Exploring the role of insect host factors in the dynamics of *Trypanosoma cruzi*–*Rhodnius prolixus* interactions

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Abstract

Members of the subfamily Triatominae, family Reduviidae, comprise a large number of insect species of which some are vectors of *Trypanosoma cruzi*, the causative agent of Chagas’ disease. This article outlines research on the process of transformation and the dynamics of developmental stages of *Trypanosoma cruzi* in the triatomine insect hosts. Special attention is given to the interactions of parasites with gut molecules, and with host developmental physiology and intestinal organization. The vector insect’s permissiveness to *Trypanosoma cruzi*, which develops in the vector gut, largely depends on the host nutritional state, the parasite strain, trypanolytic compounds, digestive enzymes, lectins, resident bacteria in the gut and the endocrine system of the insect vector. Finally, the mechanisms of these interactions and their significance for *Trypanosoma cruzi* transmission are discussed.

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Keywords: *Trypanosoma cruzi*; Triatomines; Vector; Parasites

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1. Introduction

Chagas’ disease, also called American trypanosomiasis, is a human tropical disease, which is endemic in large areas of South and Central America. Among the parasitic diseases, Chagas’ disease is ranked as one of the most important in Latin America in terms of social and economic impact, affecting about 18 million people, with about 100 million people living in what are considered to be high risk zones, and approximately 300,000 new cases occurring every year with around 21,000 deaths annually (Schofield, 1994; Moncayo, 2003; WHO, 2002). A few rare cases have been reported in the United States (Ryckman, 1981). The triatomine vector, the parasite and wild reservoirs all occur in the United States (Burkholder et al., 1980).

The discoverer of Chagas’ disease, the Brazilian Carlos Chagas, not only described many clinical, anatomical–pathological, and epidemiological elements, but also identified the flagellate Trypanosoma cruzi, as the etiological agent of the disease, and its insect vector as triatomines, hematophagous bugs of the order Hemiptera (Chagas, 1909).

Previously, Garcia and Azambuja (1991) and Kollien and Schaub (2000) reviewed the Trypanosoma cruzi–insect vector associations but subsequently there has been much recent research effort aimed at the understanding of both Trypanosoma cruzi–insect vector interactions and vector control. This article therefore reviews this more recent research emphasizing basic biological factors such as food supply, intestinal components, gut flora and insect physiology that may be relevant to understanding the parasite–vector interaction and that may reveal new perspectives for the control of Chagas’ disease.

2. The insect vector

The subfamily Triatominae of the family Reduviidae, which are large hematophagous bugs, contains more than 130 species, of which several are vectors or potential vectors of Chagas’ disease (Galvão et al., 2003). Most of the species, and all known vectors, occur in the New World (Lent and Wygodzinsky, 1979). Triatomines are common from the Southern United States and throughout Latin America, to Southern Patagonia, thus in 18 countries in the Western Hemisphere (WHO, 2002). One genus and several species also occur in India, where the possibility of Chagas’ disease therefore exists theoretically (Schaef er, 1998). Brief descriptions of the biology and ecology of these Indian triatomines are found in Ambrose (1999).

Observations on the ecology of Triatominae are still fragmentary, principally because target species in control processes are almost exclusively domestic. Most species spread throughout the Americas and maintain enzootic cycles involving wild mammals in a variety of biotopes. Thus, many triatomines are sylvatic and are found in the safety of burrows and nests of wild vertebrates (opossums, rodents and birds), and in rocks (especially associated with small rodents), fallen timber, hollow trees, roots, palms and bromeliads. Many species are also found in peri-domestic locations and in domestic animal houses while other triatomine species have adapted to human dwelling, and are thus domestic, becoming vectors of Chagas’ disease. Domiciliary triatomines are quite opportunistic in their host selection and feed well on humans. The most important species of Chagas’ disease vectors are Triatoma infestans, Triatoma dimidiata, Triatoma brasiliensis, Triatoma maculata, Triatoma sordida, Rhodnius prolixus, Rhodnius neglectus, Rhodnius pallescens and Panstrongylus megistus (for review see Lent and Wygodzinsky, 1979; Cruz-López et al., 2001).

3. The parasite

Trypanosoma cruzi belongs to the order Kinetoplastida and the family Trypanosomatidae. Under natural conditions, Trypanosoma cruzi infects over 100 mammalian species from different orders (Devera et al., 2003). The parasite comprises a pool of parasite populations circulating among humans, vectors, sylvatic reservoirs and domestic animals, and has a complex life cycle. Trypanosoma cruzi displays quite distinct morphological and functional forms, alternating between dividing stages (including epimastigotes present in the intestine of the insect vector, but also observed at the logarithmic phase of growth in axenic cultures, and amastigotes found in mammalian cells) and nonreplicative but infective forms (including metacyclic trypomastigotes found in the feces and urine of the insect vector and in stationary phase of growth in axenic cultures of parasites, and the bloodstream tryptomastigotes in mammals and liquid phase of cell culture of tryptomastigotes) (Hoare and Wallace, 1966; Tyler and Engman, 2001).

More recent investigations using isoenzyme analysis, riboprinting analysis, rRNA promoter activity, sequencing of minixion genes and microsatellite markers, exhibited clear evidence that Trypanosoma cruzi is not a single entity but corresponds to two highly divergent genetic subgroups, named as lineages 1 and 2 (Briones et al., 1999). Although both groups of Trypanosoma cruzi cause the human disease, Trypanosoma cruzi 2 is apparently more frequently associated with the domestic cycle while Trypanosoma cruzi 1 is more frequently related with wild mammals and sylvan triatomines (sylvatic cycle) (Devera et al., 2003). However, recent studies showed that there are only minor differences in the biological behavior of natural populations of Trypanosoma cruzi belonging to the large genetic groups Trypanosoma cruzi 1 and Trypanosoma cruzi 2 (Zalloum et al., 2005), isolated from different hosts (human, sylvatic reservoirs and vector triatomines). Multilocus enzyme electrophoresis and random amplified polymorphic DNA findings demonstrated that lineage 2 is further subdivided into five smaller types (Brisse et al., 2001).
During the invertebrate phase, Trypanosoma cruzi undergoes development into epimastigotes and then epimastigotes differentiate into metacyclic trypomastigotes (a process named metacyclogenesis), which are eventually eliminated together with feces and urine and are capable of infecting vertebrate hosts (Brêner, 1973; Garcia and Azambuja, 1991; Kollien and Schaub, 2000; Azambuja et al., 2005b). Trypanosoma cruzi cannot penetrate intact skin, only entering via mucous membranes at the eyes and the mouth or small abrasions and punctures of the skin (Schuster and Schaub, 2000). In mammals, the parasites develop intracellularly and are present in the blood after rupture of the host cells (Brêner, 1973; Garcia and Azambuja, 1991; Kollien and Schaub, 2000; Azambuja et al., 2005b).

4. Trypanosoma cruzi and insect vector interactions

The co-evolution of parasites and insects has promoted the development of a powerful and sophisticated strategy based on both insect vector and parasite mechanisms, which act to facilitate parasite development or its disruption in the invertebrate host. Many potential factors that may influence the development of Trypanosoma cruzi in its insect host have been described since the fundamental discovery of the trypanosomatid basis of this human disease by Chagas (1909) and Dias (1934). There has been much discussion concerning the Trypanosoma cruzi–triatomine vector interactions resulting from the complexity of this association and the modes of parasite transmission (Garcia and Azambuja, 1991; Kollien and Schaub, 2000; Azambuja et al., 2005b).

Before considering more details of the vector–parasite interactions, it is important to understand some aspects of the parasite life cycle in the insect host. The progress of Trypanosoma cruzi through the insect vector has evolved a biologically complex system. The development of the parasite starts when the insect host feeds on the mammalian host by sucking blood. Interactions between Trypanosoma cruzi and its insect host begin with the arrival of an infected blood meal in the insect gut.

4.1. The biological cycle

Despite the fact that the reduviid vectors of the parasite have been well identified for almost 100 years, the understanding of the insect cycle of the parasite remains remarkably limited. Although Trypanosoma cruzi and blood-sucking triatomines probably have not coevolved to facilitate the protozoan transmission (Takano-Lee and Edman, 2002), it is clear that Chagas’ disease is dependent on a high degree of interaction between the triatomine vectors and the parasites. For successful transmission, the parasite undergoes three stages of transformations in the gut of the insect vector. During feeding, the trypomastigotes forms from the blood of the infected vertebrate host are ingested by the insect (Garcia and Azambuja, 1991; Kollien and Schaub, 2000; Azambuja et al., 2005b) (Fig. 1). It is assumed that after a few days in the stomach (dilated anterior part of the midgut) of the insect, most of the bloodstream trypomastigotes transform into epimastigotes and some spheromastigotes (Brack, 1968; Schaub, 1989; Garcia and Azambuja, 1991; Kollien and Schaub, 2000). Once established in the midgut, mainly in the posterior part (small intestine), the epimastigotes divide repeatedly by binary division and can attach to the perimicrovillar membranes of the intestinal cells (Zeledon, 1997; Gonzalez et al., 1999). The posterior midgut is the gut region with the highest digestive activities and where the greatest concentration of metabolites should occur, but astonishingly it does not support the highest parasite population densities (Schaub, 1989). At later stages in the rectum, the highest parasite population densities occur, following the attachment of a proportion of the epimastigotes to the rectal cuticle and transformation into metacyclic trypomastigotes which are eliminated with the feces and urine and are able to infect the vertebrate host (Garcia and Azambuja, 1991; Kollien and Schaub, 2000; Azambuja et al., 2005b) (Fig. 1). Drastic biological alterations accompany the transformation of the epimastigotes into trypomastigotes in the vector host cycle, including the capacity of the metacyclic trypomastigotes to survive in mammals, in contrast to the epimastigotes, which are destroyed by the complement system (Nogueira et al., 1972; Fernandez-Presas et al., 2001). Analyses of the cell surface of the Trypanosoma cruzi epimastigotes have discovered the existence of differences in the carbohydrate, protein, and lipid composition in comparison with trypomastigotes, so that molecular changes in the parasite surface may protect parasites against complement in the mammalian host ( Ferguson, 1999; Previato et al., 2004). Recent results from the whole genome sequencing of Trypanosoma cruzi showed that this parasite has expanded the number of genes coding for surface molecules, including transialidase, mucin, mucin-associated surface proteins (masp) and gp63 family of metalloproteases, and it is likely that these genes have evolved to evade the host immune response (El-Sayed et al., 2005). Since some of these genes, such as members of the mucin and masp groups, are expressed in the epimastigote stage of the Trypanosoma cruzi (El-Sayed et al., 2005), then they may also function to avoid the insect immune response.

The degree of natural infectivity of a vector species to a strain of Trypanosoma cruzi is highly variable and depends upon the susceptibility of the triatomine species to become infected when feeding on an infective blood meal. Another important aspect with regard to the susceptibility of insect vectors to Trypanosoma cruzi, is the adaptation of a strain of parasite to develop more readily in a triatomine species (Garcia and Dvorak 1982; Perlowagara-Szumlewics and Carvalho-Moreira, 1994). The virulence of Trypanosoma cruzi strains in insect and mammalian hosts can also change during long-term in vitro maintenance of the
parasite (Chiari, 1974). The inability of some strains of *Trypanosoma cruzi* to develop in certain species of triatomines may depend on the intrinsic qualities of either parasite or insect vector (García et al., 1984b; Gonzalez and García, 1992). There seems to be a tendency of local insect vectors to be more susceptible to strains of *Trypanosoma cruzi* from the same geographical areas (Zeledón, 1974; Perlowagora-Szumlewics et al., 1990). It is also interesting to observe that triatomines infected with *Trypanosoma cruzi* show only slight changes in the vector behavior (Schaub, 2006).

4.2. Factors present in the insect vector gut

The establishment of *Trypanosoma cruzi* infection in the gut of the insect vector may be dependent on, and possibly regulated by, a range of biochemical and physiological factors. Since the gut of triatomines is the first environment for the transformation of *Trypanosoma cruzi*, we previously examined the possible influence of digestive enzymes on parasite development (García, 1987), developed suitable bioassays for specific aspects of triatome physiology and biochemistry in the gut (for review see García and Azambuja, 1997), and analyzed a variety of other factors, such as the parasite clone used and insect vector physiology, influencing the life cycle of *Trypanosoma cruzi* in the gut of the vector, *R. prolixus* (García and Azambuja, 1991).

After entering the gut with the blood meal, the parasites are confronted by components of the anterior and posterior midgut and products of blood digestion. These included a hemolytic factor, peptides derived from α-δ-globin and lectins, all of which may modulate the dynamics of multiplication and transformation of *Trypanosoma cruzi* in the triatome vectors gut and illustrate the complexity of the mechanisms involved (for review see García and Azambuja, 1991; Kollien and Schaub, 2000; Azambuja et al., 2005b).

Several aspects of the competition of *Trypanosoma cruzi* with its vector for nutrients, demonstrate that feeding affects not only the parasite population density but also changes the percentages of different stages observed in the rectum (Kollien and Schaub, 2000). Kollien and Schaub (1997) also showed that diuresis rather than factors from the hemolymph or digestive products induced the development of metacyclic trypomastigotes of *Trypanosoma cruzi*.

In contrast with dipteran vectors that have trypsin for the digestion of blood proteins, the triatomines use cathepsins that require acid pH in the intestinal contents. Experiments showed that in insects infected with *Trypanosoma cruzi*, although cathepsin D activity increased 1 and 3 days after the blood meal (Borges et al., 2006), feeding *R. prolixus* with the acid SH-proteinase inhibitor, pepstatin, had no effect on rates of *Trypanosoma cruzi* infection (García and Gilliam, 1980). In addition, Ursic-Bedoya and

Fig. 1. Scheme of biological cycle of *Trypanosoma cruzi* within its insect vector. The insect feeds on blood infected with trypomastigote forms which transform into epimastigotes and some spheromastigotes in the stomach (A). In the intestine, the epimastigotes multiply (B) increasing the population of parasites. In the rectum, the epimastigotes transform into metacyclic trypomastigotes (C) which are eliminated with the feces and urine.
Lowenberger (2007) showed that although cathepsin B transcript levels were specific for the midgut, there was no differential expression between insects infected with Trypanosoma cruzi and the non-infected controls.

Kollien et al. (2003) described, isolated and characterized a cDNA encoding for a lysozyme from the gut of Triatoma infestans. This lysozyme gene was expressed differentially in the various regions of the digestive tract. Thus, expression was strong in the cardia and stomach i.e., the anterior regions of the midgut, but only traces of lysozyme mRNA could be detected in the small intestine i.e., the posterior region of the midgut. Two cysteine proteases in the digestive tract of Triatoma infestans were also sequenced and characterized (Kollien et al., 2004). The relevance of these enzymes in the interaction of the insect vector with Trypanosoma cruzi is under investigation.

Others putative determinants of Trypanosoma cruzi development in the insect host are components of the vector humoral immune system, such as defensin molecules. Defensin is an antimicrobial peptide consisting of 43 residues produced in the fat body but also in the intestine and midgut of triatomines. In Rhodnius, these peptides are termed R. prolixus defensin A. The transcription of defensin is significantly enhanced in the midgut and intestine upon Trypanosoma cruzi-induced immune activation of R. prolixus (Lopez et al., 2003). However, this elevated production of defensin does not threaten the trypanosomes since the parasite colonizes the intestine and is protected from exposure to lethal concentrations of the peptide which is produced primarily in the fat body and released into the hemolymph (Lopez et al., 2003). Since R. prolixus feed on blood meals, they are missing many essential vitamins and nutrients in their diets. These substances and nutrients are supplied to them via the symbiont, Rhodococcus rhodnii, that live in the intestine (Beard et al., 2002). Defensin at abnormally high concentrations can also kill these bacterial symbionts and other resident bacteria in the gut (Azambuja et al., 2004, 2005a). However, Lopez et al. (2003) hypothesized that lower concentrations of defensins are produced in the intestine to prevent death of these symbionts and thus these peptides probably have no major influence on parasite development.

Ursic-Bedoya and Lowenberger (2007) reported the identification of immune-related molecules from the fat body and intestine of R. prolixus when challenged with Escherichia coli, Micrococcus luteus and Trypanosoma cruzi. After using suppressive subtractive hybridization to identify immune-related genes, they generated three subtracted libraries, sequenced the clones and assembled the sequences. The functional annotation revealed expressed sequence tags (ESTs) in all tissues in response to challenge with microorganisms, and included molecules such as transferrins involved in iron metabolism and in the innate immune system, nitrophorins related to nitric oxide transportation, β1–3 glucan recognition protein already identified as a pathogen-recognition molecule in Manduca sexta and other insects, and a hemolymph proteinase possibly involved in prophenoloxidase activation (Ursic-Bedoya and Lowenberger, 2007). These authors also found mucin/peritrophic-like molecules probably related to the selectively of interaction with Trypanosoma cruzi (for review see Azambuja et al., 2005b).

Reactive oxygen radicals and nitrogen intermediates may also play roles in modulating the outcome of infection by Trypanosoma cruzi in the triatomine gut. High levels of superoxide molecules in the hemolymph of R. prolixus have been shown not only to correlate with the inability of the H14 strain of Trypanosoma rangeli to survive and complete it’s life cycle but also correlated with the rapid killing of injected Trypanosoma cruzi (Whitten et al., 2001). This rapid killing of Trypanosoma cruzi in the vector hemolymph may at least partially explain why this parasite, in contrast with the closely related Trypanosoma rangeli, fails to invade the hemolymph but is confined to develop in the gut of its host. More significant are the observations on the analyses of the nitric oxide synthase (NOS) gene expression and nitric oxide (NO) levels following R. prolixus infection with Trypanosoma cruzi strain Chile 5. Results showed that Trypanosoma cruzi per os induced the upregulation and downregulation of the NOS gene and NO production according to the stage of development of the parasite in the insect host. Thus, levels of NO were high in the midgut and rectal tissues as the parasite passed through these gut regions but, since the parasites localized in the rectum, they would have been protected by the presence of the thick waxy rectal cuticle. The early upregulation of hemocyte NOS gene expression despite the absence of parasites from the hemocoel, and combined with the elevated crop and midgut NO levels, may also help to explain why Trypanosoma cruzi does not invade the hemocoel (Whitten et al., unpublished observations).

4.3. Parasite attachment in the gut of the insect vector

Developing in different regions of the insect vector gut, Trypanosoma cruzi is in contact with very distinct epithelial and cell surface constituents. One process of Trypanosoma cruzi–insect interaction involves attachment of the parasite to the gut epithelial surfaces. Nogueira et al. (pers. comm.) using videomicroscope analyzed the in vitro interaction of both epimastigotes and trypomastigotes of Trypanosoma cruzi (Dm 28c clone and Y strain) with the midgut epithelium of R. prolixus. The observations showed that both parasite developmental stages moved towards the epithelial surface. However, while trypomastigotes did not attach to the epithelium, epimastigotes attached both to the anterior (stomach) and posterior midgut (intestine) luminal surfaces. On the anterior midgut surface, epimastigotes bound through the cell body or flagellum. On the posterior midgut, only flagellar attachment to perimicrovillar membranes occurred (Gonzalez et al., 1999) (Fig. 2). These preliminary findings indicate that attachment is due to a process of recognition with the involvement of glycoisitol phospholipids (GiPLs) molecules abundantly exposed
on the epimastigote surfaces (Colli and Alves, 1999; Azambuja et al., 2005b). Kollien et al. (1998a) demonstrated that the mode of association of the *Trypanosoma cruzi* epimastigotes with the midgut surface differs fundamentally from that in the rectum. They showed that parasites resided mainly at the outer border of the midgut contents. In regions in which the perimicrovillar membrane layers were absent or only weakly developed, trypanosome bodies or flagella occasionally could be found inserted shallowly between tips of the microvilli. It has been shown that gene expression, carbohydrates and polypeptides are modified during the parasite adhesion (Bonaldo et al., 1991; Andrade et al., 1991; Dallagiovanna et al., 2001). Interestingly, the deletion of a gene encoding a surface glycoprotein of *Trypanosoma cruzi* results in detachment of the flagellum from the cell body and significantly diminishes the population of *Trypanosoma cruzi* in the vector, *R. prolixus* (Ribeiro de Jesus et al., 1993; Bassombrio et al., 2002).

In the rectal lumen, the flagellates also interdigitated with each other and on the rectal wall *Trypanosoma cruzi* intimately attaches to the rectal cuticle lining. Metacyclogenesis is almost exclusively confined to the rectum and can be correlated with attachment to the rectal wall (Kollien et al., 1998a). *In vitro* experiments also showed that attachment of epimastigotes to the walls of culture flasks is needed for differentiation into metacyclic trypomastigotes (Bonaldo et al., 1988; Schmidt et al., 1998; Schaub et al., 1999).

4.4. Kinetics of development of different strains/clones of *Trypanosoma cruzi* in the gut

Apparently, not only the kinetics of *Trypanosoma cruzi* epimastigotes multiplication but also the metacyclogenesis process is dependent on the strains and clones of the infecting parasites (reviewed by Garcia and Azambuja, 1991; Kollien and Schaub, 2000; Azambuja et al., 2005b).

*In vitro* and *in vitro* metacyclogenesis experiments with the Y and Berenice strains of *Trypanosoma cruzi* in the vectors *Triatoma pseudomaculata* and *R. neglectus* resulted in a higher percentage of metacyclics for both strains in *R. neglectus* gut than in *Triatoma maculata* (Carvalho-Moreira et al., 2003). *In vitro* experiments incubating *Trypanosoma cruzi* culture forms with extracts of different gut regions (stomach, intestine, and rectum) of both species of triatomines, demonstrated that a higher percentage of metacyclic trypomastigotes occurred in the rectal extract of *R. neglectus* in comparison with *Triatoma pseudomaculata*. The same findings were observed with *in vitro* experiments using parasites incubated with urine from each of those vectors (Carvalho-Moreira et al., 2003).

Some authors point out that in a species as heterogeneous as *Trypanosoma cruzi*, an interaction and cooperation effect among the different parasite subpopulations in the environment of the insect gut should be considered (Lana et al., 1998; Pinto et al., 1998, 2000; Lima et al., 1999).
5. Gut microbiota and Trypanosoma cruzi–insect vector interactions

In the digestive tract of some insect vectors, parasites ingested with the blood meal decrease in number before coming into direct contact with host tissues. Many factors could be responsible for this reduction in parasite populations such as digestive enzymes, lectins and others biochemical factors and temperature change. Potentially important too are large communities of naturally occurring microorganisms in the gut of the insect vectors that could have a role as determinants of parasite survival and development in insect vector hosts and, therefore, contribute to the modulation of vector competence for many important diseases (Azambuja et al., 2005a).

One elegant approach to inhibit the Trypanosoma cruzi parasite–insect vector interactions is by the use of transgenic symbiotic bacteria that attack Trypanosoma cruzi directly. After a series of successful transformations of R. rhodnii, a symbiont of R. prolixus, a pore-forming peptide of Hyalophora cecropia, which is induced in the humoral immune response and kills many Gram-positive and negative bacteria. This compound eliminates or strongly reduces the number of Trypanosoma cruzi in the insect vector (Durvasula et al., 1999; Beard et al., 2002), and therefore has potential in reducing vector competence.

Recently, Azambuja et al. (2004) opened up an exciting new research area by studying the effects of resident bacteria in the stomach of R. prolixus on erythrocyte lysis and Trypanosoma cruzi infection. Following infective blood feeding with either the Y strain or Dm28c clone of Trypanosoma cruzi, bacteria rapidly proliferated in the vector gut and the number of surviving Y strain in the stomach diminished drastically, while infection with the Dm28c clone remained stable. Hemolytic bacteria were unable to adhere to the stomach or intestinal epithelium (Gonzalez et al., 1999). Thus, manipulation of the physiological condition of the vector host such as decapitation, head transplantation, azadirachtin and edysone therapy may all influence the parasite development. Insects that received these treatments showed a distinct effect characterized by ultrastructural disorganization of the midgut epithelial cells of R. prolixus and indicated that the prothoracicotropic hormone (PTTH)–edysone pathway interferes with Trypanosoma cruzi survival and development in its vectors (Nogueira et al., 1997; Gonzalez et al., 1999) (Figs. 2 and 3).

When the R. prolixus larvae were decapitated or treated with azadirachtin, the Trypanosoma cruzi epimastigotes were unable to adhere to the stomach or intestinal epithelium (Gonzalez et al., 1999). Thus, manipulation of the physiological condition of the vector host such as decapitation, head transplantation, azadirachtin and edysone therapy may all influence the parasite development. Insects that received these treatments showed a distinct effect characterized by ultrastructural disorganization of the midgut epithelial cells of R. prolixus and indicated that the prothoracicotropic hormone (PTTH)–edysone pathway interferes with Trypanosoma cruzi survival and development in its vectors (Nogueira et al., 1997; Gonzalez et al., 1999) (Figs. 2 and 3).

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Lignans and neolignans are also widely occurring natural plant compounds and may have feeding-deterrent and insect larval growth-inhibitory properties (Garcia and Azambuja, 2004). The oral treatment with burchelin or concentrations of d-mannose were found to protect Trypanosoma cruzi and Trypanosoma rangeli diminishing the lysis caused by S. marcescens variants SM 365 and RPH. However, this carbohydrate was unable to interfere with hemolysis induced by these two bacterial variants. Castro et al. (2006) concluded that the trypanolytic effect induced by S. marcescens is inhibited by d-mannose and distinct from the hemolytic activity, suggesting that this bacterium possesses mannose-sensitive fimbriae that mediate adherence and lysis of trypanosomes. The study of bacteria in the gut of triatomine hosts provides new tools to block the development of parasites in the insect vector (Azambuja et al., 2004, 2005a).

6. Natural plant compounds and Trypanosoma cruzi–insect vector interactions

Interesting insights into the Trypanosoma cruzi–vector interactions resulted from the investigations made with the compound azadirachtin, a natural growth inhibitor from the neem tree (Azadirachta indica A. Juss), which strongly interferes with the neuroendocrine regulation of the insect hormone titers (Garcia and Rembold, 1984; Garcia et al., 1984a, 1986, 1990). This compound, given via blood meals, before, during and after infection, not only affects the development of triatomines but also the establishment of Trypanosoma cruzi infection in the gut of different species of triatomines (Garcia et al., 1989; Gonzalez and Garcia, 1992; Gonzalez et al., 1999; Kollien et al., 1998b; Kollien and Schaub, 1999) (Figs. 2 and 3).

When the R. prolixus larvae were decapitated or treated with azadirachtin, the Trypanosoma cruzi epimastigotes were unable to adhere to the stomach or intestinal epithelium (Gonzalez et al., 1999). Thus, manipulation of the physiological condition of the vector host such as decapitation, head transplantation, azadirachtin and edysone therapy may all influence the parasite development. Insects that received these treatments showed a distinct effect characterized by ultrastructural disorganization of the midgut epithelial cells of R. prolixus and indicated that the prothoracicotropic hormone (PTTH)–edysone pathway interferes with Trypanosoma cruzi survival and development in its vectors (Nogueira et al., 1997; Gonzalez et al., 1999) (Figs. 2 and 3). These results provide the first clear evidence showing the importance of the insect endocrine system in establishing the Trypanosoma cruzi infection in the vector. Investigations on the effects of decapitation, head transplantation, azadirachtin and edysone therapy show that the prothoracicotropic hormone (PTTH)–edysone pathway interferes through edysone release in the Trypanosoma cruzi survival and infection in its vector (Gonzalez et al., 1999; Cortez et al., 2002) (Figs. 2 and 3).

Lignans and neolignans are also widely occurring natural plant compounds and may have feeding-deterrent and insect larval growth-inhibitory properties (Garcia and Azambuja, 2004). The oral treatment with burchelin or
nordihydroguaiaretic acid (NDGA) during and before an infection with *Trypanosoma cruzi* epimastigotes, provides the first evidence of the effects of these compounds on the parasite development. When the infection had already established, only burchelin was able to decrease the number of flagellates in the gut (Cabral et al., 1999, 2001). Based on the highly specialized parasite–insect vector interactions, probably the biological effects of these chemicals on the insect’s physiological condition are due to interference with the hormonal homeostasis and thus inhibition of the *Trypanosoma cruzi*–insect vector interactions (Garcia and Azambuja, 2004).

7. Concluding remarks

After 30 years working with triatomines, especially with *R. prolixus*, it is clear to us that at present we know much more about the biochemistry and physiology of these insect vectors. However, regardless of the medical importance of triatomines and their suitability for experimentation, still little is understood about their relationships with trypanosomes. This article indicates the most recent literature on the complexity of the trypanosome–triatomine relationship and that research into the basic aspects of parasite–insect host interactions can point out mechanisms for the establishment of *Trypanosoma cruzi* infection, that are transmitted by hematophagous insect vectors. The demand for this kind of investigation is increasing with the necessity to find new vector control methods. Many points remain unanswered, such as (i) what other molecules are present in the digestive tract and involved as modulators of parasite multiplication by controlling the interaction with the gut epithelial cells and perimicrovillar membranes whose functions are complex and poorly understood; (ii) how do the hormones induce modification in the vector gut at the molecular level; (iii) how do genes, parasites and vector molecules interact to modulate parasite establishment in *Trypanosoma cruzi* infection; and (iv) what, if any, role do the inducible immune responses play in parasite–host interactions as they could potentially influence survival and parasite establishment in the gut of the insect vector, and (v) to what extent does the natural bacterial flora influence parasite survival and development in the vector gut?

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