches from the cytoskeleton and clusters into tau aggregates in the neuron, which are believed to cause most of the pathology associated with AD3. Aggregated tau starts accumulating first intracellularly, but as disease progresses, it is assumed to be released from affected neurons into the extracellular space, from which it can be taken up by adjacent or synaptically connected healthy neurons in a “prion-like manner”. Once internalized, the tau aggregate induces further tau aggregation *via* templated conformational change4.

According to this hypothesis, therapies capable of interrupting tau seeding might slow down or reverse the course of tau-mediated neurodegenerative disease. In support of this, mice made susceptible to tauopathy by genetic mutation and passively injected with anti-tau antibodies show reduced tau pathology and improved cognitive function5–9. However, the mechanisms by which therapeutic antibodies reduce pathology still remain elusive.

One of the proposed mechanisms is antibody-mediated uptake and clearance of pathological aggregated forms of tau by microglia, the brain’s resident immune cells. Recent publications suggest that microglia can efficiently internalize and degrade pathological tau species and this ability is enhanced by anti-tau antibodies *via* an Fc-dependent mechanism involving Fc receptors expressed on the surface of microglia and receptor mediated phagocytosis10, 11. These data identify microglia as potentially important effectors of therapeutic antibodies.

We describe herein a cell-based assay to quantitatively assess tau uptake by microglia. Data generated with this assay can help elucidating the mechanisms of action of anti-tau antibodies thus representing a useful tool to advance anti-tau antibodies to further the steps of their development as potential AD treatment.

**PROTOCOL:**

1. **BV-2 Cells Culture**

Note: Handle BV-2 cells under Biosafety Level 2 containment. The BV-2 cell line produces an enveloped recombinant ecotropic retrovirus (capable of infecting murine cells only)12; such viruses are known for their *in vitro* transforming ability and *in vivo* tumorigenic potential.

* 1. Culture BV-2 cells in high glucose Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin and 2 mM L-Glutamine (referred to as culturing medium from now on) by seeding cells at 4 x 104 cells/mL.
  2. Maintain cultures in a humidified atmosphere of 5% CO2 at 37 °C.

Note: the cells grow loosely attached and in suspension.

1. **Label Recombinant Tau Aggregates with pH-Sensitive Fluorescent Dye**

Note: Tau aggregates were prepared as described in Apetri *et al.*13 with the difference that no Thioflavin T (ThT) was added to the reaction buffer. Aggregated samples were collected in 1.5 mL centrifuge tubes. Final fluorescence signal was checked by mixing 118 µL of the pool sample with 12 µL of a 50 µM ThT solution. Aggregates were separated by centrifuging the aggregation reaction mixture at 20,000 x *g* for 1 h at 4 °C. The supernatant was analyzed by S MALS to confirm that all the monomeric tau was converted into aggregates. Pellets (tau aggregates) were snap frozen and stored in a freezer at -80 °C.

* 1. Resuspend tau aggregates in 0.1 M sodium bicarbonate buffer (NaHCO3) at pH 8.5 to a concentration of 1 mg/mL (~ 20 μM).

Note: Concentration of tau aggregates is based on the initial monomers concentration as assessed by the absorption of tau monomers at 280 nm using an extinction coefficient of 0.31 mLmg-1cm-1.

* 1. Sonicate the resuspended aggregates using a probe sonicator while keeping on ice to avoid over-heating.
     1. Use an amplitude of 65% (with sonicator of power 250 W).
     2. Perform 8 pulses of 3 s with pauses of 15 s between pulses to avoid overheating.
  2. Prepare an 8.9 mM stock solution of pHrodo dye (henceforth reference to as pH dye) in dimethyl sulfoxide (DMSO) following manufacturer’s instruction.

Note: Always prepare a fresh solution and use it only on the day it is prepared.

* 1. Add 10 moles of dye per mole of protein to a final dye concentration of 0.2 mM.
  2. Mix by gently pipetting up and down.
  3. Incubate the reaction mixture for 45-60 min at room temperature, protected from light.
  4. In the meantime, assemble a cross-linked dextran gel desalting column following manufacturer’s instructions.
  5. Equilibrate the column with 25 mL elution buffer (100 mM NaHCO3 pH 5, 3% DMSO). Discard the flow through.
  6. Add the product of the tau aggregate labeling reaction to the column in a total volume of 2.5 mL. If the sample is less than 2.5 mL, add buffer until a total volume of 2.5 mL is achieved.
  7. Let the sample enter the packed gel completely, discard the flow-through.
  8. Elute with 3.5 mL elution buffer and collect the eluate in 4 equivalent fractions in 2 mL tubes.
  9. Determine protein concentrations of the 4 fractions by bicinchoninic acid (BCA) assay.
  10. Store the labeled protein in a -20 °C freezer.

1. **Uptake Assay with Fluorescence-Activated Cell Sorting (FACS) Read-Out**
   1. **Day 1 – Seed the Cells** 
      1. Wash BV-2 cells in the flask with phosphate-buffered saline (PBS) 1x.

Note: Washing volume will vary based on the size of the cell flask used. For example, for a T175 flask, wash with 10 mL of PBS 1x.

* + 1. Detach cells by incubating with trypsin-ethylenediaminetetraacetic acid (EDTA) 0.05% at 37 °C and 5% CO2 until the cells detach from the flask (approximately 5 min).

Note: Volume of trypsin-EDTA 0.05% depends on the size of the cell flask used. For example, for a T175 flask, use 2 mL of trypsin-EDTA 0.05%.

* + 1. Resuspend cells in culturing medium containing 200 μg/mL heparin to a final concentration of 1 x 105 cells/mL.
    2. Plate 250 µL of cell suspension (2.5 x 104 cells) per well in a 96-well tissue culture flat bottom plate.
    3. Incubate plate overnight at 37 °C with 5% CO2.
  1. **Day 1 – Prepare Immunocomplexes**
     1. Thaw pH dye-tau on ice.
     2. Prepare a 250 nM solution of pH dye-tau aggregates in serum-free medium (SFM) (high glucose DMEM supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin and 200 μg/mL of heparin).
     3. Dilute antibodies in 250 nM pH dye-tau solution to desired concentrations in a 96-well plate. Seal the dilution plate and incubate over night at 37 °C.
  2. **Day 2 – Immunocomplexes Uptake**

* + 1. Remove culturing medium from BV-2 cells. Wash cells once with 100 µL room temperature PBS 1x.
    2. Transfer 125 µL of immunocomplexes to the cells using a multichannel pipette. Incubate the cells with the immunocomplexes for 2 hours at 37 °C with 5% CO2.
    3. Remove the incubation medium from the cells and discard it. Wash cells once with 100 µL room temperature 1x PBS.
    4. Treat cells with 50 µL trypsin-EDTA 0.25% for 20 min at 37 °C with 5% CO2.
    5. Add 200 µL of culturing medium and resuspend well to detach the cells. Transfer cells to a 96-well U-bottom plate. Centrifuge plate at 400 x *g* for 5 min at 4 °C.
    6. Put the plate on ice, remove culturing medium and resuspend the cell pellets in 150 µL ice cold 1x PBS.
    7. Centrifuge plate at 400 x *g* for 5 min at 4 °C.
    8. Put the plate on ice and wash cells by adding 150 µL cold PBS. Centrifuge plate at 400 x *g* for 5 min at 4 °C.
    9. Put cells on ice and wash them by adding 150 µL cold FACS buffer (1x PBS, 0.5% bovine serum albumin (BSA), 2 mM EDTA). Centrifuge plate at 400 x *g* for 5 min at 4 °C.
    10. Put cells on ice and resuspend them in 200 µL cold FACS buffer.
    11. Analyze samples immediately by FACS acquiring 2 x 104 events in the live cells gate (see step 4.1).

1. **FACS analysis**

Note: Refer to **Figure 1** for the gating strategy.

* 1. Using the forward scatter area (FSC-A) versus side scatter area (SSC-A) density plot, gate on live cells by excluding events with lower forward scatter levels (*i.e.,* debris and dead cells).
  2. Within the live cell population, use FSC-A versus forward scatter height (FSC-H) to exclude cell doublets and aggregates. This is the singlet gate.
  3. Using the events in the singlet gate, generate a pH dye single parameter histogram.
  4. Determine mean fluorescence intensity. Determine percentage of pH dye-tau positive cells by excluding negative cells as determined using BV-2 only control.

1. **Day 2 – Immunocomplexes Uptake with Microscopy Read-Out**
   1. **Day 1 – Seed the Cells** 
      1. Wash BV-2 cells in the flask with PBS 1x. Detach cells using trypsin-ethylenediaminetetraacetic acid (EDTA) 0.05% and incubating at 37 °C with 5% CO2 until the cells detach from the flask (approximately 5 min).

Note: Volume of trypsin-EDTA 0.05% depends on the size of the cell flask used (*e.g.*, for a T175 flask, use 2 mL of trypsin-EDTA 0.05%).

* + 1. Resuspend cells in culturing medium to a final concentration of 2.6 x 104 cells/mL.
    2. Plate 150 µL of cell suspension (3.9 x 103) per well in a poly-D-Lysine coated 96-well black plate with clear flat bottom.
    3. Incubate plate overnight at 37 °C with 5% CO2.
  1. **Day 3 – Prepare Immunocomplexes**

Note: Mild sonication of labelled tau aggregates prior to incubation with antibody, was performed to improve microscopy results.

* + 1. Thaw pH dye-tau on ice and sonicate using a probe sonicator while keeping on ice. Use an amplitude of 15% (sonicator power of 250 W). Perform 30 pulses of 2 s and wait 20 s between pulses.
    2. Prepare a 250 nM solution of pH dye-tau aggregates in SFM.
    3. Dilute antibodies in 250 nM pH dye-tau solution to the desired concentrations in a 96-well plate. Seal the dilution plate and incubate over night at 37 °C.
    4. Remove medium from cell plate and replace with 150 μL of culturing medium supplemented with 200 μg/mL heparin.
    5. Incubate plate overnight at 37 °C with 5% CO2.
  1. **Immunocomplexes Uptake**
     1. Remove culturing medium from the BV-2 cells. Transfer 125 µL of immunocomplexes to the cells using a multichannel pipette.
     2. Incubate the cells with the immunocomplexes for 1 h and 45 min at 37 °C with 5% CO2.

Stain cell nuclei and acidic organelles with Hoechst and Lysotracker Red respectively. Incubate the cells 15 min at 37 °C with 5% CO2.

Note: Dilute the dyes in SFM.

* + 1. Perform live-cell imaging using a high content screening confocal system. Set temperature set to 37 °C and 5% CO2. For high quality images, use a 63X water immersion objective and acquire 0.5 μm planes (20 per Z-stack) per imaged field.

**REPRESENTATIVE RESULTS:**

Aggregated recombinant tau was covalently labelled with pHrodo green dye. This dye dramatically increases its fluorescence upon its internalization in acidic organelles, thereby allowing for intracellular quantification. Labeled tau aggregates were incubated with anti-tau monoclonal antibodies. In particular, we used a chimeric version (mouse IgG1 Fc region) of CBTAU-28.1. This human antibody binds to the N-terminal insert region of tau and is able to bind *in vitro* generated tau fibrils13. In this assay, we also tested an affinity-improved version of CBTAU-28.1 – dmCBTAU-28.1. Fab fragments of CBTAU-28.1 in the parental and high-affinity mutant format and a mouse IgG1 isotype control were used as controls.

BV-2 cells were incubated with the pre-formed immunocomplexes or aggregated tau alone for 2 h in the presence of heparin to block antibody-independent uptake. After incubation, cells were trypsinized to remove the tau bound to the extracellular membrane, and were analyzed for tau uptake by flow cytometry. As we recently described13, we observed that CBTAU-28.1 variants promoted uptake of tau in BV-2 cells in a dose-dependent manner. The uptake was Fc mediated since CBTAU-28.1 Fab fragments did not increase basal tau uptake (**Figure 2**). Moreover, the high affinity dmCBTAU-28.1 antibody mediated tau uptake into BV-2 cells to a higher extent than the wild-type antibody (**Figure 2**).

Antibody-mediated tau uptake and localization of tau aggregates in the endolysosomal compartment was confirmed by confocal microscopy (**Figure 3**) where the acidic cellular compartment was stained using the LysoTracker dye. Intracellular puncta of green pH dye labeled tau aggregates were observed inside the cells that were incubated with CBTAU-28.1. Moreover, intracellular tau aggregates often colocalized with LysoTracker red thus suggesting presence of tau aggregates in the acidic organelles. CBTAU-28.1 Fab fragments did not increase tau uptake again indicating an Fc-receptor mediated internalization mechanism (**Figure 3**).

**FIGURE LEGENDS:**

**Figure 1. Gating strategy used in flow cytometry analysis to detect tau internalization by BV-2 cells.** Sample data from BV-2 only control (**A-C**), isotype control (**D-F**) and dmCBTAU-28.1 (**G-I**) are shown. BV-2 cell population was gated on a FSC-A vs SSC-A density plot excluding debris and dead cells (A, D). BV-2 cells were then further gated on a FSC-A vs FSC-H density plot to exclude cell doublets and aggregates (B, E). Single cell gate was used to generate a pH dye (FITC in these representative results) single parameter histogram and determine geometric mean fluorescence intensity. Alternatively, percentage of pH dye-tau positive cells was calculated excluding negative cells as determined by using BV-2 only control.

**Figure 2. CBTAU-28.1 mediates uptake of tau aggregates into microglial BV-2 cells.** Aggregated recombinant tau was covalently labelled with green fluorescence pH-sensitive dye and incubated with a mouse chimeric version of the human anti-tau antibody CBTAU-28.1, its affinity improved format, dmCBTAU-28.1, the corresponding Fab fragments, a mouse IgG1 isotype control antibody or no antibody (tau aggregates alone). Immunocomplexes were subsequently incubated with BV-2 cells for 2 h in the presence of heparin to block antibody-independent uptake. Uptake of immunocomplexes was assessed by flow cytometry and expressed as the geometric mean (GM) of fluorescence intensity (**A**) or percentage of tau positive (tau+) cells (**B**). Error bars in (A) indicate the standard deviation of two independent experiments, while (B) shows a single experiment.

**Figure 3. Tau aggregates are internalized by BV-2 cells and localize in cellular acidic organelles.**

Preformed tau/antibody immunocomplexes were incubated with BV-2 cells for 2 h in the presence of heparin to block antibody-independent uptake. After incubation, nuclei were stained with Hoechst (blue) and the acidic cellular compartment with LysoTracker Red dye. Live-cell imaging revealed intracellular puncta of labeled tau aggregates (green) inside the cells that were incubated with CBTAU-28.1 and dmCBTAU-28.1, but not with the isotype control. Moreover, intracellular tau aggregates often colocalized with the red dye (yellow) thus suggesting presence of tau aggregates in the acidic cellular compartment. CBTAU-28.1 Fab fragments did not increase tau uptake indicating an Fc-receptor mediated internalization mechanism. Images represent maximum intensity projections of a 20 planes Z-stack (0.5 μm planes) acquired with a 63X water immersion objective.

**DISCUSSION:**

Microglia, the resident brain’s immune cells, have been recently identified as important players in antibody-mediated therapeutic approaches for tauopathies10, 11. Antibody-mediated tau clearance by microglia, together with blocking of neuronal uptake9, inhibition or destabilization of fibril formation13, 14 and clearance of intraneuronal fibrils *via* the lysosomal pathway15, might all contribute to the anti-tau antibody efficacy observed in mouse model of tauopathy5–9.

We described here a cell-based assay to quantitatively assess tau uptake by microglia with the aim of creating an investigational tool to better characterize the mechanisms of action of anti-tau antibodies.

This assay, adapted from Funk *et al.*11, uses BV-2 cells, which are immortalized murine microglial cells. While they cannot fully be compared to primary microglial cells, they feature many of the characteristics of primary microglia, including the ability of robustly phagocytose both Aβ and tau fibrils11, 16–19. Moreover, they showed a reproducible behavior *in vitro* which made them highly suitable for assay development and quantitative studies, which require minimal experimental variability. Beside this, immortalized cell lines allow higher assay throughput and eliminate the need for animal sacrifice compared to the use of primary microglia.

The tau aggregates we used in this assay were obtained using the highly reproducible *in vitro* aggregation procedure that we recently described13, and show similar morphology to paired helical filaments (PHFs) isolated from brains of AD patients. While we did not observe any unexpected results that might have been caused by tau aggregates adherence to plastic or glass surfaces, the use of stable and well characterized tau aggregates played a crucial role in the reproducibility of this assay.

Another aspect that significantly contributed to assay reproducibility was cell density. The numbers of cells per well described in the protocol represent the optimal cell density in the described conditions.

Differently than what Funk *et al.*11 described, we labeled tau aggregates with a pH sensitive dye, pHrodo, which significantly increases its fluorescence upon internalization in acidic organelles, thus allowing for intracellular quantification. This, together with trypsin digestion of surface bound immunocomplexes and/or tau, guarantees that fluorescence signal measured by flow cytometry is the result of tau uptake rather than binding to the cellular surface. Moreover, the use of a pH sensitive dye eases detection of internalized tau aggregates in microscopy experiments without the need of digesting surface bound immunocomplexes/tau aggregates which would then requires cell re-plating and recovery.

We also further optimized the microscopy read-out of our assay, compared to what has previously been described11, by using a highly selective dye for acidic organelles (LysoTracker) in our microscopy experiments which allowed us not only to confirm antibody-mediated tau uptake, but also localization of tau aggregates in the endolysosomal compartment.

The assay we developed, has optimal specificity which results in a good experimental window allowing a strong separation between positive and negative samples. Interestingly, the assay indirectly detects differences in antibody affinity thus representing a powerful tool to study anti-tau antibody effector functions.

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**DISCLOSURES:**

The authors have nothing to disclose.

**REFERENCES:**

1. Hefti, F., Goure, W.F., Jerecic, J., Iverson, K.S., Walicke, P.A., Krafft, G.A. The case for soluble Aβ oligomers as a drug target in Alzheimer’s disease. *Trends in Pharmacological Sciences*. **34** (5), 261–266, doi: 10.1016/j.tips.2013.03.002 (2013).

2. Castillo-Carranza, D.L., Lasagna-Reeves, C.A., Kayed, R. Tau aggregates as immunotherapeutic targets. *Frontiers in bioscience (Scholar edition)*. **5** (8), 426–38, doi: S381 [pii] (2013).

3. Martin, L., Latypova, X., Terro, F. Post-translational modifications of tau protein: Implications for Alzheimer’s disease. *Neurochemistry International*. **58** (4), 458–471, doi: 10.1016/j.neuint.2010.12.023 (2011).

4. Holmes, B.B., Diamond, M.I. Prion-like properties of Tau protein: The importance of extracellular Tau as a therapeutic target. *Journal of Biological Chemistry*. **289** (29), 19855–19861, doi: 10.1074/jbc.R114.549295 (2014).

5. Giacobini, E., Gold, G. Alzheimer disease therapy--moving from amyloid-beta to tau. *Nature Reviews Neurology*. **9** (12), 677–686, doi: 10.1038/nrneurol.2013.223 (2013).

6. Asuni, A.A., Boutajangout, A., Quartermain, D., Sigurdsson, E.M. Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. *Journal of Neuroscience*. **27** (34), 9115–9129 (2007).

7. Boutajangout, A., Ingadottir, J., Davies, P., Sigurdsson, E.M. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *Journal of Neurochemistry*. **118** (4), 658–667, doi: 10.1111/j.1471-4159.2011.07337.x (2011).

8. Chai, X. *et al.* Passive immunization with anti-Tau antibodies in two transgenic models: reduction of Tau pathology and delay of disease progression. *Journal of Biological Chemistry*. **286** (39), 34457–34467, doi: 10.1074/jbc.M111.229633 (2011).

9. Yanamandra, K. *et al.* Anti-tau antibodies that block tau aggregate seeding *in vitro* markedly decrease pathology and improve cognition *in vivo*. *Neuron*. **80** (2), 402–414, doi: 10.1016/j.neuron.2013.07.046 (2013).

10. Luo, W., Liu, W., Hu, X., Hanna, M., Caravaca, A., Paul, S.M. Microglial internalization and degradation of pathological tau is enhanced by an anti-tau monoclonal antibody. *Scientific Reports*. **5**, 11161, doi: 10.1038/srep11161 (2015).

11. Funk, K.E., Mirbaha, H., Jiang, H., Holtzman, D.M., Diamond, M.I. Distinct Therapeutic Mechanisms of Tau Antibodies: Promoting Microglial Clearance *Versus* Blocking Neuronal Uptake. *Journal of Biological Chemistry*. **290** (35), 21652–21662, doi: 10.1074/jbc.M115.657924 (2015).

12. Blasi, E., Barluzzi, R., Bocchini, V., Mazzolla, R., Bistoni, F. Immortalization of murine microglial cells by a v-raf/v-myc carrying retrovirus. *Journal of neuroimmunology*. **27** (2–3), 229–37, at <http://www.ncbi.nlm.nih.gov/pubmed/2110186> (1990).

13. Apetri, A. *et al.* A common antigenic motif recognized by naturally occurring human VH5-51/VL4-1 anti-tau antibodies with distinct functionalities. *Acta neuropathologica communications*. **6** (1), 43, doi: 10.1186/s40478-018-0543-z (2018).

14. Schneider, A., Mandelkow, E. Tau-based treatment strategies in neurodegenerative diseases. *Neurotherapeutics*. **5** (3), 443–457, doi: 10.1016/j.nurt.2008.05.006 (2008).

15. Congdon, E.E., Gu, J., Sait, H.B., Sigurdsson, E.M. Antibody uptake into neurons occurs primarily *via* clathrin-dependent Fcgamma receptor endocytosis and is a prerequisite for acute tau protein clearance. *Journal of Biological Chemistry*. **288** (49), 35452–35465, doi: 10.1074/jbc.M113.491001 (2013).

16. Bocchini, V., Mazzolla, R., Barluzzi, R., Blasi, E., Sick, P., Kettenmann, H. An immortalized cell line expresses properties of activated microglial cells. *Journal of Neuroscience Research*. **31** (4), 616–621, doi: 10.1002/jnr.490310405 (1992).

17. Koenigsknecht, J. Microglial Phagocytosis of Fibrillar -Amyloid through a 1 Integrin-Dependent Mechanism. *Journal of Neuroscience*. **24** (44), 9838–9846, doi: 10.1523/JNEUROSCI.2557-04.2004 (2004).

18. Kopec, K.K., Carroll, R.T. Alzheimer’s β-Amyloid Peptide 1-42 Induces a Phagocytic Response in Murine Microglia. *Journal of Neurochemistry*. **71** (5), 2123–2131, doi: 10.1046/j.1471-4159.1998.71052123.x (2002).

19. Marsh, S.E. *et al.* The adaptive immune system restrains Alzheimer’s disease pathogenesis by modulating microglial function. *Proceedings of the National Academy of Sciences of the United States of America*. **113** (9), E1316-1325, doi: 10.1073/pnas.1525466113 (2016).