OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Proteomic Selection of Immunodiagnostic Antigens for *Trypanosoma congolense*

Jennifer R. Fleming[1☯], Lalitha Sastry[1☯], Thomas W. M. Crozier[1], Grant B. Napier[2], Lauren Sullivan[1], Michael A. J. Ferguson[1]*

1 Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee, United Kingdom, **2** Global Alliance for Livestock Veterinary Medicines, Doherty Building, Pentlands Science Park, Edinburgh, United Kingdom

Abstract

Animal African Trypanosomosis (AAT) presents a severe problem for agricultural development in sub-Saharan Africa. It is caused by several trypanosome species and current means of diagnosis are expensive and impractical for field use. Our aim was to discover antigens for the detection of antibodies to *Trypanosoma congolense*, one of the main causative agents of AAT. We took a proteomic approach to identify potential immunodiagnostic parasite protein antigens. One hundred and thirteen proteins were identified which were selectively recognized by infected cattle sera. These were assessed for likelihood of recombinant protein expression in *E. coli* and fifteen were successfully expressed and assessed for their immunodiagnostic potential by ELISA using pooled pre- and post-infection cattle sera. Three proteins, members of the invariant surface glycoprotein (ISG) family, performed favorably and were then assessed using individual cattle sera. One antigen, Tc38630, evaluated blind with 77 randomized cattle sera in an ELISA assay gave sensitivity and specificity performances of 87.2% and 97.4%, respectively. Cattle immunoreactivity to this antigen diminished significantly following drug-cure, a feature helpful for monitoring the efficacy of drug treatment.

Citation: Fleming JR, Sastry L, Crozier TWM, Napier GB, Sullivan L, et al. (2014) Proteomic Selection of Immunodiagnostic Antigens for *Trypanosoma congolense*. PLoS Negl Trop Dis 8(6): e2936. doi:10.1371/journal.pntd.0002936

Editor: Armando Jardim, McGill university, Canada

Received January 5, 2014; **Accepted** April 28, 2014; **Published** June 12, 2014

Funding: This work was supported by the Global Alliance for Livestock Veterinary Medicine (GALVmed) with funding from the UK Government's Department for International Development (DFID) as part of GALVmed's Animal African Trypanosomosis Programme (DFID Programme: Controlling African Animal TRYPANOSOMOSIS (AAT) (Aries code 202040-101). LS was supported by an MRC Centenary Award, TWMC by a Wellcome Trust PhD studentship and MAJF by a Wellcome Trust Senior Investigator Award (WT101842IAI). The proteomics and computation facilities used in this work are supported by Wellcome Trust strategic awards 100476 and 097945. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: m.a.j.ferguson@dundee.ac.uk

☯ These authors contributed equally to this work.

Introduction

Animal African Trypanosomosis (AAT) presents a severe problem for agricultural development in sub-Saharan Africa. The economic loss in cattle production is estimated to be between US$ 1 billion per annum [1–3], increasing to US$ 5 billion when secondary costs are taken into consideration [1,3,4]. It is estimated that around 60 million cattle are at risk in endemic regions [1,4]. In addition, AAT affects many other domestic livestock such as pigs, camels, goats, sheep and horses. There are no vaccines and treatment is usually via intramuscular administration using trypanocides, either diminazene (therapeutically) or isometamidium (prophylactically). Current diagnostics methods are laborious (microscopy), expensive (PCR) and generally impractical for use in the field, at the point of treatment.

AAT is caused by several species of protozoan parasites of the genus trypanosoma, including *T. congolense*, *T. brucei*, *T. vivax* and *T. evansi*. [1]. *T. congolense* is the most pathogenic and main causative agent of cattle AAT and is transmitted in Africa by tsetse flies of the genus Glossina [1,5]. Symptoms of *T. congolense* AAT include anaemia, weakness, weight loss and, in most untreated cases, death [5,6]. These symptoms are often used to clinically diagnose AAT, although they are congruent with many other anaemia causing diseases prevalent in the endemic regions including babesiosis, anaplasmosis, hemonchosis, and theileriosis. Wrong diagnosis is costly and counter productive to efficient treatment.

Currently, specific diagnostics rely on microscopic detection of the parasites, the laboratory detection of specific antibodies or the detection of parasite DNA by PCR [5,6]. A card agglutination test (CATT), such as that used to detect human African trypanosomosis in the field, is available for *T. evansi* infections [5] but is not applicable to *T. congolense* and *T. vivax* infections. Diagnosis in the field for these pathogens currently relies on whole cell lysate tests that suffer from antigen instability, reproducibility and specificity problems [5].

With this in mind, we set out to discover new diagnostic antigens for *T. congolense* that might be compatible with ELISA assays and subsequent development into lateral flow devices.

In order to identify antigens for recombinant production and test development, we utilised a proteomic approach similar to that recently used to identify invariant surface glycoprotein (ISG) 65 as a potential diagnostic antigen for human African trypanosomosis [7]. Briefly, this method involves loading identical amounts of parasite whole-cell detergent lysates onto identical amounts of immobilized IgG isolated from the same animals before and after experimental infection and then comparing, by label-free quantitative proteomics, the proteins subsequently eluted from the immobilized IgGs and selecting those >100-fold more highly