Cytokine Production in Microglial Cells: A Target for Alzheimer's Disease Therapeutics

Connie Linardos[1,2], Morgan Bertrand[1,3], Allison Regan[1,2], Evan Chandlee[1,2], Paige Braden-Kuhle[1,2], Michael Chumley[1,3], and Gary Boehm[1,2]

[1]Neurobiology of Aging Collaborative [2]Department of Psychology and [3]Department of Biology at Texas Christian University Fort Worth, TX 76129

Alzheimer's Disease (AD) is a neurodegenerative disease that primarily affects elderly populations. AD engenders memory loss and cognitive decline, and its prevalence is rapidly growing. It is estimated that 14 million Americans will have AD by the year 2050. Therefore, it is imperative for researchers to examine the underlying biological mechanisms responsible for AD. Previous research has demonstrated that chronic inflammation is linked to the hallmark AD pathology, amyloid beta (Aβ). Aβ is a protein that disrupts neuronal communication and increases the production of effector proteins called pro-inflammatory cytokines. Microglia function like immune cells in the brain, and when they are activated by inflammatory triggers, such as Aβ, they secrete pro-inflammatory cytokines. Although cytokine release is initially a healthy response, excess cytokine production is harmful to the brain and exacerbates AD pathologies. Prior research has demonstrated that pro-inflammatory cytokines are upregulated in the serum of AD patients. Therefore, cytokines are a crucial target for AD therapeutics. The current experiment examined the temporal inflammatory response of microglial cells following lipopolysaccharide (LPS) insult. LPS is a component of common bacteria and can induce inflammation in microglial cells. We treated cells with several different concentrations of LPS and assessed cytokine production at several different timepoints. To do this, we collected cell supernatant (secretions) and measured multiple cytokines using an ultrasensitive electrochemiluminscent assay. Data collected from these experiments will be used in many future studies of potential therapeutics and dietary supplements. In fact, data from these experiments will be used by current and future departmental honors students to determine the optimal treatments and times for their experiments. This project is incredibly relevant because AD is currently the 6th leading cause of death in the United States. Data collected will help us pinpoint proper testing procedures for therapeutic compounds that are developed.

Introduction

- Over 6 million Americans currently have Alzheimer's disease (AD), and that number is only expected to rise in the coming years.
- AD pathology involves chronic inflammation in the brain (LaFerla et al., 2007).
- Brain microglial cells react to inflammatory triggers, injury, or infection, by secreting pro-inflammatory cytokines in the central nervous system (Block & Hong, 2005).
- Although acute inflammation is beneficial, chronic inflammation in the brain further exacerbates AD pathologies (Horvath et al., 2008; Stansley et al., 2012).
- We are interested in examining the temporal regulation of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, in microglia following an inflammatory insult.
- Here we used bacterial lipopolysaccharide (LPS) to induce microglial cell activation and cytokine production.
- Understanding the temporal regulation of cytokine production will allow us to optimize future experiments that investigate potential therapeutic compounds.

Methods

- Immortalized BV2 microglial cells were cultured in a cell incubator at 37 ºC and 5% CO_2. Cells were grown in complete medium and passaged around 80% confluency.

Treatment	Concentrations (µg/mL)
Lipopolysaccharide (LPS)	0, 0.0005, 0.005, 0.05, 0.5, 5.0

Figure 1. ELISA was utilized to measure cytokine production following LPS treatment in BV2 cells. Cells were treated with LPS (0 µg/mL – 5µg/mL) at varying treatment durations (2, 4, 8, 12, and 24 hours).

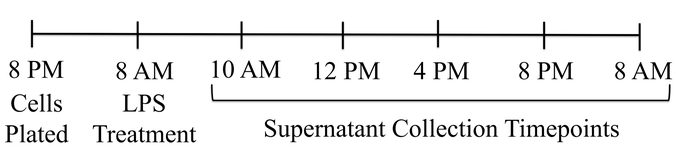

Figure 2. Timeline of BV2 cell treatment and supernatant collection.

Results

TNF-alpha (pg/mL) in BV2 Cell Supernatant Following LPS Treatment

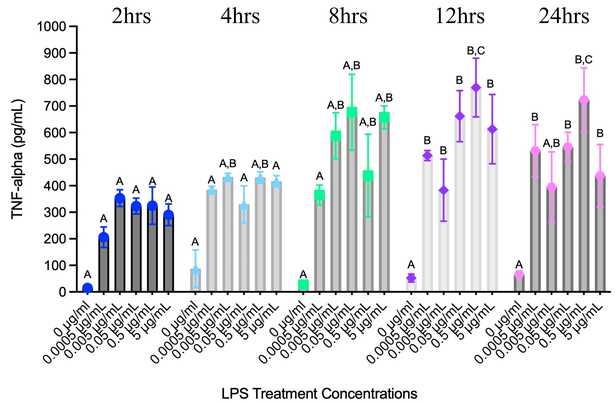

Figure 3. ANOVA revealed a significant main effect of LPS treatment ($p \leq 0.001$) and treatment duration ($p \leq 0.001$) on TNF-alpha production in BV2 microglial cells. Additionally, there was a significant interaction between LPS treatment and treatment time ($p = 0.036$). Bars represent mean ± SEM. N's = 3.

IL-6 (pg/mL) in BV2 Cell Supernatant Following LPS Treatment

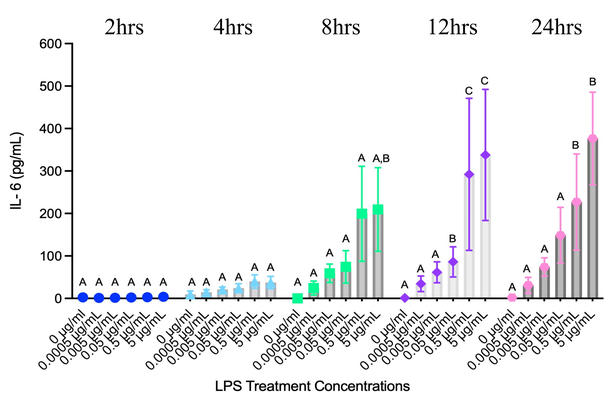

Figure 3. ANOVA revealed a significant main effect of LPS treatment ($p \leq 0.001$) and treatment duration ($p = 0.003$) on IL-6 production in BV2 microglial cells. Bars represent mean ± SEM. N's = 1–3.

Conclusions

- LPS treatment and treatment duration induced an inflammatory response in BV2 microglial cells.
- TNF-alpha production elevated with increasing LPS concentrations and treatment duration at 4, 8, 12, and 24 hours.
- IL-6 production continually increased with higher LPS concentrations and longer treatment durations at 8, 12, and 24 hours.
- There was no main effect of LPS treatment and treatment time on IL-1β production in BV2 microglial cells (graph not shown).
- Previous studies have also demonstrated that BV2 cells do not secrete IL-1β (Stansley et al., 2012).

Future Directions

- Data will be used to determine which concentrations of LPS should be administered to cells in future experiments such as assessing the therapeutic effects of cannabidiol (CBD) and potent small molecules created by Dr. Kayla Green (TCU Chemistry).
- Transition from analyzing cytokine production in BV2 microglia to monocytes isolated from mice injected with LPS.
- Assessing how cytokine production from LPS would differ in an immortalized macrophage line as opposed to a microglia line.

References

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