Behavioral and Physiological Response to Immune Challenge in *T.s. parietalis*

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Abstract

Fever responses to an immune challenge have been studied in endothermic creatures, but studies of the equivalence is lacking in ectotherms. In this study, heterophil counts were shown to be significantly higher in individuals immune challenged with LPS when compared to a control group. This is indicative of an innate immune response even though neither a behaviorally induced fever nor a difference in the red blood cell to white blood cell ratio was not found.

Introduction

The immune system is crucial in any organism and expensive to maintain, forcing organisms to balance their energy expenditures between immunity, reproduction, growth and development[1]. These trade-offs have been well studied and are generally understood in mammals and birds, but our understanding of the immune system and energy budgets in ectothermic vertebrates is limited[1]. In response to foreign antigens, animals will produce a variety of innate immune cells, particularly white blood cells (WBCs)[2]. Heterophils, cells from the innate branch of the immune system, are responsible for rapid, non-specific action against the foreign antigen[1] and indicate innate immune response. Red-sided garter snakes (*Thamnophis sirtalis parietalis*) provide a well studied ectothermic vertebrate model ideal for this experiment. Previous experiments have shown that *T.s. parietalis* display an observable response to a simulated immune challenge using lipopolysaccharide (LPS), an antigen isolated from the membrane of gram negative bacteria, in the form of modified mating behavior[3].

Figure 1. Male red-sided garter snakes (Thamnophis sirtalis parietalis) from the Interlake region of Manitoba, Canada.

Materials and Methods

In this study, 44 male *T.s. parietalis* were used. Of those, 22 were injected with LPS and 22 were injected with phosphate buffered saline (PBS) to serve as a control. Each individual was injected with a total volume of 0.0063 mL/g body weight and allowed 24 hours to mount an immune response while in a temperature gradient chamber. Internal body temperatures were determined for each snake 24 hours after injection using an internal probe. Blood samples (~0.1mL whole blood) were then collected in order to analyze the ratio of red blood cells to immune cells and to count individual heterophils. Each slide was stained with Camco One-Step Quik Stain following manufacturers guidelines. Images were taken of each slide at a magnification of 400x and blood cells were counted using ImageJ software. All analyses were completed using R statistical software.

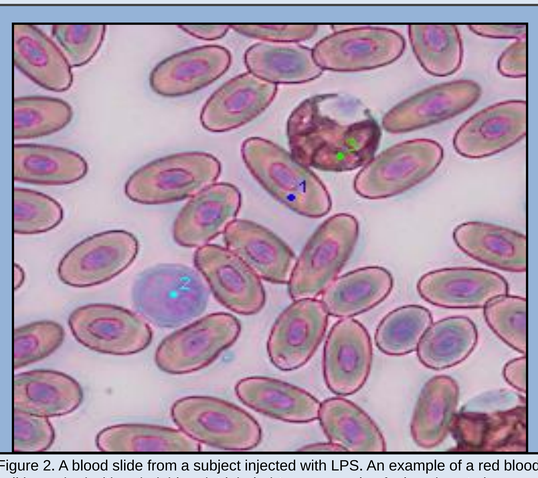

Figure 2. A blood slide from a subject injected with LPS. An example of a red blood cell is marked with a dark blue dot labeled 1, an example of a lymphocyte is marked with a light blue dot labeled 2, and a heterophil is marked with a green dot labeled 3.

Results

Average body temperature was not statistically significant (p-value = 0.1143) between the LPS and PBS groups (Figure 3).

Red blood cell/white blood cell ratio was not statistically significant (p-value = 0.9919) between the two groups (Figure 4).

Heterophil counts were statistically significant between LPS injected individuals and PBS injected individuals (p-value = 1.24×10^{-7}) (Figure 5).

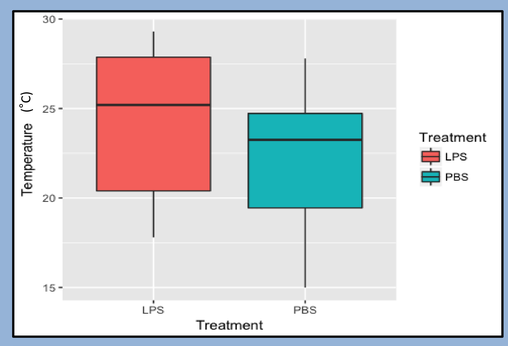

Figure 3. Average internal body temperatures (° C) 24 hours post injection for the LPS and control (PBS) groups.

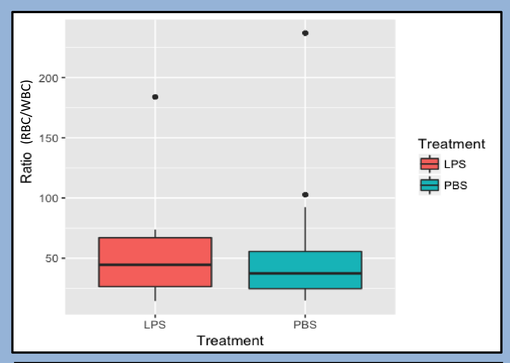

Figure 4. Average ratio of red blood cells (RBC) to white blood cells (WBC) 24 hours post injection for the LPS and control (PBS) groups.

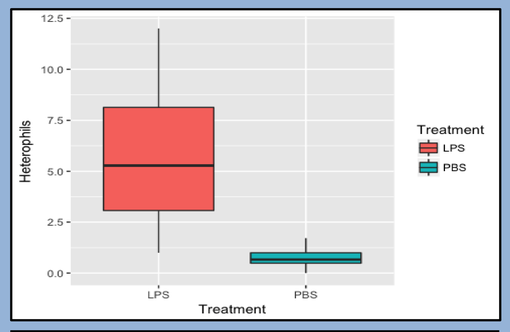

Figure 5. Average heterophil count 24 hours post injection for the LPS and control (PBS) groups.

Conclusions

While heterophil count was the only statistically significant result from this experiment (p-value = 1.24×10^{-7}) the other two results found were still of value. The behavioral component of this experiment is suggestive, but inconclusive (p-value = 0.11), and therefore worth further investigation. Also, because heterophils were part of the overall WBC count for the RBC/WBC ratio, but no significant difference was found in the ratio between groups (p-value = 0.99), it is reasonable to assume only the innate branch of the immune system is being up regulated in this LPS immune challenge.

While the behavioral data collected in this experiment was not statistically significant, and therefore gives no evidence to support behavioral fever, the combination of innate immune response and a difference of 2°C in the average body temperatures between groups indicates that this could be a result of small sample size. That temperature difference could be biologically significant for ectotherms and repeating this experiment with a larger sample size could reveal a significant difference in average body temperature.

To expand on this experiment, further research will include a repeat of behavioral trials using a larger sample size for each group and performing bacteria killing assays (BKA) on blood samples taken from each individual. Additionally, initial BKA samples will be taken before injections, giving a baseline for the post-injection assay and allowing any already immunocompromised individuals to be removed from the behavioral study. These individuals will be identified by abnormally high starting levels of bacterial killing ability (implying increased levels of innate immune components) from the initial BKA analyses to insure no individuals are already facing an immune challenge.

References

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Acknowledgments

Thanks to the College of Science for awarding me the SURE Grant and special thanks to E. Bentz, W.G. Dennis, J. Ortega, E. Uhrig, and S. VanBrocklin for their contributions in experimental design and data collection.