

Alcohol and immune suppression in mice

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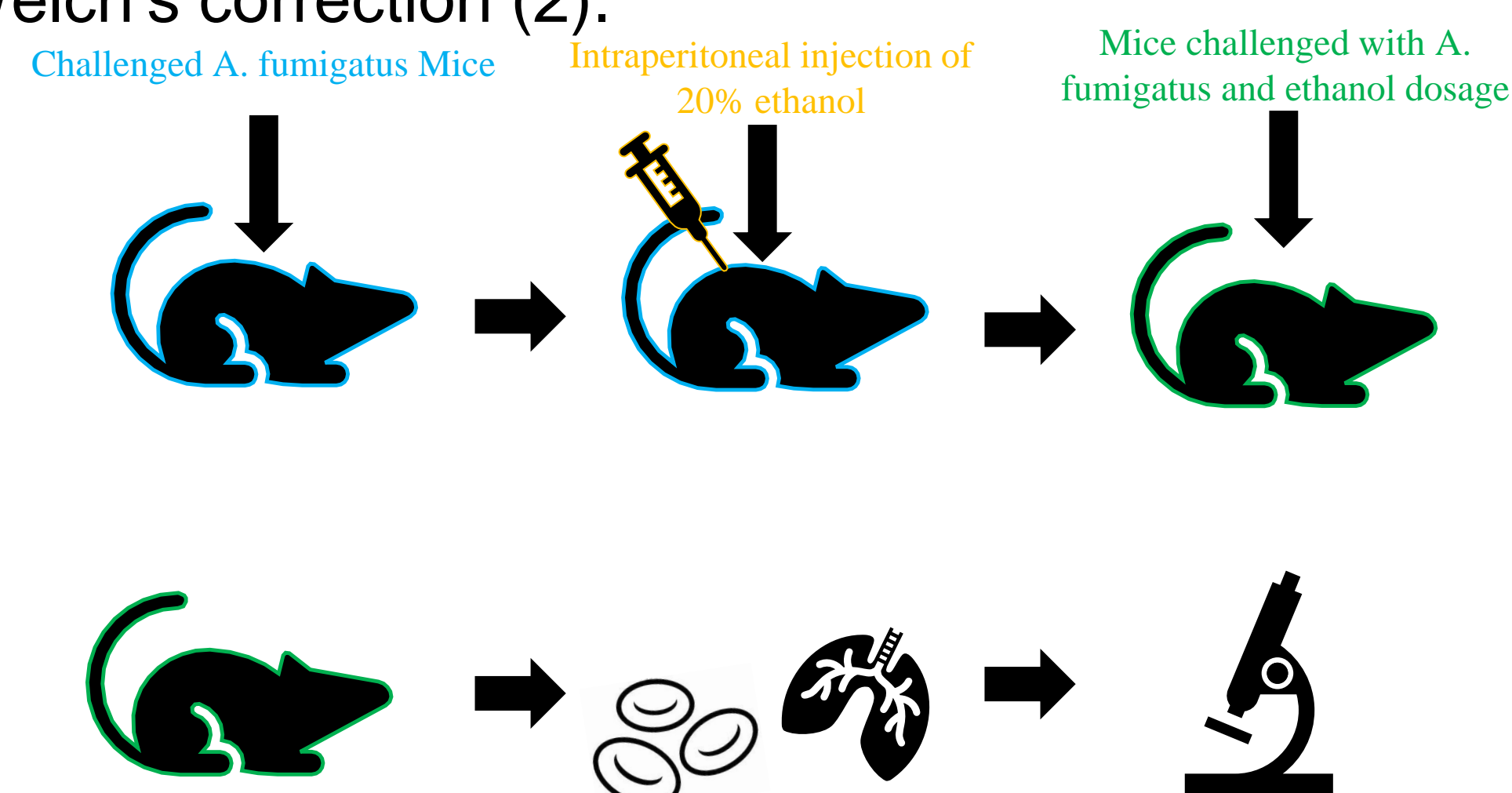
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Background Information

Alcohol has been linked to the malfunction of bodily processes and homeostatic mechanisms; studies show that heavy alcohol consumption causes damage to organs and increases susceptibility to infection (1). This has several repercussions on bodily function and the extent to which the body is damaged is not widely understood. This study tests the effect of alcohol consumption on the immune response of immunocompromised mice. Mice were exposed to *Aspergillus Fumigatus*, a fungus that causes infection within the lungs, therefore compromising the immune system of the mice. Mice were then injected with various doses of ethanol in order to determine the effect that the alcohol inhibits the immune response to the infection caused by *A. Fumigatus*. Immune response was measured by measuring the amount of White Blood cells (WBC) present in the blood as higher WBC count indicates that the body is mounting an immune response against a pathogen. The purpose of this study was to determine if the ethanol impaired the immune response to infection caused by *A. Fumigatus*.

Methods

Mice (C57BL/6) were obtained from the Immunobiology lab at MSUM, these mice were challenged with *A. fumigatus* spores once a week for three weeks and were caged separately. Mice were maintained on a standard laboratory diet and were housed in a controlled environment with a light/dark cycle. Acute ethanol intoxication was induced by intraperitoneal injection of 20% ethanol once a week for three weeks(4). Bronchoalveolar lavage samples were taken and stained cytopinned slides were done at Day 0, Day 3, and Day 28. Additionally, lungs were set in Neutralized Buffered Formalin, embedded in paraffin, sectioned and stained with Periodic Acid Schiff stain and Sirius Red stain for counts on mucus/goblet cell formation and collagen production. Cell counts were obtained at Day 0, Day 3, and Day 28 for macrophages, neutrophils, and lymphocytes. All results are expressed as the mean \pm the standard error of mean. GraphPad Prism 8 software was used to calculate statistics; differences between groups were tested with Two-way ANOVA and Welch's correction (2).



Exposed once a week for three weeks

Cell counts obtained at Day 0, Day 3, Day 28

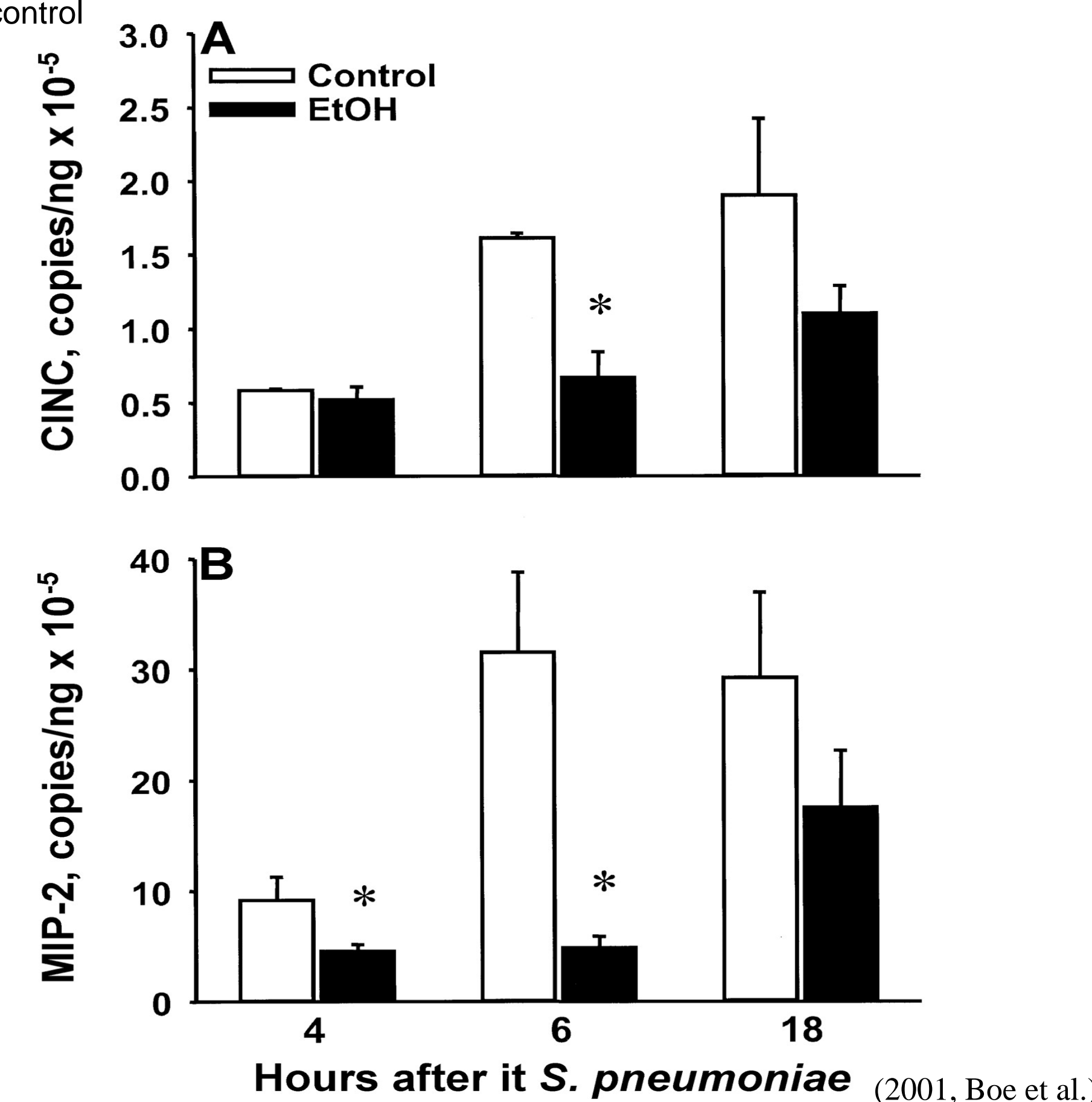
Results

Ethanol doses applied to mice are expected to overall result in a weaker immune response to *A. fumigatus* inhalation caused infections. We expect to find this due to research finding ethanol's effect on TLR signaling within peritoneal macrophages and cytokine expression. The weaker immune response will result in lower counts of lymphocytes present in comparison to native mice on our cell counts. For the Sirius red stain, we expect to find an increase of goblet cell metaplasia.

Excessive acute ethanol doses within mice exposed to *A. fumigatus* is expected to influence the innate and adaptive immune system as well as the body's other defenses towards infection. For the immune system response, acute doses of high ethanol will suppress TLR signaling. Research in mice has found that peritoneal macrophage TLR3 signaling was affected by the suppression of the degradation of IL-1R associated kinase 1 activated by a TLR3 ligand (3). This results in reduced uptake of cytokines which suppresses cellular signaling. Further results of this research showed that the release of IL-12 was significantly affected by peritoneal macrophages. The lack of induction of IL-12 into the peritoneal cavity affects the cellular communication of monocytes to T-cells for activation (3).

Within the lungs, research involving *Streptococcus pneumoniae* and acute ethanol intoxication has shown to suppress lung chemokine production. Alcoholism is associated with increased frequency and severity of pneumococcal pneumonia (4). The effect of ethanol was shown in rats to affect neutrophils and macrophages in suppressing chemokine production after infection and causes a delayed response which increases the frequency and severity of infection (4).

Figure 1. Cytokine-induced neutrophil chemoattractant (CINC; A) and macrophage inflammatory protein-2 (MIP-2; B) mRNA levels in bronchoalveolar lavage fluid cells of cyclophosphamide-treated control and alcohol-intoxicated (EtOH) rats after intratracheal (it) infection with *Streptococcus pneumoniae*. Data are mean \pm SEM (n=6–12). *P<.05 vs. time-matched control



Discussion & Conclusion

From our study we have found that by administering different levels of ethanol for a set period of time to exposed *A. fumigatus* mice causes decreased immunity. Due to the ethanol being administered into the mice by intraperitoneal injection of 20% ethanol once a week for three weeks their immune system is lowered even more. With ethanol being administered, cells including macrophages, neutrophils, and lymphocytes were counted on Days 0, 3, 28 to record how the immune system was functioning. We will see lower amounts of WBC's from the mice due to the innate immune response being reduced due to ethanol. We also will include a Bronchoalveolar lavage test where there will be a stain slides will be collected on days 0, 3, and 28. Additionally, a Periodic Acid Schiff stain and Sirius Red stain will be collected by taking the lungs and setting them in a Neutralized Buffered Formalin. From those stains we then will be looking at the mucus/goblet cell formation and collagen production and gaining a count on how the ethanol was affecting their production sites. All of these results thus show that increased amounts of ethanol can have a negative impact on how the body's immune system works. Due to the fact that these tests have shown lowered amounts of production in the cells that were tested on the separate dates listed. So, those who may consume alcohol on a regular basis may have a harder time getting over infections or disease due to their body being compromised. Proving that alcohol can have a major role in decreasing the body's immunity in everyday life.

References

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