Characterisation of the chronic infection-driving trypanosomes in cattle through single cell transcriptomics

Authors

E Briggs[1*]; S Larcombe[1*]; E Paxton[2]; KR Matthews[1]; L Morrison[2];
[1] Institute of Immunology & Infection Research, University of Edinburgh, UK; [2] The Roslin Institute, University of Edinburgh, UK

Abstract

African trypanosomes cause livestock and human disease across sub-Saharan Africa. Although Human African Trypanosomiasis is on track for elimination, Animal African Trypanosomiases (AAT) remains a significant economic burden that causes ~US$4.5 billion losses in annual GDP due to the associated wasting disease, as well as significant animal suffering. AAT in cattle, Nagana, results in 3 million livestock deaths alone each year.

Most trypanosome research has focused on laboratory-adapted *Trypanosoma brucei* 'monomorphic' lines that proliferate indefinitely in culture and generate unrealistic acute infections. Recently there has been increased focus on 'pleomorphic' parasites more relevant to field infections, which exhibit quorum sensing whereby 'slender forms' develop to arrested 'stumpy forms' as parasite density increases. Stumpy forms are pre-adapted for tsetse fly vector transmission. Recently, we have found that in *Trypanosoma brucei* chronic stage infections of mice (several weeks) when parasitaemia in the blood remains high, the overwhelming majority of parasites in the bloodstream are non-proliferating forms pre-adapted for transmission to the tsetse. Additionally, 'intermediate' forms that have undergone cell cycle arrest and express stumpy-associated transcripts, but had not undergone full morphological change associated with stumpy forms, were evident in chronic infection. These forms may be key to understanding how trypanosomes establish and maintain chronic infections.

In cattle chronic infections are the norm, but at tens of magnitude lower blood parasitaemia than that observed in mouse models. Thus, cattle infections are an essential foundation for exploring the roles of slender, stumpy and 'indeterminate' forms in chronicity and providing insight into the infection status dominant in the field. Two calves were infected with pleomorphic *T. brucei* and parasitaemia was followed over 60 days. Single cell transcriptomics was performed at four discrete time points from early, mid and late infections, together capturing the transcriptomes of 37,602 individual parasites. Coupled with microscopy, we find populations of parasites that are highly similar to both slender and stumpy forms to be prevalent in the blood throughout infection. Although stumpy-like forms isolated from cattle do not have the classical stumpy morphology frequently observed in mouse models, we find these forms have transcriptomic profiles most similar to mature stumpy forms. Dividing parasites were infrequently observed through-out infection, consistent with lower blood parasitaemia. Finally, comparison of in vitro generated slender and stumpy forms with both mouse and cattle derived *T. brucei* revealed host-specific transcriptomic changes.