Recrudescence Induced by Cyclophosphamidine of Chronic *Trypanosoma cruzi* Infection in Mice is Influenced by the Parasite Strain

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Reactivation of chronic chagasic patients may occur upon use of immunosuppressive drugs related to kidney or heart transplantation or when they are affected by concomitant HIV infection. This recrudescence, however, does not occur in all chagasic patients exposed to immunosuppressive agents. We therefore investigated the influence of Trypanosoma cruzi strains in the recrudescence of the parasitism in mice at the chronic phase treated with cyclophosphamide, an immunosuppressant that blocks lymphocytes DNA synthesis and therefore controls B cells response. A large variation was detected in the percentages of newly established acute phases in the groups of mice inoculated with the different strains. We suggest that reactivation of chronic T. cruzi infections is influenced by the parasite intrinsic characteristics, a phenomenon that might occur in the human disease.

Key words: *Trypanosoma cruzi* - strains - immunosuppression - cyclophosphamide

*Trypanosoma cruzi* infection in humans is characterized by an acute phase with patent parasitemia which lasts one to two months followed by a lifelong chronic phase with subpatent parasitemia and scarce tissue parasites. Recrudescence of the parasitism in the chronic phase may occur upon administration of immunosuppressive agents. Thus chronic chagasic patients submitted to heart or kidney transplantation treated with immunosuppressors drugs are liable to present reactivation of the *T. cruzi* infection (Stolf et al. 1987). Acquired immunodeficiency syndrome (AIDS) is also an important hazard for chronic chagasic patients and an increasing number of patients with concomitant HIV and Chagas disease display new acute phase infection characterized by *T. cruzi* meningoencephalitis, myocarditis and patent parasitemia (Rocha et al. 1994).

Interesting, this iatrogenic renewal of the acute phase does not occur in all chronic chagasic patients exposed to immunosuppressive agents. Barousse et al. (1980) carried out complete autopsies of six chronic chagasic patients who received immunosuppressive drugs such as prednisone and cyclophosphamidine (Cy) for treatment of concurrent diseases but no evidence of reactivated parasitism was detected. Lopez Blanco et al. (1992) reported that nine chronic chagasic patients recipients of transplanted kidneys and submitted to immunosuppressive drugs have not also shown evidence of parasitism during prolonged follow-up.

Since the course of experimental Chagas disease is strongly influenced by *T. cruzi* strains and responses of the host immunity (Krettli & Brener 1976), we decided to investigate the phenomenon of recrudescence in groups of mice chronically infected with eight different strains treated with Cy, an immunosuppressant of the humoral response. Distinct patterns of parasitemia emerged from the experiments, suggesting that the model herein described may contribute to a better knowledge of immunosuppression in the human disease.

**MATERIALS AND METHODS**

*T. cruzi strains and mice inoculation* - Table I identifies the eight strains, their origin and provides information on the inocula as well as the number of days of infection of the mice used in the experiments. Female albino Swiss mice, 18-20g, were inoculated by intraperitoneal route with either *T. cruzi* bloodstream forms (BTry) or metacyclic trypomastigotes (MTry) obtained by cultivating in Liver Infusion-Tryptose (LIT) medium parasites recently isolated by hemoculture from infected mice. Each strain was inoculated in 30 mice and then 20 were treated with Cy and 10 kept for control. The mice inoculated with the different strains were kept in the laboratory for six to eight months. Before Cy administration the persistence of ongoing *T. cruzi* infection was confirmed by hemocultures carried out by inoculating 0.2-0.4 ml of blood collected from mice orbital venous sinus into two tubes containing 5 ml of LIT medium that were incubated at 26-28°C and examined microscopically for living flagellates after 30-60 days. The number of BTry in the
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The percentage of reactivation of the Cy treated mice and the number of parasites at the peak of parasitemia compared with the control values were used as criteria for considering the infection recrudescence. Fig. 1 shows the percentages of reactivation in the groups of chronically infected mice inoculated with different *Trypanosoma cruzi* strains immunosuppressed by Cy. The infection recrudescence occurred in a range of 90.3% to 10.7% according to the strain used. Table II shows the numbers of blood forms in the peak of parasitemia in mice treated with Cy. Although the standard deviation in the outbred mice is rather high, the differences between mice treated with Cy and the untreated animals are highly significant and corresponds to Fig.1. Figs 2A-D show patterns of parasitemia curves in immunosuppressed and untreated mice groups. They show that there is no correlation between the subpatent or residual levels of parasitemia from the untreated animals and the parasitemia of the immunosuppressed mice. Mice inoculated with Buriti and J strains (Figs 2A, B) which show low numbers of circulating parasites by fresh blood examination display, when submitted to Cy treatment, peaks of parasitemia of, respectively, 80,000 and 3,000 BTry/5 µl. On the other hand, the groups of mice inoculated with the VL-10 and CL strains (Figs 2C, D) which induce steady subpatent parasitemia present, when immunosuppressed in the same conditions, peaks of parasitemia, of respectively 400 and 30,000 BTry/5 µl.

As control we also investigated whether the different levels of parasitemia induced by Cy in the chronically infected mice were due to intrinsic differences in the replication rate of BTry from the different strains. Thus, naive mice treated with Cy according to Materials and Methods had been inoculated with 10^5 BTry from VL-10 and CL strains. Fig. 3 shows that both strains yielded similar curves of parasitemia characterized by excels of parasitemia from the untreated animals and the parasitemia of the immunosuppressed mice.

### RESULTS

The percentage of reactivation of the Cy treated mice and the number of parasites at the peak of parasitemia compared with the control values were used as criteria for considering the infection recrudescence. Fig. 1 shows the percentages of reactivation in the groups of chronically infected mice inoculated with different *Trypanosoma cruzi* strains immunosuppressed by Cy. The infection recrudescence occurred in a range of 90.3% to 10.7% according to the strain used. Table II shows the numbers of blood forms in the peak of parasitemia in mice treated with Cy. Although the standard deviation in the outbred mice is rather high, the differences between mice treated with Cy and the untreated animals are highly significant and corresponds to Fig.1. Figs 2A-D show patterns of parasitemia curves in immunosuppressed and untreated mice groups. They show that there is no correlation between the subpatent or residual levels of parasitemia from the untreated animals and the parasitemia of the immunosuppressed mice. Mice inoculated with Buriti and J strains (Figs 2A, B) which show low numbers of circulating parasites by fresh blood examination display, when submitted to Cy treatment, peaks of parasitemia of, respectively, 80,000 and 3,000 BTry/5 µl. On the other hand, the groups of mice inoculated with the VL-10 and CL strains (Figs 2C, D) which induce steady subpatent parasitemia present, when immunosuppressed in the same conditions, peaks of parasitemia, of respectively 400 and 30,000 BTry/5 µl.

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TABLE II
Parasitemia in chronically infected mice treated with cyclophosphamide (Cy) and controls

<table>
<thead>
<tr>
<th></th>
<th>Buriti</th>
<th>CL</th>
<th>J</th>
<th>Colombiana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitemia</td>
<td>55.360±54.064×a</td>
<td>30.289±26.919</td>
<td>8.158±4.403</td>
<td>2.157±2.620</td>
</tr>
<tr>
<td>Generoso</td>
<td>84±183b</td>
<td>0</td>
<td>106±212</td>
<td>145±112</td>
</tr>
<tr>
<td>Parasitemia</td>
<td>384±362</td>
<td>371±253</td>
<td>156±380</td>
<td>46±66</td>
</tr>
<tr>
<td>Generoso</td>
<td>0</td>
<td>39±54</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a: mean number of trypomastigotes in the peak of parasitemia in mice submitted to Cy treatment. b: mean number of trypomastigotes in the peak of parasitemia in mice not submitted to Cy treatment.

Fig. 2: curves of parasitemia in mice chronically infected with different Trypanosoma cruzi strains and immunosuppressed by cyclophosphamide as well as sham-treated controls. Strains: A: Buriti; B: J; C: VL-10; D: CL.

Fig. 3: curves of parasitemia of normal mice immunosuppressed by cyclophosphamide and inoculated with 10⁴ BTry of VL-10 and CL Trypanosoma cruzi strains.

tremely high numbers of BTry, strongly suggesting that the differences in the percentages of recrudescence on the groups of the immunosuppressed mice do not depend on the replication rate of the BTry.

DISCUSSION

Recrudescence of the chronic chagasic patients is caused by various factors that interfere with the steady immune response mounted by T. cruzi. They are in general immunosuppressive cytotoxic drugs or concurrent infections of T. cruzi and HIV involving a decline of CD4 cells. Due to the difficulties to mimic all situations that induce immunosuppression we decided to use in our experiments Cy, a suppressor of B lymphocyte function (Cupps et al. 1982) that demonstrated to reactivate T. cruzi in-


REFERENCES


