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FDA U.S. FOOD & DRUG ADMINISTRATION

Dual scRNA-seq Analysis Reveals Rare and Uncommon Parasitized Cell Populations In Chronic *L. donovani* Infection

Pacheco-Fernandez Thalia, FDA/CBER/OBRR; Karagiannis Konstantinos, Moderna, Cambridge MA; Gannavaram Sreenivas, FDA/CBER/OBRR; Verma Chaitenya, Wexner Medical Center, OSU; Bhattacharya Parna, FDA/OHTVII/CDRH; Nakhasi Hira L, FDA/CBER/OBRR; Satoskar Abhay R, Wexner Medical Center, OSU

SUMMARY

Visceral leishmaniasis is a lethal disease caused by the parasite *Leishmania donovani*. Relapse is observed in endemic regions, although the mechanism is still unknown. Its possible that a few parasites remain dormant in the host until the immune system becomes permissive for them to reemerge. Phagocytic cells are known hosts of Leishmania parasites, but it is unclear whether other cell types can be infected.

By using unbiased scRNA-seq to simultaneously analyze host cell and *Leishmania donovani* transcriptomes we were able to identify the parasitized cells in spleen and bone marrow during chronic infection.

This dual-scRNA-seq methodology allowed the detection of rare parasitized populations:

In spleen: mainly monocytes and macrophages; while megakaryocytes, basophils and NK cells are found unexpectedly infected.

In the bone marrow: mainly Hematopoietic Stem Cells (HSCs), but also cycling basal cells, eosinophils, and macrophages were found infected.

INTRODUCTION

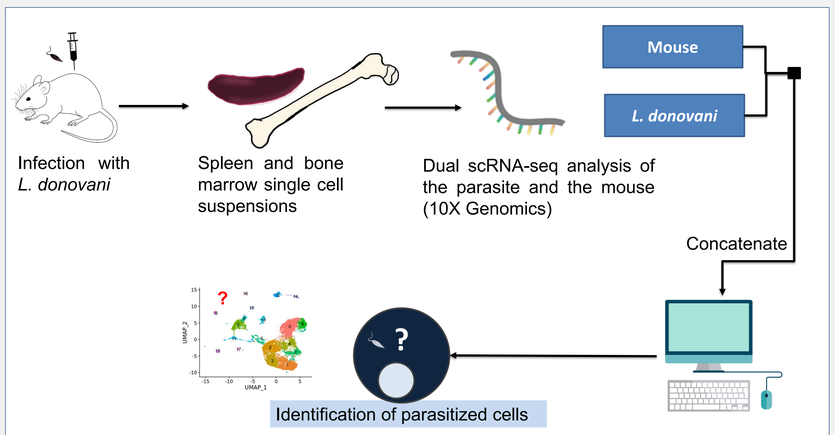
What is known:

- Visceral leishmaniasis is a deadly disease endemic in tropical countries.
- *Leishmania donovani*, the causative parasite, is known to remain in the host's body even after apparent cure.
- Phagocytic cells are the main host for *L. donovani*
- Visceral leishmaniasis patients often relapse when their immune system shift to a permissive profile and the parasite re-emerges.

What is unknown:

- What are the cells sustaining the chronic infection of *L. donovani?*
- Are phagocytes the only cells capable of getting infected?

METHODOLOGY



What is new?

Dual-scRNA-seq precisely identified the murine cells where the parasite transcripts were also detected in the chronic stage of infection that enabled annotation of rare and unexpected cell populations harboring the parasite.

RESULTS

I. Identification of parasitized clusters and identity of such clusters

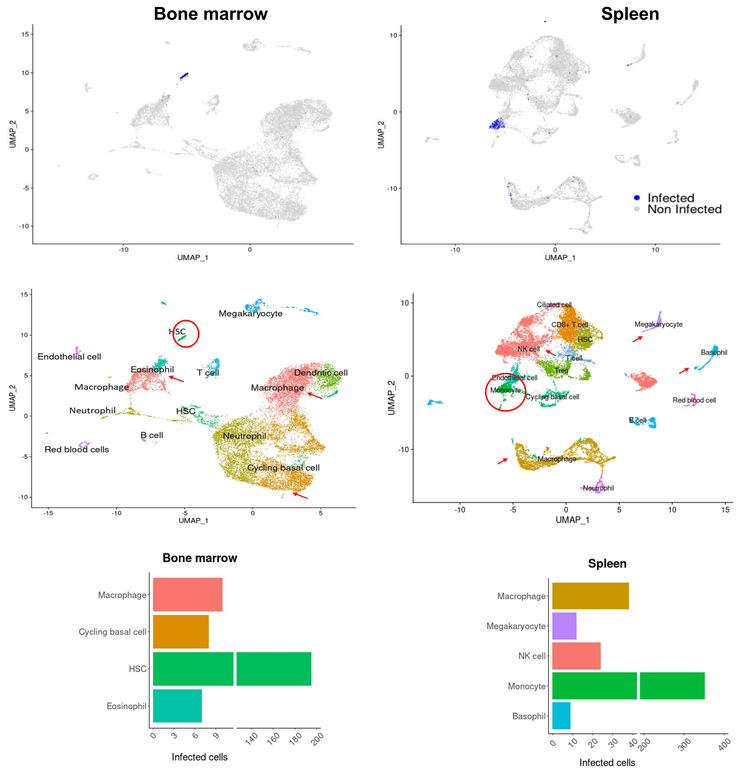


Fig. 1. UMAPs showing (upper) the clusters where *L. donovani* infected cells were detected. Then UMAPs (middle) show the clusters after cell identification. At bottom, bar graphs show the number of infected cells per cluster.

II. Differential gene expression in parasitized cells

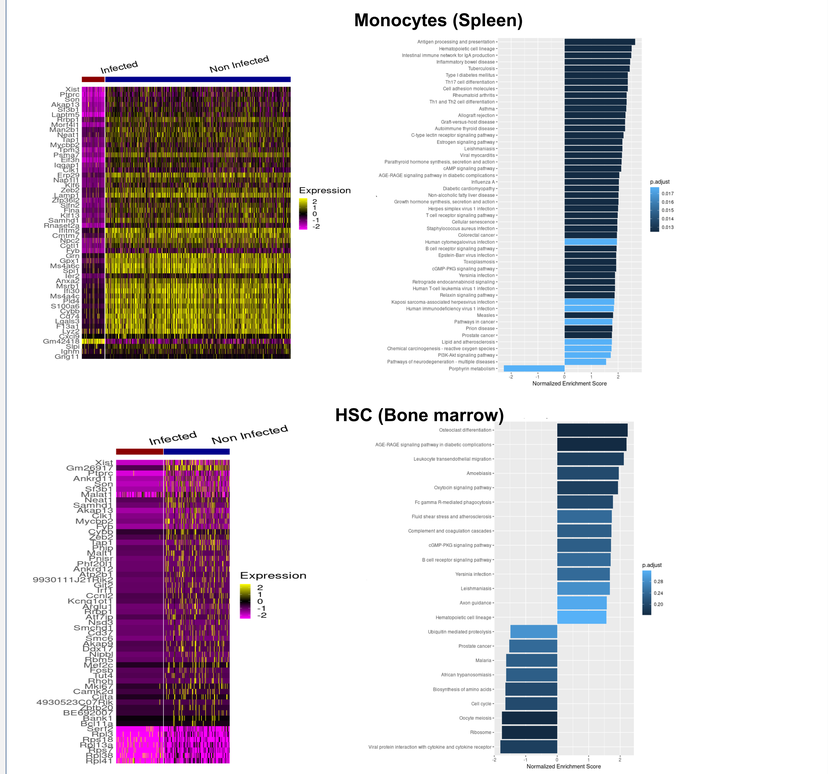


Fig. 2. Heatmaps (left) of differentially expressed genes between the infected and non infected monocytes from the spleen (up) and HSCs from the bone marrow (bottom). GSEA analysis (right) showing select signaling networks enriched in *L. donovani* infected Monocytes (C) and HSCs

III. Detection of *L donovani* parasites in HSCs expressing phagocytic receptors-Validation of dual scRNASeq

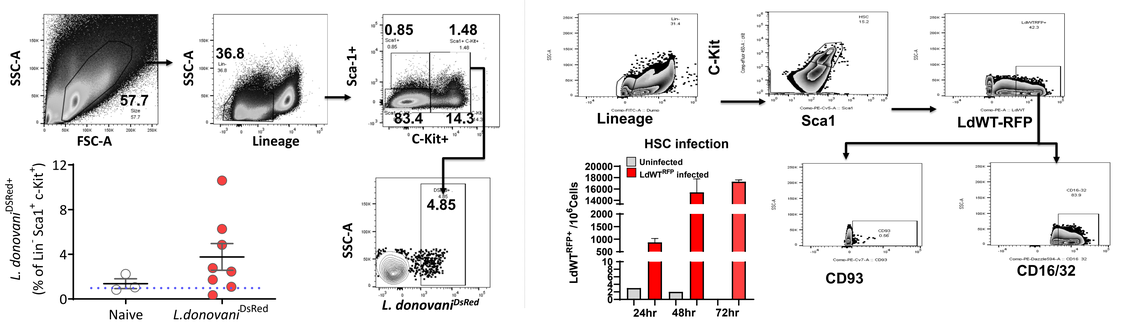
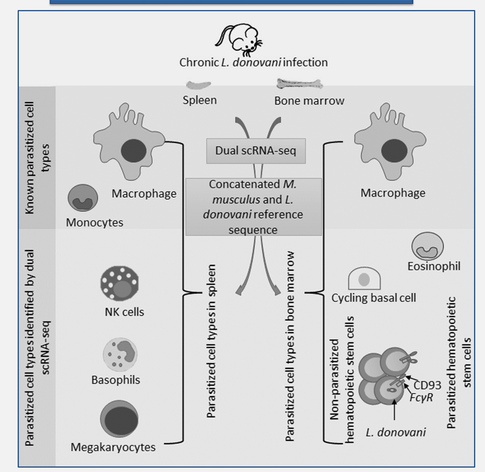


Fig. 3. Parasitized HSCs were identified *in vivo* (left) using fluorescent *L. donovani*^{DsRed} as Lin- Sca1+ C-Kit+ DsRed+ cells. Infected cells *in vitro* (right) show an increased expression of the phagocytic receptors CD16/32 and CD93.

GRAPHICAL SUMMARY



CONCLUSIONS

HSCs can become parasitized and serve as hosts for *L. donovani* during chronic infection, but also other unexpected cells, which were only identified without *a priori* knowledge using a high sensitivity method such as dual-scRNAseq.

Detection of latent infection is important for understanding of pathogenesis and screening of blood donors that may be asymptomatic.