Identifying neutrophils in immunocompetent mice infected with *Mycobacterium abscessus*

Sanchez-Hidalgo A[1], De La Cuadra L[2].

Cell and molecular biology program[1], Clinical sciences department[2]. Colorado State University

INTRODUCTION

*Mycobacterium abscessus (*Mabs), a bacteria prevalent in the environment targets patients with weakened immune system or preexisting lung conditions such as cystic fibrosis or chronic obstructive pulmonary disease. Regardless of the therapy, Mabs can develop high levels of resistance to antibiotics, preventing a complete clearance of the infection.

Macrophages are crucial reservoir of Mabs; however, recent investigations have highlighted the critical role of neutrophils in this infection. These cells play a key role as the first line of defense against pathogens in the respiratory tract and are predominant in the bronchial lumen. The role of neutrophils on NTM infection, however, is still controversial. While neutrophils could restrict the growth of NTM, they are also associated with the aggravation of clinical symptoms, such as bronchitis to bronchiectasis.

The goal of this mini-project is to identify neutrophil populations in uninfected and M.abcessus-infected C57BL/6 mice by using flow-cytometry, while learning to use the Aurora Cytex flow cytometer and FlowJo software for data analysis.

OBJECTIVES

1. Identify neutrophil populations in the lungs of C57BL/6 mice under uninfected and *Mycobacterium abscessus*-infected conditions using flow cytometry.
2. Compare neutrophil and macrophage profiles between infected and uninfected mice to assess immune cell recruitment and activation during *M. abscessus* infection.

EXPERIMENTAL STRATEGY

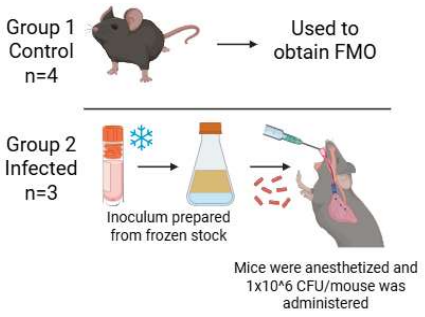


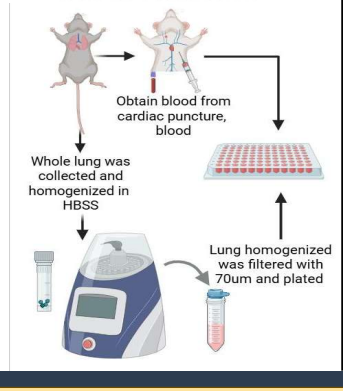
Figure 1. Mice were humanely euthanized following approved ethical guidelines

ACKNOWLEDGEMENTS

1. Gonzalez-Juarrero Lab for kindly provide all the supplies and lab space to perform these experiments.
2. Anne Chenchar for providing some of the mice used on these experiments
3. All members of the MIP730 class for their feedback

METHODOLOGY

SAMPLE COLLECTION



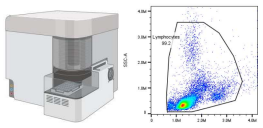
STAINING PROTOCOL

Zombie NIR (Near InfraRed)

FC Block. reduce nonspecific binding of Abs to Fc receptors on cells.

MARKER	FLUOROPHORE	CLONE
MertK	BV711	Rat
CD11C	BV605	Hamster IgG
Ly-6C	PE/Dazzle 594	Rat IgG2c,k
CD45	Spark Blue 550	Rat IgG2b, k
Ly-6G	APC/fire 750	Rat IgG2a, k

READING



Cytex *aurora* 4 lasers:
V, B,Y/G, R
Compensation beads
Biolegend 424601

ANALYSIS

FLOWJO Version10.4

GATING STRATEGY

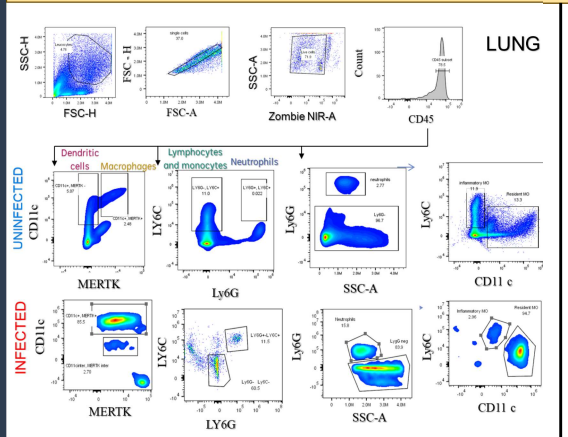


Figure 2. Gating strategy of CD11*c*, Mertk, Ly6C and Ly6G performed for lung samples

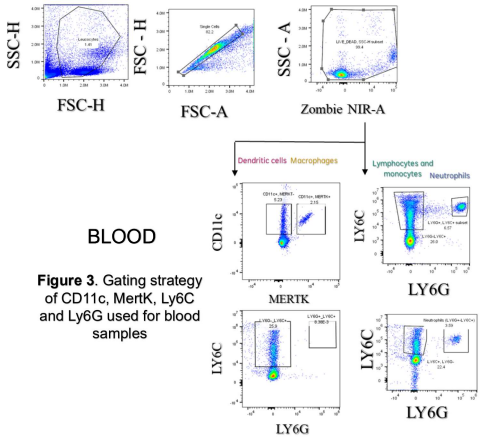


Figure 3. Gating strategy of CD11c, MertK, Ly6C and Ly6G used for blood samples

CONCLUSIONS

The aim of this study was to understand the use of flow cytometry and the analysis of the data obtained through FlowJo, which was highly successful in highlighting the challenges of sample processing, including the considerable time required for data analysis. However, this study provided valuable insights for improving the experiment, including the addition of markers to enable a more in-depth characterization of the cell populations involved in the infection mechanism in both mouse lung and blood.

The characterization of the neutrophil population was obstructed by the extremely low presence of live cells in the sample. The first explanation could be an error on laboratory processing of the sample, a second explanation could include a depletion of neutrophils during infection, however, other studies have shown that M. abscessus infection leads to an upregulation of neutrophil migration to the lungs.

LIMITATIONS

- Small sample size significantly reduced the statistical power of the study, affecting the accuracy of the results.
- Markers selected for this experiment were insufficient to successfully characterize the intended cell populations in the samples and to optimize a flow cytometry panel, such as CD11b, CD62L for neutrophil activation and CxCR2 (neutrophil aging)
- Limited experience and exposure to this type of experiment may have influenced data quality and contributed to variability between the samples.

RESULTS

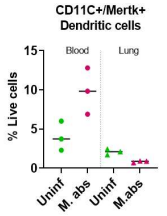


Fig 4. Left. mDendritic cells (mDC)/Alveolar Macrophages (AM) CD11c+/Mertk+

These cells are known to phagocyte apoptotic cells and prevent inflammation, contributing to immune modulation.

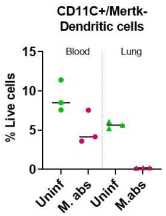


Fig 5. Right. Dendritic cells CD11c+/Mertk-

Antigen presentation, immune activation, higher in lungs of infected mice.

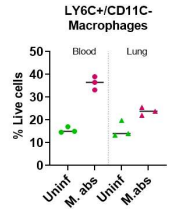


Fig 6. Left. LY6C+/CD11C- Inflammatory macrophages

High expression of Ly6C+ macrophages could be associated with activated due to inflammatory state

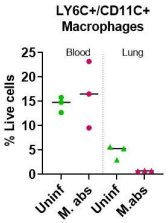


Fig 7. LY6C+/CD11C+ Resident macrophages

Decreased presence of this cells in blood which could indicate migration of macrophages likely to the lung

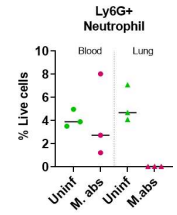


Fig LY6G+ Neutrophils

First line of defense, highly reactive, known to produce Reactive oxygen Species (ROS), NETs formation. Our neutrophil isolation was low on infected animals due to lower viability

REFERENCES

Arlt E, Kindermann A, Fritsche AK, Navarrete Santos A, Kielstein H, Bazwinsky-Wutschke I. A Flow Cytometry-Based Examination of the Mouse White Blood Cell Differential in the Context of Age and Sex. Cells. 2024 Sep 20;13(18):1583. doi: 10.3390/cells13181583. PMID: 39329764; PMCID: PMC11430320.

Baumann, Z., Wiethe, C., Vecchi, C. M., Richina, V., Lopes, T., & Bentires-Alj, M. (2024). Optimized full-spectrum *flow cytometry* panel for deep immunophenotyping of murine lungs. *Cell Reports Methods*, *4*(11).

Some images were created with Biorender.