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Immune Cell Responses to Amyloid-Beta Proteins: What is the Extent and Implications of Amyloid-Beta Induced Cytotoxicity in Microglial Cells and Peripheral Blood Mononuclear Cells?

Annie Cao

Alzheimer's disease (AD) is a neurodegenerative disease with cognitive and physical symptoms. Scientists generally accept the amyloid cascade hypothesis as the defining focus of AD pathology. Past studies have indicated that Aβ oligomers induced cytotoxicity in immune cells, but the role of Aβ monomers has remained largely unexplored. The purpose of this study is to confirm the cytotoxicity exhibited by Aβ oligomer and immune cell interactions, and to discover whether Aβ monomer interactions will produce a similar response. We used lyophilized Aβ peptides to generate monomers and oligomers, and then added them into cultured BV2 microglial cells and peripheral blood mononuclear cells (PBMCs). After incubation, we used a Glo-Assay procedure to measure Aβ induced cytotoxicity based on cell viability. Ultimately, it was found that Aβ oligomers were more prone to induce cytotoxicity than monomers. The results imply that PBMCs show a greater sensitivity to Aβ proteins than microglial cells.

Keywords: Alzheimer's disease, amyloid-beta, microglia, PBMCs

Introduction and Lit Review

Alzheimer's disease (AD) is a progressive neurological disease that is marked by cognitive and mental decline. According to the Alzheimer's Association, a leading non-profit healthcare organization in global AD funding and research, it is the most common form of dementia and is the sixth leading cause of death in the United States. As of 2016, over 5.4 million Americans have been diagnosed with AD. By 2050, the number is expected to nearly triple. Along with other forms of dementia, AD treatments cost $236 billion nationally (Alzheimer's Association, 2016). In terms of both mortality rates and financial costs, AD has proven to be a costly disease. Despite its prevalence in many areas of society, AD is often overlooked, especially when compared to illnesses such as cancer and various cardiovascular diseases.

AD is most prevalent among people above 65 years of age, but symptoms can manifest among younger age groups. Although the primary risk factor associated with the illness, symptoms of the disease differ from those of the natural aging process. In addition to memory loss, chronic memory lapses, delayed cognitive responses, AD patients progressively lose the ability to perform daily functions, and in moderate to severe cases, become dependent upon a caregiver (Mayo Clinic Staff, 2015). AD is burdensome not only to the patient and their loved ones but also to caregivers. It disrupts the daily lives of those affected and presents itself as both an emotional and financial burden. In addition to aging, factors such as genetics and lifestyle choices also play a role in determining an individual's likelihood to be affected by AD. Unlike some illnesses triggered by viruses or bacteria, researchers are not able to define a strict combination of variables

that stimulate the growth of AD. Because of this inability to identify what causes AD, virtually everyone is at risk to be affected by the disease in one way or another. This is one of the many reasons why AD has is such a complex but necessary issue to explore.

Despite the millions of dollars of annual contributions to AD research, no cure for the disease has yet been found. Currently, the US Food and Drug Administration (FDA) has approved two classes of drug treatments known as cholinesterase inhibitors and memantine to alleviate the cognitive symptoms of AD (Alzheimer's Association, n.d.). According to the National Institute on Aging, a department of the National Institutes of Health (NIH), these drugs regulate neurotransmitters and offer temporary relief for memory and cognitive issues. These medications only address the symptoms that manifest among patients and are unable to eliminate or reverse the damaging effects of the disease. They also cause side effects such as vomiting and nausea, and the effectiveness of each medication can vary greatly from patient to patient (Alzheimer's Association, n.d.). Many clinical trials testing immunization therapies and physical and cognitive training are also being explored (NIH, n.d.). In order for a truly effective treatment to be found for AD, however, it is necessary to target the underlying causes of the disease. In doing so, greater measures can be taken to prevent the first indications of AD from occurring.

The wide variety of factors that may influence an individual's risk of AD are largely debated, but its physiological effects on the brain are more established. Since AD is a debilitating neurological illness, it poses the greatest damage on neurons in the brain. When compared to a healthy brain, an AD-ridden brain is typically smaller in size due to brain atrophy (deterioration of the brain) from a loss of neurons. Abnormal protein deposits known as amyloid plaques and tau tangles have also been found to appear in brain, hindering communication between neurons that later leads to cell death (NIH, 2016). The majority of existing and ongoing AD research is focused on why these deposits form in the brain and what actions can be taken to clear them and possibly lessen the severity of cognitive and physical symptoms.

The most accepted and discussed theory regarding the cause of AD symptoms is the amyloid cascade hypothesis, which defines amyloid-beta (Aβ) plaques as the central focus of AD pathology. Aβ plaques are composed of Aβ peptides, which are generated from amyloid precursor proteins (APPs). Under some pathological conditions, APPs develop into plaques through what is known as APP processing. The large volume of published papers detailing the process is a significant indicator of the acceptance of the hypothesis in the scientific community. A publication by Richard O'Brien from the Johns Hopkins Bayview Medical Center and Phillip Wong from the Johns Hopkins University School of Medicine (2011) defines APP as a naturally occurring transmembrane protein with a large ectodomain that protrudes past the cell membrane and an intracellular C-terminus. In APP processing, the ectodomain is first cleaved by the protease alpha-secretase (α-secretase) or beta-secretase (β-secretase). Cleavage by α-secretase results in the soluble amyloid precursor protein-alpha (sAPPα) fragment. The function of the sAPPα is not clear, but many studies have suggested that it has neuroprotective effects in the brain (Chow et. al., 2010). After the sAPPα is cut off, a piece of the original protein called the carboxyl terminal fragment-alpha (CTFα) remains embedded in the cell membrane. The protease gamma-secretase (γ-secretase) then cleaves the CTFα into an APP intracellular domain (AICD), which is believed to play a role in gene transcription and signal transduction, and a soluble P3 peptide , whose role is largely unknown (Zhang et al., 2011). The α-secretase cleaved sAPPα leads to what is known as a non-amyloidogenic, or non-plaque forming, pathway. In the amyloidogenic pathway, as explained in a publication by a team of biomedical researchers from Xiamen University and the Sanford-Burnham Institute for Medical Research (2011), APP is cleaved by β-secretase rather than α-secretase. The product, the soluble amyloid precursor protein-beta (sAPPβ), is detached, and the carboxyl terminal fragment-beta (CTFβ) is left in the membrane. Like CTFα, CTFβ is cleaved by γ-secretase. The resulting fragments are what distinguish the sAPPα non-amyloidogenic pathway from the sAPPβ amyloidogenic pathway. An AICD is formed again, but this time, an Aβ peptide, rather than the P3 peptide, is generated. The Aβ peptide is longer and more complex than the P3 peptide. Over time, the sticky Aβ peptides aggregate to form simple, monomeric proteins known as Aβ monomers. These monomers then combine to form higher isoforms

called oligomers. As oligomers congregate, Aβ fibrils form. Groups of Aβ fibrils are referred to as plaques. With each aggregation, the protein becomes increasingly insoluble. The insoluble Aβ plaques situate themselves at the synapses between neurons in the brain and block communication between cells. This disrupts the natural course of nerve impulses as they carry signals throughout the brain, leading to mental decline.

Despite the broad acceptance of the amyloid cascade hypothesis, the theory has undergone much criticism. A study by Sanjay Pimplikar from the Lerner Research Institute's Department of Neurosciences (2008) focused on analyzing the shortcomings and gaps of the hypothesis. Pimplikar (2008) noted that although AD progression is typically associated with advanced plaque development, plaque levels do not actually correspond with the severity of mental symptoms. Consequently, as found by a group of neuroscientists from the University of Southampton in a phase I clinical trial, the clearance of plaques has not been shown to reverse or diminish cognitive activity (Holmes et al., 2008). Post mortem brain scans of both human and mouse models have revealed that some patients with impaired memory do not exhibit plaque growth while conversely, other patients were found to have plaque deposits without any implications of mental decline (Pimplikar, 2008). Echoing these concerns, a publication by a group of researchers from the Neuroscience Department of the Garvan Institute of Medical Research (2014) noted that it would not be accurate to label Aβ plaques as the primary cause of synapse destruction in the brain due to the uncertainties still surrounding the approach. They cite clinical failures and the difficulty of translating data from studies done on mice models to humans as reasons for concern (Morris, Clark, & Vissel, 2014).

While it is evident that the amyloid cascade hypothesis is not a flawless argument, it remains the most heavily supported by numerous publications. Many studies focus AD treatment on the basis of this hypothesis. The abnormality of Aβ peptides in the brain is a glaring indicator of a compromised immune system (Jóźwik et al., 2012). Under normal conditions, the human body's immune system is able to combat illnesses ranging from the common cold to severe infections. A feasible explanation for the body's inability to combat the physiological changes brought by AD is the impairment of the immune system. It has already been established that the Aβ protein, in its many forms, has noticeable effects on the brain. By observing these and comprehending immune responses from the brain, the path to repairing the immune system may become clearer.

The primary immune cells in the central nervous system (CNS) are known as microglial cells (or microglia). As described by Hiroaki Wake and R. Fields, from the Nervous System Development and Plasticity Section of a human development institute, the cells are responsible for neural development through phagocytic clearance of dead cells and are crucial to the preservation of a healthy brain. Additionally, microglia have been found to possess the potential to respond to virtually any disturbance of the CNS, including the growth of Aβ plaques (Nicola & Perry, 2015). Like the validity of the amyloid cascade hypothesis, the implications of activated microglia are widely debated. Emerging evidence has raised controversy over whether or not microglial cells have neurodegenerative or neuroregenerative properties. The reason for this controversy may stem from the changing physiology of the immune cells in aging human bodies.

When stimulated by foreign or invasive substances, microglia become "activated." Activated microglia are known to secrete chemokines, cytokines, and inflammatory proteins, which are biological substances that aid in the defense against diseases in the nervous system (Solito & Sastre, 2012). As with a typical immune response, the secretion of these chemicals is intended to yield neuroprotective effects on the CNS. Because the strength of the immune system declines with age, however, these immune responses are sometimes unnecessarily prolonged and eventually show evidence of neurotoxicity.

A comprehensive review of roughly twenty studies by Wake and Fields (2011) has suggested that with increasing age, factors such as oxidative stress and amplified inflammatory responses have been observed. One reference concluded that rather than support the immune system, the cytokine production of older microglia actually decreased cognitive function. This would explain the adverse effects of microglia in AD brains. Due to mitochondrial changes in the cells, the once beneficial agents of immune response had negative impacts in the brain. These progressive observations, far more than accidental data, are supported by

the findings of Egle Solito and Magdalena Sastre from medical and neuroscience-based research institutes in London (2012). In a similar compilation of various resources, Solito and Sastre recognized that the products emitted by microglia in aged and AD patients had altered expressions compared to those from healthy participants in a study. In translating this knowledge to AD, it was hypothesized that in the early stages of the disease, microglia acts as a protector and in some cases reduced the aggregation of Aβ. As the severity of AD increases, however, microglia eventually are more harmful than beneficial through their release of the previously mentioned cytokines and chemokines. The belief that microglia loses its protective function in AD is known as the "microglial dysfunction hypothesis" and the similar but distinct "neuroinflammatory hypothesis" attributes exaggerated inflammatory responses as the catalyst for AD (Solito & Sastre, 2012). No matter which theory may be more accurate, both exemplify the altered role of microglia in AD. These findings show the need for more research to better determine how and why these CNS immune cells lose their defensive functions with age.

Apart from microglial cells, the human body also houses peripheral blood mononuclear cells (PBMCs), a type of peripheral cell derived from blood. PBMCs contain white blood cells such as lymphocytes and monocytes, which form the main defensive immune system outside of the neuroimmune system (Arosio et al., 2014). The role of PBMCs in the peripheral nervous system (PNS) is comparable to the role of microglia in the CNS. Although PBMCs and microglia are two distinct classes of cells, multiple studies have shown that PBMCs are capable of differentiating into microglial cells. Akiyama et al.'s study referenced by Aaron Lai and JoAnne McLaurin of the Department of Laboratory Medicine and Pathobiology at the University of Toronto (2012) identified that these peripheral cells were able to pass the blood-brain barrier (BBB) and undergo cell differentiation, the process by which the function of a cell becomes specialized or changes (Akiyama et al., 1996). The differentiation of PBMCs into microglia presents the possibility of PBMCs being used as a biomarker , in addition to the already established microglial cells, in studying AD pathology. Even more revolutionary is the suggestion that differentiated PBMCs may be more effective in the phagocytosis of Aβ deposits than microglia. The study reported by Lai and McLaurin implied that PBMCs, not microglia, were responsible for the deterioration of Aβ. Furthermore, Alain Simard and his colleagues the Department of Anatomy and Physiology at Laval University confirmed this speculation through an *in vivo* study and cited that PBMC monocytes are indeed able to cross the permeable BBB and differentiate into microglia. Simard et al. (2006), like Akiyama et al., hypothesize that most of the observed reduction in protein deposits in the brain can be attributed to PBMCs rather than microglia, and even those microglia may likely be differentiated cells rather than resident immune cells.

As shown by the vast array of studies centered on the analysis of microglia and PBMCs, understanding immune reactions to Aβ deposits is essential to unlocking the mystery behind AD treatment. These findings pointed the focus of this research paper towards the observation of the response of the body's central immune cells, microglia and PBMCs, to Aβ deposits, the main obstacle in AD treatment. There was found to be a relative lack of research done on PBMC interactions with Aβ proteins. Microglial cell responses, on the other hand, have been more thoroughly studied and documented. The goal of this paper is to confirm the cytotoxic effects of Aβ oligomers on microglial cells, discover if the same effect is induced by Aβ monomers, and determine if these cytotoxic patterns persist in PBMCs. It is predicted that the findings will support current knowledge of Aβ-induced toxicity in microglial cells and show the same or greater cytotoxic response from PBMCs induced by Aβ.

Methods

One publication by Ma et al. (2016), a group of researchers from various biomedical and biotechnological universities around the nation, for example, investigated the neuroprotective effects of a standardized phenolic-enriched maple syrup extract on Aβ aggregation and also looked at Aβ induced neurotoxicity and inflammation in microglial cells. The intent of the study was to determine whether or not a compound of maple syrup would reduce Aβ fibrillation in an animal model. Although the focus of the maple syrup extract as a preventative substance for AD progression is

irrelevant to this research paper, the process by which Ma et al. measured the effects of Aβ on microglia proved to be inspirational. The researchers observed the BV2 microglial cell response to Aβ fibrils. For the purposes of this study, Aβ monomers and Aβ oligomers were used in place of fibrils. Monomers and oligomers are less developed than fibrils. By replicating a similar experiment with a less complex version of the same protein, more insight will be gained as to during which stage of Aβ development the protein first exhibits cytotoxicity. Another modification of Ma et al.'s study was the exclusion of oxidative stress, nitric oxide species measurements, and cytokine levels as indicators of cytotoxicity. The measuring of cell viability (amount of living cells) was kept, but a Glo-Assay procedure was to be used in place of the MTT assay. The addition of PBMCs is another change made to the referenced study. Using PBMCs as well as microglia will show which immune cell makes greater contributions to the advancement or delay of Aβ progression.

Microglial Cell Culture

A combination of different scientific experiments was used in this study. Frozen BV2 mouse microglial cells were used from a previously established cell line. Mice microglia were used rather than human microglia due to lab availability and the commonplace method of using mouse models in science. BV2 cells were used rather than primary microglial cells due to its high cell proliferation and convenience. Primary microglial cells harvested from a living organism is ideal, but doing so would raise ethical concerns and is a more time consuming method. Stored BV2 cells were used due to better characterization of the cells and the ease of data replication. The cells were retrieved from liquid nitrogen storage and were reconstituted in 10mL cell culture solution containing Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) in a 25 cm^2 flask. The flask was then incubated in a CO_2 incubator at 37°C for cell proliferation. The cells were observed under the microscope and maintained daily until 80% confluency was reached for adequate cell growth, about 48 hours. The medium was poured out and the flask was rinsed with a trypsin/EDTA solution. About 3 mL of new trypsin/EDTA was added and the flask was placed back into the incubator for about five minutes until the trypsin had lifted the cells from the surface of the flask. This was determined by observation. The cells were pipetted in and out to ensure even dispersion of the cells in the solution and to detach the cells from each other. Culture medium was added until the total volume of the solution reached 30 mL. The solution transferred to a 50 mL tube. A 10 µL sample was aliquoted (divided) from the tube and mixed with 10 µL of trypan blue dye. Gloves were worn and special caution was taken to avoid direct contact with trypan blue, due to its tendency to stain. A hemocytometer was used to count the number of cells in the sample. 100 µL of the BV2 cell solution at a concentration of $5x10^5$ cells/mL were added into each well of a 96-well plate. The plate was placed back into the incubator overnight.

PBMC Preparation

Blood cells ordered from a blood bank were re-suspended with RPMI-1640 (a culture medium) at a 1:1 volume with the buffy coat. Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Hypaque gradient centrifugation of the re-suspended blood cells. The cells were then placed into vials ($1x10^7$ cells per vial) and frozen. To thaw the PBMC vials rapidly, they were placed in a 37˚C degree water bath. The contents were then transferred into pre-labeled 50 mL conical centrifuge tubes with 30 mL RPMI-1640 in order to allow the cells to suspend in the buffer as rapidly as possible. The cells were placed in the centrifuge at1500 rpm for 5 minutes so the contents could conjugate into a pellet. The supernatant (remaining solution at the top of the tube) was decanted and the pellets were un-clumped to be suspended with 10 mL of RPMI-1640 containing 10%FBS and 1% P/S. The cell concentration was counted using a hemocytometer after being stained with trypan blue. The cell concentration was adjusted to $1x10^6$ cells/mL and add 100 µl to each well of a 96 well plate and keep at 37°C and 5% CO_2 overnight. The cells were treated with 100 µl of 2x monomeric Aβ and oligomeric Aβ at the designated concentrations, the process of which is described in the following Monomeric and Oligomeric Drug Treatment section.

Aβ preparation

Monomeric Aβ preparation.

Lyophilized $A\beta_{1-42}$ peptide (from Biomer Technology, Cat: 1409-rPEP-02) was first thawed at room temperature for 30 minutes to avoid condensation from forming upon opening the tube. The contents of the vial were then suspended in pre-chilled HFIP (1,1,1,3,3,3-Hexafluoro-2-popanol) solution at 1 mM. The Aβ solution was kept in a rotator at room temperature for 24 hours until transparent and colorless (or until they were completely dissolved). The solution was placed on ice for 10-15 minutes. 10 µl of the solution was then quickly aliquoted in pre-labeled, pre-chilled tubes and stored in -80 °C, or centrifuged in Speedvac at 1000g to evaporate all traces of HFIP for future use.

Oligomeric Aβ preparation.

HFIP pretreated $A\beta_{1-42}$ peptides prepared from the previous monomeric Aβ preparation procedure were reconstituted in DMSO solution to a stock concentration of 5mg/ml. They were diluted with PBS to 62.5 ng per reaction in 10 µl with 1xPBS. A 10 µl of mixture of drug and Aβ peptide were held in a low-retention tube and constantly rotated on a rotator in a 37°C incubator for 48 hours.

Monomeric and Oligomeric Drug Treatment

The 96-well plate was retrieved from the incubator from the microglial cell culture procedure. One tube each of prepared monomeric and oligomeric $A\beta_{1-42}$ peptide were dissolved in DMSO stock (5 mg/mL) and diluted to a concentration of 20 µmol into new Eppendorf tubes. Each protein was assigned 6 tubes. The proteins then underwent a series dilution from 10 µmol to 0.156 µmol. 100 µmol of the Aβ from each tube of the varying concentrations was pipetted and mixed into the designated well on the 96-well plate. Three non-treatment controls containing only cell culture medium were set up on each plate. The plate was then incubated for 24 hours. After incubation, 100 µl of solution from each well was collected and stored in new tubes and placed in -80°C storage for future analysis. 50 µl glow assay solution was pipetted into each well and the plate was placed into a plate reader.

This procedure was repeated with the 96-well plate set up and incubated in the PBMC Preparation section. All of the data gathered from the plate reader was plotted using the GraphPad Prism 8.0 software.

Results and Analysis

Aβ Induced Cytotoxicity In BV2 Cells

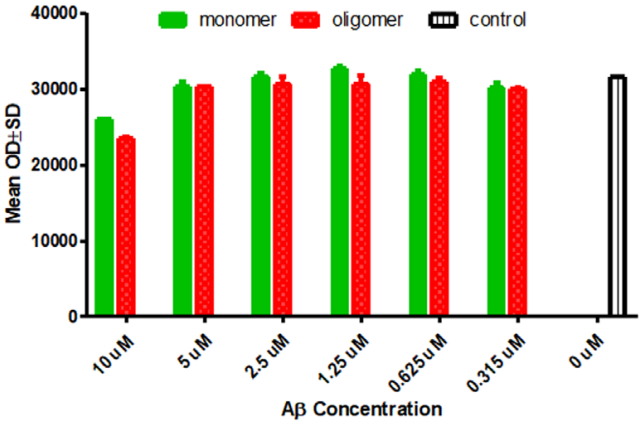

Figure 1. Graph of Glo-Assay results with microglia. This figure displays the results of Aβ induced cytotoxicity in BV2 cells. OD stands for optical density, which refers to the amount of light absorption of a substance. A higher OD value corresponds with a greater amount of living cells (higher cell viability).

The x-axis of *Figure 1* shows the increasing concentration fo Aβ from the twofold series dilution. The y-axis values represent cell viability. A higher y-axis value corresponds with a higher volume of living cells, while a lower value signifies a larger number of dead cells. The unfilled control bar to the far right of the graph indicates cell viability of the well without Aβ. Values below the control value, shown with a horizontal line drawn across the graph, was interpreted to indicate cytotoxicity. The results represented in the graph show indications of cytotoxicity for both monomeric and oligomeric Aβ at 10 µM ($p<0.05$) when compared to the control. Between the monomeric and oligomeric Aβ, the oligomeric Aβ exhibited a stronger toxicity ($p<0.05$). At 5 µM, both isoforms of the protein continued to show toxicity when compared

to the control ($p<0.05$). As the Aβ concentration decreased, the level of cytotoxicity generally declined. At 1.25 μM, the monomeric Aβ was not shown to induce cytotoxicity but the oligomeric form continued to show toxicity compared to the control.

Aβ Induced Cytotoxicity In PBMCs

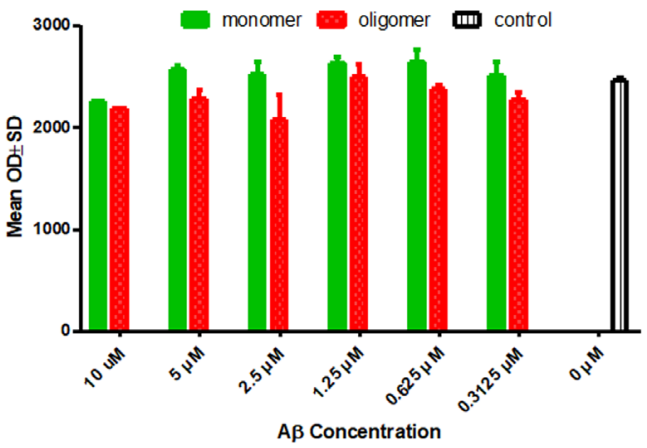

Figure 2. Graph of Glo-Assay results with PBMCs. This figure displays the results of Aβ induced cytotoxicity in PBMCs. OD stands for optical density, which refers to the amount of light absorption of a substance. A higher OD value corresponds with a greater amount of living cells (higher cell viability).

The graph in *Figure 2* displays the Glo-Assay results of Aβ induced cytotoxicity in PBMCs. Here, the cytotoxic effects of oligomeric Aβ compared to monomeric Aβ are more evident than in *Figure 1*. Similar to the BV2 graph, both forms of the protein indicated toxicity compared to the control well at 10 μM ($p<0.05$). For the remaining concentrations, the monomeric form showed no indication of toxicity while the oligomers continued to show cytotoxicity, except at 1.25 μM, which may be attributed to human error in the process of conducting the methods.

Results and Discussion

From the data, it can be concluded that BV2 cells tend to exhibit cytotoxicity to only higher concentrations of both monomeric and oligomeric Aβ. At low concentrations, a nonexistent or negligible level of toxicity was observed. PBMCs showed a higher sensitivity to Aβ. At the highest concentration, both monomeric and oligomeric Aβ induced toxicity, and oligomeric Aβ continued to do so at lower concentrations. It can be inferred that at low concentrations, Aβ can enhance cell proliferation instead of toxicity when reacting to PBMCs.

The data shows that PBMCs are more sensitive to Aβ proteins, which supports existing findings that PBMCs are more efficient at clearing Aβ deposits. However, this may have occurred because BV2 is a transformed mouse microglial cell that is resistant to the effects of Aβ toxicity. PBMC is a primary cell from human blood that has undergone fewer changes and is therefore more inclined to change. Despite these factors, the data suggest that more focus should be centered on the role of PBMCs in AD due to its higher sensitivity to Aβ peptides. These findings also confirm that in the environment of an AD brain, Aβ, when formed into oligomers, can induce toxicity to microglia and PBMCs.

The BV2 microglial cells used in this study are representative of the central immune system in the brain. The PBMCs represent the peripheral immune system. Since AD is a disease largely attributed to aging that leads to immunosenescence, or the degradation of the immune system, it is important to analyze the immune system's role in combatting AD. In the peripheral blood system, monocytes from PBMCs possess the ability to migrate into the brain and differentiate into microglial. Therefore, peripheral monocytes may also serve as peripheral blood markers for AD. It is very important to study both microglial and PBMC sensitivity to Aβ (monomeric and oligomeric). By discovering more about immune cell reactions to abnormal bundles such as Aβ plaques, scientists can know how to create more effective treatments that will not just alleviate symptoms but eliminate them as well.

Limitations and Future Directions

Due to time constraints, a Glo-Assay procedure measuring cell toxicity was the only procedure run on the BV2 cells and PBMCs. Additional time to develop the project, along with a more extensive background knowledge of AD and immunology and a wider availability of lab resources, would have allowed for a more in-depth and complex study. My lack of education

concerning the mechanisms of this topic was also a limiting factor on the types of research projects I could pursue.

As stated by Arosio et al. (2014), the role of PBMC in this context is largely unexplored. Microglia and PBMC function remains a grey area that many researchers are still gathering more information about. Contrasting findings have proven that the two types of immune cells have both beneficial and detrimental impacts in AD patients. Further directions for this topic of exploration would be to conduct a more comprehensive study of how immune cells react to Aβ peptides. Measuring and comparing the levels of cytokines emitted by activated microglia and PBMCs would indicate which specific substances are responsible for the change of immune cells from beneficial to harmful agents. Studying a greater number of biomarkers to prove Aβ induced toxicity can lead to a more complete knowledge of the mechanisms behind AD pathology. This field of immune-based treatment is not just limited to the area of AD research but could also translate to encompass immunotherapy for a broad spectrum of diseases. By unlocking how the immune system reacts to foreign diseases, further steps can be taken to combat these illnesses.

References

About Alzheimer›s Disease: Treatment. (n.d.). Retrieved February 2, 2017, from https://www.nia.nih.gov/alzheimers/topics/treatment

Alzheimer's Disease Fact Sheet. (2016, August). Retrieved February 27, 2017, from https://www.nia.nih.gov/alzheimers/publication/alzheimers-disease-fact-sheet#changes

"Alzheimer's Disease: Unraveling the Mystery." *U.S National Library of Medicine*. U.S. National Library of Medicine, Sept. 2008. Web. 2 Nov. 2016.

Akiyama H, Kondo H, Mori H, et al. The amino-terminally truncated forms of amyloid beta- protein in brain macrophages in the ischemic lesions of Alzheimer's disease patients. *Neuroscience Letters*. 1996; 219(2):115–118. http://dx.doi.org/10.1016/S0304-3940(96)13197-9

Arosio, B., D'Addario, C., Gussago, C., Casati, M., Tedone, E., Ferri, E., ... & Mari, D. (2014). Peripheral blood mononuclear cells as a laboratory to study dementia in the elderly. *BioMed Research International*, *2014*.

Chow, V. W., Mattson, M. P., Wong, P. C., & Gleichmann, M. (2010). An Overview of APP Processing Enzymes and Products. *Neuromolecular Medicine*, 12(1), 1–12. http://doi.org/10.1007/s12017-009-8104-z

Gomez-Nicola, D., & Perry, V. H. (2015). Microglial dynamics and role in the healthy and diseased brain: A paradigm of functional plasticity. *The Neuroscientist*, 21(2), 169–184. http://doi.org/10.1177/1073858414530512

Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A., ... & Zotova, E. (2008). Long-term effects of Aβ 42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *The Lancet*, *372*(9634), 216-223.

Jóźwik, A., Landowski, J., Bidzan, L., Fülop, T., Bryl, E., & Witkowski, J. M. (2012). Beta-amyloid peptides enhance the proliferative response of activated cd4+cd28+ lymphocytes from alzheimer disease patients and from healthy elderly. *PLoS ONE*, 7(3), e33276. http://doi.org/10.1371/journal.pone.0033276

"Latest Alzheimer's Facts and Figures." *2016 Alzheimer's Disease Facts and Figures*. Alzheimer's Association, 29 Mar. 2016. Web. 1 Nov. 2016.

Latest Medication for Memory Loss. (n.d.). Retrieved February 26, 2017, from http://www.alz.org/alzheimers_disease_standard_prescriptions.asp

Ma, H., DaSilva, N. A., Liu, W., Nahar, P. P., Wei, Z., Liu, Y., ... & Shaikh, Z. A. (2016). Effects of a standardized phenolic-enriched maple syrup extract on β-amyloid aggregation, neuroinflammation in microglial and neuronal cells, and β-amyloid induced neurotoxicity in caenorhabditis elegans. *Neurochemical Research*, *41*(11), 2836-2847.

Morris, G. P., Clark, I. A., & Vissel, B. (2014). Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. *Acta Neuropathologica Communications*, *2*(1), 135.

Mayo Clinic Staff (2015) "Alzheimer's Disease." *Symptoms and Causes - Alzheimer's Disease*. Mayo Foundation for Medical Education and Research. http://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/dxc-20167103

O'Brien, R. J., & Wong, P. C. (2011). Amyloid precursor protein processing and Alzheimer's disease. *Annual Review of Neuroscience*, 34, 185–204. http://doi.org/10.1146/annurev-neuro-061010-113613

Pimplikar, S. W. (2009). Reassessing the amyloid cascade hypothesis of Alzheimer's disease. *The International Journal of Biochemistry & Cell Biology*, *41*(6), 1261-1268.

Simard, A. R., Soulet, D., Gowing, G., Julien, J. P., & Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron*, *49*(4), 489-502 http://dx.doi.org/10.1016/j.neuron.2006.01.022

Solito, E., & Sastre, M. (2012). Microglia function in Alzheimer's disease. *Frontiers in Pharmacology*, 3, 14. http://doi.org/10.3389/fphar.2012.00014

Stansley, B., Post, J., & Hensley, K. (2012). A comparative review of cell culture systems for the study of microglial biology in Alzheimer's disease. *Journal of Neuroinflammation*, *9*(1), 115.

Wake, H., & Fields, R. D. (2011). Physiological function of microglia. *Neuron Glia Biology*, 7(1), 1–3. http://doi.org/10.1017/S1740925X12000166

Zhang, Y., Thompson, R., Zhang, H., & Xu, H. (2011). APP processing in Alzheimer's disease. *Molecular Brain,* 4, 3. http://doi.org/10.1186/1756-6606-4-3

Endnotes

1 A transmembrane protein refers to a protein that is partially protruding out of a cell and partially covered inside the cell. The ectodomain defines the section exposed outside of the cell and the C-terminus refers to the part remaining inside the cell (O'Brien & Wong, 2011).

2 A protease is an enzyme involved in the breakdown of proteins.

3 Peptides are the simplest structures that combine to form proteins.

4 Isoforms refer to variations of related proteins.

5 Phagocytosis is the process by which cells in the body engulf bacteria.

6 A biomarker is any measurable indicator that shows medical health (Strimbu & Tavel, 2010).

7 Lyophilization is the freeze-drying of biological substances.