Vaccination with epimastigotes of different strains of Trypanosoma rangeli protects mice against Trypanosoma cruzi infection

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In our laboratory, we have developed a model of vaccination in mice with Trypanosoma rangeli, a non-pathogenic parasite that shares many antigens with Trypanosoma cruzi. The vaccinated mice were protected against infection with virulent T. cruzi. The goal of the present work was to study the protective activity of strains of T. rangeli of different origin, with the aim of analysing whether this protective capacity is a common feature of T. rangeli. BALB/c mice were vaccinated with live or fixed epimastigotes of two T. rangeli strains, Choachi and SC-58. Vaccinated (VM) and control mice (CM) were infected with virulent T. cruzi, Tulahuen strain. The results showed that the levels of parasitemia of VM, vaccinated with the two strains of T. rangeli were significantly lower than those developed in CM. The survival rate of VM was higher than that CM. Histological studies revealed many amastigote nests and severe inflammatory infiltrates in the heart and skeletal muscles of CM, whereas in the VM only moderate lymphomonocytic infiltrates were detected. Altogether, the results of the present work as well as previous studies show that the antigens involved in the protection induced by T. rangeli are expressed in different strains of this parasite. These findings could prove useful in vaccine preparation.

Key words: vaccination - Chagas disease - Trypanosoma rangeli - Trypanosoma cruzi

Infection with Trypanosoma cruzi, the protozoan agent of Chagas disease, affects nearly 16 million people, and 75-90 million people are exposed in Latin America (Coura 2007). In several Latin American countries, there is another trypanosome, T. rangeli, which can infect humans but is non-pathogenic (Guhl & Vallejo 2003). Both parasites share endemic areas and vectors, and the two have a strong antigenic relationship (Basso et al. 1987, Stevens et al. 1999).

As with many other parasitic diseases, there is no effective vaccine for humans yet available, despite numerous studies performed with different antigenic materials. Approaches ranging from subcellular fractions to recombinant antigens and even plasmid DNA-encoding antigens of T. cruzi have been used (Taibi et al. 1995, Costa et al. 1998, Wrightsman & Manning 2000, Planelles et al. 2001, Garg & Tarleton 2002). In our laboratory, we have developed a model of vaccination for mice with T. rangeli, in which mice were protected by fixed epimastigotes of a Colombian strain of T. rangeli. The immunization induced a strong reduction in parasitemia and mortality levels when compared with non-vaccinated infected mice (Basso et al. 1991).

The aim of this work was to analyze the evolution of T. cruzi infection in mice that were previously vaccinated with live and fixed epimastigotes of two strains of T. rangeli of different geographical origins, to determine if the protection against T. cruzi infection is a common feature across different T. rangeli isolates.

MATERIALS AND METHODS

Animals - Balb/c mice were used and maintained in our laboratory under standard conditions

Parasites - The Tulahuen strain of T. cruzi was maintained through weekly subcutaneous inoculations of Balb/c albino mice. Bloodstream trypomastigotes used for challenging inoculations were obtained by cardiac puncture on day 14 post infection (pi). The SC-58 and Choachi strains of T. rangeli, kindly provided by the Laboratório de Protozoologia, Univ. Fed. Sta. Catarina, Brazil, were used. The epimastigote forms were cultured in LIT monophasic medium. Parasites were harvested in the exponential phase of growth and the immunization material was prepared in two different ways: (i) live epimastigotes (L-EPI) and (ii) fixed epimastigotes (F-EPI); these were obtained as previously described (Basso et al. 1991). Briefly, parasites were washed three times in PBS and centrifuged at 10,000 g at 4°C/ 20 min. Then, they were fixed with glutaraldehyde (0.1%) for 15 min at room temperature, following 15 min at 4°C and washed again. Just before immunization, 1x10^9 parasites/ml were emulsified with saponin.

Vaccination and infection schedule - Groups of 7-8 Balb/c mice (3 weeks old) were vaccinated with epimastigotes of SC-58 or Choachi strain, as previously described (Introini et al. 1998, Ceravetta et al. 2003). Group (a): mice were inoculated intraperitoneally with a volume
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0.2 ml containing $1 \times 10^7$ live epimastigotes 28 days before. Group (b): mice received subcutaneous injections in the thigh of 0.2 ml of antigenic emulsion containing $1 \times 10^8$ fixed epimastigotes 28, 21, 7 days before. Group (c): mice received 0.2 ml of PBS (control group).

All mice were infected at day 0 by intraperitoneal inoculation of 1,500 blood trypomastigotes of *T. cruzi*. The parasite counts were performed in a Newbauer chamber, using 0.85% NH$_4$Cl as diluent (Hoff 1974). The survival rates were checked daily until day 40 pi.

All experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in ILAR (1996).

**Histological studies** - Mice from all experimental groups were sacrificed via ether anaesthesia. The heart, spleen, and quadriceps skeletal muscle were immediately removed, fixed in buffered 10% formalin (pH 7.0) and embedded in paraffin. Sections, 5 µm thick, of each organ were collected at a systematic distance of 10 sections apart. A total of 300 µm per organ were analyzed. All sections were stained with hematoxylin and eosin. At least 25 areas from each section were checked for parasites and histopathology under a 40X objective in a blind study.

**Statistical analysis** - The experimental data were analysed using Student's $t$ Test. All the results were considered statistically significant at a level of $p < 0.05$.

**RESULTS**

**Parasitemia** - Fig. 1 shows the results of a representative experiment performed in animals vaccinated with the L-EPI or F-EPI *T. rangeli* Choachi strain and in non-vaccinated mice (control group), where all of the groups were infected with *T. cruzi*. As can be seen, by the 10th day pi, there were no differences in parasitemia levels developed by the experimental and control groups. On the 20th day, both the L-EPI and F-EPI vaccinated groups presented significantly lower parasitemia levels than the control group ($p < 0.01$). This behavior was also observed at the 25th day pi. Similar results were observed with *T. rangeli* SC-58 strain (Fig. 2). Also in this case, the parasitemia levels of the vaccinated groups were lower than those in the control group ($p < 0.01$).

More than 90% of the mice vaccinated with both strains of *T. rangeli*, live or fixed, survived after being challenged with *T. cruzi*. At day 30 pi, the parasites were no longer detected in the circulation of the vaccinated mice. In contrast, 62-75% of the animals of the control group died within 30 days pi. A representative experiment performed with F-EPI of Choachi strain can be seen in Fig. 3.

![Fig. 1](image1.png) Levels of parasitemia in mice vaccinated with the Choachi strain of *T. rangeli* (F-EPI and L-EPI) and unvaccinated (control mice). Each time point represents the mean ± SEM of 3–4 mice of a representative experiment. Asterisks indicate significant differences between vaccinated and infected groups (Student $t$ test $p < 0.01$).

![Fig. 2](image2.png) Levels of parasitemia in mice vaccinated with the SC 58 strain of *T. rangeli* (F-EPI and L-EPI) and unvaccinated (control mice). Each time point represents the mean ± SEM of 3–4 mice of a representative experiment. Asterisks indicate significant differences between vaccinated and infected groups (Student $t$ test $p < 0.01$).

![Fig. 3](image3.png) Cumulative mortality in mice vaccinated with F-EPI of Choachi strain of *T. rangeli* and in unvaccinated control mice infected with *T. cruzi*. 

Fig. 1: levels of parasitemia in mice vaccinated with the Choachi strain of *T. rangeli* (F-EPI and L-EPI) and unvaccinated (control mice). Each time point represents the mean ± SEM of 3–4 mice of a representative experiment. Asterisks indicate significant differences between vaccinated and infected groups (Student $t$ test $p < 0.01$).
Histological analysis of the hearts of the control mice revealed the typical histopathological alterations compatible with acute chagasic myocarditis, with numerous nests of amastigotes of *T. cruzi* and numerous lymphomononuclear inflammatory infiltrates of 10 or more cells (Fig. 4A, B). In comparison, the histological studies of the hearts of some of the mice vaccinated with the L-EPI *T. rangeli* SC-58 strain revealed myocarditis in the subendocardia, myocardia and epicardia, with zones of lymphomononuclear cells infiltration, but no amastigote nest (Fig. 4C, D). The skeletal muscle samples of the control mice exhibited disorganization of the tissue architecture, necrosis, and large nests of *T. cruzi* amastigotes (Fig. 5A B), while in the vaccinated mice, the skeletal muscle showed inflammatory infiltrates which consisted mainly of mononuclear cells. Again, no amastigote nest could be detected (Fig. 5C, D). Similar results were observed in the animals vaccinated with L-EPI of Choachi strain. In all mice vaccinated with F-EPI of both strains, the inflammatory infiltrates were milder in the heart and skeletal muscle samples.

The spleens of the mice immunized with live or fixed *T. rangeli* of both strains displayed an increased T-cell area, and also revealed a dramatically increased weight with respect to the control group. The weight ratio was expressed as spleen weight in mg/total body weight in grams. For the SC-58 strain, the results were: F-EPI: 22.09 ± 0.80; L-EPI: 19.44 ± 2.33; controls: 7.83 ± 0.42 (n = 3 of each group; p < 0.001).

Tissues from the surviving control animals (infected only with *T. cruzi*) sacrificed in the chronic phase showed linear infiltrates of mononuclear cells and fragmentation of cardiac muscle fibers. The skeletal muscle showed evidence of necrosis and foci of mononuclear cells infiltrates. While the majority of immunized mice were histologically normal, some of them showed mild cardiac alterations. In some cases, the skeletal tissue revealed discrete mononuclear cell infiltrates. No amastigotes were found in either the control or in the vaccinated mice (data not shown).

**DISCUSSION**

In previous studies (Basso et al. 1991, Cervetta et al. 2002, Basso et al. 2004), we demonstrated that immunization with fixed epimastigotes of *T. rangeli* elicited B- and T-cell-specific responses to *T. cruzi*, as well as a particular pattern of cytokines and a strong reduction in the mortality rate among infected mice. Indeed, more than 95% of the mice survived a lethal *T. cruzi* infection and displayed a strongly reduced burden of parasites during the acute phase; this was associated with high levels of antibodies and cellular response, elevated serum levels of IL-12 and IFNγ, low levels of pro-inflammatory cy-
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Fig. 5: Histological sections of skeletal muscles. A, B: Control groups show nests of amastigotes (thin arrows) and mononuclear cells infiltration (thick arrow); C, D: Representative sections from mice vaccinated with L-EPI of *T. rangeli* SC-58 strain. Infected mice show focal mononuclear cell infiltrates (thick arrows). No amastigote nest was observed (A, C: 100X; B, D: 400X).

tokines (IL-6 and TNFα) and normal levels of IL-10. The immunization induced an adequate balance of Th1 and Th2 responses, which are both necessary for the induction of a protective response.

In our present work, we demonstrated that the protection elicited by *T. rangeli* is a common feature displayed by at least three strains of *T. rangeli* of different geographical origins. In fact, in this study, we observed that immunization with epimastigotes of both the Brazilian strains of *T. rangeli* SC-58 as well as the Colombian Choachi strain is sufficient to protect mice against a virulent *T. cruzi* strain, as was demonstrated with another Colombian strain (Basso et al. 1991). The vaccinated mice displayed low parasitemia, increased survival rate, absence of tissue amastigote nests in the acute period and an absence of histopathological lesions in the chronic period. The fact that this protection could be induced by strains of different origins is important for future vaccination strategies. These results are in accord with those reported by Zuñiga et al. (1997) and Palau et al. (2003). Moreover, using another experimental model, Araujo et al. (1999) have demonstrated that vaccination of BALB/c mice with a combination of killed *Leishmania* promastigotes with BCG before challenging with *T. cruzi* prolonged their survival rate and decreased their parasitemia levels. It is interesting to note that the level of protection in mice depended on the number of *T. rangeli* used for immunization. In fact, as has been demonstrated in previous work (Cervetta et al. 2003), the minimum protective doses were $10^7$/mouse with L-EPI and $10^8$/mouse with F-EPI.

Although both immunogens (L-EPI and F-EPI) were able to induce protection, we assume that the use of live parasites in future human vaccines will be restricted due to the increased number of immunocompromised patients. Moreover, in our work, we have observed differences in histological studies that have revealed increased inflammatory infiltrates in animals vaccinated with live parasites. Taking these results into account, we have decided to continue our research on vaccination employing dead parasites.

At present, it is not known if infection with *T. rangeli* may change the course of infection with *T. cruzi* in humans. This does not exclude the possibility that an exposure to *T. rangeli* may elicit a particular humoral and/or cellular immune response that confers some degree of protection against subsequent infection with *T. cruzi* (Palau et al. 2003, Caballero et al. 2007).

In conclusion, the results of the present work show that the antigens involved in the protection induced by *T. rangeli* are expressed in different strains of this parasite; this could be an advantage for developing future vaccination strategies for Chagas disease. In this sense, it will be also useful to advance in the identification of those protective antigens in order to use purified or synthesized molecules of *T. rangeli* in protection assays.
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