Lymphangiogenesis is not sustained during non-healing cutaneous leishmaniasis caused by *L. amazonensis*

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ABSTRACT

Cutaneous leishmaniasis (CL) is a spectrum of diseases caused by *Leishmania* parasites causing 1-2 million new cases each year. Based on the species of parasite and the host immune status, CL can manifest as healing skin lesions or non-healing disease leading to permanent disfigurations. Although the parasites species and immune response to infection can dictate disease, other factors that restrict healing are unknown. Here we focus on the role of the lymphatics during non-healing CL, specifically lymphangiogenesis or the creation of new lymphatic vessels. Given that the lymphatics drain infiltrating cells, soluble mediators, and fluid away from the site of infection and play a role in lesion resolution during healing CL, we characterized the lymphatics, investigated the presence of lymphangiogenic mediators, and the immune response during non-healing CL caused by *L. amazonensis* infection.

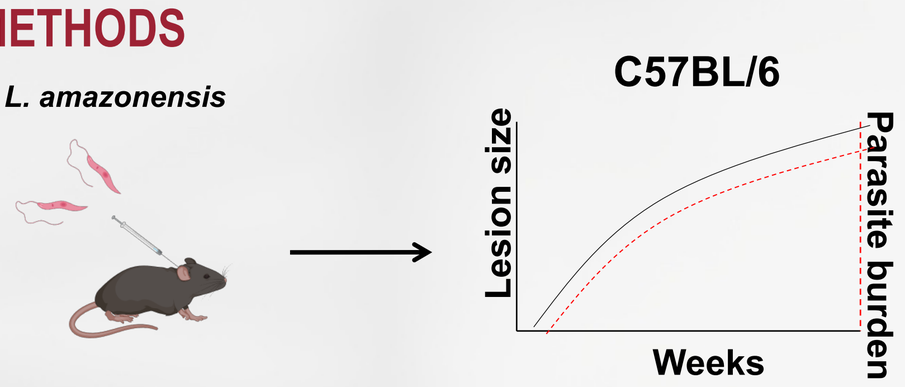
BACKGROUND

The lymphatic vasculature regulates inflammation during infection, transporting soluble antigen and antigen presenting cells to the lymph nodes and actively modulating inflammatory immune cell exit from the site of infection. Specifically, the VEGF-A / VEGFR-2 signaling pathway has been implicated in lesion resolution during murine self-healing CL caused by *L. major*, mediating lymphangiogenesis—allowing immune cells infiltrating to the site of infection to drain out through the lymphatics. Additionally, the transcription factor, HIF-1α, is responsible for transcription of VEGF-A in macrophages at the site of infection. Blocking VEGFR-2 or depleting HIF-α specifically in myeloid cells leads to inefficient lesion resolution. Although there is a well-appreciated function for the lymphatics during self-healing CL there is no work investigating the lymphatics during non-healing CL

HYPOTHESIS

Lymphatic remodeling leads to lesion resolution which will be attenuated in mice infected with species of *Leishmania* causing non- healing disease.

METHODS



C57BL/6 mice were infected with 100,000 *L. amazonensis* parasites in the ear intradermally. Lesions were measured weekly to assess disease progression. At 6 and 12 wpi tissue was taken for RT-PCR, immunofluorescence microscopy, and flow cytometry to assess lymphangiogenesis.

RESULTS

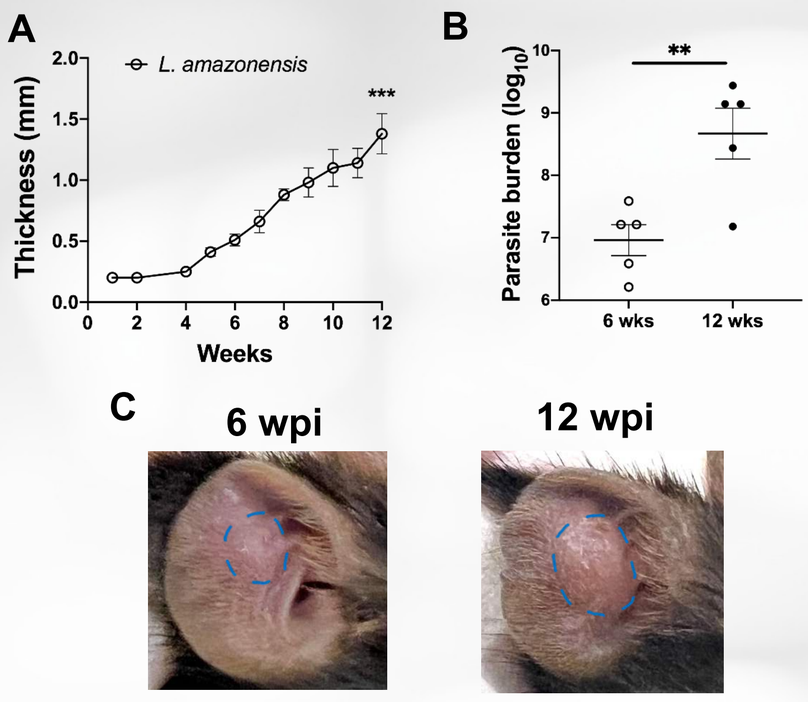


Figure 1: *L. amazonensis* infection causes progressive non-healing CL. (A) Mice were infected with 100,000 *L. amazonensis* parasites and disease was monitored weekly. (B) At 6 and 12 wpi tissue was taken to perform limiting dilution assays to quantify parasite burden. (C) Representative lesions are shown from mice infected with *L. amazonensis* at 6 and 12 wpi.

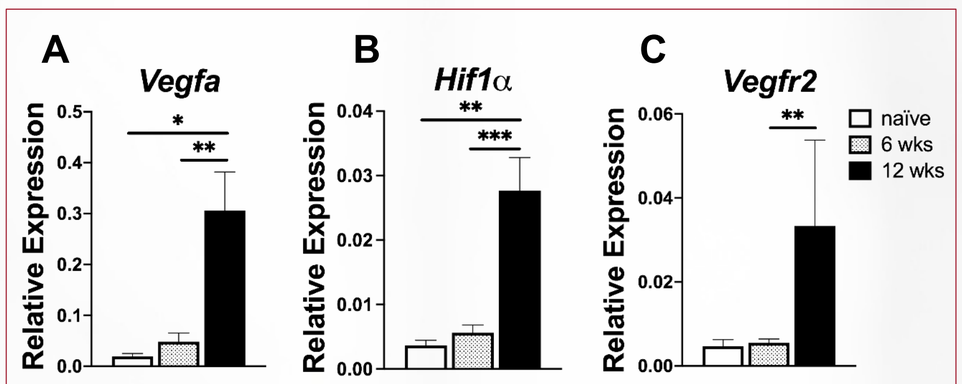


Figure 2: Expression of lymphangiogenic mediators occurs late during infection with *L. amazonensis*. (A-C) Expression levels of *Hif1α, Vegfa, Vegfr2* at 6 and 12 wpi in ear tissue are shown during infection of C57BL/6 mice with *L. amazonensis*

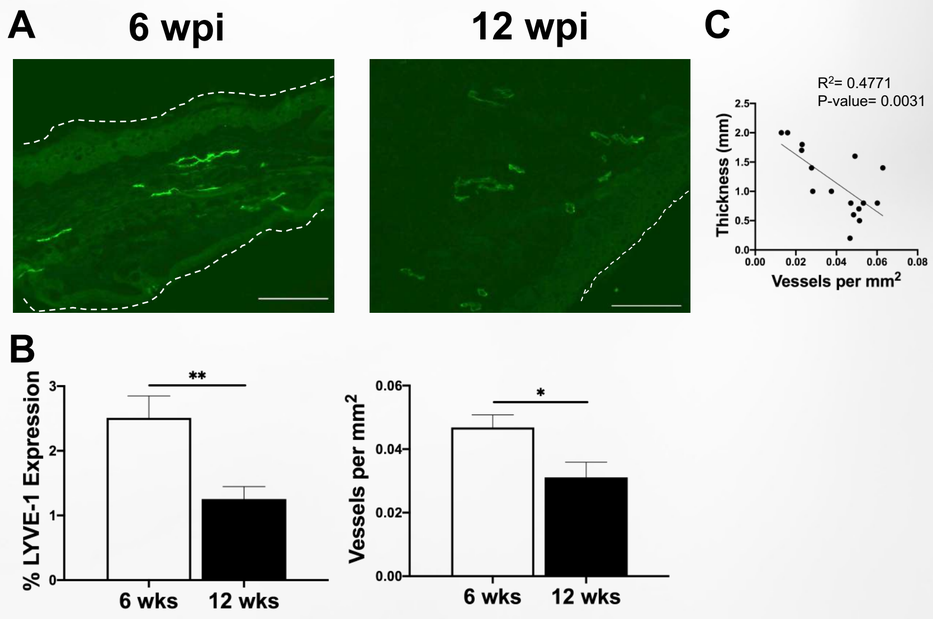


Figure 4: Lymphangiogenesis and vessel expansion are attenuated during non-healing CL due to *L. amazonensis* infection (A) Ear sections were stained with anti-LYVE-1 antibody and (B) %LYVE-1 was determined using IF microscopy and analysis via Velocity software at both 6 and 12 wpi. Experiments are pooled from two independent experiments (n=10). (C) Correlation analysis of ear thickness and vessel density of all animals at 6 and 12 wpi, (n=16).

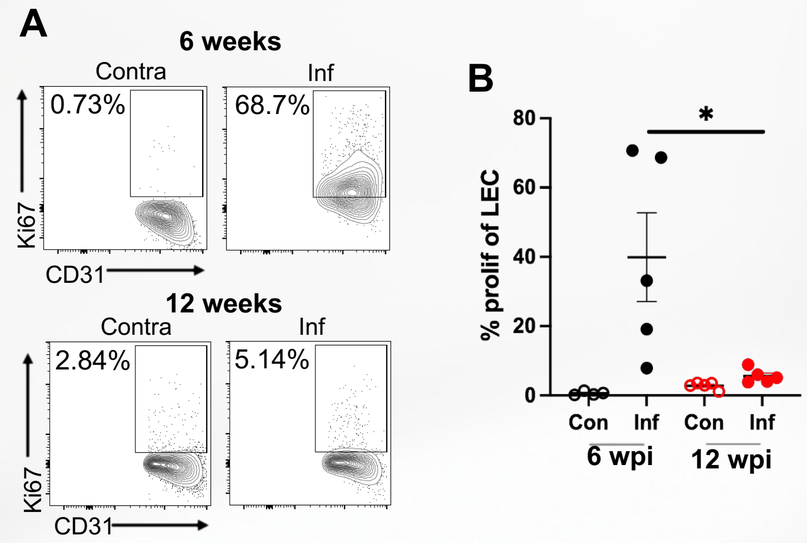


Figure 5: LEC proliferation decreases over time. Flow cytometry was performed on ear tissue from 6 and 12 wpi. Proliferation of LECs was determined by gating on live cells, singlets, CD45⁻CD31⁺Podo⁺Ki67⁺. (A) Representative flow cytometry plots of Ki67 of CD45⁻CD31⁺Podo⁺ are shown. (B) Quantification of percentages of proliferating LECs is shown.

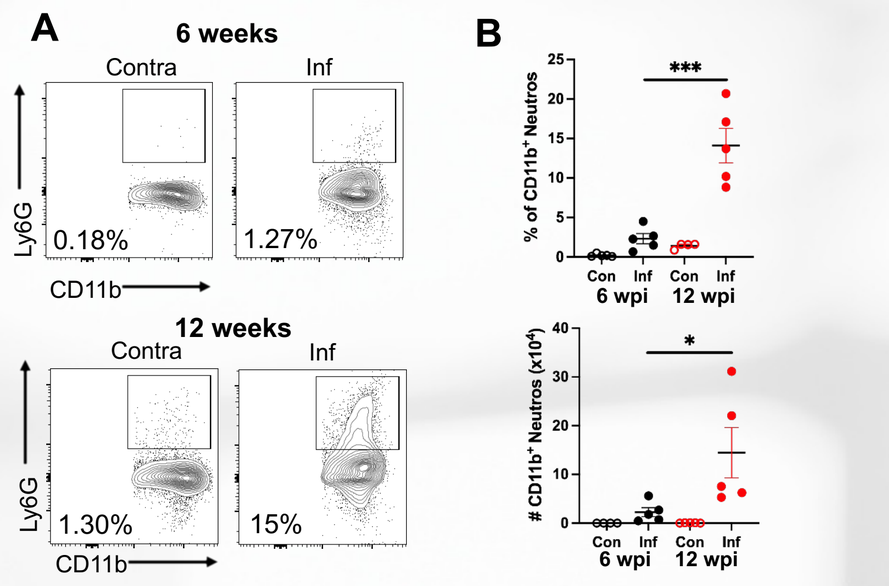


Figure 6: Neutrophils are elevated in the ear later during *L. amazonensis* infection. Ear tissue was collected at 6 and 12 wpi and prepped for downstream analysis by Flow Cytometry. Neutrophil populations were determined by gating on live cells, singlets, CD45⁺CD11b⁺Ly6G⁺SiglecF⁻. (A)Representative flow cytometry plots are shown for LyG⁺ of CD45⁺CD11b⁺. (B) Quantification of percentages and numbers of neutrophils.

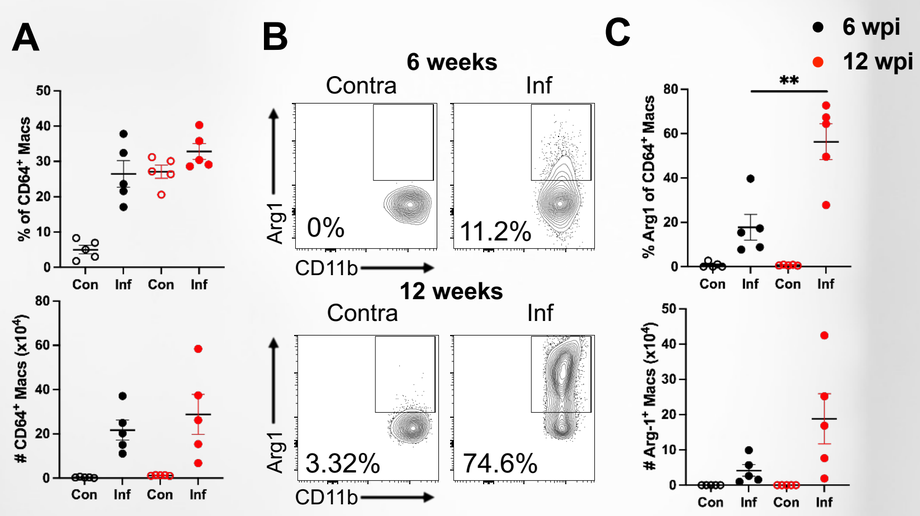


Figure 7: The environment at the infection site is distinct later during infection. Macrophage populations and phenotype were determined by flow cytometry analysis. (A) CD64⁺ populations were determined by gating on live cells, singlets, CD45⁺CD11b⁺. (B-C) Macrophage phenotype was determined by quantifying the percentage and numbers of Arg-1 of CD45⁺CD11b⁺CD64⁺ cells.

CONCLUSIONS

- **Non-healing progressive CL due to *L. amazonensis* infection shows attenuated lymphangiogenesis.**
- **An accumulation of neutrophils is seen in the infection site**
- **With less avenues of exit from the infection site, it is likely decresed lymphangiogenesis and heightened neutrophil influx contributes to non-healing disease**
- **This indicates impaired lymphatic remodeling contributes to persistent lesions in patients.**
- **Impaired lymphangiogenesis is a new mechanism of immunopathology for patients with CL and offers a potential new therapeutic target to improve healing and lesion resolution in patients**

REFERENCES

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