The Effects of Alcohol on Microglial Phagocytosis
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Abstract
- Microglia, which are the innate immune cells of the central nervous system (CNS), are involved in normal brain function and have been linked to CNS disorders such as multiple sclerosis and traumatic brain injuries (TBIs).
- As a key player in the inflammatory response, microglia remove cellular debris by phagocytosis (i.e., uptake of cellular debris).
- Alcohol is one of the most used and abused drugs in the United States. It affects all organ systems in the body and especially alters functioning of the central nervous system.
- Given the high prevalence of alcohol use and the prominent role of microglia in normal and pathological brain function, the objective of this study was to determine the effects of alcohol on microglial phagocytosis.

Methods
- BV2 microglia cells were maintained in DMEM containing 10% fetal bovine serum with 100 units/ml of penicillin, 0.1 mg/ml of streptomycin and 250 mg/ml of amphotericin B (i.e., complete media) at 37°C in an atmosphere of 95% air: 5% CO₂.
- For experiments, cells were plated (0.3 x 10⁶ cells/plate) onto poly-lysine coated 35mm tissue culture plates and allowed to attach overnight.
- To measure phagocytosis, BV2 cells were incubated in complete media containing 30 x 10⁶ Fluoresbrite® YGCarboxylate Microspheres (1.0 μm, Polysciences Inc., Warrington, PA) in the absence and presence of 100mM alcohol. Prior to this treatment, the BV2 cells were incubated in DMEM alone for 1 hour at 37°C in an atmosphere of 95% air: 5% CO₂.
- Before use, the fluorescent microspheres were washed in phosphate-buffer saline (PBS) and incubated in a bath sonicator for 10 minutes in PBS containing 3 mg/ml bovine serum albumin (BSA) to reduce non-specific binding. The fluorescent microspheres were collected by centrifugation (13,000 xg for 5 minutes) and incubated in a bath sonicator for 10 min in either complete media (opsonized) or DMEM alone (unopsonized).
- After the BV2 cells were incubated for 1 hour at 37°C or 4°C, plates were placed on ice and the media was removed. The BV2 cells were rinsed 3X with cold PBS and fixed by a 15 minute incubation in 4% paraformaldehyde (PFA) in PBS. After the removal of PFA, the BV2 cells were rinsed 3X in PBS.
- Bright-field and fluorescent images of the cells were captured using an Olympus IX70 inverted fluorescence microscope (OX-40 objective) equipped with a Olympus mercury bulb and a FITC filter plus a Hamamatsu orca-ER CCD camera. The images were analyzed using ImageJ software from the National Institute of Health (NIH).
- For each experimental condition, 1-2 plates (presence or absence of alcohol) were used and 6-8 randomly selected fields per plate were captured. For the experiments, 229-520 cells were analyzed per plate.

Results
- The number of unopsonized microspheres per BV2 cell was determined at 37°C in the presence and absence of 100mM alcohol. Data are expressed as mean ± SEM (N=7-8). The data are plotted as mean ± SEM (N=7-8).
- Analysis of the data by 2-way repeated measure ANOVA indicated no statistically significant difference in the phagocytic index between the control and alcohol-treated cells.
- Alcohol caused a statistically significance increase the percentage of BV2 cells that were engaged in phagocytosis of the unopsonized microspheres. Alcohol did not alter specific binding of the microspheres.
- The data are plotted as mean ± SEM (N=7-8). **: P = 0.003 (Student’s t test)

Conclusions
- The present study demonstrates that alcohol stimulates microglial phagocytosis of unopsonized, but not opsonized microspheres. The data suggest an effect of alcohol on phagocytosis mediated by scavenger or integrin receptors, but not the complement or Fc receptors. Interestingly, a previous report using radiolabeled bacteria (E.coli) rather than fluorescent microspheres found that alcohol inhibited phagocytosis.
- Aside from the effect of alcohol on phagocytosis, the study found no statistically significant differences in the phagocytic index of opsonized microspheres between the control and alcohol-treated cells. (Data not shown)

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References