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Lysophosphatidylcholine mediates neutrophil activity through early metabolic modulation following immunization with a live-attenuated Leishmania vaccine

Markle, Hannah L[1]; Pacheco-Fernandez, Thalia[1]; Bhattacharya, Parna[2]; Sun, Jinchun[3]; Azodi, Nazli[1]; Kitthanawong, Grace[1]; Hobson, Caroline[1]; Beger, Richard[3]; Gannavaram, Sreenivas[1]; Nakhasi, Hira L[1]

[1]Food and Drug Administration/Center for Biologics Evaluation and Research/Office of Blood Research and Review/Division of Emerging & Transfusion Transmitted Diseases/Laboratory of Emerging Pathogens, Silver Spring, Maryland, USA
[2]Food and Drug Administration/Office of Health Technology 7/Center for Devices and Radiological Health, Silver Spring, Maryland, USA
[3]Food and Drug Administration/National Center for Toxicological Research, Jefferson, Arkansas, USA

Abstract

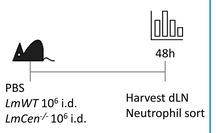
Leishmaniasis, a spectrum of diseases caused by *Leishmania* parasites, is spread by sand fly vectors and blood transfusions. Towards discovering a safe and effective human vaccine against leishmaniasis, a *centrin* gene deleted mutant of the *Leishmania major* parasite (*LmCen*$^{-/-}$) was developed. Upon vaccination, the metabolite lysophosphatidylcholine (lysoPC), which is implicated in the functions of neutrophils, was recruited to the site of *Leishmania* infections. LysoPC is a by-product of phospholipase and can control the activity of *Leishmania major*, which is a dendritic cell. By studying the role of lysoPC in neutrophil recruitment and activity, the immune mechanism of protection induced by *LmCen*$^{-/-}$ parasites can be elucidated.

Introduction

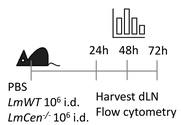
Clinical manifestations of *Leishmania* infections include cutaneous (CL), mucocutaneous (MCL), and visceral leishmaniasis (VL). Its associated morbidity and mortality warrants a pressing need to develop effective tools to ensure the safety of the national blood supply and help curb the spread of *Leishmania* infections. Preclinical studies have shown that immunization with *LmCen*$^{-/-}$ parasites induces protection from CL and VL, however, the mechanism of protection behind *LmCen*$^{-/-}$ immunization has not been fully detailed. Towards this goal, untargeted metabolomic analysis of neutrophils isolated from infected mice was performed. Results of the early metabolic immune regulation following immunization with *LmCen*$^{-/-}$ parasites will be discussed.

Materials and Methods

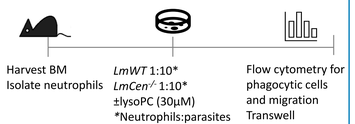
Neutrophil sorting for mass spectrometry:
C57Bl/6 mice were inoculated with LmWT or *LmCen*$^{-/-}$ parasites intradermally. At 48 hours post-infection, 2.5-3x10^6 parasitized and non-parasitized neutrophils were sorted from ear to draining lymph nodes (dLN) by flow cytometry and quenched immediately. Untargeted metabolomic analyses were performed on the neutrophil populations using mass spectrometry.



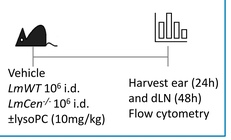
Neutrophil influx to dLNs: Neutrophil migration to dLNs was measured via flow cytometry at 24, 48, and 72 hours post-intradermal inoculation with *LmWT* and *LmCen*$^{-/-}$.



In vitro assays: Transwell assays were performed on parasitized neutrophils to measure migration in the presence and absence of exogenous lysoPC. Simultaneously, in vitro phagocytosis and migration were measured by flow cytometry.



In vivo neutrophil activation: Neutrophil activation with and without LysoPC supplementation was measured in the ear and dLNs via flow cytometry.



Results and Discussion

Mass spectrometry revealed significant enrichment of the bioactive lipid, lysophosphatidylcholine (LysoPC) in neutrophils isolated from *LmCen*$^{-/-}$-infected mice compared to naïve or *LmWT*-infected mice. Peak neutrophil influx to dLNs occured at 48 hours post-infection. Transwell assays revealed enhanced neutrophil migration in presence of exogenous lysoPC in both *LmWT* and *LmCen*$^{-/-}$ infections. In vitro infection of neutrophils revealed increased phagocytic cells and migrated neutrophils measured by LFA-1 expression in the *LmCen*$^{-/-}$ group compared to *LmWT*. LFA-1 expression was enhanced in the *LmCen*$^{-/-}$ group supplemented with lysoPC. In addition, neutrophils in the *LmCen*$^{-/-}$ group expressed greater CXCR2 in the ear, and greater CXCR2 and CXCR4 in the dLNs, compared to *LmWT* when supplemented with lysoPC.

Neutrophils sort selected from *LmCen*$^{-/-}$ infection show high levels of lysoPC

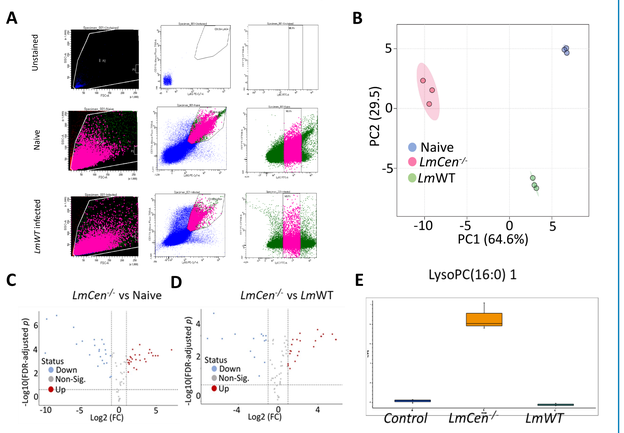


Figure 1. (A) Neutrophils were sort selected (CD11b, Ly6G+ and Ly6C^{-int}) from the dLNs of naïve, *LmWT* or *LmCen*$^{-/-}$ infected mice 48hrs post-infection for metabolomic studies. (B) Dimensionality reduction of the pooled metabolomic dataset via Principal Component Analysis (PCA) shows three distinct clusters correlating to *LmWT*, *LmCen*$^{-/-}$, and naïve infections. (C-D) Significant features selected by volcano plots for the *LmCen*$^{-/-}$ vs naïve and the *LmCen*$^{-/-}$ vs *LmWT* comparisons with log transformed fold change (x) 2 and t-test thresholds (y) 0.05, which, coupled with the PCA graphs, indicate distinct metabolomic signatures under infected and immunized conditions. (E) LysoPC levels in naïve, *LmCen*$^{-/-}$, and *LmWT* groups, depicting exclusive upregulation in *LmCen*$^{-/-}$.

LmCen$^{-/-}$ infection results in a greater neutrophil influx to dLNs

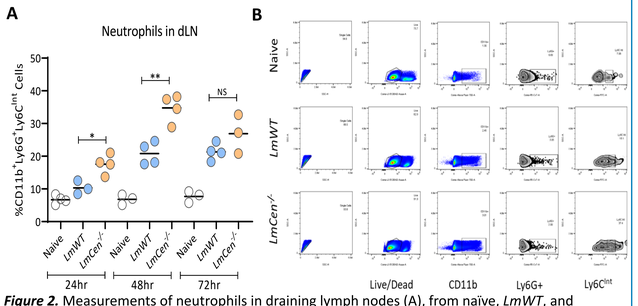


Figure 2. Measurements of neutrophils in draining lymph nodes (A), from naïve, *LmWT*, and *LmCen*$^{-/-}$ mice at 24, 48, and 72 hours. After 48 hours, peak proliferation of neutrophils was seen in the lymph nodes. (B) The percent composition of cells in the neutrophils from the draining lymph nodes at the 48hr mark was measured using flow cytometry.

Supplementation of lysoPC enhances phagocytosis and migration of neutrophils

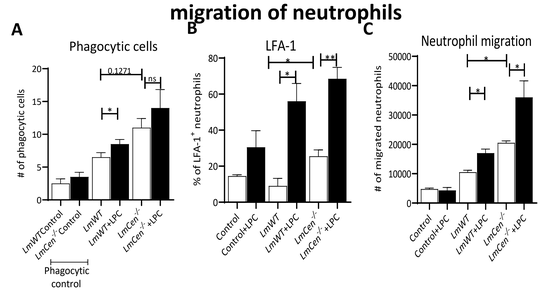


Figure 3. Transwell assays of neutrophils isolated from BM. Increased numbers of phagocytic cells were seen in *LmCen*$^{-/-}$ parasitized neutrophils compared to *LmWT*. More phagocytic cells were also seen when supplementing *LmWT* with lysoPC. Greater neutrophil migration was seen in the *LmCen*$^{-/-}$ group compared to *LmWT*, including during supplementation with lysoPC. Expression of LFA-1 was greater in the *LmCen*$^{-/-}$ group compared to *LmWT,* with expression upregulated with lysoPC supplementation.

Supplementation of lysoPC results in neutrophil activation

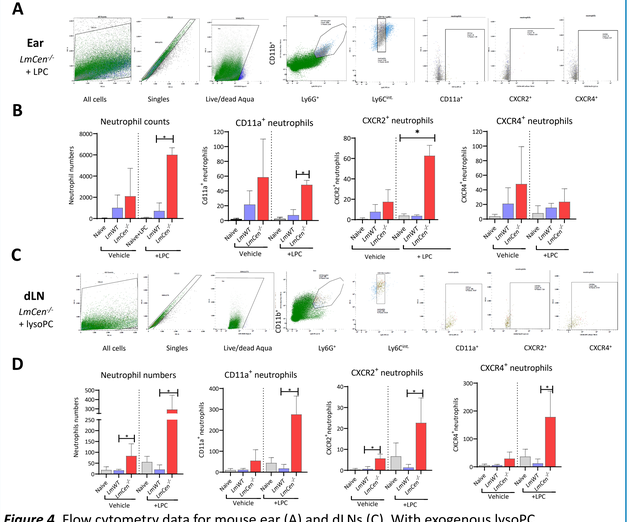


Figure 4. Flow cytometry data for mouse ear (A) and dLNs (C). With exogenous lysoPC supplementation following infection, greater neutrophil migration from the ear to dLN was seen. Neutrophil counts and activation (CXCR2+) are greater in the *LmCen*$^{-/-}$ group when supplemented with lysoPC in the ear (B). The same is seen in the dLNs, with upregulated CXCR4+ expression (D).

Conclusion

The enrichment of lysoPC and other bioactive lipids in neutrophils isolated from *LmCen*$^{-/-}$ infected mice in comparison to *LmWT* and naïve controls, suggests that lysoPC could play an important immunoregulatory role in vaccine immunity. Recruitment of neutrophils to the lymphoid organs, including the draining lymph nodes is known to be mediated by leukotriene B4-CXCL12 signaling. The enrichment of lysoPC in neutrophils and enhanced neutrophil migration following lysoPC supplementation points to a potentially novel signaling mechanism utilized by *LmCen*$^{-/-}$ parasites. Additionally, once neutrophils migrate to the lymph nodes, lysoPC seems to play a functional role in the activation of neutrophils. Future studies will explore the effect of lysoPC supplementation on CD4+ T cell response in vaccinated animals, thus assigning a definitive role of lysoPC in vaccine immunity. Further studies are also needed to investigate the role of lysoPC in the production and expression of inflammatory cytokines, which will be assessed by qRT-PCR following the isolation of neutrophils from infected mice.

References

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