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Absence of CD4$^+$ T Lymphocytes, CD8$^+$ T Lymphocytes, or B Lymphocytes Has Different Effects on the Efficacy of Posaconazole and Benznidazole in Treatment of Experimental Acute *Trypanosoma cruzi* Infection$^\nabla$

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We investigated the influence of CD4$^+$ T lymphocytes, CD8$^+$ T lymphocytes, and B lymphocytes on the efficacy of posaconazole (POS) and the reference drug benznidazole (BZ) during treatment of acute *Trypanosoma cruzi* infection in a murine model. Wild-type mice infected with *T. cruzi* and treated with POS or BZ presented no parasitemia, 100% survival, and 86 to 89% cure rates, defined as the percentages of animals with negative hemocultures at the end of the observation period. CD4$^+$-T-lymphocyte-knockout (KO) mice infected with *T. cruzi* and treated with BZ or POS controlled parasitemia during treatment, although circulating parasites reappeared after drug pressure cessation, leading to only a 6% survival rate and no cure. CD8$^+$-T-lymphocyte-KO mice infected with *T. cruzi* and treated with POS or BZ had intermediate results, displaying discrete parasitemia after the treatment was ended, 81 and 86% survival, and cure rates of 31 and 66%, respectively. B-lymphocyte-KO mice infected with *T. cruzi* and treated with BZ relapsed with parasitemia 1 week after the end of treatment and had a 67% survival rate and only a 22% cure rate. In contrast, the activity of POS was much less affected in these animals, with permanent suppression of parasitemia, 100% survival, and a 71% cure rate. Our results demonstrate that abrogation of different lymphocytes' activities has distinct effects on the efficacy of POS and BZ in this experimental model, probably reflecting different parasite stages preferentially targeted by the two drugs and distinct cooperation patterns with the host immune system.

Chagas' disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects approximately 10 to 15 million inhabitants in Latin America (33, 45). Substantial advances in controlling this disease have been made by combating the triatomid vector with insecticides and blood transfusions, particularly in the Southern Cone countries (9). Nevertheless, Chagas' disease remains a public health problem, since the progress of such control programs in Latin America is uneven. Furthermore, the disease is now appearing in countries where it has not been endemic due to migration of infected individuals, most of them in chronic stages of the disease (8). To date, only two drugs are clinically available for the specific treatment of this disease: 2-nitroimidazole (benznidazole [BZ]; Rochagan; Roche) and 5-nitrofuran or nifurtimox (NFX; Lampit; Bayer). Both drugs possess limited efficacy during the acute phase (up to 80%) and especially in the chronic (8 to 20%) phase of infection (7). Furthermore, use of these drugs leads to frequent undesirable side effects, which can lead to discontinuation of treatment (7). The presence of *T. cruzi* strains that are naturally resistant to both BZ and NFX (13) may account for the low cure rates observed for some treated patients, even those in the acute phase of infection. However, little is known about the influence of host parameters on therapeutic failures. One of the major factors potentially influencing the efficacy of treatment for Chagas' disease is the possible cooperative action between the drug effects and the host immunological response, which is particularly important in the context of immunosuppressed patients (3, 21, 24, 31, 34).

Host resistance to infection with *T. cruzi* is dependent on both innate and adaptive immune responses. During the first week of infection in mice, *T. cruzi* induces a host cellular response (4) to control parasite replication. Activation of cell-mediated immunity occurs through induction of interleukin-12 (IL-12) (1) and gamma interferon (IFN-γ) (40), which actively participate in macrophage activation during innate immune responses. Adaptive immune responses, which are mainly mediated by CD4$^+$ T lymphocytes, seem to be the most effective means of controlling parasite replication (4). CD4$^+$ T lymphocytes promote activation of macrophages and proliferation of CD8$^+$ T and B lymphocytes (36). CD8$^+$ T lymphocytes are involved in IFN-γ production and have specific cytolytic activity toward infected host cells, controlling intracellular parasite replication (17). B lymphocytes participate in infection control, producing immunoglobulins involved in opsonization and phagocytosis, as well as complement-mediated lysis (4). CD4$^+$-T-lymphocyte (28, 29, 36), CD8$^+$-T-lymphocyte (35, 39), and B-lymphocyte (26) deficiencies have all been shown to render

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experimental mammalian hosts hypersusceptible to *T. cruzi* infection, increasing parasitemia and mortality rates.

Details of the cooperative therapeutic effects of drugs and the host immune system on parasitic diseases such as avian malaria (34), murine schistosomiasis (10), and canine visceral leishmaniasis (25) have been reported in the literature. For Chagas' disease, immune system activation with recombinant IL-12 has been shown to enhance the efficacy of BZ chemotherapy in experimental acute models (18). Indeed, IFN-γ- and IL-12-knockout (KO) mice demonstrated a reduced response to BZ treatment compared with wild-type (WT) mice (27). Furthermore, increased IFN-γ levels have been observed in patients treated and cured with BZ and NFX, compared with those who were not cured (2).

Novel antifungal triazole derivatives, developed for the treatment of invasive fungal infections, have arisen as an alternative treatment for Chagas' disease. These drugs are potent and selective inhibitors of *T. cruzi* ergosterol synthesis, which is essential for parasite growth and survival. In addition, they possess pharmacokinetic properties particularly suited to the control of this disseminated intracellular infection. Several triazole derivatives have been experimentally tested, including D0870 (20), posaconazole (POS) (19, 43), ravuconazole (44), albaconazole (15), and TAK-187 (42). In particular, POS (Schering-Plough Research Institute) was recently registered for the prophylaxis and treatment of invasive fungal infections in the European Union, Australia, and the United States. These drugs have also been shown to possess potent in vitro and in vivo anti-*T. cruzi* activities, curing acute and chronic parasitological mouse infections, even those caused by BZ-resistant strains (19). Therefore, POS is considered a rational candidate for clinical trials in Chagas' disease patients (41).

Recently, we demonstrated that the activity of POS in a murine model of acute Chagas' disease is much less dependent on IFN-γ than is that of BZ (11). The goal of the present work was to investigate the influence of T and B lymphocytes on POS and BZ anti-*T. cruzi* activity in a murine model of acute infection.

MATERIALS AND METHODS

Animals. C57BL/6 mice knocked out for major histocompatibility complex class II ($CD4^+$ T lymphocyte KO), the gene for the β-2 microglobulin protein ($CD8^+$ T lymphocyte KO), or the gene for the heavy chain of immunoglobulin μ (B lymphocyte KO), as well as C57BL/6 (WT) mice, were used in the present investigation. The animals were provided by the Departamento de Imunologia, Faculdade de Medicina de Ribeirão Preto (FMRP/USP, São Paulo, Brazil). WT and KO male mice between 8 and 10 weeks of age were infected with *T. cruzi* and maintained under standard conditions of isolation at the animal house of the Centro de Pesquisa René Rachou, in Belo Horizonte, Minas Gerais, Brazil. All procedures involving the use of animals in the present study were performed according to the Ethical Principles in the Use of Laboratory Animals supplied by the Brazilian College of Animal Experimentation (Cobea) and the *Guide for the Care and Use of Laboratory Animals* (20a). In this regard, we applied the "3Rs Principle" (replacement, reduction, and refinement), which specifies that experiments may not be performed if another scientifically satisfactory method of obtaining the desired result that does not entail the use of animals is reasonably and practically available. Moreover, the experiments were designed to avoid or reduce distress and unnecessary pain and suffering for the experimental animals.

Mouse infection and parasitemia determination. WT and KO mice were infected via intraperitoneal injection with 5,000 blood trypomastigote forms of the Y strain of *T. cruzi*, which had been maintained in our laboratory through serial passages in Swiss-Webster mice. Infection was confirmed 4 days postinfection (dpi) and followed up to the 60th day by examination of fresh blood collected from the tails of mice. Parasitemia was monitored daily from the 4th to the 16th dpi and thereafter every other day up to the 60th dpi (3).

Treatment of infected mice. On the 4th dpi, WT and KO mice began receiving oral treatment with POS (20 mg/kg of body weight/day, administered in two daily doses) or BZ (100 mg/kg/day, once a day), and the treatment continued for 20 consecutive days. BZ was dissolved in water containing gum arabic, and POS was suspended in 2% methylcellulose and 0.5% Tween 80. *T. cruzi*-infected, untreated, and vehicle-treated WT and KO mice were used as controls. Mice infected with the Y strain of *T. cruzi* were divided into the following experimental groups: WT nontreated ($n = 20$), BZ treated ($n = 29$), and POS treated ($n = 18$); $CD4^+$ T lymphocyte KO nontreated ($n = 15$), BZ treated ($n = 16$), and POS treated ($n = 16$); $CD8^+$ T lymphocyte KO nontreated ($n = 11$), BZ treated ($n = 29$), and POS treated ($n = 16$); B lymphocyte KO nontreated ($n = 12$), BZ treated ($n = 18$), and POS treated ($n = 14$).

Hemoculture. Hemoculture was used as an indicator of parasitological cure. On the 60th dpi, mice with no parasitemia, as observed by optical microscopy, were aseptically bled via the venous orbital sinus, and a volume of 0.5 ml of blood was drawn from each mouse. Blood samples were distributed into two tubes containing 5 ml of liver infusion tryptose medium (6). The tubes were incubated for 30 to 60 days at 28°C and then microscopically examined for parasite detection. In this work, negative direct fresh blood microscopic examination plus negative hemoculture was used as a semiquantitative measure of the relative effects of the drugs on the parasite load of animals in the different experimental groups. However, this methodology has limited sensitivity (40 to 80%) (23), and it can be concluded only that the animals' parasitemia levels fell below the detection limit of the method.

Statistical analyses. Means and standard deviations of parasitemia levels were calculated with Microsoft Excel (Windows). Comparisons between parasitemia graphs for POS- and BZ-treated mice were carried out using the nonparametric Mann-Whitney method, since the data were asymmetric. The cure rates of infected, treated, and nontreated mice were compared using the chi-square test and the Bonferroni method with the Minitab software package (Minitab Inc., State College, PA). Survival analysis was carried out using the nonparametric Kaplan-Meier method and log rank test, implemented with the Aabel v.2.4.2r software package for Mac (Gigawiz Ltd. Co., Oklahoma). Mean survival times were compared with the Tukey-Kramer test using the same package. Differences were considered significant when P was <0.05.

RESULTS

C57BL/6 WT mice infected with *T. cruzi* are highly responsive to both POS and BZ treatments. The mean parasitemia levels of WT mice infected with the *T. cruzi* Y strain, untreated (control) or treated with BZ or POS, are shown in Fig. 1A. The WT mice exhibited patent parasitemia by 4 dpi; parasitemia reached a peak at 9 dpi and then became undetectable by 16 dpi. POS- and BZ-treated mice had subpatent parasitemia throughout the entire observation period, after which they were submitted to hemoculture; the animals in each experimental group exhibiting a decrease in parasitemia below the detection limit of these methods are listed as cured below and in Table 1 (see Materials and Methods). All infected, untreated WT mice (control) were dead by 28 dpi (mean survival time, 20.5 ± 4.9 days) (Fig. 2A). In contrast, all treated animals survived throughout the observation period (Fig. 2A). Cure rates were 0% for untreated WT mice and 86 or 89% for WT mice treated with BZ or POS, respectively (Table 1). There were significant differences in parasitemia, mortality, and cure rates between POS- or BZ-treated and untreated mice ($P < 0.001$). However, no significant differences were observed between POS- and BZ-treated WT mice.

$CD4^+$-T-lymphocyte-KO mice are more susceptible to *T. cruzi* infection than are WT animals and are significantly less responsive to BZ and POS treatment. $CD4^+$-T-lymphocyte-KO mice were highly susceptible to *T. cruzi* infection: untreated animals attained peak parasitemia levels that were

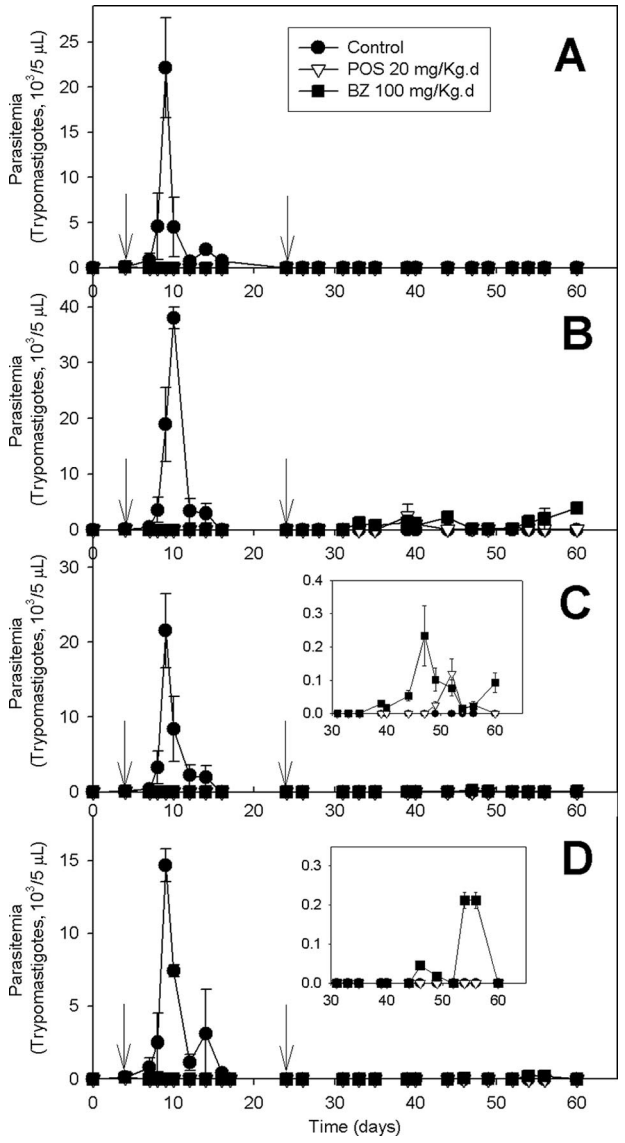

FIG. 1. Parasitemia of WT (A), $CD4^+$-T-lymphocyte-KO (B), $CD8^+$-T-lymphocyte-KO (C), and B-lymphocyte-KO (D) mice infected with the Y strain of *Trypanosoma cruzi*. Closed circles, nontreated mice; closed squares, BZ-treated mice; open inverted triangles, POS-treated mice. Arrows indicate the start and end of the treatment. The insets in panels C and D show the parasitemia levels on an expanded scale, from 30 to 60 days. For details, see Materials and Methods.

twofold higher than those of WT animals (Fig. 1B), and all died at 15 dpi (Fig. 2B). Treatment with BZ or POS maintained subpatent parasitemia levels throughout the treatment (from 4 to 24 dpi), but the infection reactivated 7 days after the end of BZ treatment and 11 days after the end of POS treatment, as evidenced by the presence of parasites in the bloodstream, and persisted intermittently throughout the rest of the observation period (Fig. 1B). The reactivation was intense and was associated with only 6% survival by 60 dpi among both groups of drug-treated animals (Fig. 2B; mean survival times, 53.5 ± 6.7 and 50.7 ± 6.7 days for BZ and POS, respectively). No significant differences in survival were observed by the log-rank test among drug-treated animals ($P > 0.3$), but both treated groups demonstrated significantly greater survival rates than did untreated animals ($P < 0.001$). Moreover, no mice were cured in either the BZ- or the POS-treated $CD4^+$-T-lymphocyte-KO groups (Table 1).

$CD8^+$-T-lymphocyte-KO mice are more susceptible to *T. cruzi* infection than are WT animals but are responsive to both POS and BZ treatment. *T. cruzi*-infected and untreated $CD8^+$-T-lymphocyte-KO mice exhibited peak parasitemia at 9 dpi (Fig. 1C), similar to that of WT mice (Fig. 1A), and yet the mean survival time (15.4 ± 1.8 days [Fig. 2C]) was significantly shorter than that of WT animals (Fig. 2A). Parasitemia levels were subpatent for *T. cruzi*-infected $CD8^+$-T-lymphocyte-KO mice treated with either POS or BZ during the entire treatment period (Fig. 1C). Thereafter, low levels of circulating parasites were observed 15 days after the end of BZ treatment and 25 days after the end of POS treatment (Fig. 1C, inset). Reactivation of the infection was observed in 22% of the BZ-treated mice and in 13% of the POS-treated mice, and parasitemia persisted intermittently throughout the remainder of the observation period. The survival rates at 60 dpi were 0% for $CD8^+$-T-lymphocyte-KO untreated mice, 86% for BZ-treated mice, and 81% for POS-treated mice (Fig. 2C and Table 1), with mean survival times of 58.8 ± 3.0 and 59.6 ± 1.5 days for BZ-treated and POS-treated animals, respectively. Both treated groups had significantly higher rates of survival than did untreated animals ($P < 0.001$), but no significant differences in survival were observed between the treated animals ($P > 0.5$). The cure rates for treated $CD8^+$-T-lymphocyte-KO mice were 66% and 31% for BZ and POS treatments, respectively.

B-lymphocyte deficiency reduces the efficacy of BZ treatment but not that of POS. *T. cruzi*-infected and untreated B-lymphocyte-KO mice exhibited peak parasitemia at 9 dpi (Fig. 1D), similar to that of WT untreated mice (Fig. 1A), although the mean survival time (15.3 ± 0.78 days) was again significantly shorter than that of WT animals (Fig. 2D). Twenty-two days after the end of BZ treatment, the infection reactivated, as demonstrated by relapsed parasitemia (Fig. 1D, inset) in 56% of the BZ-treated mice. This relapse was associated with a decreased survival rate among these animals, which reached 67% by the end of the observation period (Fig. 2D). In contrast, no relapse of patent parasitemia was observed in infected B-lymphocyte-KO mice treated with POS, as demonstrated by a 100% survival rate for these animals during the observation period (Fig. 1D and 2D). There was a statistically significant difference in the survival not only of both treated groups compared with untreated animals ($P < 0.001$ [Table 1]) but also between POS- and BZ-treated animals ($P = 0.02$). The superior performance of POS in these animals was also reflected in the parasitological cure rates, which were 22% in BZ-treated mice and 71% in POS-treated mice (Table 1).

DISCUSSION

Previous investigations using humans and experimental models have demonstrated the importance of the host immune system in the efficacy of chemotherapeutic treatments against parasitic diseases (31, 34). In the present work, we investigated the effects of T- or B-lymphocyte deprivation on the anti-*T.*

TABLE 1. Parasitological cure and survival rates of WT and CD4$^+$-T-lymphocyte-, CD8$^+$-T-lymphocyte-, and B-lymphocyte-KO mice infected with the Y strain of *T. cruzi* and treated with POS or BZ

Mouse group	Result for treatment:								
	No treatment			BZ			POS		
	No. of mice surviving at 60 dpi/ total no. (%)	Mean survival time (days) ± SD	No. of mice cured/total no. (%)[a]	No. of mice surviving at 60 dpi/ total no. (%)	Mean survival time (days) ± SD[b]	No. of mice cured/ total no. (%)[a]	No. of mice surviving at 60 dpi/total no. (%)	Mean survival time (days) ± SD[b]	No. of mice cured/ total no. (%)[a]
WT	0/20 (0)	20.5 ± 4.9	0/20 (0)	29/29 (100)	>60	25/29 (86)	18/18 (100)	>60	16/18 (89)
CD4$^+$ T-lymphocyte KO	0/15 (0)	15	0/15 (0)	1/16 (6)	53.7 ± 6.6	0/16 (0)	1/16 (6)	50.5 ± 6.6	0/16 (0)
CD8$^+$ T-lymphocyte KO	0/11 (0)	15.4 ± 1.8	0/11 (0)	25/29 (86)	58.8 ± 3.0	19/29 (66)	13/16 (81)	59.6 ± 1.5	5/16 (31)
B-lymphocyte KO	0/12 (0)	15.3 ± 0.78	0/12 (0)	12/18 (67)	59.3 ± 1.5	4/18 (22)	14/14 (100)	>60	10/14 (71)

[a] Proportion of mice with negative parasitemia upon fresh blood examination and hemoculture after chemotherapy.
[b] Results are biased because the mean survival times were calculated assuming the death (failure) of the survivors to be exactly 60 dpi, providing an underestimation of the true mean survival times when the observation period was limited.

cruzi activity of POS and BZ in a murine model of acute Chagas' disease. Our results demonstrated substantial participation of the host immune system in the antiparasitic activity of both drugs.

BZ is currently the most frequently available drug for the treatment of Chagas' disease in countries where it is endemic. Previous work has shown that the efficacy of BZ is markedly reduced in immunosuppressed mice (27). Although the mechanism of BZ activity has not been clearly established, evidence suggests the involvement of reductive stress, in which reduced nitroradicals react with nucleic acids, proteins, and other macromolecules, forming stable covalent adducts (41). Because the effectiveness of BZ depends on host immune system activity, this could pose a limitation in the use of this drug, mainly in the treatment of immunodeficient and immunosuppressed Chagas' disease patients. However, several clinical studies have shown that BZ retains significant anti-*T. cruzi* activity in those patients, although no parasitological cures have been reported (12, 30, 32). Thus, one of the objectives for improved specific treatment of Chagas' disease is the ability of the drug to retain its activity even when the host is immunosuppressed. This possibility has already been suggested for POS (20, 41): POS treatment of cyclophosphamide-immunosuppressed mice infected with BZ-susceptible and BZ-resistant *T. cruzi* strains revealed trypanocidal activity similar to that observed in non-immunosuppressed mice (20). Furthermore, POS cure rates were consistently higher than those obtained with BZ treatment in the same experimental model. More recently, we have shown that the anti-*T. cruzi* activity of POS is less dependent on IFN-γ than that of BZ (11).

The results of the present work confirm that both POS and BZ effectively increase survival and induce parasitological cure in WT mice with acute *T. cruzi* infection. They also indicate that the antiparasitic activity of both drugs is reduced in lymphocyte-deficient mice; lymphocyte-KO mice infected with *T. cruzi* demonstrated higher susceptibility to infection, with increased levels of parasitemia and earlier mortality compared with those of WT mice (Fig. 1 and 2). CD4$^+$-T-lymphocyte-KO mice treated with either POS or BZ had a 100% rate of infection reactivation a few days after the end of treatment, a very low survival rate (6%), and no evidence of parasitological cure

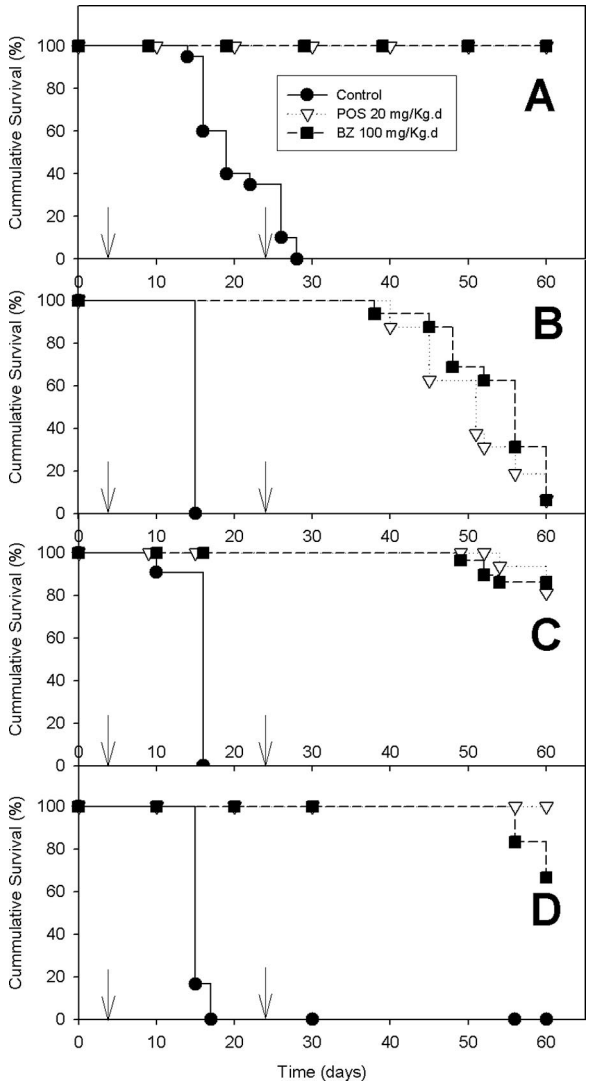

FIG. 2. Survival of WT (A), CD4$^+$-T-lymphocyte-KO (B), CD8$^+$-T-lymphocyte-KO (C), and B-lymphocyte-KO (D) mice infected with the Y strain of *Trypanosoma cruzi*. Closed circles, nontreated mice; closed squares, BZ-treated mice; open inverted triangles, POS-treated mice. Arrows indicate the start and end of the treatment. For details, see Materials and Methods.

(Fig. 1 and 2; Table 1). The essential requirement of $CD4^+$ T lymphocytes for drug efficacy may be due to their crucial helper function in $CD8^+$-T-lymphocyte and B-lymphocyte activation, antibody production, and direct release of important cytokines, such as IFN-γ (4). Our results are in accordance with those previously described (28, 29) showing that *T. cruzi*-infected $CD4^+$-T-lymphocyte-depleted mice demonstrate increased parasitemia levels and mortality rates. Several studies have confirmed that a deficiency in this immunological component leads to a reduced overall host immunological response and increased tissue parasitism (14, 28, 29, 37, 38). Strict dependence on $CD4^+$ T lymphocytes for an effective chemotherapeutic response is particularly worrisome when chronic *T. cruzi*-infected patients are coinfected with human immunodeficiency virus or in other contexts in which immunosuppression develops.

$CD8^+$-T-lymphocyte-KO mice were also markedly less resistant to *T. cruzi* infection than were WT animals but more than $CD4^+$-T-lymphocyte-KO animals. Other authors have also demonstrated that animals with deficiencies in the production of β-2 microglobulin show high parasitemia levels and die earlier than do WT-infected animals due to a deficient inflammatory response compared with that of WT mice (38). In this work, relatively low levels of infection reactivation and mortality were observed among treated $CD8^+$-T-lymphocyte-KO mice compared with $CD4^+$-T-lymphocyte-KO mice, but the level of parasitological cures dropped significantly in comparison with WT mice, particularly among POS-treated animals (Table 1). The efficacy of both antiparasitic treatments in increasing the survival of $CD8^+$-T-lymphocyte-KO mice may indicate important contributions of other components of the adaptive immune system in controlling the initial stages of infection, especially during BZ treatment. In this case, $CD8^+$ T lymphocytes are probably most necessary during later stages of infection. Disease cure by BZ during the chronic phase of disease has recently been associated with the presence of a stable $CD8^+$-T-lymphocyte population, which exhibits characteristics of memory central cells (5). This population is antigen independent, resulting in strong host protection, even against a new challenge. In contrast, there was a large decline in $CD4^+$-T-lymphocyte levels after treatment during the chronic phase, demonstrating that this population degrades faster and that treatment is more effective during early stages of the disease. Other investigations have also shown that BZ treatment enhances the $CD8^+$-T-lymphocyte response, building up resistance to reinfection (22). However, the presence of functional $CD8^+$ T lymphocytes seems to be essential for the eradication of parasites in mice treated with POS. The higher dependence of POS activity on the presence of $CD8^+$ T lymphocytes may reflect its specific activity against the intracellular (amastigote) stages, which require de novo ergosterol biosynthesis for survival and proliferation and are targeted by $CD8^+$-T-lymphocyte cytolytic cells. B lymphocytes are important for the host immune response in controlling the late acute phase of *T. cruzi* infections, enabling circulating parasites to be removed. However, the humoral immune response resulting from B-lymphocyte activation during the acute phase of Chagas' disease is mostly nonspecific (4), suggesting that B lymphocytes play a secondary role during this phase of the infection. *T. cruzi*-infected but untreated B-lymphocyte-KO mice exhibited parasitemia levels similar to those of WT infected mice, but their rate of mortality was greatly enhanced and the efficacy of BZ treatment was markedly reduced. In contrast, the antiparasitic action of POS was much less affected, with full survival and cure rates close to those of WT treated animals (Table 1). The greater dependence of BZ activity on B lymphocytes might be related to the BZ mechanism of action, as BZ treatment induces nonspecific damage to its target cells that could expose parasite-specific antigens and induce the B-lymphocyte-mediated humoral immune response (16). The phagocytic activity of mouse peritoneal macrophages against *T. cruzi* has been shown to increase in infected mice treated with BZ compared with nontreated animals (16). Additionally, BZ may preferentially target extracellular (trypomastigote) forms of the parasite, which are targets of the antibody-mediated immune response, while POS acts selectively against the proliferative amastigote stages (see above), which are typically not susceptible to antibodies due to their intracellular location.

In conclusion, our results indicate that $CD4^+$ T lymphocytes are essential for the in vivo trypanocidal activity of both BZ and POS, probably due to the activating mechanism required for the adaptive cellular immune response, which is the main source of intracellular parasite control. POS activity was more dependent on active $CD8^+$ T lymphocytes than was that of BZ. In contrast, the activity of BZ was highly dependent on B lymphocytes, while POS activity was almost completely retained in their absence. The varying dependence of POS and BZ activity on distinct lymphocyte types may reflect the different parasite life stages targeted by the two drugs and their patterns of cooperation with the host immune system.

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