Available online at www.sciencedirect.com
ScienceDirect
Microbial Pathogenesis 43 (2007) 22–36
MICROBIAL PATHOGENESIS
www.elsevier.com/locate/micpath

Trypanosoma cruzi cell invasion and traffic: Influence of *Coxiella burnetii* and pH in a comparative study between distinct infective forms

Maria Cecília Fernandes[1], Carolina L'Abbate[1], Walter Kindro Andreoli, Renato Arruda Mortara*

Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo-Escola Paulista de Medicina, Rua Botucatu, 862, 6º andar, São Paulo, SP 04023-062, Brazil

Received 26 September 2006; received in revised form 6 February 2007; accepted 8 February 2007
Available online 12 March 2007

Abstract

Previous studies have shown that *Coxiella burnetii*, an intracellular bacterium that resides within acidified vacuoles with secondary lysosomal characteristics, is an effective modulator of the intracellular traffic of trypomastigote forms of *Trypanosoma cruzi*. In addition, vacuolar and cellular pH are related to fusion events that result in doubly infected phagosomes. *T. cruzi*, the etiological agent of Chagas' disease, occurs as different strains grouped in two major phylogenetic lineages: *T. cruzi* I, associated with the sylvatic cycle, and *T. cruzi* II, linked to the human disease. In this work we compared extracellular amastigotes (EA), metacyclic trypomastigotes (MT) and tissue culture derived trypomastigotes (TCT) belonging to *T. cruzi* I or *T. cruzi* II for their ability to invade and escape from their parasitophorous vacuole (PV), in Vero cells or Vero cells harboring the bacterium, *C. burnetti*. Distinct invasion patterns were observed between different infective stages and between infective forms of different strains. Studies on the transference kinetics revealed that pH modulates the intracellular traffic of each infective stage, but this influence is not exclusive for each phylogenetic group. Endosomal to lysosomal sequential labeling with EEA-1 and LAMP-1 of the PV formed during the entry of each infective form revealed that the phagosome maturation processes are distinct but not strain-dependent. Due to their low hemolysin and *trans*-sialidase activities, MTs are retained for longer periods in LAMP-1 positive vacuoles. Our results thus suggest that despite the contrasting invasion capabilities, parasites of distinct phylogenetic group behave in similar fashion once inside the host cell.

Keywords: *Trypanosoma cruzi*; *Coxiella burnetii*; Amastigote; Trypomastigote; Cell invasion; Phagosome

1. Introduction

In the present study the co-infection model (*Trypanosoma cruzi* and *Coxiella burnetii*) was used in an attempt to evaluate a possible interference of the bacteria vacuole on the invasion and intracellular traffic of distinct infective forms of *T. cruzi* and gain insights on the mechanisms responsible for higher infectivity of particular strains and forms. *T. cruzi* is the etiological agent of Chagas' disease and occurs as different strains or isolates that may be grouped in at least two major phylogenetic lineages: *T. cruzi* I, linked to the sylvatic cycle of the parasite and other mammalian hosts; and *T. cruzi* II, associated with the domestic cycle and human disease [1–3]. Metacyclic trypomastigote (MT) forms derived from the differentiation of epimastigotes, in the rectum of triatomine vectors, invade a variety of mammalian cells, where they escape from the parasitophorous vacuole, differentiate into amastigotes and replicate in the cytosol of the infected cell. Amastigotes then develop into trypomastigotes that initiate another round of infection. Amastigotes may occasionally be found extracellularly derived from the premature lyses of infected cell [4–7], or through extracellular differentiation of trypomastigotes [8,9]. These extracellular amastigotes (EA) can invade professional or non-professional phagocytes, where they survive and sustain the parasite's life cycle [10,11].

*Corresponding author. Tel.: +55 11 5579 8306; fax: +55 11 5571 1095.
E-mail address: renato@ecb.epm.br (R.A. Mortara).
[1]Both authors contributed equally to the work.

0882-4010/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micpath.2007.02.005

The efficiency in entering non-phagocytic mammalian cells may vary widely between *T. cruzi* forms and strains belonging to different phylogenetic lineages. MT belonging to *T. cruzi* II are known to be more infective than *T. cruzi* I trypomastigotes [12–14] while the inverse is often seen with EA. Extracellularly generated amastigotes of *T. cruzi* I strains display greater infectivity than those of *T. cruzi* II strains [15,16]. Differences between both trypomastigote forms have also been described regarding their infectivity towards cells, the expression of surface components such as sialic acid acceptor [17], and the molecules mobilized during host cell invasion [18,19]. Once invasion has been accomplished, lysosomal markers are found in parasitophorous vacuoles of both trypomastigote forms [20–22] and amastigote forms [21,23]. The escape process, in order to reach the cytoplasm and continue the infection, involves Tc-Tox hemolysin [24] and *trans*-sialidase activity [25] present in all infective stages.

Most intracellular bacterial pathogens inhabit replication vacuoles that exhibit a wide range of interactions with the endocytic pathway [26]. It has been previously demonstrated that *T. cruzi* parasitophorous vacuole can interact with other vesicular compartments like *C. burnetii* large acidic vacuole [22,27,28]. *C. burnetii* is an obligate intracellular gram-negative bacterium and the causative agent of human Q fever [29]. Once inside cells, this bacterium forms large vacuoles with lysosomal characteristics, by acquisition of hydrolases and lysosomal markers (LAMP-1 and LAMP-2) creating the acidic compartment required for its replication [30,31]. Protozoan–bacterial co-infections using *C. burnetii* have resulted in doubly infected or single infected phagosomes. *Leishmania* (*L.*) *amazonensis* infection of CHO cell lines containing *C. burnetii* clearly showed that the two microorganisms could share the same intracellular space [28,32]. Fibroblast double-infection with *C. burnetii* and *T. gondii* resulted in minimal co-localization [33], indicating that this intracellular pathogen interacts with endosomal–lysosomal pathways. Recent studies in our laboratory revealed that *C. burnetii* is an effective modulator of the intracellular traffic of CL strain trypomastigote forms and that pH is related to fusion events, which resulted in doubly infected phagosomes. Significant differences in the recruitment of endosomal–lysosomal markers were also reported between metacyclic and tissue-culture trypomastigote (TCT) [22].

We chose to examine the interaction of *T. cruzi* with an intracellular pathogen that requires complete subversion of the endocytic pathway for its growth and explores the only intracellular niche capable of establishing the moderately acidic pH to sustain its metabolism. Considering that *T. cruzi* parasitophorous vacuoles interact with the endosomal–lysosomal pathway and at some point are destined to fuse with lysosomes, co-infection could become a valuable tool to observe the responses of different infective forms and strains to such a modulator agent.

We compared parasites of distinct infective stages of *T. cruzi* I and *T. cruzi* II groups in their ability to invade host cells, infected or not with *C. burnetii*, and to escape from the parasitophorous vacuole. The presence of the bacteria had distinct effects on the invasion of trypomastigote forms of different strains, but had no significant influence on the intracellular traffic of any infective form indicating that, once inside the host cell, parasites with distinct infectivities behave in similar ways.

2. Results

2.1. Invasion of Vero cells infected or not with C. burnetii by different forms of T. cruzi

It has been previously described that the ability of *T. cruzi* to invade host cells is very diverse among strains and forms [12–16]. We first compared the infectivity of MT, TCT and EA of two different parasite strains belonging to the two major phylogenetic groups: *T. cruzi* I (G) and *T. cruzi* II (Y). A comparison between the infectivity of distinct forms of G and Y strains confirmed previous findings [15,16] that *T. cruzi* I EA (G strain) were much more infective than when compared to type *T. cruzi* II EA (Y strain) (Fig. 1). In addition regardless of the phylogenetic group, EA presented higher infectivity towards Vero cells than MT or TCT (Fig. 1).

Most double infection studies concentrate on the observation of the behavior of one pathogen in the presence of another parasitizing the same cell. In this study we used a well-established co-infection model in our

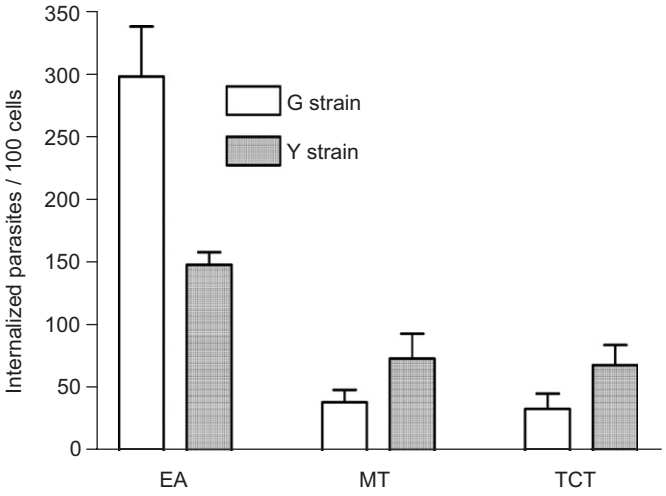

Fig. 1. Extracellular amastigotes (EA) of *T. cruzi* strains of distinct phylogenetic groups invade Vero cells in higher rates than metacyclic trypomastigotes (MT) or tissue derived trypomastigotes (TCT). Vero cells were exposed to *T. cruzi* infective forms for 90 min, coverslips were stained with Giemsa and the number of internalized parasites was determined under light microscopy. Parasite:cell ratio for G strain MT and TCT was 20:1, for G strain EA 10:1, for Y strain MT and TCT was 10:1 and for 30:1 for Y strain EA. As indicated in Materials and Methods, the relative parasite:cell proportions were adjusted in order to obtain sufficient intracellular parasites to be scored. Differences between EA and MT/TCT for each strain and between strains are statistically significant ($p < 0.05$).