Examining LPS-induced inflammatory cell recruitment in tissue-regenerating rodents

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Background

Evidence of tissue regeneration in mammals is rare. African spiny mice (*Acomys* spp.) have been identified as genuine regenerators of cartilage, skin, muscle, nerves, and adipose tissue (Seifert *et al.*, 2012). Evolutionary evidence suggests pro-inflammatory responses, which promote cell-mediated immunity and fibrosis, trade off with regeneration (Mescher & Neff, 2005; Bely & Nyberg 2009; Eming *et* al., 2009; King *et al.*, 2012). *Acomys* represent a mammalian system with which to address this hypothesis. In this study, we compared cellular and humoral immunity and inflammatory cell recruitment during challenge with lipopolysaccharide (LPS) in regenerating and non-regenerating mice. We found regenerators to exhibit increased bacterial killing by serum alone, indicating a humoral bias. We also observed reduced recruitment of inflammatory cells in the blood and spleen of regenerators after LPS administration. This suggests regenerating mice may mount weaker pro-inflammatory responses to this mitogen than non-regenerators, supporting our hypothesis.

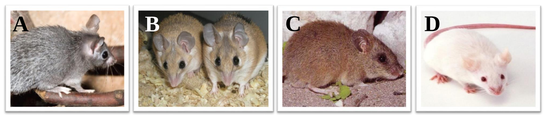
Hypothesis

Regeneration trades off with pro-inflammatory immunity

"Anti-inflammatory"	"Pro-inflammatory"
Wound healing	Pathogen clearance
Regeneration	Tissue damage
Humoral responses	Cellular responses

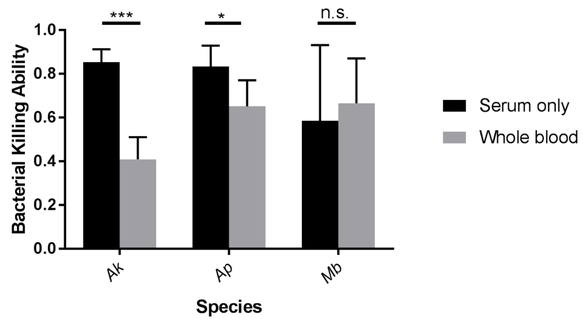
Methods

Acomys percivali (*Ap*, Fig. 1A) *A. kempi* (*Ak*, Fig. 1B) and a non-regenerating, wild sympatric species, *Myomyscus brockmani* (*Mb*, Fig. 1C), were wild-caught at the Mpala Research Centre in Kenya. Six *Ak* and *Ap*, two *Mb*, and six lab *Mus musculus* – Swiss webster (*Mm*, Fig. 1D) were injected with 1.6mg/kg of LPS intraperitoneally. An equal number of individuals from each species received a saline injection. After six hours, blood and tissue were harvested for bacterial killing assays using whole blood and serum, blood smears, and spleen histology.

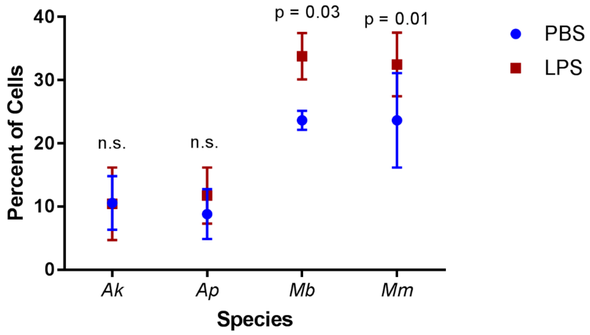


▲ **Figure 1.** Rodent species in this investigation, including regenerators *Ap* (A) and *Ak* (B) and non-regenerators *Mb* (C) and *Mm* (D).

Results

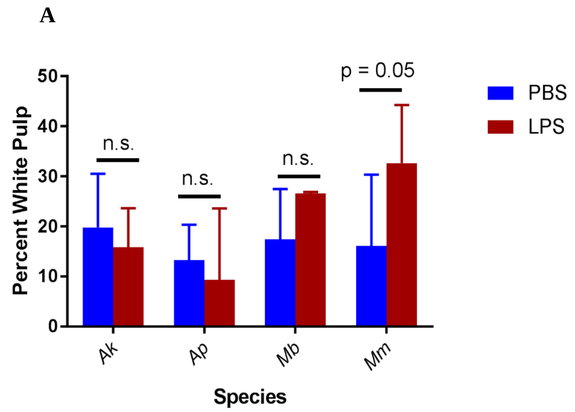


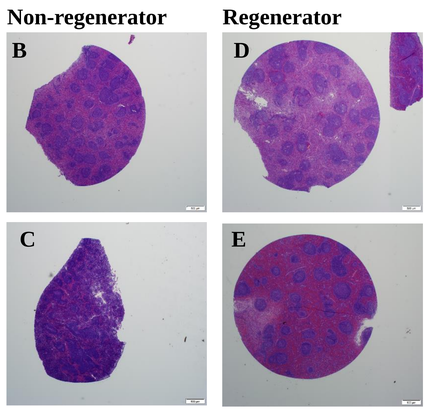
▲ **Figure 2. *E. coli* killing ability of whole blood and serum from wild mice prior to treatment.** Serum alone was more effective at killing *E. coli* than whole blood in *Ak* (Student's T, t = 9.32, p < 0.0001) and *Ap* (Student's T, t = 2.97, p = 0.0141). In non-regenerators *(Mb)*, there was no difference in killing ability of whole blood versus serum (Student's T, t = -0.28, p = 0.8051).



▲ **Figure 3. Neutrophils in the blood following treatments.** In non-regenerators, LPS treatment resulted in increased proportions of neutrophils. Post-hoc principal component analysis (PCA) revealed this to be significant for both *Mm* (Student's t-test, t=3.14, p=0.0105) and *Mb* (Student's t, t=5.29, p=0.0339). In regenerators, LPS treatment did not change neutrophil frequencies as confirmed by post-hoc PCA for *Ak* (Student's t-test, t= -1.02, p=0.3329) and *Ap* (Student's t-test, t=0.21 , p=0.8354).

▼ **Figure 4. White blood cells in the spleen following treatments.** H&E staining revealed poorly defined follicles and increased white pulp in spleens of non-regenerating mice given LPS (A, C) compared to mice given PBS (A, B). Spleens of regenerating mice had well-defined follicles and comparable white pulp levels following PBS (A, D) and LPS (A, E) treatments. These results were not statistically significant; a power analysis revealed this to be a sample size issue.





Summary

- **African spiny mice that can regenerate tissue exhibit robust humoral immune defenses to bacteria that are not significantly aided by cell-mediated defenses in the blood.**
- **Compared to related mice that do not regenerate, *Acomys* spp. recruit fewer inflammatory cells to the blood and spleen during challenge with LPS.**
- **These results support the hypothesis that regeneration trades off with pro-inflammatory immunity, but additional experiments are needed for confirmation.**

References

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