Adhesion of *Trypanosoma evansi* to Red Blood Cells (RBCs): Implications in the Pathogenesis of Anaemia and Evasion of Immune System

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Received date: January 23, 2017; **Accepted date:** February 06, 2017; **Published date:** February 11, 2017

Abstract

Background: *Trypanosoma evansi* is the etiological agent of trypanosomosis, a disease of domestic (horses, cattle and goats) and wild (capybara, vampires) animals characterized by anaemia, degeneration, necrosis and inflammatory processes. This disease is of great concern because it produces growth retardation, loss of body weight, low production of animal proteins and diminished fertility and traction power. Because anaemia is considered the most characteristic symptom, the aim of this study was to assess the effects that *T. evansi* adhesion to erythrocytes has on their morphology, surface oligosaccharides profiles and trypanosome surface antigens.

Methods: Blood and tissues samples from mice experimentally infected, were studied using scanning (SEM) and transmission (TEM) electron microscopes and lectin's histochemistry (sConA, sWGA, PNA, LFA, etc.). Furthermore immunoprecipitation of *T. evansi* radio iodinated surface antigens with specific anti-hosts and anti-trypanosome sera was performed and antigens analysed by electrophoresis and autoradiography.

Results: *T. evansi* adhesion to erythrocytes as well as changes in the morphology of them, were frequent findings. SEM studies showed that adhesion of bloodstream trypomastigotes of *T. evansi* to mature erythrocytes and reticulocytes occurred through its flagellum, undulating membrane or cellular body and were mediated by filopodia. Minute pores and filamentous material were sometimes observed on erythrocytes membrane at the point of adhesion to the trypanosome. Oligosaccharides changes of RBCs glycocalix were characterized by a marked decrease in Man/Glc, terminal GalNAc and terminal Neu5Ac labelling accompanied by an increase in labelling of terminal GlcNAc. There were not labelling changes with the other lectins assayed. Preliminary immunoprecipitation studies of *T. evansi* radio iodinated surface antigens with anti-host sera, showed the presence of a of 45 kDa antigen from erythrocyte and host plasma on [illegible] *evansi*uscts: T.¡evansM

Glucose; GlcNAc: N-Acetyl Glucosamine; α- and β-GalNAc: α- and β-N-Acetyl Galactosamine; D-Gal: D-Galactose; D-Fuc: D-Fructose; Neu5Ac: N-Acetylneuraminic Acid; GalNAc: N-Acetyl Galactosamine; RαE: Rabbit Anti-Mouse Erythrocyte Membrane Serum; RαP: Rabbit Anti-Mouse Plasma Serum; RαT: Rabbit Anti-*T. evansi* serum; MαT: Mouse Anti- *T. evansi* serum; NR: Normal Rabbit Serum; NM: Normal Mouse Serum; DEAE-Cellulose: Diethyl-Amino-Ethyl Cellulose; KRT: Krebs Ringer Tris Bufer; PMSF: Phenyl Methyl Sulphonyl Fluoride; SDS-PAGE: Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis; CPM: Counts Per Minute; $^{125}\alpha$

mice were evaluated daily from tail vein blood by Brenner method [14]. T e deep circulation blood to be used in SEM studies was obtained by cardiac puncture using EDTA as anticoagulant. Samples of liver, spleen and adrenal glands to be studied by TEM were obtained from anesthetized animals. All the samples used were obtained at terminal stage of infection (parasitemia=10^9

Preparation of Plasma Membrane Fractions from *T. evansi* and Studies of Surface Antigens Composition

Changes in composition of *T. evansi* surface antigens during experimental infections of mice were determined by preparation of a radio iodinated cell membrane fraction, and inmunoprecipitation of *T. evansi* surface antigens with specifc antisera and Protein A-agarose. Radio-iodination of surface proteins with ^{125}I was performed with 2×10^8 intact blood trypomastigotes obtained by DEAE-cellulose chromatography, using Iodogen method [21]. Plasma membrane fractions from bloodstream trypomastigotes (2×10^8) were obtained afer 1 hour lysis at 4ºC in Krebs Ringer Tris (KRT) pH 7.4 containing 1% Triton X-100, 1 mM phenyl-methyl-sulphonyl-fuoride (PMSF) and 1 mM EDTA (ethylene diamine tetraacetic acid) followed by centrifugation for 30 minutes at 38,000 x g. Immunoprecipitation with specifc antisera was performed overnight at 4ºC by mixing 50 µl of radio iodinated fractions with 40 µl of specifc antisera in 10 µl of 50

type connection (Figure 3) seems to be favoured by flopodia in the vicinity of the contact area, whose emission begins with formation of small bubbles on the RBC surface.

Figure 4: Emission of flopodia during *T. evansi*-RBC adhesion. Panel A: High magnifcation scanning micrograph showing the formation of RBC flopodia (→←) on its surface in the intimate area of *T. evansi* (◊) adhesion to RBC (○). Notice the apparent fusion of membrane (*) from *T. evansi* fagellar-pocket ( ); (•) fagellum; Bar: 720 nm. Panel B: Filopodia mediated adhesion of *T. evansi* (◊) to an abnormal RBC (○). Notice the binding of a flopodium emitted from RBC with another one from undulating membrane of *T. evansi* (→←); (*) Wartened surface; Flagellum (•); Bar: 5 µm.

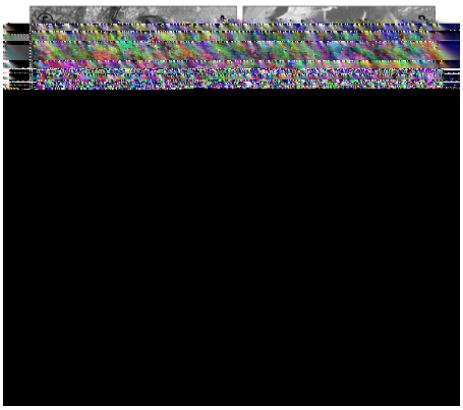

Figure 5: Transmission Electron Micrographs of *T. evansi* interactions with RBCs. Panel A: Interaction of a trypomastigote (◊) with RBCs (*) in a blood vessel of adrenal gland. Notice the presence of an electron dense material at trypanosome-RBC limits (○); (•) Flagellum; (Δ) Mitochondrion, ( ) Cell nucleus with nucleolus; (□) Cortical cell; Bar: 0.70 µm. Panels B, C and D: Freeze-fracture replicas of *T. evansi* adhesion to RBCs. Panel B and C:

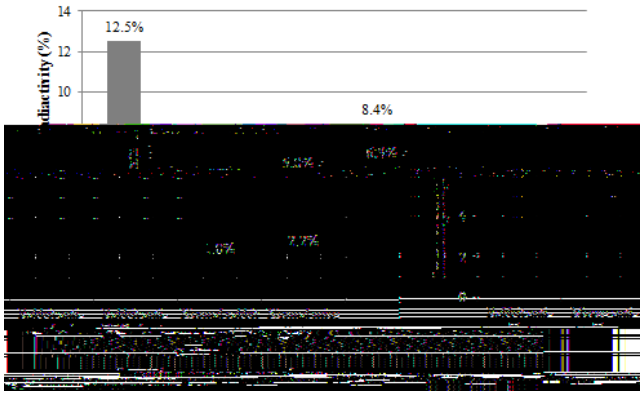

Figure 8: Percentage of immunoprecipitated radioactivity by different anti- host, anti-*T. evansi* and normal sera against ^{125}I-labelled surface antigens of *T. evansi*.

implies removal of this saccharide residues from erythrocyte glycocalix

macrophages and Kupffer cells from hepatic sinusoids, leading to the abnormal extravascular erythrophagocytosis observed in this work and responsible for the subsequent anemia developed [47,48].

Detection of a 45 kDa polypeptide from RBC and host plasma in plasma membrane fraction of *T. evansi* provides antigenic evidences about the occurrence of an unusual mechanism of immune evasion in Africa trypanosomes. The mechanisms that trypanosomes of brucei-group have developed to escape immune surveillance are currently under investigation in many laboratories, because their elucidation may provide new approaches to the prevention of the diseases that parasites cause [49] . One possible way of evasion supported by results herein presented is the uptake of host antigen by trypanosomes. This phenomenon of masking of *T. evansi* surface antigens by host proteins has been extensively studied and documented in schistosomiasis. In this regard, the acquisition of mouse antigens, human A, B, and H blood group antigens, glycolipids from erythrocytes, serum factors and mouse histocompatibility antigens have been demonstrated in *Schistosoma mansoni* and offer an effective mechanism for antigenic mimicry of host and subsequent escape from the immune response [50,51].

This mechanism would allow *T. evansi* to evade the immune system by a different mechanism than the antigenic variation previously described by Perrone et al. [52] for Venezuelan isolates as well as for *T. evansi* and other African trypanosomes [49,53]. Masking of host antigens, such as immunoglobulins has been described for bloodstream forms of many trypanosomes such as *T. congolense*, *T. brucei gambiense, T. brucei rhodesiense, T. cruzi*, *T. musculi* and *T. lewisi*. Other host proteins, such as albumin, have also been detected on surface of protozoa and some trypanosomes species. In this respect it has been suggested that surface immunoglobulins are responsible for the persistence of *T. lewisi* in the bloodstream of rats.

The acquisition of host antigens by *T. evansi* could be achieved by absorption from host plasma but also during *T. evansi*-RBC surface interactions and adhesion. In this regard, freeze-fracture replicas results showed areas in which trypanosome-RBC contacts appear as continuity regions between the plasma membrane of *T. evansi* and RBC. A similar feature of the adhesion phenomenon has also been described during cytoadherence of *Trichomonas vaginalis* to epithe

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