Recrudescence Induced by Cyclophosphamide 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 immunosuppressor 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 immunosuppressor 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 cyclophosphamide (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.

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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 immunosuppressor 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

TABLE I

Trypanosoma cruzi strains used in immunosuppression experiments

T. cruzi strains	Origin	Inoculum	Days of infection
CL	*Triatoma infestans* from R.G. Sul, Brazil (Brener & Chiari 1963)	50 BTry	225
Y	Acute phase patient, São Paulo, Brazil (Pereira da Silva & Nussenzweig 1953)	30 BTry	185
Buriti	*T. infestans* from R.G. Sul, Brazil (Filardi & Brener 1987)	2 x 10^5 MTry	245
VL-10	Chronic chagasic patient from Minas Gerais, Brazil (Schlemper Jr et al. 1983)	50 BTry	205
Generoso	Acute phase patient from Minas Gerais, Brazil (Filardi & Brener 1987)	2 x 10^5 MTry	218
SC-28	Isolated from a marmoset, Santa Catarina, Brazil (Steindel 1993)	2 x 10^5MTry	225
J	Acute phase patient from Minas Gerais, Brazil (Filardi & Brener 1987)	50 BTry	218
Colombiana	Patient from Colombia (Federici et al 1964)	50 BTry	256

BTry: blood forms; MTry: culture metacyclic trypomastigotes

immunosuppressed and control mice was daily determined according to Brener (1961), starting in the second week of treatment and pursue until the animals death.

Cy administration - Cy (Pravaz Division, Abbott Laboratories) was diluted in saline and injected weekly with doses of 200 mg/kg. Treatment was extended for four weeks. For the experiments in which normal mice were immunosuppressed, single doses of 50 mg/kg of Cy was given two days before inoculation, followed by three weekly doses post infection. For this experiment groups of 10 female albino Swiss mice, 18-20 g were inoculated with 10^4 Btry from CL and VL-10 strains by intraperitoneal route.

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 *T. 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 10^4 BTry from VL-10 and CL strains. Fig. 3 shows that both strains yielded similar curves of parasitemia characterized by ex-

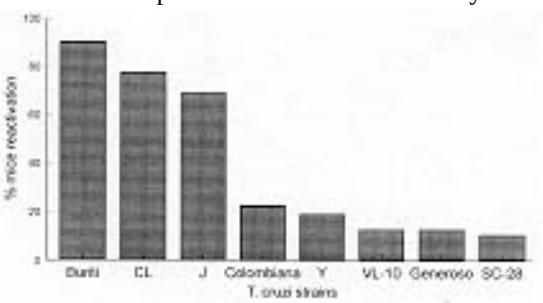

Fig. 1: percentages of mice with reactivated infection induced by cyclophosphamide in groups of mice chronically infected with different *Trypanosoma cruzi* strains.

TABLE II

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

Buriti	CL	J	Colombiana
55.360± 54.064[a]	30.289± 26.919	8.158±4.403	2.157± 2.620
84± 183[b]	0	106± 212	145± 112
Generoso	VL-10	SC-28	Y
384± 362	371± 253	156± 380	46± 66
0	39± 54	0	0

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.

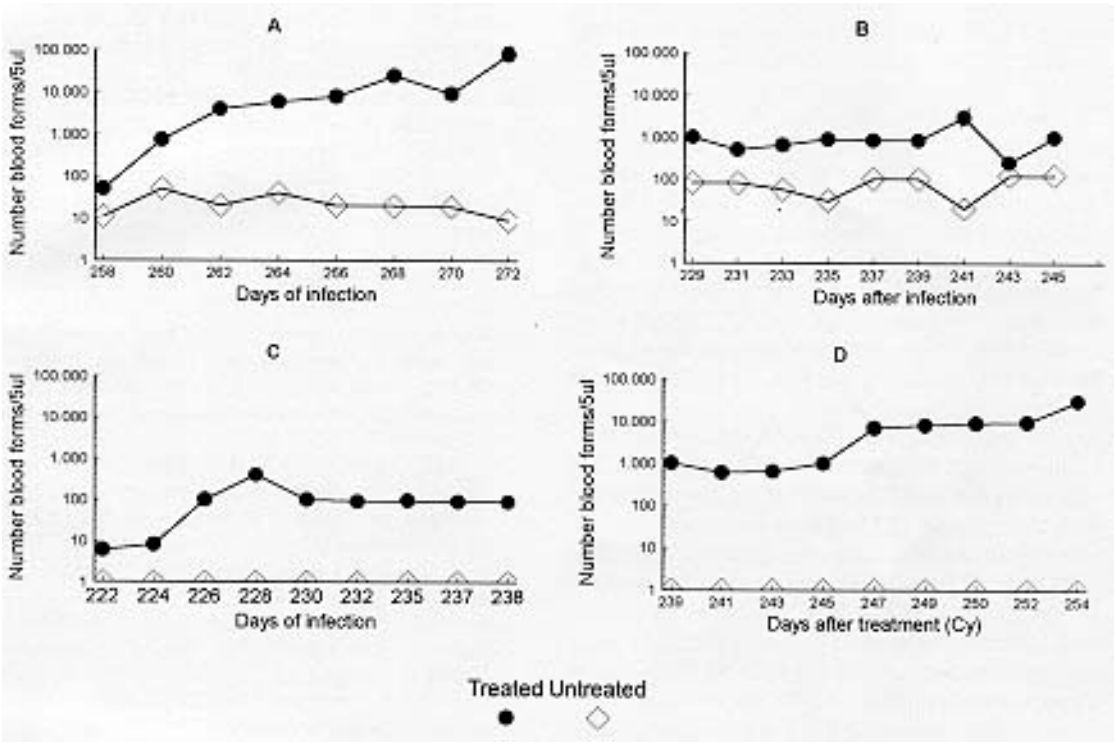

Fig. 2: curves of parasitemia in mice chronically infected with different *Trypanosoma cruzi* strains and immunosuppressed by cyclophosphamide as well as sham-treated controls. Strains: A: Buriti; B: J; C: VL-10; D: CL.

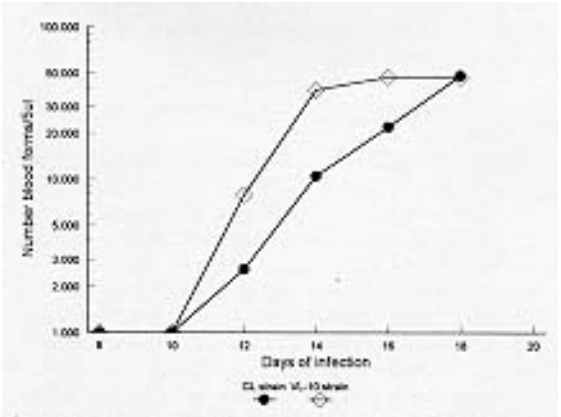

Fig. 3: curves of parasitemia of normal mice immunosuppressed by cyclophosphamide and inoculated with 10^4 BTry of VL-10 and CL *Trypanosoma cruzi* strains.

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-

fections (Brener & Chiari 1971). The data herein confirm that an immunosuppressor factor that affects humoral response induces new outbreaks of acute Chagas disease in chronically infected mice, and also shows that *T. cruzi* strains react differently to the immunosuppressive effect. Krettli and Brener (1976) demonstrated that *T. cruzi* Y strain blood trypomastigotes incubated with homologous and heterologous specific anti-serum had their infectivity decreased whereas CL strain trypomastigotes remained unchanged with no decline of the infectivity. More evidences of the differences of *T. cruzi* strains *vis a vis* the specific acquired immunity was reported by Krettli (1977) who infected with *Plasmodium berghei* mice chronically infected with Y and CL *T. cruzi* strains and has shown that a strong immunosuppressive effect and recrudescence occurred in the CL-infected animals whereas the immune response against Y strain remained unchanged. The immunodeficiency observed in the rodents has been attributed to the depletion of B cells that occur in this malaria model (Krettli & Nussenzweig 1974). These experiments performed with the "polar strains" Y and CL described by Brener (1977) have been now extended to a higher number of *T. cruzi* populations investigated under standardized conditions.

The variability in the percentage of mice reactivation is not induced by the inoculation of different development *T. cruzi* stages, namely, blood forms or metacyclic trypomastigotes from LIT culture. Buriti and Generoso strains, inoculated with 2 x 10^5 metacyclic trypomastigotes yielded in the LIT medium displayed, respectively, 90.3% and 12.9% of immunosuppressed mice, whereas in groups of mice inoculated with 50 bloodstream trypomastigotes of CL and VL-10 strains the percentages of reactivated mice were, respectivelly, 77.4% and 12.9%. Moreover, no correlation could be established between the multiplication rate of blood forms from different strains and the results observed in the immunosuppressed chronically infected mice.

Taken together the data herein shown strongly suggest that this parasitism variability depends on intrinsic characteristics of the different *T. cruzi* strains. It has not yet been determined if this also occurs in immunodeficient chronic chagasic patients. Joint clinical and experimental investigations are worth to be carried out considering the increasing occurrence of *T. cruzi* recrudescence in human disease.

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