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Altering the motility of *Trypanosoma cruzi* with rabbit polyclonal anti-peptide antibodies reduces infection to susceptible mammalian cells

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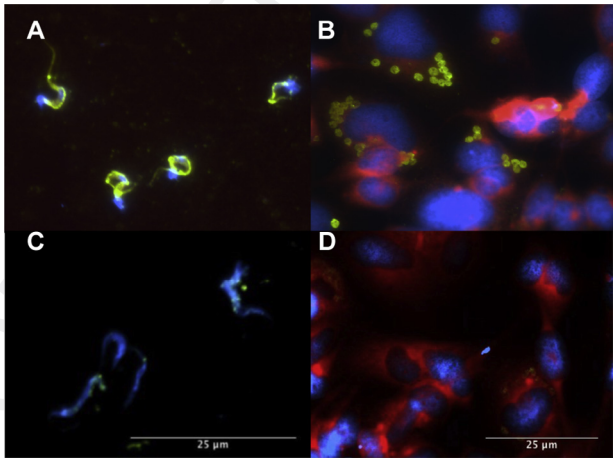
HIGHLIGHTS

- The immunogenicity of a peptide derived from *Trypanosoma cruzi* was increased.
- Polyclonal anti-peptide antibodies recognize live *T. cruzi* forms.
- Anti-TcTLE peptide antibodies reduce *T. cruzi* infection to human astrocytes.
- An imaging system tracks the parasite's flagellum and estimates their reduction speed due to antibodies
- Reducing trypomastigote motility with anti-*T. cruzi* polyclonal antibodies.

GRAPHICAL ABSTRACT



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ABSTRACT

Trypanosoma cruzi's trypomastigotes are highly active and their incessant motility seems to be important for mammalian host cell infection. The kinetoplastid membrane protein-11 (KMP-11) is a proteinexpressed in all parasite stages, which induces a cellular and humoral immune response in the infected host, and is hypothesized to participate in the parasite's motility. An N-terminal peptide from KMP-11, termed K1 or TcTLE, induced polyclonal antibodies that inhibit parasitic invasion of Vero cells.

Abbreviations: CFSE, carboxyfluorescein diacetate succinimidyl ester; FIS, FISEAIIHVLHSR peptide; KMP-11, kinetoplastid membrane protein 11; TcTLE, *Trypanosoma cruzi* TLEEFSAKL KMP-11 derived peptide (formerly K1).

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The goalof this study was to evaluate the motility and infectivity of *T. cruzi* when exposed to polyclonal anti-TcTLE antibodies. Rabbits were immunized with TcTLE peptide along with FIS peptide as an immunomodulator. ELISA assay results showed that post-immunization sera contained high titers of polyclonal anti-TcTLE antibodies, which were also reactive against the native KMP-11 protein and live parasites as detected by immunofluorescence and flow cytometry assays. Trypomastigotes of *T. cruzi* were incubated with pre- or post-immunization sera, and infectivity to human astrocytes was assessed by Giemsa staining/light microscope and flow cytometry using carboxyfluorescein diacetate succinimidyl ester (CFSE) labeled parasites. *T. cruzi* infection in astrocytes decreased approximately by 30% upon incubation with post-immunization sera compared with pre-immunization sera. Furthermore, trypomastigotes were recorded by video microscopy and the parasite's flagellar speed was calculated by tracking the flagella. Trypomastigotes exposed to post-immunization sera had qualitative alterations in motility and significantly slower flagella (45.5 μm/s), compared with those exposed to pre-immunization sera (69.2 μm/s). In summary, polyclonal anti-TcTLE serum significantly reduced the parasite's flagellar speed and cell infectivity. These findings support that KMP-11 could be important for parasite motility, and that by targeting its N-terminal peptide infectivity can be reduced.

1. Introduction

With eight to nine million people chronically infected with *Trypanosoma cruzi*, Chagas disease remains a public health challenge for countries along Latin America (Hotez et al., 2008). The acute phase of the disease, characterized by a high parasitemia, is largely undiagnosed due to the presence of constitutional symptoms. Although controlled by a competent immune system, parasites are not completely eliminated and their persistence induces a symptomatic chronic disease in nearly 30% of infected individuals. Cardiomyopathy and gastrointestinal enlargement are the two main chronic clinical outcomes (Rassi et al., 2012). Anti-parasitic therapy is used during the acute phase of the disease, but treatment during the chronic phase is largely supportive (Perez-Molina et al., 2009).

Host-cell infection by *Trypanosoma cruzi* is a complex process involving molecular and kinetic factors from both the host cell and the parasite. Many parasitic proteins are involved in the interaction with host cells (de Souza et al., 2010), among them trans-sialidases (TS) are important for parasite attachment to the cell membrane (Buschiazzo et al., 2012). *In vitro* cellular infection models have shown that parasite attachment is followed by endocytosis and formation of a parasitophorous vacuole, which interacts with organelles of the endosomal–lysosomal pathway, from where the parasite actively escapes to the cellular cytoplasm (Ferreira et al., 2006; de Souza et al., 2010; Woolsey and Burleigh, 2004). Endocytosis of the parasite seems to depend on intracellular calcium influx on the host cell, probably through an undetermined parasite-derived calcium agonist (Rodriguez et al., 1995).

The parasite's intense flagellum beating seems to favor a close contact with the cell targeted for infection. Indeed, inhibition of ATP synthesis and metabolic starvation of the parasite significantly reduced the infection on susceptible cells (Martins et al., 2009; Schenkman et al., 1991). After the parasite's apposition and attachment, cytoskeletal reorganization of the host cell is important for *T. cruzi* endocytosis in both phagocytic and non-phagocytic cells, as demonstrated by the inhibition of actin polymerization with cytochalasin D (Ferreira et al., 2006). Other internalization mechanisms such as caveolin-dependent endocytosis, also participate in *T. cruzi* infection (Barrias et al., 2013; Romano et al., 2012).

In *Trypanosoma brucei,* the agent of African trypanosomiasis, antigenic variation and the incessant motility allow the parasites to evade the host immune responses, enabling them to survive in a harsh environment such as the vertebrate's bloodstream (Heddergott et al., 2012). Indeed, the movement of *T. brucei* trypomastigote's in a viscous fluid (such as blood) generates a hydrodynamic drag force, which displaces surface-bound antibodies along the plasma membrane toward the flagellar pocket of the parasite where the antibodies are removed by endocytosis (Engstler et al., 2007). Although such motility-dependent mechanisms have not been described in *T. cruzi*, this parasite also displays a similarly fast motion.

The kinetoplastid membrane protein-11 (KMP-11) is a highly conserved protein of *T. cruzi* expressed during all stages of the parasite's life cycle. Although its functional role remains elusive, it is postulated that KMP-11 participates in motility due to its close association with the microtubular structure of the parasite's cytoskeleton (Thomas et al., 2000). KMP-11 induces humoral and cellular immune responses in animal infection models and humans (Cuellar et al., 2009; Thomas et al., 2001). The TcTLE peptide (TLEEFSAKL), also known as K1, is located in the N-terminal region of KMP-11 and has been identified as a CD8+ T cell epitope-restricted to HLA*0201 (Diez et al., 2006). The TcTLE peptide and KMP-11 protein can be recognized by human CD8+ T cells and also by antibodies from chronic chagasic patients (Diez et al., 2007; Flechas et al., 2009; Lasso et al., 2010).

We have previously shown that immunization of rabbits with the TcTLE peptide produces polyclonal antibodies that recognize live forms of *T. cruzi* and reduce parasite invasion in Vero cells (Diaz-Soto et al., 2012). However, the low antibody titers obtained prevented further assessment of their potential functional properties. Here, we successfully increased the anti-TcTLE antibody titers by co-immunizing with the FIS peptide, which is a 13-mer peptide derived from sperm-whale myoglobin that helps to increase the immunogenicity of small peptides (Prieto et al., 1995). Trypomastigotes were then exposed to rabbit polyclonal anti-TcTLE antibodies, and their motility as well as capacity to invade susceptible human astrocyte cells was evaluated.

2. Materials and methods

2.1. Peptides and recombinant protein

TcTLE (TLEEFSAKL) and FIS (FISEAIIHVLHSR) peptides were obtained by solid phase synthesis (SPS) using the Fmoc technique (Atherton et al., 1981) and purified by high performance liquid chromatography (HLPC). Cloning and purification of recombinant KMP-11 was carried out as described previously (Thomas et al., 2001).

2.2. Rabbit immunization and control sera

Three male New Zealand 2 month-old white rabbits were immunized three or four consecutive times every 10 days with 50 μg with TcTLE plus FIS peptides (2 animals) or FIS alone (1 animal), along with a 1 mL of 1:1 mix of complete and incomplete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO, USA). The rabbits were maintained with food and water provisions *ad libitum*, and during manipulation they were sedated with 2 mg/Kg IM of ketamine

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