Characterizing the Effect of the Extravascular Environment on *Trypanosoma brucei* Antigenic Diversity

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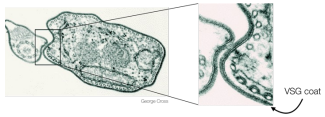
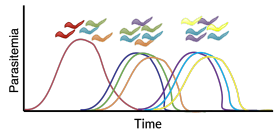
BACKGROUNDS

***Trypanosoma brucei (T. brucei)* is a single-celled parasite causing African Sleeping Sickness (HAT)**

Antigenic Variation: *T. brucei* Evades Immune Detection by Switching Variant Surface Glycoprotein(VSG) Coat



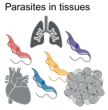
T. brucei is entirely **extracellular** and covered in an **immunodominant VSG coat**, with each parasite expressing a single VSG, making it the primary immune target.

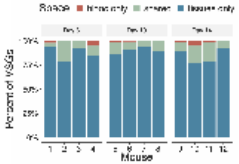


T. brucei **periodically switches** its VSG coat, allowing itself to evade immune detection and effectively avoid host immune responses, thus ensuring its persistent survival within the host space.

Extravascular Spaces: Primary Reservoir of Antigenic Diversity

Recent studies suggest that **tissues, rather than the bloodstream, are the primary sites of antigenic variation**, challenging the traditional focus on parasites in the bloodstream.





More than 85% of VSGs are found exclusively within extravascular spaces.

OBJECTIVES

Characterizing the Extravascular Environment

Q What triggers VSG switching and antigenic variation in the extravascular environment of *T. brucei*?

Optimize Extracellular Fluid (EF) Extraction from Infected Mice

- Minimize contamination from blood and cytoplasmic proteins.
- Enable downstream analyses such as TMT proteomics and ELISA.

METHODS

Centrifuge Down EF from Infected Mice Tissues

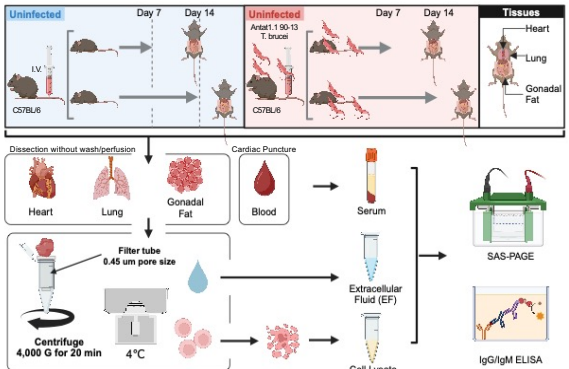


Figure 1: Methodology for Extracellular Fluid Extraction and Analysis. Tissues of interest were dissected from infected mice, and extracellular fluid was obtained by **centrifugation using cellulose acetate membrane filter tubes.**

RESULTS

Unique Protein Distribution in Extracted EF

: Protein bands resent **in serum** but absent in EF
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EF: Extracellular Fluid, **S:** Serum, **CL:** Cell Lysate

Post Infection

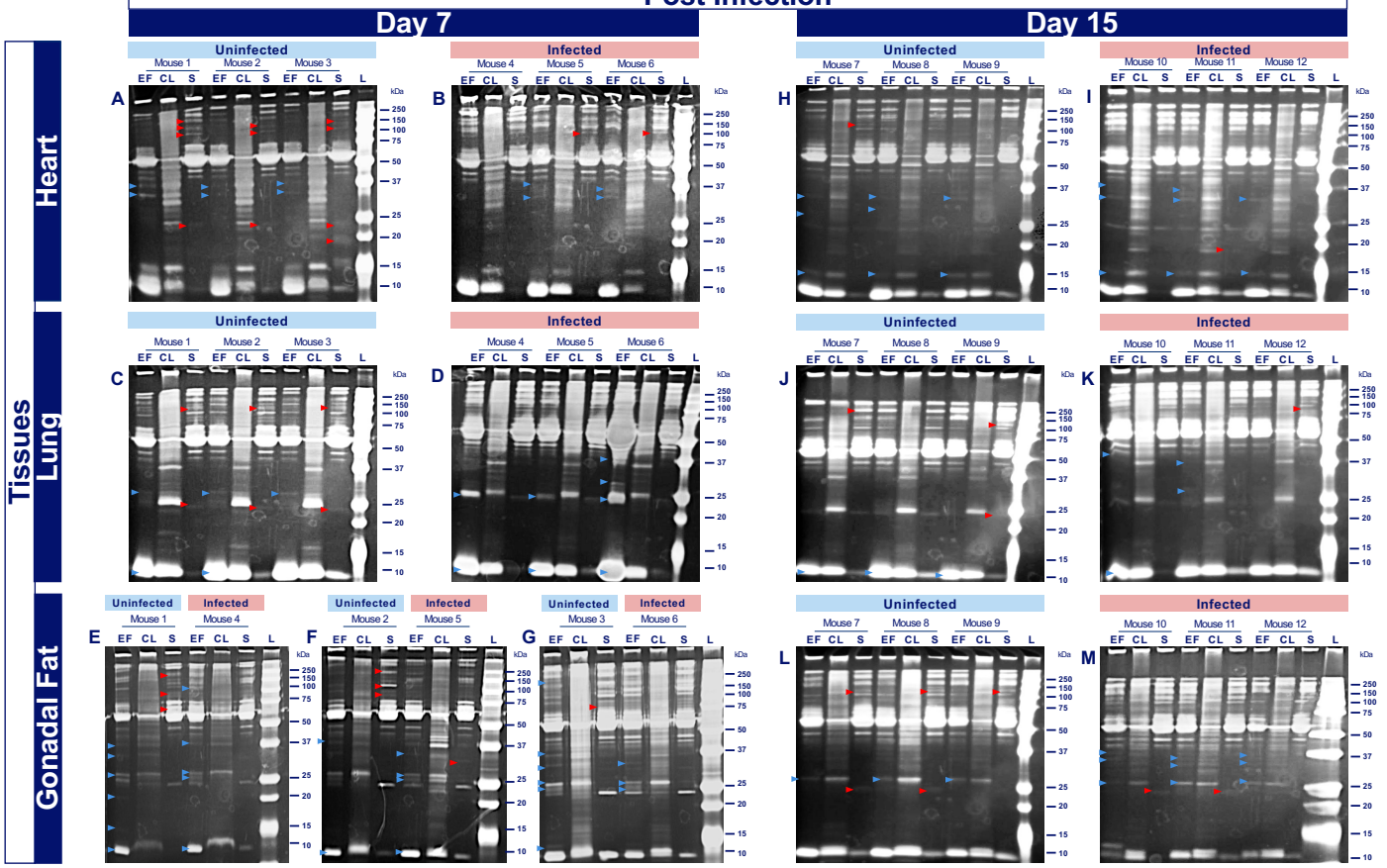

Figure 2: Silver-stained SDS-PAGE analysis of protein distribution in extracellular fluid, cell lysate, and serum. Panels A to D were loaded with 10 µg of protein per lane, and panels E to M with 5 µg per lane. The gel concentration was 12%, and electrophoresis was conducted at 110V for 100 minutes. Red arrows indicate bands present in EF but absent in serum.

- **IgG:** ~50 kDa, ~25 kDa
- **IgM:** 75 ~78 kDa, 25 kDa
- **Albumin:** ~65 kDa

Parasite Number Over Time

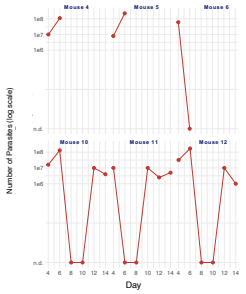

Figure 3: Parasitemia measured every other day from mouse tail blood samples.

EF Volume to Tissue Weight Ratio

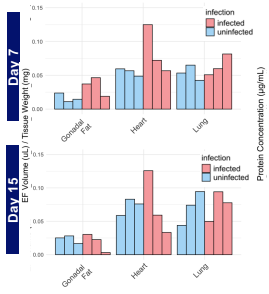

Figure 4: Amount of EF extracted via centrifugation, normalized to the weight of the dissected organ.

EF Protein Concentration

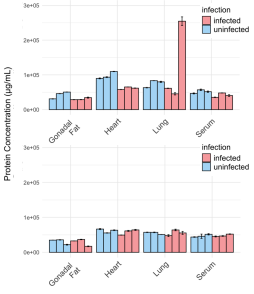

Figure 5: Protein concentration in extracted EF measured by Pierce BCA Assay.

ELISA

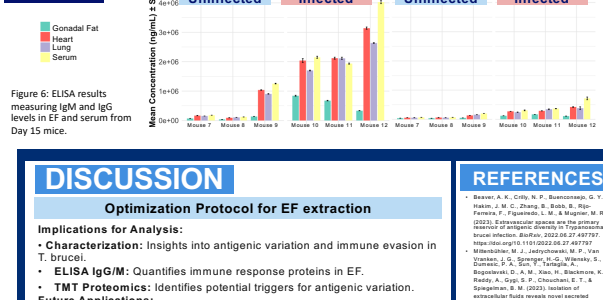

Figure 6: ELISA results measuring IgM and IgG levels in EF and serum from Day 15 mice.

DISCUSSION

Optimization Protocol for EF extraction

Implications for Analysis:

- **Characterization:** Insights into antigenic variation and immune evasion in T. brucei.
- **ELISA IgG/M:** Quantifies immune response proteins in EF.
- **TMT Proteomics:** Identifies potential triggers for antigenic variation.

Future Applications:

- Applicable to other diseases involving extracellular pathogen interactions.

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