Biological Factors Involving *Trypanosoma cruzi* Life Cycle in the Invertebrate Vector, *Rhodnius prolixus*

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Trypanosoma cruzi, the ethiologic agent of Chagas disease (Chagas 1909), is transmitted through triatomine vectors during the meal on the invertebrate host. The parasite displays quite distinct morphological and functional forms, alternating between replicative stages (epimastigotes in the vector midgut and amastigotes in vertebrate cells) and infective, non-dividing forms (metacyclic trypomastigotes in the vector, which are deposited together with feces and urine, and bloodstream trypomastigotes in mammals) (for revision see Brener 1973, Zeledon 1974, Garcia & Azambuja 1991, 1996, Gonzalez et al. 1998a).

Because of the importance of Chagas disease in Latin America a great effort has been devoted to develop an experimental model that could imitate basic features on *T. cruzi* life cycle in the invertebrate vector. Thus, we developed in our laboratory the *Rhodnius prolixus* model to study several parameters related to the high degree of interaction between *T. cruzi* and invertebrate vector. Basically, this model implies in feeding of larvae and adults of *R. prolixus*, through a special membrane feeding apparatus, on blood containing different strains/clones of *T. cruzi* and, at different intervals, determination of the number of parasite in the gut, urine and feces of the vector (see Garcia & Azambuja 1991, 1996, Gonzalez et al. 1998a, 1999). Herein, we will describe experiments, using this useful parasite-vector model, to elucidate some mechanisms involved in the interaction *T. cruzi*-triatomine insect and also link insect factors with the success or the failure of strains/clones of parasites to establish the infection in the digestive tract of the vector.

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TRYPANOSOMA CRUZI AND TRIATOMINE FACTORS

The *T. cruzi*-triatomine host interaction is complex and mediated by several parasite and insect factors (Garcia & Azambuja 1991, 1996, Gonzalez et al. 1998a). The parasite multiplies and differentiates in the triatomine gut. If bloodstream trypomastigotes are ingested by the vector, the first transformation into epimastigotes occurs in the stomach and initiates few hours after parasite ingestion. The rate of differentiation and development of the parasite in the vector gut depends on the strains/clones of parasites and vector species (Garcia et al. 1984a). The transformation of epimastigotes to metacyclic trypomastigotes occurs in the entire gut but predominantly in the rectum (Brener 1973, Zeledon 1974, Garcia & Azambuja 1991, 1996). Similarly, the metacyclogenesis of different *T. cruzi* strains/clones are related to the nature of the parasite and its susceptibility to a triatomine species (Garcia et al. 1986a).

The establishment of *T. cruzi* infection in the gut of the invertebrate host is affected by several insect factors. Between these factors include a crop lytic factor which was purified, characterized as a basic peptide which lysed the erythrocyte membrane, releasing free hemoglobin for digestion (Azambuja et al. 1983). The crop hemolytic factor lysed many strains/clones of parasites, with different lysis kinetics (Azambuja et al. 1989a, Mello et al. 1996). For instance, *in vitro* experiments with epimastigotes of different *T. cruzi* strains/clones demonstrated that some flagellates are more resistant than others to the action of this lysing agent (Azambuja et al. 1989a,b). Thus, Y strain of *T. cruzi* is more susceptible to the incubation with this factor than Dm28c clone of this parasite. *In vivo* experiments confirmed these findings, i.e., Y strain of *T. cruzi* has low levels of development in

Rhodnius and Dm28c clone of this parasite develops quite well in the vector (Garcia & Azambuja 1991). Also, lectins are factors involved in the establishment of the *T. cruzi* infection in the insect vector. Mello et al. (1996) demonstrated that infectivity in the gut is more complex correlating with agglutination of the parasite strain/clone by the gut extract. Thus, DM28c clone of *T. cruzi* is agglutinated and achieves a high infectivity levels while Y strain of the parasite, in contrast, is not agglutinated and fails to develop in the vector gut. These data indicate that gut lectins and crop hemolytic factor can be important determinants of infectivity in the parasite-triatomine vector interaction. Several aspects on this subject are discussed by Azambuja et al. (present Symposium).

Fraidenraich et al. (1993) isolated peptides from the hindgut of *Triatoma infestans* resulting from the digestion of a component of the α^D-globin chain of chicken blood that are capable to induce the metacyclogenesis of *T. cruzi in vitro*. Later, Garcia et al. (1995) demonstrated that differentiation of epimastigotes into metacyclic trypomastigotes in the insect's intestine is obtained if either hemoglobin or peptides corresponding to residues 30-49 and 35-73 of α^D-globin chain are added to an infective plasma diet. They postulated that globin fragments, released by proteolytic enzymes attacking hemoglobin, modulate the dynamics of *T. cruzi* transformation from epimastigotes into metacyclic trypomastigotes in the vector's gut.

TRYPANOSOMA CRUZI AND TRIATOMINE NEUROENDOCRINE INTERACTION

Garcia and Rembold (1984) and Garcia et al. (1984b) demonstrated that azadirachtin, an insect growth inhibitor, blocks the ecdysis in *R. prolixus*. Along with the inhibitory effect on moulting, azadirachtin also prolongs the duration in the stage followed by death (Garcia et al. 1984b, 1990). Garcia et al. (1986b, 1987) described that the sensitive period to azadirachtin in *R. prolixus* is coincident with the head critical period (HCP), i.e., the period that the insect´s brain is essential for inducing the moulting (for details see Garcia et al. 1990). Clearly, azadirachtin decreases the ecdysteroid levels in the hemolymph (Garcia et al. 1986b, 1987) and blocks synthesis of the new cuticle, which is dependent on the ecdysteroid hormones (Garcia et al. 1986a). As a consequence of these facts ecdysial stasis as induced by azadirachtin is reversed by treatment of *R. prolixus* larvae with ecdysone (Garcia & Rembold 1984). Interestingly, *in vitro* experiments indicated that only high concentration of azadirachtin directly affects the production of ecdysteroids by the prothoracic glands *in vitro* (PGs) but physiological dose does not (Garcia et al. 1987). Therefore, we postulated that azadirachtin diminishes the production of prothoracicotropic hormone (PTTH, brain hormone) that stimulates the production of ecdysteroids rather than affects directly ecdysteroid synthesis in the PGs. Garcia et al. (1990), using head-transplant techniques and measuring the ecdysteroid levels in the hemolymph, confirmed this hypothesis. They demonstrated that heads obtained from insects treated with azadirachtin transplanted into control headless insects decrease the ecdysteroid levels in the hemolymph. In converse experiments, heads taken from control insects implanted into brainless azadirachtin-treated insects enhance the levels of these hormones in the hemolymph (Garcia et al. 1990). Thus, they suggested that larvae of *R. prolixus* treated with azadirachtin decreases the synthesis and release of PTTH, consequently affecting ecdysteroid production in the PGs (Garcia et al. 1990). All these facts imply that the insect is not able to ecdysis to the next instar (Garcia et al. 1984b, 1986b, 1987).

On the other hand, Nogueira et al. (1997) demonstrated that azadirachtin causes drastic changes in the epithelial cell organization of the midgut, which, after a blood meal, is normally composed of epithelial cells and an associated membrane named perimicrovillar or extracellular membrane. These modifications included: clumps of the microvilli, disorganization of the extracellular membrane layers, and alteration in the organization of the basal portion of the epithelial cells. According to Garcia et al. (1991) these midgut alterations are due to extensive changes in the neuroendocrine system induced by azadirachtin. Moreover, Gonzalez et al. (1998b) demonstrated, using decapitation, head transplantation, and ecdysone therapy, that insects decapitated before HCP present the same midgut alterations observed in azadirachtin-treated insects. Also, they showed that head transplantation and ecdysone therapy partially reverse the decapitation effect on the development of the extracellular membranes. Thus, Gonzalez et al. (1998b) postulated that a brain factor (possibly PTTH) may be a factor responsible for the midgut cell organization in *Rhodnius*.

Furthermore, it was postulated that azadirachtin can affect not only the triatomine but also *T. cruzi* development. Garcia's group demonstrated that azadirachtin applied at different intervals before, during, or after *T. cruzi* infection, reduces the number of parasites in the gut (Garcia et al. 1989a,b). A simple dose of azadirachtin is able of preventing infection or re-infection of the vector by the parasites for long time (Gonzalez & Garcia 1992). After feeding *R. prolixus* with blood infected with *T. cruzi* clone Dm28c without azadirachtin, the

number of parasites increases 15 fold within three weeks of infection, but in the azadirachtin fed insects the infection drops within the same period to 14%, and after 30 days no parasites can be detected (Garcia et al. 1989a,b). In addition, to the total loss of the ingested parasites, a later infection with *T. cruzi* is inhibited for up to 120 days at least (Gonzalez & Garcia 1992). Finally, a direct toxic effect on the parasite can be nearly excluded, becasue *T. cruzi* cultivated in LIT-medium are not affected by annaddition of azadirachtin. *T. cruzi* also develops normally if blood is incubated with azadirachtin and then injected into a mouse (Garcia et al. 1989a,b).

Taken together, the effect of azadirachtin, decapitation, head transplantation and ecdysone therapy on the gut organization and the action of azadirachtin on the development of *T. cruzi* in the gut, we suggested that the changes induced on the gut epithelium by blood feeding may be responsible for the establishment of *T. cruzi* infection in the insect vector. Recently, Gonzalez et al. (1999) explored the techniques of decapitation and head transplantation on the development of *T. cruzi* in *R. prolixus*. They demonstrated that decapitation as well as azadirachtin treatment induce a diminishing of the parasite infection. In converse experiments, head transplantation and ecdysone therapy reestablish the development of *T. cruzi* infection in headless and azadirachtin-treated larvae. They also observed a direct relationship between the development of extracellular membrane layers in the gut and the growth of *T. cruzi* (Gonzalez et al. 1999). They postulated that a neurohormone, possibly PTTH, has an important role in maintaining, directly or indirectly, the ultrastructural organization of the *R. prolixus* gut, and that the extracellular membranes are involved in the establishment and development of *T. cruzi* in the gut of this vector. Other experiments related to the hypothesis on interaction of the neuroendocrine system, gut organization and *T. cruzi* development in the triatomine vector are under way in our laboratory giving us a new possible strategy to combat Chagas disease.

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