Mugnier Lab
Bloomberg School of Public Health
Johns Hopkins University
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Characterizing the Effect of the Extravascular Environment on *Trypanosoma brucei* Antigenic Diversity

Fuminori Tanizawa

Trypanosoma brucei is a protozoan parasite responsible for human and animal African trypanosomiasis, diseases that pose significant economic and public health challenges in sub-Saharan Africa. This extracellular parasite evades the host's immune system through a sophisticated mechanism of antigenic variation, periodically "switching" its dense variant surface glycoprotein (VSG) coat. This switching renders previously generated anti-VSG antibodies ineffective, allowing the parasite to persist within the host.

While traditional research has focused on parasites in the bloodstream, recent studies indicate that the primary reservoirs of antigenic diversity are in the extravascular spaces—areas between cells within tissues. These findings suggest that specific features of the extravascular environment might facilitate or trigger VSG switching. Understanding these potential triggers is therefore crucial for gaining insights into the parasite's immune evasion and persistence mechanisms.

To investigate potential triggers for antigenic variation in extravascular spaces, we aimed to characterize the extracellular fluid (EF) extracted from infected mice. In our study, we optimized the method to extract EF for downstream analyses. We dissected infected mice and collected tissues, including adipose tissue, heart, and lung, at two-time points: day 7 and day 15. The tissues were centrifuged on a cellulose acetate membrane filter at various speeds to extract EF while minimizing contamination from cytoplasmic proteins released by tissue damage. Additionally, we tested different techniques, such as washing and perfusion, to identify the optimal method for obtaining EF without blood contamination. After optimizing the protocol, SDS-PAGE confirmed that the EF contained distinct protein components compared to cell lysate and serum.

Our results demonstrated that specific centrifugation speeds and dissection techniques effectively minimized contamination, providing a reliable EF extraction method. This optimized protocol enables accurate and standardized characterization of EF, potentially advancing our understanding of the extravascular environment's role in *Trypanosoma brucei* infection and potentially other diseases. Further analysis, such as TMT proteomics to identify the protein components of EF and ELISA to quantify the amount of antibodies exerting pressure on parasites, can contribute to investigating the mechanism in antigenic variation of parasites. This method potentially aids in identifying novel therapeutic targets and understanding pathogen-host interactions, ultimately contributing to the development of new treatments for various infections and diseases.