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Influence of the endosymbiont of *Blastocrithidia culicis* and *Crithidia deanei* on the glycoconjugate expression and on *Aedes aegypti* interaction

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Abstract

Blastocrithidia culicis and *Crithidia deanei* are trypanosomatid protozoa of insects that normally contain intracellular symbiotic bacteria. The protozoa can be rid of their endosymbionts by antibiotics, producing a cured cell line. Here, we analyzed the glycoconjugate profiles of endosymbiont-harboring and cured strains of *B. culicis* and *C. deanei* by Western blotting and flow cytometry analyses using lectins that recognize specifically sialic acid and mannose-like residues. The absence of the endosymbiont increased the intensity of the lectins binding on both trypanosomatids. In addition, wild and cured strain-specific glycoconjugate bands were identified. The role of the surface saccharide residues on the interaction with explanted guts from *Aedes aegypti* gut was assessed. The aposymbiotic strains of *B. culicis* and *C. deanei* presented interaction rates 3.3- and 2.3-fold lower with the insect gut, respectively, when compared with the endosymbiont-bearing strains. The interaction rate of sialidase-treated cells of the wild and cured strains of *B. culicis* and *C. deanei* was reduced in at least 90% in relation to the control. The interaction of *B. culicis* (wild strain) with explanted guts was inhibited in the presence of mucin (56%), fetuin (62%), sialyllactose (64%) and α-methyl-D-mannoside (80%), while in *C. deanei* (wild strain) the inhibition was 53%, 56%, 79% and 34%, respectively. Collectively, our results suggest a possible involvement of sialomolecules and mannose-rich glycoconjugates in the interaction between insect trypanosomatids and the invertebrate host.

Keywords: *Crithidia*; *Blastocrithidia*; Sialic acid; Glycoconjugates; Cellular interaction; Endosymbiont; Lectin; *Aedes aegypti*

1. Introduction

Blastocrithidia culicis and *Crithidia deanei* are parasitic trypanosomatid protozoa of insects that normally contain intracellular symbiotic bacteria. Insect trypanosomatids have been used as laboratory models for biochemical and molecular studies because they are easily cultured under axenic conditions [1] and contain homologues of virulence factors of pathogenic trypanosomatids [2,3]. Recently, trypanosomatids not normally infectious to humans were isolated from immunosuppressed patients causing either visceral and/or cutaneous

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leishmaniasis-like lesions [4]. In addition, insect trypanosomatids are being studied as a model in symbiotic relationships, since some of them present bacterial endosymbionts in the cytoplasm, which divide synchronously with the host cell and are integrated into the physiology of the parasite [5].

In trypanosomatids, the possibility of elimination of the endosymbiont by antibiotic treatment (cure) has increased the interest in the study of endosymbiont-harboring species, since several bacterium–protozoan interactions can be analyzed by the comparison of endosymbiont-containing and endosymbiont-free populations of the same species [5]. The available data in the literature indicate that the presence of the endosymbiont induces morphological changes, interferes with several aspects of the metabolism of the trypanosomatid [2,3,5–7] and modulates the surface properties of the protozoan, such as exposition of carbohydrates residues [8–12].

The composition of the cell surface is of primary importance in the cellular response to environmental stimuli and, in this context, glycoconjugates are important for specific recognition between parasites and host cells, which can be mediated by the interaction of carbohydrate-binding proteins (lectin-like molecules) [13–18]. Lectins are non-catalytic proteins that bind specifically to carbohydrate residues and are widely distributed in nature. The specific reactions between these molecules have become valuable tools to study the insertion, fate, distribution and function of glycoconjugates on and in parasites [18]. Several agglutinins of plant origin have been used in these studies, such as Concanavalin A (Con A), elderberry bark lectin (*Sambucus nigra*, SNA), *Maackia amurensis* agglutinin (MAA) and the lectin isolated from *Limax flavus* (LFA) [19]. Lectin-like molecules on insect guts have also been described as responsible for recognition of parasite glycoconjugates [20].

In this work, we report the comparison of cell surface glycoconjugates from the wild and cured strains of the insect trypanosomatids *B. culicis* and *C. deanei* by flow cytometry using fluorescein isothiocyanate (FITC)-labeled lectins and Western blotting analysis with peroxidase-labeled lectins (LFA, SNA, MAA and Con A). In an effort to reveal a functional role for the cell surface glycoconjugates, we showed for the first time the possible involvement of these molecules in the adhesion process of *B. culicis* and *C. deanei* wild and cured strains to explanted guts from adult *Aedes aegypti* female mosquitoes.

2. Materials and methods

2.1. Parasites and cultivation

The endosymbiont-harboring and endosymbiont-free strains of *B. culicis*, as well as the bacterium-free strain of *C. deanei* were kindly provided by Dr. Maria Cristina M. Motta (Instituto de Biofísica Carlos Chagas Filho, UFRJ, Brazil). The endosymbiont-containing counterpart strain of *C. deanei* (ATCC 30255/CT–IOC 044) was donated by Dr. Maria Auxiliadora de Sousa (Coleção de Tripanossomatídeos, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil). The parasites were grown at 28 °C in 3.7% (w/v) brain heart infusion (BHI) medium supplemented with 2 mg% (w/v) hemin and 2% (v/v) heat-inactive fetal bovine serum (FBS).

2.2. Sialidase treatment

The trypanosomatids (1.0×10^7 cells/ml) were collected at the log growth phase and incubated for 1 h at 37 °C in the presence of sialidase from *Vibrio cholerae* (Sigma) at 0.2 U/ml in 50 mM phosphate buffer, pH 6.0 [21]. The viability of the organisms throughout the experiment was assessed by mobility, trypan blue dye exclusion and by monitoring lactate dehydrogenase (LDH), an intracellular enzyme, in the supernatant from the incubation systems [3].

2.3. Flow cytometry analysis

For flow cytometry analysis, both sialidase-treated and untreated cells were incubated in the presence of the FITC-labeled lectins LFA, SNA, MAA, Con A (EY Lab) or peanut agglutinin (PNA) from *Arachis hypogaea* (Sigma) at 125 μg/ml for 1 h at room temperature. After incubation, the parasite-associated fluorescence was excited at 488 nm and quantified on a fluorescence-activated cell sorter (FACS) FACSCalibur (BD Bioscience, USA). The specificity of the binding was checked by the incubation of the cells in the presence of each lectin (LFA, SNA, MAA, Con A and PNA) and its specific sugar inhibitor(s) purchased from Sigma (2.0 μg/ml fetuin plus 2.0 μg/ml sialyllactose, 2.0 μg/ml fetuin, 2.0 μg/ml sialyllactose, 0.2 M α-methyl-D-mannoside and 0.1 M D-galactose, respectively). At least 50,000 cells were analyzed per sample with four repeats per experiment [21,22].

2.4. Flagellate extracts and electrophoresis

Parasites (1.0×10^8 cells) were collected at the log growth phase and disrupted by sonication lysis at 4 °C following centrifugation at 5000*g* for 10 min at 4 °C. The supernatants obtained after centrifugation correspond to the parasite cellular extracts [23]. Samples containing the equivalent to 5.0×10^6 cells were added to 10 μl of sample buffer (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 0.002% bromophenol blue) and mixed with 10% (v/v) β-mercaptoethanol, followed by heating at 100 °C for 5 min [24]. Alternatively, samples were incubated with sialidase (0.2 U/ml) for 1 h and