Immunity in *Rhodnius prolixus*: Trypanosomatid-vector Interactions

P Azambuja/+, D Feder, CB Mello*, SAO Gomes, ES Garcia

Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil  *Departamento de Biologia Geral, Universidade Federal Fluminense, Niterói, RJ, Brasil

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Many insects respond to a bacterial infection with the stimulation of distinct cellular and humoral defense system, that cooperate in a more or less integrated way to decrease the chance of microorganisms becoming pathogens. Cellular reactions include phagocytosis, nodule formation, and in some cases encapsulation and other factors related to immune system as prophenoloxidase (proPO) system, lectin, lysozyme and induced peptides such cecropin, attacin and other factors (Ratcliffe & Rowley 1979, Dunn 1986, Boman & Hultmark 1987). Feder et al. (1997) demonstrated, when *Rhodnius prolixus* was challenged with *Enterobacter cloacae*, the importance of the effects of diet components on the immune reactivity. For example, plasma diet induced immune depression. Ecdysone therapy counteracted the immune depression in *Rhodnius* larvae fed on plasma diet alone (Feder et al. 1997).

In spite of the extensive research conducted over the last few years on the molecular bases of these responses, the regulation on the blood sucking insect defense reaction against parasite remains relatively poor understood. Many trypanosomatides develop their cycles in the hemocel and/or digestive tract of the insect vector. While in *R. prolixus* the development of *Trypanosoma cruzi* (causative agent of Chagas disease) is confined to the gut lumen, *T. rangeli* develops in the gut but clearly invades the hemolymph and survives free in the blood or inside the hemocytes (Brener 1972, D’Alessandro 1976, Garcia & Azambuja 1991).

The importance of the vector immune system as an essential component of the parasite-insect vector relationship has recently been recognized (Molyneux et al. 1986, Kaaya 1989, Ingram & Molyneux 1991, Mello et al. 1995). In this paper we present findings to support the hypothesis that the vector immune system may have a role in the trypanosomatid-triatomine interaction.

**TRIATOMINE REACTIONS AGAINST T. CRUZI**

Isola et al. (1981, 1986) suggested the involvement of factors from the intestine in the development of *T. cruzi*. The parasite develops within the digestive tube of the vector where the presence of digestive enzymes and a hemolytic factor produce a potentially hostile environment for this parasite (Azambuja et al. 1983, Garcia 1987, 1989a,b). Recently, Mello et al. (1995) studied the course of infection of *T. cruzi* (clone Dm28c) and its interaction with hemolymph components of *R. prolixus*. The main conclusion of this paper was that *T. cruzi* had no division, did not induce trypanolytic and antibacterial molecules, but induced high lysozyme and nodule formation levels when inoculated in the hemolymph. Furthermore, the number of *T. cruzi* in the hemolymph was directly correlated with PO activities which decreased soon after the parasite disappear. It seems that *T. cruzi* has no ability to escape from some immune reactions of the hemolymph.

Mello et al. (1996) also made a differential *in vivo* and *in vitro* study of three strains of *T. cruzi* in the gut and hemolymph of *R. prolixus*. Basically, they demonstrated that both *T. cruzi* strains Dm28c and CI successfully infected the gut as evidenced by over than 50% of insects having infective forms in feces and urine a month after feeding with parasites. Concomitantly, both of these strains were agglutinated but no lysed by the crop homogenates. By contrary, *T. cruzi* Y strain, showed no agglutination, but some lysis in the crop, and consequently rapidly disappeared from the gut, as already described by Azambuja et al. (1989 a,b). After in-
oculating into the hemocel, only the Cl strain survived and maintained with high number of parasite circulating. That strain was also the only one which agglutinated in the hemolymph. The other two strains, Dm28c and Y, rapidly disappeared from the hemocel probably due to the action of the cellular immune reaction. Finally, these author demonstrated that carbohydrate on the parasite surfaces had differences between the three strains. Therefore, gut and hemolymph lectins and parasite surface carbohydrates could be important determinants of infectivity in the trypanosomatid triatomine interaction. It is difficulty of making generalizations about such parasite-host interactions without taking into consideration many strains of parasites tested. The activity of agglutinins found in the gut tissues and hemolymph of Rhodnius was also tested using rabbit erythrocytes (Ratcliffe et al. 1996). They demonstrated that crop, midgut, hindgut, and crude hemolymph contained hemagglutinins. The agglutinins were produced by the vector rather than absorbed and concentrated from the blood meal. The gut extracts obtained from insects fed on rabbit plasma had strong activity despite the fact that rabbit plasma failed to agglutinate the erythrocytes. Carbohydrate and glycoprotein inhibition studies of the crop, midgut, and hindgut also failed to detect a simple sugar which inhibited the agglutination of these tissues. The only inhibitory compounds for any of the gut hemagglutinins or crop parasite agglutinin were sugars linked to p-nitrophenol such as p-nitrophenyl-α-D-galactopyranoside which effectively inhibited all the agglutinins. Also, p-nitrophenol without linkage to any sugar residue was also inhibitory. Inhibition studies with the crude hemolymph agglutinin demonstrated, in agreement with Pereira et al. (1981), that galactose containing sugars, including galactose, lactose, D-galactosamine, and methyl-D-galactopyranoside were inhibitory. An SDS-PAGE gel of the hemolymph lectin following elution from the galactose-bound, Sepharose 6B minicolumn, demonstrated a single band of the lectin with molecular weight of 40 kDa.

INTERACTION BETWEEN T. RANGELI AND R. PROLIXUS

As we already described, T. rangeli develops in the gut, invades and survives in the hemolymph and thus it is probably recognized by the immune system of the insect vector. Tobie (1968, 1970), Takle (1988) and Mello et al. (1995) demonstrated that following inoculation into the hemolymph of Rhodnius, T. rangeli survives and multiplies. In vitro experiment of R. prolixus hemolymph from insects inoculated with T. rangeli, there were further consistent change in erythrocyte agglutination nor induction of antitrypanosome activity. However, levels of PO, lysozyme, and hemocyte numbers in the hemolymph significantly enhanced after inoculation of this parasite. Nodule formation increased during the entire experiment indicating that T. rangeli although recognized and enclosed within the cellular defenses, is capable to survive and to utilize the cells involved for multiplication (Takle 1988).

Pereira et al. (1981) and Gregorio and Ratcliffe (1991b) described lectins in the crop, midgut, and hemolymph of R. prolixus able to agglutinate trypanosomes. Gregorio a Ratcliffe (1991b) also reported a wider distribution of lectins in T. infestans, than in Rhodnius, and postulated that these molecules may have imparted to Triatoma into refractoriness to infect with T. rangeli. An alternative candidate for immune modulation of T. rangeli invasion is the proPO. The proPO, an inactive precursor of PO found in the plasma fraction of hemolymph (Pye 1974) or in the hemocytes (Leonard et al. 1985) of invertebrates, can be activated by proteases, such as trypsin and chymotrypsin, bacteria, and fungi, or by their cell wall components (Ashida et al. 1983). In R. prolixus, the activation of the proPO pathway was observed in insects inoculated with bacteria or trypanosomatids (Azambuja et al. 1986, 1989a,b, Gregorio & Ratcliffe 1991b, Mello et al. 1995, Feder et al. 1997). Gregorio and Ratcliffe (1991a), using in vitro activation of the proPO system, showed that the T. rangeli infection of R. prolixus, at least in part, may have been related to the suppression of the activation of proPO in the presence of the parasite. Mello et al. (1995) demonstrated that the rate of T. rangeli development in the hemolymph of R. prolixus linearly enhanced within four days of infection. Numerous short epimastigotes of T. rangeli were present until day two in the hemolymph but after this time, they disappear to be replaced by a massive colonization by long epimastigotes. Recently, basing on these findings, Gomes et al. (1999) studied the in vivo and in vitro activation of the proPO in R. prolixus infected with short and long epimastigotes of T. rangeli, separately. The in vitro activation of the proPO pathway was low in the absence of fat body, hemolymph, and both parasites. A higher PO activity was observed when short, but not long, epimastigotes of T. rangeli were incubated with hemolymph, fat body, and the substrate L-DOPA. Similarly, the PO activity of hemolymph taken from infected insects with long epimastigotes showed a low activity if compared with hemolymph obtained from insects inoculated with short parasites. Based on these results, Gomes et al. (1999) suggest that
(a) factor(s) in the hemolymph as well as in the fat body may be released by the presence of short epimastigotes of *T. rangeli* and which results in the activation of the proPO system in *R. prolixus*. Gomes et al. (data not published) demonstrated that the factor(s) must be proteases detected in the hemolymph of insects which were fed on, or inoculated with, short epimastigotes of *T. rangeli*. In this case they were not observed in the fat body. No protease activity could be observed in both hemolymph and fat body taken from insects inoculated with, or fed on, long epimastigotes. In support of these findings Mello et al. (1999) demonstrated that in *R. prolixus* hemocyte monolayers, *T. rangeli* is able of inducing hemocyte/parasite clump formation. They also observed that purified hemolymph galactoside-binding lectin markedly increased the formation of clumps by *T. rangeli* in *R. prolixus* hemocyte monolayers, consequently with an enhance in clump size and hemocyte aggregation. This enhancement of nodule formation was specifically inhibited by addition of the galactose ligant for the lectin. Furthermore, pure lectin affected the motility and survival of short epimastigotes, but no the long ones, when they were incubated *in vitro*. Based on the present findings, we postulate that differential activation of the proPO pathway and the cellular reaction induced by short and long epimastigotes of *T. rangeli* is responsible for differences in the development and establishment of the parasites in the hemolymph of *R. prolixus*. Thus, we delineate, for the first time, an *in vivo* mechanism related to immune reactions that is involved in the infection of *T. rangeli* in the vector.

REFERENCES


