

INVESTIGATION ON THE POSSIBILITY OF SPONTANEOUS CURE OF MICE INFECTED WITH DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI*

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SUMMARY

Seventy Swiss mice chronically infected with different strains of *Trypanosoma cruzi*, with persistently negative parasitemia on routine blood examination were parasitologically investigated to find out whether spontaneous cure occurred. Duration of infection varied from 90 to 250 days in the initial phase of this investigation. Parasitological tests consisted of daily direct blood examination performed during at least 25 days, followed by xenodiagnosis and subinoculation of blood into newborn mice. Mice that persisted negative were treated with Cyclophosphamide with one dose of 250 mg/kg of body weight and then investigated by direct blood examination, xenodiagnosis and subinoculation. A second dose of 250 mg/kg b. w. was given to the persistently negative mice. With one single exception, all mice showed positive parasitological tests in the different stages of the present investigation and we conclude that spontaneous cure did not occur in this group, which is representative of the chronic infection with different strains of *T. cruzi*.

KEYWORDS: *Trypanosoma cruzi*; Spontaneous cure; Chronic infection; Murine model; Cyclophosphamide.

INTRODUCTION

In experimental *T. cruzi* infection of mice, a percentage of animals can survive the acute infection and lapse into a chronic phase. Persistently negative parasitemia that represents a subpatent infection, could be taken as an indication of autocure. However it is difficult to demonstrate a real parasitological cure in chronically infected mice, due to the paucity of parasites present during this phase. Even repeated peripheral blood examination can fail to disclose a subpatent infection. Positive serology (indirect immunofluorescence test) does not confirm the presence of a patent infection since it can be positive in parasitologically cured animals². Furthermore, the complement mediated lysis test, depending on the strain, can be negative even in parasitologically positive mice⁴. However, the

possibility of a spontaneous cure could be considered when daily parasitological blood examination together with different parasitological tests such as xenodiagnosis, subinoculation of blood into newborn mice, and hemoculture show persistently negative results.

In the present investigation mice chronically infected with *T. cruzi*, with negative parasitemia by routine examination were evaluated to test the possibility of spontaneous cure. Besides an accurate and repeated direct search of parasites in peripheral blood, the above mentioned parasitological tests, were performed. In persistently negative animals, an immunosuppressive treatment with high doses of Cyclophosphamide (CY), known to stimulate parasite multiplication¹¹ was used.

Results have shown that autocure had not occurred in these mice.

MATERIAL AND METHODS

Seventy Swiss mice chronically infected with different strains of *T. cruzi*, were used in the present investigation. All the animals were previously submitted to direct examination of peripheral blood for the search of trypomastigotes with negative results.

Strains of *T. cruzi*: Fifty two mice were infected with Type II strains (12 SF, 21 SF and 20 Mam) from São Felipe-BA and Mambai-GO, respectively and 18 mice with the Colombian strain, Type III, according to the classification of ANDRADE ¹. Table 1 shows the distribution of the animals according to the parasite strain.

Inocula: 4×10^3 to 10^5 blood forms were the inocula for the animals infected with Type II strains and 10^4 blood forms for those infected with the Colombian strain (Type III).

Duration of infection: Considering the initial phase of this investigation, the lapse of time between the inoculation and the parasitological evaluation was variable: 90, 120 and 240 days for the animals infected with type II strains and 120 days for those infected with the Colombian strain (Type III). For the subsequent stages in which mice were treated with CY, duration of infection is shown in Table 1.

Search of trypomastigotes in peripheral blood: Daily direct blood examination was performed under cover slips 22 x 22 in 50 microscopic fields 400 X, during 25 days. Animals persistently negative were submitted to the following parasitological tests: 1) Xenodiagnosis with five 3rd to 5th stage nymphs of

Rhodnius prolixus; 2) subinoculation of blood (0.1 µl) into suckling mice.

Treatment with Cyclophosphamide (Enduxan: Pravaz-Abbot Laboratories), was performed in those mice persistently negative in direct blood examination, xenodiagnosis and subinoculation. CY was administered in one or two doses of 250 mg/kg.b.w. intraperitoneally. After the first dose of CY the animals were submitted to the same procedures above described for the search of trypomastigotes in peripheral blood; the second dose was administered to those persistently negative after the first treatment with CY.

Serology - Indirect immunofluorescence (IIFT) test was performed using culture forms of *T. cruzi* as antigen and antimouse fluorescein conjugated gamma-globulin (dilution 1:80) according with CAMARGO ⁶.

RESULTS

With one single exception, all mice have yielded positive parasitemia with at least one of the different parasitological tests used (carried out as presented in Table 1). Patent parasitemia was disclosed in 44/70 mice by daily blood examination, during 25 days. In 9 mice xenodiagnosis revealed the subpatent infection. From 16 persistently negative animals, 8 became positive after the 1st dose of CY (6 by direct blood examination and 2 by xenodiagnosis) and 8 after the 2nd dose of CY (4 by direct blood examination and 4 by xenodiagnosis). One mouse persisted negative to parasitological tests.

Indirect immunofluorescence tests performed in the initial phase were all positive and the titres

TABLE 1
General data on mice chronically infected with *T. cruzi* submitted to parasitological tests.

<i>T. cruzi</i> strains	Number of mice	Duration of infection (days)		
		DBE* XENO	1 st CY** treatment	2 nd CY*** treatment
12 SF	33	90-240	115-265	140-290
21 SF	6	240	265	290
20 MAM	13	90	115	140
COLOMB.	18	120	145	170

* DBE (direct blood examination) and xenodiagnosis

** First treatment with Cyclophosphamide (250 mg/kg b. w.)

*** Second treatment with Cyclophosphamide (250 mg/kg b.w.) in those persistently negative.

varied from 1:40 to 1:1280. The only mouse that persisted parasitologically negative until the end of the investigation disclosed a positive IIFT with titre of 1:1280.

DISCUSSION

In the present study, mice chronically infected with *T. cruzi*, showing negative parasitemia by routine examination, became positive either after direct blood examination daily performed during at least 25 days, or to xenodiagnosis and/or subinoculation of blood into suckling mice, before or after treatment with high doses of CY, sufficient to determine immunosuppression¹⁰. All mice have disclosed a positive serological test at initial evaluation. This was not considered as sufficient to confirm the parasitological positivity since previous studies have shown that mice submitted to chemotherapy, with negative parasitological tests (subinoculation, hemoculture or xenodiagnosis) can persist with positive IIFT for long periods of time after treatment^{2,3}.

In humans, positive conventional serology has been considered by different authors as a positive indication of patent infection. Its negatization has been taken as an indication of spontaneous cure in untreated patients, as reviewed by ZELEDON et al¹⁴, who registered three cases of spontaneous cure, based on the negatization of conventional serology. His cases fit well with the concept of cure already established for the patients submitted to chemotherapy, as registered by CANÇADO⁷. In animals, THOMAZ & DEANE¹³ referred the occurrence of spontaneous cure in marsupials, based on negative tests for lytic antibodies. KRETTLI et al⁸, claim that complement mediated lysis is the only serological test that can detect epitopes on the membrane of living trypomastigotes. However, according to these same authors, an anti-complementary serum activity can be responsible for false negative results. In mice, a previous study has shown that a percentage of parasitologically positive animals disclosed negative complement mediated lysis tests⁴. In conclusion, we can not evaluate an auto-cure in mice based on the serological tests. As have been referred, cured mice can persist with positive IIFT for more than six months after treatment, while presenting *T. cruzi* antigens in dendritic cells of the spleen⁵. In such cases, only different and simultaneously performed parasitological tests can disclose a subpatent infection. Treatment with suppressor dose of CY determined a reactivation of

chronic infection, and in the present study, this contributed to the diagnosis of apparently negative cases. Similarly, a reactivation of chronic Chagas' cardiopathy has occurred in patients submitted to heart transplantation, during immunosuppressive therapy¹² and appeared as an important complication in patients with AIDS⁹. These aspects show the importance of the subpatent parasitemia that occurs in the chronically infected patients, in the indeterminate form of Chagas' disease.

From the 70 mice submitted to this study, 69 showed patent parasitemia either through a detailed and intensive application of parasitological tests or after the use of high doses of CY. We can conclude that spontaneous cure did not occur in this group of animals that are representative of the infection with different strains of *T. cruzi*, different inocula and different periods of infection. Since similar procedures are not suitable to be applied to man, reports of auto-cure in human *T. cruzi* infection should be taken with caution.

RESUMO

Investigação da possibilidade de cura espontânea de camundongos infectados com cepas diferentes de *Trypanosoma cruzi*

Setenta camundongos Suíços, cronicamente infectados com diferentes cepas do *Trypanosoma cruzi*, cuja parasitemia se manteve negativa ao exame de rotina do sangue periférico, foram investigados parasitologicamente com o objetivo de verificar se houve cura espontânea dos mesmos. A duração da infecção variava entre 90 e 250 dias quando os camundongos foram inicialmente investigados. Os testes parasitológicos usados foram: pesquisa direta de parasitos no sangue periférico, feita diariamente, durante pelo menos 25 dias, seguida de xenodiagnóstico e subinoculação do sangue em camundongos recém-nascidos. Os camundongos que mostraram resultados negativos foram tratados com uma dose de Ciclofosfamida de 250 mg/Kg peso corporal e submetidos subsequentemente a xenodiagnóstico e subinoculação de sangue em camundongos recém-nascidos. Uma segunda dose de Ciclofosfamida, de 250 mg/Kg peso corporal foi aplicada aos camundongos persistentemente negativos. Com apenas uma exceção, todos os camundongos cronicamente infectados tiveram testes parasitológicos positivos em diferentes etapas da presente investigação. Concluímos que neste grupo representativo da fase crônica da infec-

ção por diferentes cepas do *T. cruzi*, não ocorreu cura espontânea.

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