Towards an understanding of the interactions of *Trypanosoma cruzi* and *Trypanosoma rangeli* within the reduviid insect host *Rhodnius prolixus*

PATRÍCIA AZAMBUJA[1], NORMAN A. RATCLIFFE[2] and ELOI S. GARCIA[1]

[1]Department of Biochemistry and Molecular Biology, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz
Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil
[2]Biomedical and Physiologial Research Group, School of Biological Sciences, University of Wales
Swansea, Singleton Park, Swansea, SA28PP, United Kingdom

ABSTRACT

This review outlines aspects on the developmental stages of *Trypanosoma cruzi* and *Trypanosoma rangeli* in the invertebrate host, *Rhodnius prolixus*. Special attention is given to the interactions of these parasites with gut and hemolymph molecules and the effects of the organization of midgut epithelial cells on the parasite development. The vector insect's permissiveness to *T. cruzi*, which develops in the vector gut, largely depends on the host nutritional state, the parasite strain and the molecular interactions with trypanolytic compounds, lectins and resident bacteria in the gut. *T. rangeli* invades the hemocoel and once in the hemolymph, can be recognized and activates the defense system of its insect vector, i.e., the prophenoloxidase system, phago-cytosis, hemocyte microaggregation, superoxide and nitric oxide activity and the eicosanoid biosynthesis pathway. Taken together, these findings not only provide a better understanding of the interactions para-site - insect vector, but also offer new insights into basic physiological processes involved in the parasites transmission.

Key words: Trypanosomes, *Rhodnius prolixus*, vector, parasites.

INTRODUCTION

The co-evolution of parasites and insects has pro-moted the development of a powerful and sophisti-cated strategy based on both physiological and bio-chemical vector and/or parasite mechanisms, which act to facilitate parasite development or its disrup-tion in the invertebrate host. The debate concerning the *Trypanosoma cruzi* and *T. rangeli*- triatomine vector interactions results from their complexity and modes of parasite transmission (Garcia and Azam-buja 1991, Kollien and Schaub 2000). Like *T. cruzi*, *T. rangeli* infects humans and a wide range of other mammals and has a similar geographic distribution (Guhl and Vallejo 1998).

Many factors are believed to contribute to the establishment of trypanosomes in the gut of the in-vertebrate host. However, differences in the biolog-ical cycles between both parasites exist: *T. rangeli*, but not *T. cruzi*, invades the hemocoel and physico-chemical barriers such as the gut membranes, which

are important to *T. cruzi* development, but not to *T. rangeli* in the digestive tract (Gonzalez et al. 1999, Azambuja and Garcia, personal observation).

This review outlines research on the developmental aspects of *Trypanosoma cruzi* and *Trypanosoma rangeli* in the insect vector, which may reveal new perspectives for the control of Chagas disease.

TRYPANOSOMA CRUZI AND VECTOR INTERACTIONS

Before considering more details of the vector – parasite interactions, it is important to understand some aspects of the parasite life cycle in the invertebrate host. Points of contact between *T. cruzi*, the etiologic agent of Chagas disease, and its triatomine vectors begin with ingestion of the infective blood meal. Blood feeding is essential for the parasite growth in all instars and in the adult insect (Kollien and Schaub 1998). Interactions between *T. cruzi* and its vector insects begin with the arrival of an infected blood meal in the insect gut. 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). After a few days in the stomach (anterior part of the midgut) of the insect most of the bloodstream trypomastigotes transform into epimastigotes and some spheromastigotes (Garcia and Azambuja 1991, Kollien and Schaub 2000). Subsequently, mainly in the intestine (posterior part of the midgut), the epimastigotes divide repeatedly by binary division and can attach to the perimicrovillar membranes in the intestinal cells (Gonzalez et al. 1999). At later stages in the rectum, a proportion of the epimastigotes attach to the rectal cuticle and transform into metacyclic trypomastigotes that are eliminated with the feces and urine and are able to infect the vertebrate host (Garcia and Azambuja 1991, Kollien and Schaub 2000).

Since the gut of triatomines is the first environment for the establishment of *T. cruzi* infection, we studied the possible influence of digestive enzymes on parasite development (Garcia 1987), developed suitable bioassays for specific aspects of triatomine physiology and biochemistry in the gut (for review

see Garcia and Azambuja 1997), and analyzed a variety of factors, such as the clone used and feeding with azadirachtin, influencing the life cycle of *T. cruzi* in the gut of the vector, *R. prolixus* (Garcia and Azambuja 1991).

Results showed that feeding *R. prolixus* with the inhibitor of acid SH-proteinase, pepstatin, had no effect on rates of *T. cruzi* infection (Garcia and Gilliam 1980). Also, trypomastigotes from single-cell-isolates of two clones of *T. cruzi* produced different rates of growth because of different population doubling times of the parasites and only one clone underwent epimastigote to metacyclic trypomastigote transformation (Garcia and Dvorak 1982). Results therefore indicate that not only the kinetics of *T. cruzi* epimastigote multiplication but also the transformation to metacyclic trypomastigotes depend on the strains and clones of the infecting parasite (Garcia et al. 1984b). In addition, the passage of homogeneous strains of *T. cruzi* through the invertebrate host gut was identical with the host was unable to modify any biological, biochemical or genetic characteristics of the parasite (Garcia et al. 1986b).

Although many factors are thought to be important to the establishment of *T. cruzi* infection in the gut of the vector, only a few molecules have been implicated, such as a stomach lytic factor (Azambuja et al. 1983, 1989), lectins (Pereira et al. 1981, Mello et al. 1996) and hemoglobin fragments (Garcia et al. 1995), all of which have been tested directly against the parasite. The work of Pereira et al. (1981) reported lectins in the digestive tube and hemolymph of *R. prolixus* and suggested that these molecules could be involved in the development of *T. cruzi* in the triatomine bug. Mello et al. (1996) demonstrated differences in the development of three strains of *T. cruzi* in the gut of *R. prolixus* and related the infectivity of these to the ability of the digestive tube extract to agglutinate these parasites *in vitro*. Interestingly, lectins agglutinated the Dm28c clone of *T. cruzi* which achieved high infectivity levels while, in contrast, the Y strain of the parasite which was not agglutinated, was lysed and failed to develop in the vector gut (Azambuja et al.

1989, Mello et al. 1996, 1999).

Frainderaich et al. (1993) demonstrated that metacyclogenesis of *T. cruzi* is promoted *in vitro* by an α^D-globin-derived peptide present in hemoglobin corresponding to residue 1-40 from the amino terminus found in the gut of *Triatoma infestans*. Synthetic peptides having the amino terminal globin sequences and containing conserved domains spanning amino acid residues 30 to 40 are recognized by a surface receptor in epimastigote cells and stimulate *T. cruzi* adenylyl cyclase (Frainderaich et al. (1993). Garcia et al. (1995) also studied *in vivo* in *R. prolixus*, the effects of hemoglobin and synthetic peptides carrying α^D-globin fragments on both the growth and transformation of *T. cruzi* epimastigotes into metacyclic trypomastigotes. This differentiation in the insect gut occurred when hemoglobin and synthetic peptides corresponding to residues 30-49 and 35-73 of the α^D-globin were added to the plasma diet. However, synthetic peptide 41-73 did not induce differentiation of epimastigotes even in the presence of the two former peptides so that peptide 41-73 appeared to block the action of these stimulatory peptides. In addition, the whole hemoglobin molecule was shown to be a very important blood component for the growth of parasites (Garcia et al. 1995). These data identified an unusual molecular mechanism, which modulates the dynamics of transformation of epimastigotes into metacyclic trypomastigotes in the triatomine vectors gut.

Recently, Azambuja et al. (2004) opened an exciting new research area by studying the effects of resident bacteria in the stomach of *R. prolixus* on erythrocyte lysis and *T. cruzi* infection. Following feeding, bacteria rapidly multiplied and the number of surviving Y strain of *T. cruzi* in the stomach declined drastically, while infection with Dm28c clone remained stable. Hemolytic bacteria were isolated and identified as *Serratia marcescens* biotype A1a (referenced as RPH), which produces the pigment, prodigiosin. *In vitro* experiments, comparing incubation of RPH or *S. marcescens* SM365, a prodigiosin pigment producer, or *S. marcescens* DB11, a none pigmented variant, as a control, with erythro-

cytes and *T. cruzi* demonstrated that: (i) at 0°C or 30°C, both SM365 and RPH reduced the populations of Y strain, but not of the DM28c clone, and DB11 was unable to lyse either *T. cruzi* strain; and (ii) all three strains of *S. marcescens* were able to lyse erythrocytes (Azambuja et al. 2004). These results suggest that *S. marcescens* trypanolytic activity from the SM365 and RPH strains is distinct from the hemolytic activity and that prodigiosin is an important factor for the trypanolytic action of the bacteria in the gut of the vector. The study of bacteria in the gut of invertebrate hosts may be important and provide new tools to block the development of parasites in the insect vector.

One of the more interesting outcomes of the *T. 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, 1986a, 1990). This compound not only affects the development of triatomines but also the establishment of *T. 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. 1998).

Nogueira et al. (1997) analyzed the process of interaction of epimastigotes and trypomastigotes of *T. cruzi* (Dm 28c clone and Y strain) with the intestinal epithelium of *R. prolixus in vitro*. Their observations showed that both parasite developmental stages move towards the epithelial surface. However, while trypomastigotes did not attach to the epithelial surface, all epimastigotes strongly attached through the flagellum, preferentially at the periphery of the cells. When the *R. prolixus* larvae were decapitated or treated with azadirachtin, the *T. cruzi* epimastigotes were unable to adhere to the stomach or intestinal epithelium (Gonzalez et al. 1999). This may result from the effect of these treatments on factors present in the perimicrovillar membrane, which interact with glycoinositol phospholipids (GIPLs) molecules abundant on the epimastigote plasma

membrane and involved in the process of parasite insect host-interaction (Garcia and Azambuja, un-published data). Thus, manipulation of the physio-logical condition of the vector host may affect the development of *T. cruzi*, with decapitation, head trans-plantation, azadirachtin and ecdysone therapy con-sequently all influencing the parasite development. Insects that received these treatments showed a dis-tinct effect characterized by ultrastructural disorga-nization of the midgut epithelial cells of *R. prolixus* and indicated that the prothoracicotropic hormone (PTTH)-ecdysone pathway interferes with *T. cruzi* survival and development in its vectors (Gonzalez et al. 1999). These results provide the first clear evidence showing the importance of the insect en-docrine system in establishing *T. cruzi* infection in the vector.

TRYPANOSOMA RANGELI AND VECTOR INTERACTIONS

T. rangeli, is a parasite generally considered non-pathogenic to humans and animals but can be infec-tive to triatomines (Watkins 1971). Little is known about the life cycle of *T. rangeli* in the vertebrate hosts, although infections in humans and wild an-imals have been reported (Guhl and Vallejo 1998). After ingestion as trypomastigotes, *T. rangeli* differ-entiates into epimastigotes and multiplies in the gut of its insect vector (usually *Rhodnius prolixus*) and commonly invades the hemolymph to continue its growth, and complete its development in the sali-vary glands, where metacyclogenesis takes place (Watkins 1971, Hecker et al. 1990, Garcia et al. 1994, Mello et al. 1995). Interestingly, insects with salivary glands infections pierce the host skin more frequently draw less blood and at a slower rate than controls when feeding on mammals (Garcia et al. 1994). Additionally, infected salivary glands signif-icantly reduced levels of both apirase activity and re-active nitrogen groups. It seems that salivary gland infection of *T. rangeli* manipulates the vector abil-ity to locate blood vessels by affecting the salivary antihemostatic properties, thus enhancing the pos-sibility of intradermal inoculation of parasites into

the mammalian host (Garcia et al. 1994).

Other important compounds involved in the parasite – vector interactions are lectins. These com-pounds not only block carbohydrates exposed on the parasite surface of but also inhibit the attachment of the *T. rangeli* in both midgut (Oliveira and de Souza 1997) and salivary glands (Basseri et al. 2003). Af-ter *T. rangeli* invasion of the hemolymph from the midgut, the parasites are taken up by the *R. pro-lixus* hemocytes and divide therein before escaping and invading the salivary glands (Tobie 1970). It is not clear if the invasion of parasites in the hemo-cytes is mediated solely by phagocytosis or by active invasion by the parasite in a parasitophorous vac-uole. The latter hypothesis was observed by trans-mission electron microscopy studies, which showed that plasmatocytes were able to ingest epimastigote forms of the parasite, which were then found within a parasitophorous vacuole (de Oliveira and de Souza 2003).

Once in the hemolymph, *T. rangeli* can be recognized and activates the defense system of its insect vector. Several aspects of the humoral and cellular immune responses have been investigated in *T. rangeli* – triatomine interactions, including lyso-zymes and trypanolytic activity (Mello et al. 1995, Gomes et al. 1999), prophenoloxidase (proPO) acti-vation (Mello et al. 1995, Gomes et al. 1999, 2003, Garcia et al. 2004a), phagocytosis and hemocyte mi-croagglutination (Mello et al. 1995, Garcia et al. 2004a,b), hemolymph agglutination (Mello et al. 1996), and superoxide and nitric oxide generation (Whitten et al. 2001).

Studies carried out on the activation of the proPO system of *R. prolixus* infected with short and long epimastigote forms of *T. rangeli* demonstrated that short, but not long, epimastigotes activated the formation of melanin (Gomes et al. 1999). Whitten et al. (2001) also studied the immune response of *R. prolixus in vivo* following the inoculation of two strains and two developmental forms of *T. rangeli*. The results showed that after 24 hr the H14 strain, which failed to multiply and invade the salivary glands, stimulated significantly higher levels of

prophenoloxidase, superoxide and nitrites than the Choachi strain, which rapidly multiplied in the hemolymph to invade the salivary glands for transmission to the vertebrate host. Usually, short form epimastigotes stimulated greater superoxide and proPO responses than long epimastigotes in both parasite strains. In addition, when the NADPH oxidase inhibitor, N-ethylmaleimide, or the inducible nitric oxide synthase inhibitor, S-methyl isothiourea sulfamide, are injected into groups of *R. prolixus* they caused significantly higher insect mortalities after inoculation with parasites of either strain compared with those of uninfected control insects (Whitten et al. 2001). This indicates that both NADPH oxidase and nitric oxide synthase activity may be involved in the immune response of *R. prolixus* infection by *T. rangeli*. In *Rhodnius prolixus*, NOS gene expression and nitrite levels are modulated in the stomach, midgut and fat body following *in vivo* challenge with *T. cruzi* or *T. rangeli*. Elevations in nitrite activity in the gut wall occur during the response to infection by both species of trypanosomes but are particular high with *T. cruzi*, which could explain why this parasite fails to cross the midgut wall into the hemocele (Whitten et al., unpublished data).

In many insects species, cellular immune responses to pathogens depend on two kinds of reactions: (i) nodulation and encapsulation and (ii) phagocytosis by hemocytes. Nodule formation involves hemocyte degranulation and activation of the proPO system, attachment of the developing nodule to host tissues and the beginning of phagocytic activity by the plasmatocytes (Ratcliffe and Rowley 1979).

It was shown that in *R. prolixus* hemocyte monolayers, both *T. cruzi* and *T. rangeli* are capable of inducing hemocyte/parasite clump formation. A purified galactoside-binding lectin markedly enhanced the activation of clump formation by *T. rangeli* in *R. prolixus* hemocyte monolayers, with an increase in clump size and hemocyte aggregation (Mello et al. 1999). Takle (1988) and Mello et al. (1995) described the aggregation process, following

injection of *T. rangeli* and *T. cruzi* into *R. prolixus*. The purified hemolymph lectin also significantly reduced the motility and survival of *T. rangeli* culture short forms, but not the long forms, when they were incubated *in vitro* (Mello et al. 1999). In normal infections, a few days after inoculation of *T. rangeli* short forms into the hemolymph of the insect, they disappear to be replaced by a massive colonization by long forms (Mello et al. 1995) and it may be that the hemolymph lectin mediates this process. There is little evidence for melanization of the hemocyte microaggregation, despite Alvarenga et al. (1990) having described melanized nodules in response to abiotic or biological challenges. Apparently, the *T. rangeli* long forms suppress the proPO system that produces melanin (Gomes et al. 1999, 2003).

Recently, our laboratory investigated the effects of eicosanoid biosynthesis inhibitors on hemocyte microaggregation and proPO-activating system in the hemolymph, and on parasitemia and mortality of *R. prolixus* infected with *T. rangeli*. Hemocoelic injection of *T. rangeli* epimastigotes into insects that were previously fed on blood containing an inhibitor of phospholipase A2, dexamethasone, a specific inhibitor of the cyclooxygenase pathway, indomethacin, and a non-selective lipoxygenase inhibitor, NDGA, reduced hemocyte aggregation, attenuated the proPO system in the hemolymph and enhanced parasitemia and mortality induced by the parasite challenge (Garcia et al. 2004a). These authors demonstrated that the effects obtained by dexamethasone administered orally were counteracted by inoculation of the insects with arachidonic acid. It seems that *Rhodnius*' immune response to a parasite infection is modulated by a physiological system that includes eicosanoid biosynthesis pathways. Garcia et al. (2004b) also examined the effect of feeding *R. prolixus* larvae on blood containing *T. rangeli* on the cellular immune system, focusing on aggregation reactions in the hemolymph when the insect is subsequently challenged by inoculation with the same parasite. This is an interesting model since the double infections (oral and hemocoelic inoculation) are similar to what occurs in nature. They

showed a reduced number of hemocyte aggregations in the hemolymph, enhanced numbers of parasites in the hemolymph as well as increased mortality in these insects. All these effects were counteracted by the combined inoculation of *R. prolixus* with *T. rangeli* together with arachidonic acid (Garcia et al. 2004b). They concluded that oral infection with *T. rangeli* inhibits the release of arachidonic acid, which is a mediator of hemocyte aggregation reactions in the hemolymph of *R. prolixus* infected with *T. rangeli* (Garcia et al. 2004a,b).

CONCLUSIONS

The above mini review indicates the complexity of the trypanosome – triatomine relationship and indicates that research into basic aspects of parasite – invertebrate host interactions can reveal subtle mechanisms for the establishment of parasite infection, that are transmitted by blood-feeding insect vectors. Regardless of the medical importance of triatomines and their high suitability for experimentation, remarkably little is understood about their interactions with trypanosomes. The demand for this kind of study is increasing daily with the need to find new methods of vector control. Many points remain to be answered, such as: (i) what other molecules are present in the digestive tract and involved as modulators of parasite infection by controlling the interaction with the gut epithelial cells and perimicrovillar membranes whose functions are complex and poorly understood; (ii) how do the eicosanoid pathways and hormones induce immune depression in the vector at the molecular level; (iii) how do genes, parasites and vector molecules interact and impact the superoxide and nitric oxide and other reactive radicals; and (iv) what, if any, role do the inducible antimicrobial peptides play as they could potentially influence survival and parasite establishment in the host vector.

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RESUMO

Esta revisão destaca aspectos dos estágios do desenvolvimento de *Trypanosoma cruzi* e *Trypanosoma rangeli* em seu hospedeiro invertebrado, *Rhodnius prolixus* Atenção especial é dada às interações desses parasitas com moléculas presentes no tubo digestivo e na hemolinfa e efeitos da organização das células epiteliais sobre o desenvolvimento do parasita. A susceptibilidade do inseto vetor ao *T. cruzi*, o qual desenvolve no tubo digestivo, dependem do estado nutricional do hospedeiro, da cepa do parasita e de interações moleculares com compostos tripanolíticos, lectinas e bactérias residentes no tubo digestivo. O *T. rangeli* invade a hemocele e pode ser reconhecido e ativar o sistema de defesa do inseto vetor, ou seja, o sistema profenoloxidase, fagocitose, microagregação hemocitária, atividade de superóxido e óxido nítrico e a via de biossíntese dos eicosanóides. Estes fatos fornecem uma compreensão melhor das interações parasita-inseto vetor, e também oferecem novas idéias sobre os processos básicos envolvidos na transmissão parasitária.

Palavras-chave: Tripanosomas, *Rhodnius prolixus*, vetor, parasitas.

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