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BRIEF COMMUNICATION
CRYPTIC INFECTIONS IN MICE WITH THE *Trypanosoma cruzi* CL-14 CLONE

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KEYWORDS: *Trypanosoma cruzi*; CL-14 clone; Infectivity.

The CL-14 clone was obtained from *Trypanosoma cruzi* CL strain by CHIARI[1] and under certain culture conditions can present high metacyclogenesis rates, but its metacyclics have been considered non-infective for mice, as evaluated by parasitemia, hemoculture and xenodiagnosis, and also unable to determine tissue parasitism or histopathological lesions, despite heavy inocula used in attempts to infect the animals[2,3]. Otherwise, mice inoculated with those metacyclics showed high resistance when challenged with a virulent *T. cruzi* strain. Then, this clone was considered a good antigen to study acquired immunity against *T. cruzi*, as well as a potential candidate for development of a vaccine against this parasite[2,3].

At the first step of this work, we examined whether metacyclics of the CL-14 clone were susceptible to complement-mediated lysis, since this could be an explanation of its noninfectivity. Then, culture stages of this clone grown in Yaeger's liver infusion-tryptose medium (LIT) overlaying blood agar base (NNN) were incubated for 30 min at 37 °C with 25% fresh undiluted guinea-pig serum. However, like other *T. cruzi* isolates[4], the CL-14 clone epimastigotes were lysed and its metacyclics were not. Subsequently, some experiments were carried out to verify whether metacyclics recovered from the complement lysis assay had become infective for mice. The results obtained led us to review the CL-14 clone infectivity using also culture-derived metacyclics which had not been incubated with fresh guinea-pig serum. In all experiments, CL-14 clone metacyclics proceeded from NNN+LIT medium, without passage in any metacyclogenesis inducer medium. Throughout this work, 0.2-1 x 10^6 metacyclics were intraperitoneally inoculated into 10-18 g male BALB/c mice. From the 6th to the 60th day post-inoculation (p. i.), or until the mice were killed for hemocultures or used for xenodiagnosis, about 5µl of tail blood from each animal were, at least 3 times a week, examined under the optical microscope (X400). Hemocultures and xenodiagnoses were followed for a time longer than accompanied by LIMA *et al.*[2].

No patent parasitemia was found in mice inoculated with the CL-14 clone, confirming previous results from LIMA *et al.*[2]. Subsequently, mice at the 13-29th day p. i. were bled by heart puncture and their blood seeded in NNN+LIT medium. The hemocultures were examined at least on the 30th, 60th, 75th and 100th days after seeding. Four out of 13 hemocultures (30.8%) obtained from mice inoculated with complement-treated metacyclics were positive for typical *T. cruzi* stages (epimastigotes and sometimes metacyclics). This positivity was confirmed on the 60th, 75th and 100th cultivation days. Three out of 12 hemocultures (25%) proceeding from mice inoculated with metacyclics not treated by complement also gave positive results, but only on the 100th day. In this experiment, 7 negative hemocultures were followed by a longer time, remaining negative by the 150th day, but one of them presented typical *T. cruzi* stages on the 240th day. In these hemocultures, the LIT medium had been supplemented with additional 10% fetal calf serum.

Xenodiagnoses were carried out by feeding 5 nymphs of *Panstrongylus megistus* directly on each mouse inoculated with the CL-14 clone. Five mice were used in this test, all of them on the 42nd day post inoculation with metacyclics not treated by active guinea-pig serum. Each group of 5 triatomines fed on the same mouse was placed in separate flasks, the bugs being maintained by feeding on normal mice at 30-day intervals until 90 days. Ten days later, *i.e.* 100 days after the bugs had fed on mice inoculated with the CL-14 clone, they were dissected and their mid- and hind-gut contents examined under the optical microscope (X400). Epimastigotes (at very low number) were only found in two bugs fed on the same mouse. Coprocultures were also attempted from pooled intestinal contents of triatomines from each group. However, positive culture was only achieved from intestinal contents of that group which had the above-mentioned infected bugs.

Experiments were also performed to verify whether the CL-14 clone reisolated in axenic culture after passage in mice had become able to determine patent parasitemia. Then, two groups of 6 mice were inoculated with metacyclics from hemocultures obtained from animals infected either with complement-treated parasites or with untreated ones. No bloodstream trypomastigotes were observed in these mice by microscopic examination until the 30th day p. i.; hemocultures were not done.

Our results evidence the occurrence of cryptic infections in mice inoculated with the CL-14 clone, those usually detected later on by hemoculture or xenodiagnosis. The possibility is considered that, although only a very low number of parasites being present in mice

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infected by CL-14 clone, they could be sufficient to elicit immunological mechanisms accountable for the strong protection observed in mice inoculated with this clone and then challenged with a virulent *T. cruzi* strain[2]. It is worthy mentioning that, under our experimental conditions, culture-derived metacyclics from another clone of the CL strain (CL Brener) determined patent parasitemia and 100% mortality in mice.

RESUMO

Infecções subpatentes em camundongos pelo clone CL-14 do *Trypanosoma cruzi*

A infectividade do clone CL-14 do *Trypanosoma cruzi* para camundongos foi revista utilizando-se como inóculo metacíclicos de cultura em NNN+LIT, pré-incubados ou não com complemento de cobaio. Nos animais inoculados não observamos parasitemia patente, mas a presença do parasito foi confirmada em 30% deles (9/30) através de hemocultivo ou xenodiagnóstico, este examinado aos 100 dias. A positividade das hemoculturas pôde ser evidenciada a partir dos 60 dias quando procederam de camundongos inoculados com metacíclicos tratados com complemento. Nos demais hemocultivos a positividade foi constatada aos 100 dias ou posteriormente. Um reisolado do CL-14 também não determinou parasitemia patente em camundongos até 30 dias após a inoculação. Estes achados são discutidos em relação à proteção imunológica observada em camundongos inoculados com este clone.

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