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Trypanosoma cruzi: Genetic diversity influences the profile of immunoglobulins during experimental infection

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ABSTRACT

The clonal evolution model postulated for *Trypanosoma cruzi* predicts a correlation between the phylogenetic divergence of *T. cruzi* clonal genotypes and their biological properties. In the present study, the linkage between phylogenetic divergence of the parasite and IgG, IgG1, IgG2a and IgG2b response has been evaluated during the acute and chronic phases of the experimental infection. Eight laboratory-cloned stocks representative of this phylogenetic diversity and including the lineages *T. cruzi* I (genotypes 19 and 20), *T. cruzi* II (genotype 32) and *T. cruzi* (genotype 39) have been studied. The results showed that the pattern of humoral immune response was correlated with *T. cruzi* genotype, and that stocks included in genotype 20 were responsible for the high IgG response in the acute and chronic phases. Moreover, *T. cruzi* I lineage was more efficient in over-expressing all subclasses of specific anti-parasite IgG than either *T. cruzi* II or *T. cruzi* lineages. Curiously, the alteration in the pattern of antibodies induced by Benznidazole treatment was related to the phase of the infection but not to the genotype of the parasite. The data suggest that genotypes of *T. cruzi* are able to drive levels/subclasses of specific IgG, hence giving rise to further concerns about the sensitivity of serological assays in the diagnosis of human Chagas disease.

1. Introduction

The heamoflagelate parasite *Trypanosoma cruzi* is the causative agent of Chagas disease that is endemic to Central and South American (World Health Organization, 2005). *T. cruzi* undergoes extensive morphological and biochemical changes during its evasion of the mammalian immune system, and this host–parasite interaction culminates in severe heart disturbances leading to a low quality of life and, ultimately, to death (Brener and Gazzinelli, 1997; Teixeira et al., 2002).

In individuals with an appropriate epidemiological background, a diagnosis of Chagas disease may be confirmed on the basis of positive results in at least two different serological tests such as indirect immunofluorescence, indirect haemagglutination and immunoenzymatic assays (World Health Organization, 1991; Ferreira and de Avila, 1995; Ministério da Saúde, 1998; Zauza and Borges-Pereira, 2001; Rocha et al., 2007). In terms of humoral response, immunoglobulin G (IgG) subclasses have been found to be prevalent in all positive serological tests for Chagas disease (Cerban et al., 1993; Hernández-Becerril et al., 2001) and, more recently, have also been associated with secondary clinical physiological disturbances (Zauza and Borges-Pereira, 2001; Cordeiro et al., 2001; Escobar et al., 2006). IgG antibody subclasses, especially IgG1 and IgG3, exhibit high prevalence in all clinical degrees of Chagasic cardiomyopathy, whilst the IgG2 subclass is the main antibody associated with severe cardiac enlargement (Cerban et al., 1993; Escobar et al., 2006). A plausible hypothesis for the involvement of these IgG subclasses of anti-*T. cruzi* antibodies involves the postulation of a mixture of Th1- and Th2-like responses under continuous chronic antigenic stimulation, since the presence of the parasite or its antigen during the chronic phase is a well-defined concept (Jones et al., 1993; Elias et al., 2003).

The clinical manifestations and variations in the immune response observed during Chagas disease are not well understood but are believed to be associated with genetic variability in the host or the parasite. Several studies involving *T. cruzi* infection have confirmed that genetic diversity is correlated with the intrinsic characteristics of the parasite such as virulence, drug resistance, parasitemia, tropism toward specific organs, pathological alterations and capacity to induce host mortality (de Diego et al., 1998; Toledo et al., 2002, 2003; Mejia and Triana, 2005). The concept of genetic diversity in *T. cruzi* is generally supported by studies on different strains and stocks isolated from vertebrate

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and invertebrate hosts during distinct cycles of parasitic infection. Based on the isoenzyme profiles of parasite stocks, a complex multiclonal structure has been proposed and division of the species into 43 distinct genetic groups, zymodemes, natural clones or clonets has been suggested (Tibayrenc et al., 1986).

A further important aspect linked to genetic diversity is the susceptibility of parasites to the two pharmacological therapies that are currently available to treat human Chagas disease, namely, Benznidazole (BZ; Roche, São Paulo, Brazil) and Nifurtimox (Bayer, Leverkusen, Germany). It is reported that 56.0% of *T. cruzi* strains are susceptible to BZ, while 16.82% are partially susceptible and 27.1% are resistant to the drug (Filardi and Brener, 1987; Guedes et al., 2002; Toledo et al., 2003). Genetic variation thus appears to be an evolutionary strategy that enables parasites to survive specific chemotherapies.

Serologic examinations are routine tests in the diagnosis of Chagas disease, and knowledge of the levels of immunoglobulins is clearly essential in order to interpret the results obtained. In the present work, the linkage between phylogenetic divergence of the parasite and humoral immune response has been investigated through the evaluation of the levels of anti-*T. cruzi* IgGs in untreated and BZ treated mice during the acute and chronic phases of infection with different phylogenetic lineages of *T. cruzi*.

2. Materials and methods

2.1. Trypanosoma cruzi stocks

Eight standard *T. cruzi* clones were used in this study, namely, Gamba cl1 and SP104 cl1 (genotype 19), Cuica cl1 and P209 cl1 (genotype 20), IVV cl4 and MVB cl8 (genotype 32), and Bug 2148 cl1 and MN cl2 (hybrid genotype 39). These clones are representative of the major clonets (widespread clonal genotypes) 19, 20, 39 and 32 (Tibayrenc et al., 1986, 1993). Genotypes 19 and 20 are very closely related and are classified as *T. cruzi* I lineage, whilst genotype 32 (classified as *T. cruzi* II) and genotype 39 (classified as *T. cruzi*) (Anonymous, 1999) are closely related to each other but distantly related to genotypes 19 and 20. The clones were obtained from different geographical areas in Latin America and from various hosts of the domestic and wild transmission cycles. All stocks were cloned by micromanipulation and characterised by isoenzyme profile and randomly amplified polymorphic DNA analyses (Tibayrenc et al., 1993; Barnabé et al., 2000). All procedures involving experimental animals were carried out in compliance with the guidelines issued by the Colégio Brasileiro de Experimentação Animal (2006).

2.2. Experimental animals and infection procedure

A total of 260 female Swiss mice (18–20 g each) were obtained from the Animal Facility at the Universidade Federal de Ouro Preto, Minas Gerais, Brazil, and maintained in a temperature-controlled room with access to water and food *ad libitum*. Twenty mice were retained as the non-infected control group whilst the remaining experimental animals were randomly divided into eight groups of 30 individuals, each of which was inoculated via intraperitoneal injection with 10,000 blood trypomastigote forms of *T. cruzi* clones. Inocula were obtained from Swiss mice that had been previously infected with a large number of metacyclic trypomastigotes obtained from late stationary phase cultures on liver infusion tryptose (LIT) medium. The number of parasites in each inoculum was determined according to the method of Toledo et al. (2003). The period up to 2 months after inoculation was considered to be the acute phase and during this time parasitemia was detectable by examination of freshly collected blood samples. Three months or more after infection the experimental animals presented sub-patent parasitemia and this period was considered to be the chronic phase.

2.3. Treatment with Benznidazole

Following confirmation of infection, the 30 inoculated animals within each of the eight groups were further subdivided into three subgroups of 10 animals. All of the subgroups 1 were treated orally (by gavage) during the acute phase with a daily dose of 100 mg of BZ/kg body weight starting on day 10 after infection and continuing for 20 consecutive days. Subgroups 2 were treated with a similar dose of BZ during the chronic phase starting on day 120 after infection. Subgroups 3 and the 20 non-infected animals received no medication. Serum samples were collected on day 60 for animals treated in the acute phase and on day 170 for animals treated in the chronic phase. All samples were stored at −80 °C until required for the evaluation of IgG levels.

2.4. Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed according to the method of Volter et al. (1976). Briefly, wells were sensitised with *T. cruzi* antigen (prepared by alkaline extraction of Y strain harvested at the exponential growth stage in LIT medium (Ostermayer and Castro, 1997), binding antibodies were detected using peroxidase-labelled anti-mouse IgG, IgG1, IgG2a and IgG2b isotype-horseradish peroxide conjugates (Bethyl Laboratories, Montgomery, AL, USA), and absorbances were read at 490 nm. In order to optimise serum dilution, samples were titrated from 1:20 to 1:1240 and assays were performed with four samples, each presenting high, medium and low antibody reactivities, in parallel with four samples derived from non-infected mice. A good discrimination was observed at 1:100, and this dilution was employed in all further assays. The cut-off value was taken as the mean ± 2 standard deviations of the negative sample ($n = 20$).

2.5. Statistical analysis

Percentage of positive serological test between groups was compared by χ^2 test with $2 \times k$ contingency tables. The IgG, IgG1, IgG2a and IgG2b serum levels variance among experimental groups were analysed using ANOVA and *t* student with correction of Bonferroni test in multiple comparisons. ANOVA and *t* student multiple comparison procedure was also used to analyse the levels of immunoglobulin in sera derived from mice infected with different *T. cruzi* stocks and treated with BZ during the acute and chronic phases of experimental infection. A p value <0.05 was considered to indicate statistical significance. All analyses were performed with the aid of the statistical program PRISM (GraphPad, San Diego, CA, US).

3. Results

3.1. Correlation of positive serological tests with different genotypes of Trypanosoma cruzi

During the acute phase, IgG antibodies and IgG1, IgG2a and IgG2b subtypes were detected in sera from mice infected with stocks classified as *T. cruzi* I (genotypes 19 and 20), *T. cruzi* II (genotype 32) and *T. cruzi* (genotype 39) lineages (Fig. 1A). The proportion of positive tests, however, was variable among animals infected with *T. cruzi* stocks of different genotypes. Typically, higher percentages of positive reactions were observed in serum samples of animals inoculated with stocks belonging to genotypes 19

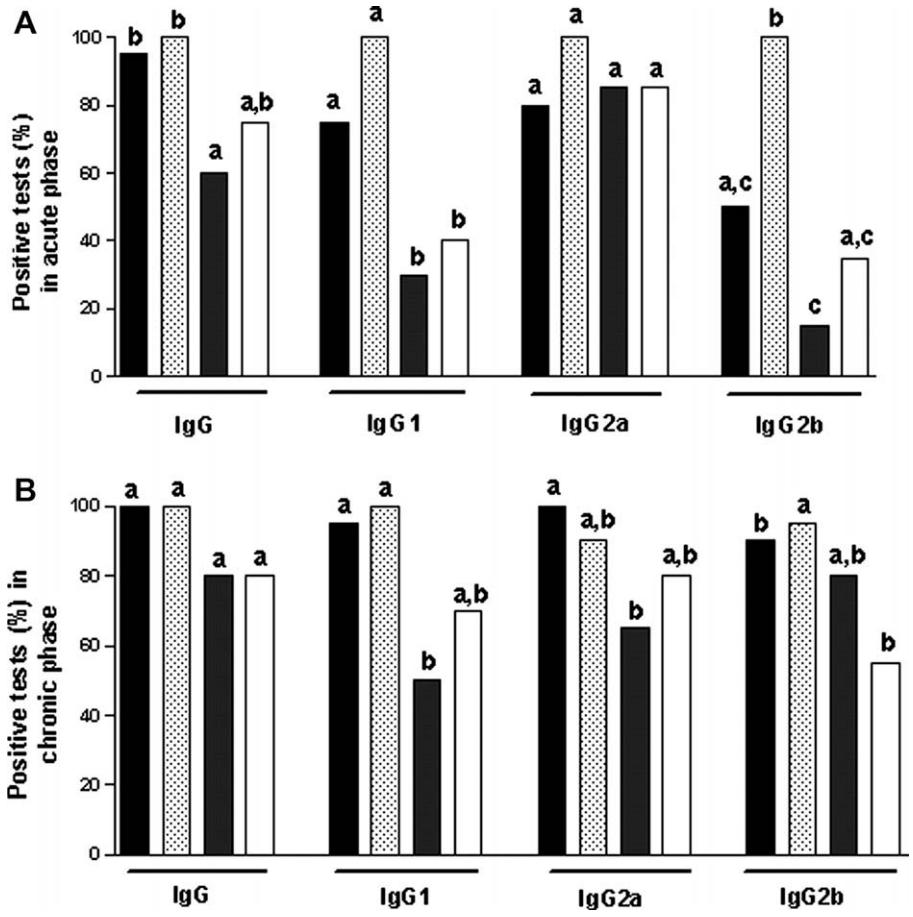

Fig. 1. Percentage of positive serological assays, distributed according to the subtypes of immunoglobulin G, in sera derived from mice infected with different *T. cruzi* lineages. ELISA assays were performed with anti- *T. cruzi* antibodies (IgG) during the acute (A) and chronic (B) phases of infection. Mice were infected with genotypes 19 and 20 (*T. cruzi* I lineage), genotype 32 (*T. cruzi* II) and genotype 39 (*T. cruzi*). The statistical analysis was performed using χ^2 test with $2 \times k$ contingency tables. (a), (b) and (c)—different letters indicate significant difference and same similar values among *T. cruzi* lineage.

and 20 in comparison with genotypes 39 and 32, although such differences appeared to be more evident with respect to IgG1 and IgG2b subtypes.

During the chronic phase, higher percentages of positive tests for IgG subtypes were observed in comparison with the acute phase (Fig. 1B). Again, larger proportions of IgG1 positive tests were associated with genotypes 19 and 20 in comparison with genotypes 39 and 32. In addition, the percentages of IgG2a and IgG2b positive tests were similar in mice infected with all genotypes except for genotype 39 (in respect of IgG2a) and genotype 32 (in respect of IgG2b).

3.2. Genetic diversity of Trypanosoma cruzi influences the profile of immunoglobulins during experimental infection

The correlation between the phylogenetic variance of the parasite and the serum levels of IgG, IgG1, IgG2a and IgG2b during the acute and chronic phases of experimental Chagas disease were analysed with respect to three levels of comparison: (i) clones of the same genotype, (ii) clones of different genotypes, and (iii) larger phylogenetic subdivisions (i.e. *T. cruzi* I, *T. cruzi* II and *T. cruzi*).

In order to determine the role of the different clones of each *T. cruzi* genotype in the outcome of humoral immune response, levels of IgG, IgG1, IgG2a and IgG2b antibodies were evaluated by ELISA in serum samples derived from non-treated mice inoculated with Gamba cl1 and SP104 cl1 (genotype 19), Cuica cl1 and P209 cl1 (genotype 20), IVV cl4 and MVB cl8 (genotype 32), and Bug 2148 cl1 and MN cl2 (hybrid genotype 39). The levels of IgG, IgG1, IgG2a and IgG2b antibodies were similar ($p > 0.05$) among animals infected with *T. cruzi* clones included in the genotypes 19, 20 and 39 in both the acute and chronic phases of the disease. Similar results were also obtained for animals infected with clones IVV cl4 and MVB cl8 (genotype 32) with respect to IgG1, IgG2a and IgG2b levels. In contrast, levels of IgG antibodies were significantly higher in the sera of animals infected with clone IVV cl4 compared with those observed in animals infected with the clone MVB cl8 in both the acute ($p = 0.001$) and the chronic ($p = 0.01$) phases of infection.

These data show that there is a good agreement between serum antibodies levels in animals inoculated with *T. cruzi* clones included in the same genotype. It was then of interest to determine whether the pattern of IgG antibodies would be correlated with the genotype of the parasite. During the acute phase, the serological pattern was clearly different among animals infected with genotype 20 compared with the other *T. cruzi* genotypes. In this phase, similar levels of antibodies levels were generally detected in sera from animals infected with 19, 39 and 32 genotypes ($p > 0.05$) (Fig. 2A, C, E and G). In contrast, levels of IgG, IgG1, IgG2a and IgG2b detected in sera of animals inoculated with *T. cruzi* stocks from genotype 20 were significantly larger ($p < 0.05$) than those observed with other genotypes.

Interestingly, the profile of IgG immunoglobulins observed during the chronic phase was dissimilar to that recorded in the acute phase. However, a marked influence of *T. cruzi* genotype on the levels of antibodies was also observed in the chronic phase (Fig. 2B, D, F and H). In general terms, two distinct patterns of antibody induc-

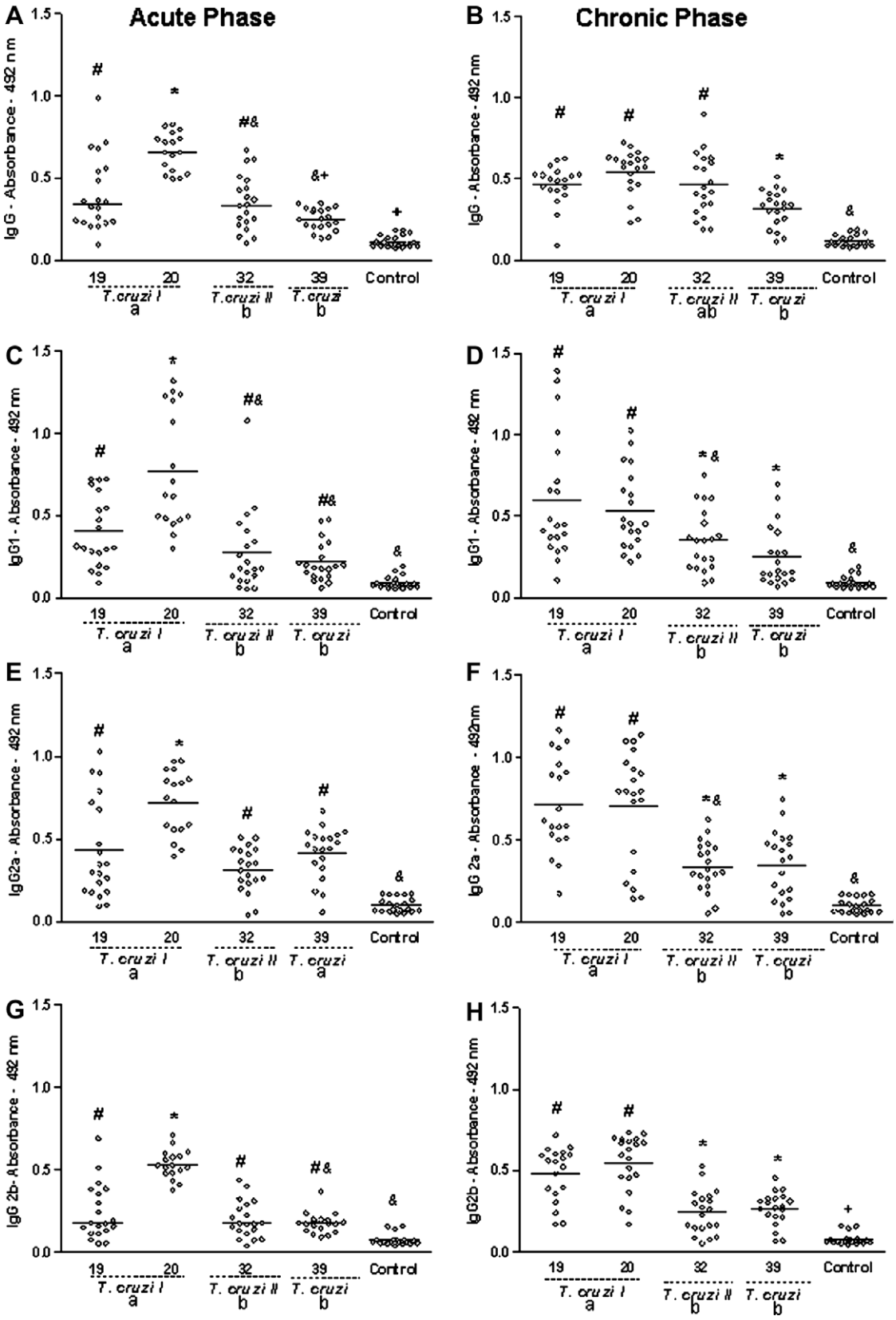

Fig. 2. Levels of immunoglobulin IgG (A and B) and subtypes IgG1 (C and D), IgG2a (E and F) and IgG2b (G and H) in sera derived from mice infected with different *T. cruzi* lineages, namely, *T. cruzi* I (genotypes 19 and 20), *T. cruzi* II (genotype 39) and *T. cruzi* (genotype 32), during the acute and chronic phases of experimental infection. Results shown are the absorbance at 492 nm for all subclasses of immunoglobulin. The statistical analysis was performed using ANOVA and *t* student with correction of Beferroni test in multiple comparisons and a p value <0.05 was considered to indicate statistical significance. (#), (∗), (&), (+)—Different symbols indicate significant difference and same similar values among genotypes of *T. cruzi*. (a), (b)—different letters indicate significant difference and same similar values among *T. cruzi* lineage.

tion were observed. In the first, high levels of all immunoglobulin subtypes were detected in sera of mice inoculated with clones belonging to genotypes 19 and 20. In the second, lower levels of antibodies were recorded in the sera of animals inoculated with *T. cruzi* stocks from genotypes 39 and 32. Only for total IgG were equal levels of antibodies induced by genotypes 19, 20 and 32 (Fig. 2B).

It is thus clear that the pattern of humoral immune response is correlated with the genotype of *T. cruzi*. In order to evaluate if this pattern is also correlated with the genetic lineage of *T. cruzi*, the results were analysed considering the lineage of *T. cruzi* I (genotypes 19 and 20), *T. cruzi* II (genotype 32) and *T. cruzi* (hybrid genotype 39). It was verified that during the acute phase of infection, the absorbance values associated with IgG, IgG1, IgG2a and IgG2b detected in sera of animals inoculated with parasites belonging to *T. cruzi* I lineage were higher than those observed in animals infected with *T. cruzi* II or *T. cruzi* lineages (Fig. 2A, C, E and G). On the other hand, *T. cruzi* II and *T. cruzi* lineages induced similar levels of IgG, IgG1 and IgG2b subclasses. Interestingly, the absorbance values associated with IgG2a in serum samples of mice infected with *T. cruzi* lineage were similar to those induced by *T. cruzi* I lineage.

In the chronic phase, levels of all subtypes of antibodies evaluated were correlated significantly with the lineage of *T. cruzi* (Fig. 2B, D, F and H). During this phase of the infection, clones belonging to the *T. cruzi* I lineage induced higher levels of IgG1, IgG2a and IgG2b antibodies than parasites of *T. cruzi* II and *T. cruzi* lineages. Only IgG levels were similar in sera from animals infected with *T. cruzi* I and *T. cruzi* II lineages (Fig. 2B).

3.3. IgG levels in benznidazole treated animals

Following 30 days of treatment with BZ, a significant reduction in the levels of IgG, IgG1, IgG2a and IgG2b was observed in all animals infected with *T. cruzi* I lineage (genotypes 19 and 20) during the acute phase (Table 1). A similar pattern was observed with genotypes 32 (*T. cruzi* II) and 39 (*T. cruzi*), with the exception that the pre-treatment level of IgG1 subtype was maintained following BZ treatment during the acute phase of infection. On the other hand, when administration of BZ started during the chronic phase, only the IgG levels were significantly altered. Thus the IgG levels detected in sera of the BZ-treated animals infected with genotypes 19, 32 and 39 were significantly smaller than those animal infected with the same genotypes but non-treated (p values were 0.049, 0.013 and 0.042, respectively). In this phase of the infection similar levels of the IgG1, IgG2a and IgG2b were generally detected in sera from BZ-treated and non-treated animals infected with all genotypes, with the exception of the animals infected with genotype 19 that showed a significant decrease ($p = 0.09$) in the IgG1 levels after the BZ treatment.

Taking into consideration all of the results of the serological assays, it may be concluded that early BZ treatment is efficient in reducing antibody levels in animals infected with the *T. cruzi* genotypes evaluated, although only small alterations in antibody levels were observed in mice treated during the chronic phase.

4. Discussion

A precise and accurate diagnosis of human Chagas disease allows close follow-up and/or early clinical intervention in those individuals presenting moderate or severe heart disturbances. However, owing to the difficulty of identifying circulating trypomastigotes during the chronic phase (Gomes et al., 1999, 2001; Castro et al., 2002; Marcon et al., 2002), diagnosis of the disease is usually performed by the application of serological or molecular methods. Serological assays are used routinely in centres of diagnosis throughout Latin American where human Chagas disease remains a prevalent infection. The efficiency of these assays basically depends on the intensity and the specificity of the circulating anti-*T. cruzi* antibodies. However, the vast majority of the data reported in the literature reveals a variable sensitivity in parasitological, molecular and, most importantly, serological methods (Marcon et al., 2002; Malan et al., 2006). This intriguing variance among diagnostic assays contributes to the significant number of false positive or negative results and appears to be a consequence of the large genetic variability that exists within the *T. cruzi* population (Myamoto et al., 2006).

Most of the clonal stocks employed in the present study were classified in the *T. cruzi* I (genotypes 19 and 20), *T. cruzi* II (genotype 32) and *T. cruzi* (genotype 39) lineages. Interestingly, these genotypes were previously described by their capacity to modify biological parameters in experimental models such as tissue and blood parasite detection, mortality, intensity of inflammatory process (Toledo et al., 2002) and, more recently, sensitivity of the polymerase chain reaction (PCR) assay on blood samples (Myamoto et al., 2006). All of the similarities established among the described parameters were found to be closely related to the specific genotypes. In the present study, we have demonstrated that the percentages of positive tests, together with levels of immunoglobulin antibodies detected, also varied according to the specific genotype of the parasite. Moreover, these parameters represent an

Table 1
Mean immunoglobulin absorbance levels in serum samples derived from mice infected with clones from different genetic groups (genotypes 19, 20, 32 and 39) of *T. cruzi* and maintained untreated or treated with Benznidazole during the acute or chronic phase of infection

T. cruzi genotype	IgG		IgG1		IgG2a		IgG2b	
	NT[a]	T[b]	NT	T	NT	T	NT	T
Acute phase								
19	**0.41 ± 0.17**	**0.18 ± 0.05**	**0.41 ± 0.22**	**0.14 ± 0.07**	**0.43 ± 0.20**	**0.13 ± 0.06**	**0.25 ± 0.14**	**0.09 ± 0.05**
20	**0.65 ± 0.06**	**0.25 ± 0.08**	**0.83 ± 0.20**	**0.21 ± 0.07**	**0.69 ± 0.08**	**0.18 ± 0.18**	**0.54 ± 0.09**	**0.17 ± 0.10**
32	**0.25 ± 0.05**	**0.14 ± 0.01**	0.22 ± 0.12	0.24 ± 0.15	**0.42 ± 0.14**	**0.16 ± 0.06**	**0.18 ± 0.05**	**0.10 ± 0.05**
39	**0.36 ± 0.11**	**0.25 ± 0.07**	0.28 ± 21	0.24 ± 0.18	**0.32 ± 0.14**	**0.21 ± 0.10**	**0.20 ± 0.11**	**0.12 ± 0.03**
Chronic phase								
19	**0.47 ± 0.12**	**0.43 ± 0.15**	**0.61 ± 0.33**	**0.35 ± 0.16**	0.73 ± 0.21	0.70 ± 0.22	0.44 ± 0.16	0.48 ± 0.17
20	0.55 ± 0.14	0.54 ± 0.06	0.54 ± 0.21	0.55 ± 0.19	0.75 ± 0.33	0.81 ± 0.07	0.52 ± 0.10	0.55 ± 0.17
32	**0.32 ± 0.10**	**0.24 ± 0.05**	0.25 ± 0.13	0.26 ± 0.16	0.35 ± 0.21	0.33 ± 0.09	**0.19 ± 0.7**	**0.27 ± 0.11**
39	**0.47 ± 0.15**	**0.34 ± 0.12**	0.36 ± 0.19	0.46 ± 0.31	0.34 ± 0.13	0.30 ± 0.08	0.19 ± 0.10	0.25 ± 0.13

Data represent mean values ± standard deviation ($n = 10$) of absorbance at 492 nm for all subclasses of immunoglobulin. Within each row, values shown in **bold type** for the separate immunoglobulins are significantly different ($p < 0.05$) with respect to the non-treated and treated group of animals. The statistical analysis was performed using ANOVA and t student multiple comparison procedure. p Value <0.05 was considered to indicate statistical significance.

[a] NT non-treated animals.

[b] T treated animals.

important and, until now, unreported issue in troubleshooting serological tests in areas that are endemic and non-endemic for Chagas disease.

Whilst all antibody subtypes attained significant and stable absorbance values in the chronic phase of Chagas disease, the levels of IgG2a, IgG1 and IgG were consistently elevated in comparison with IgG2b. Typically, the specific humoral response against *T. cruzi* becomes apparent around 2 weeks following inoculation, increases up to 30 days after infection, and subsequently persists through all of the chronic phase (Rowland et al. 1992). However, administration of medication could alter the profile of specific antibodies and thus the expression of IgG subclasses in a murine model was evaluated with respect to the genetic variability of *T. cruzi* and to specific chemotherapy with BZ.

T. cruzi I lineage was more efficient in over-expressing all subtypes of specific anti-parasite IgG than either *T. cruzi* II or *T. cruzi* lineages, a feature that seemed to be associated with the genotypes within each lineage. Thus, genotype 20 appeared to maintain clones with high IgG responsiveness in the acute and chronic phases, while genotypes 39 and 32 exhibited the lowest levels of absorbance of IgGs. It has been previously demonstrated that genotypes from *T. cruzi* I lineage present higher levels of blood parasites in comparison with those of *T cruzi* II (Toledo et al., 2002). The intensity of circulating blood parasites could be explained, in part, by the capacity of the host immune system to induce more or less immunoglobulin against parasite antigens.

The correlation between clinical forms of Chagas disease and IgG isotype levels is strongly speculated in the literature but is not clearly defined. A number of studies support the existence of an association between IgGs and the severity of the different clinical forms of the disease in humans (Morgan et al., 1996; Cordeiro et al. 2001; Michailowsky et al., 2003) and in experimental models (Rowland et al. 1992; Spinella et al., 1992; Giordanengo et al., 2000; Guedes et al. 2008). On the other hand, some researchers have been unable to detect any differences in the levels of these immunoglobulins amongst individuals presenting different clinical manifestations (Cerban et al., 1993), while others have found higher levels of IgG2 antibodies in the sera of individuals with cardiac and digestive disturbances (Morgan et al., 1996; Cordeiro et al., 2001; Hernández-Becerril et al., 2001). Additionally, the indeterminate form of Chagas disease is reportedly associated with high IgG1 levels (Cordeiro et al., 2001).

While the possible correlation between tissue lesions and IgG levels was not evaluated in the present study, the data suggest that higher levels of IgG1 during the acute and chronic phases of infection may be associated with parasite control in genotypes 19 and 20 of *T. cruzi*, but not in genotype 39. This hypothesis was supported by Toledo et al. (2002) in their evaluation of Balb/C mice infected with genotypes 19, 20 and 39. These authors detected increased blood and tissue parasitism in animals infected with stocks from genotypes 19, 20 and 39 in the acute phase, while in the chronic phase only stocks from clone 39 were correlated with high parasitism.

Evaluation of the alteration by BZ of the IgG profiles of animals infected with different *T. cruzi* genotypes showed that during the acute phase almost all IgG subtypes, with the exception of IgG1 associated with genotypes 32 and 39, were reduced following administration of the medication. These results reflect a positive interference of BZ on the host immune response during the acute phase of infection that was probably due to circulating parasite resolution. However, some authors have demonstrated that BZ treatment may also modify the synthesis of macrophage inflammatory mediators such as nitric oxide, IL-6, IL-10 and TNF-alpha (Revelli et al., 1999). Thus, together with its trypanocidal activity, BZ may also affect the balance between pro- and anti-inflammatory mediators with important consequences for the course of *T. cruzi* infection.

Consideration of the data presented here relating to the genetic diversity of *T. cruzi* and the associated capacity to drive the IgG profile in infected mammalian hosts, whether under specific chemotherapy or not, generates a further concern relating to the serological diagnosis of Chagas disease especially during the chronic phase of infection when blood parasite levels are very low. In Latin America, a large number of individuals are infected with *T. cruzi* and a further 40 million are considered to be at risk of infection (Dias et al., 2002; World Health Organization, 2005). Although different clones and genotypes of the parasite are involved in these infections, *T. cruzi* I is generally observed in wild mammals and sylvan triatomines whereas *T. cruzi* II is usually found in humans (Fernandes et al., 1998). Longitudinal studies of chronic chagasic populations have been performed by a number of researchers during the last decade and the application of the PCR assay has revealed a large percentage of positive results in serologically-negative individuals (Gomes et al., 1999; Castro et al., 2002; Dias et al., 2002). These results support the hypothesis that some strains of *T. cruzi* may not induce a higher production of specific antibodies, an analysis that has been referenced by Brenière et al. (1989) and in this study.

In summary, it is suggested that genetic variability in *T. cruzi* might not only drive pathological disturbances in the mammalian host but may also coordinate the intensity of specific IgGs during the acute and chronic phases of the disease.

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References

Anonymous, 1999. Recommendations from a satellite meeting. Memórias do Instituto Oswaldo Cruz 94, 429–432.

Barnabé, C., Brisse, S., Tibayrenc, M., 2000. Population structure and genetic typing of *Trypanosoma cruzi*, the agent of Chagas disease: a multilocus enzyme electrophoresis approach. Parasitology 120, 513–526.

Brener, Z., Gazzinelli, R.T., 1997. Immunological control of *Trypanosoma cruzi* infection and pathogenesis of Chagas' disease. International Archives of Allergy and Immunology 114, 103–110.

Brenière, S.F., Carrasco, R., Revollo, S., Aparicio, G., Desjeux, P., Tibayrenc, M., 1989. Chagaś disease in Bolívia: clinical and epidemiological features and zymodeme variability of *Trypanosoma cruzi* strains isolated from patients. American Journal of Tropical Medicine and Hygiene 41, 521–529.

Castro, A.M., Luquetti, A.O., Rassi, A., Rossi, G.G., Chiari, E., Galvão, L.M., 2002. Blood culture and polymerase chain reaction for the diagnosis of the chronic phase of human infection with *Trypanosoma cruzi*. Parasitology Research 88, 894–900.

Cerban, F.M., Gea, S., Menso, E., Voteero-Cima, E., 1993. Chagaś disease: IgG isotypes against *Trypanosoma cruzi* cytosol acidic antigens in patients with different degrees of heart damage. Clinical Immunology and Immunopathology 67, 25–30.

Colégio Brasileiro de Experimentação Animal, 2006. Legislação e ética. Available at: http://www.cobea.org.br/. Accessed on March 2008.

Cordeiro, F.D., Martins-Filho, O.A., Costa Rocha, M.O., Adad, S.J., Corrêa-Oliveira, R., Romanha, A.J., 2001. Anti-*Trypanosoma cruzi* immunoglobulin G1 can be a useful tool for diagnosis and prognosis of human Chagas disease. Clinical and Diagnostic Laboratory Immunology 8, 112–118.

de Diego, J.A., Palau, M.T., Gamallo, C., Penin, P., 1998. Relationships between histopathological findings and phylogenetic divergence in *Trypanosoma cruzi*. Tropical Medicine and International Health 3, 222–233.

Dias, J.C.P., Silveira, A.C., Schofield, C.J., 2002. The impact of Chagas disease control in Latin America. Memórias do Instituto Oswaldo Cruz 97, 603–612.

Elias, F.E., Vigliano, C.A., Laguens, R.P., Levin, M.J., Berec, C., 2003. Analysis of the presence of *Trypanosoma cruzi* in the heart tissue of three patients with chronic Chagas' heart disease. American Journal of Tropical Medicine and Hygiene 46, 242–247.

Escobar, A.L., Fernandez-Gomez, R., Peter, J.C., Mobini, R., Hoebeke, J., Mijares, A., 2006. IgGs and Mabs against the beta2-adrenoreceptor block A-V conduction in mouse hearts: A possible role in the pathogenesis of ventricular arrhythmias. Journal of Molecular and Cellular Cardiology 40, 829–837.

Fernandes, O., Souto, R.P., Castro, J.A., Pereira, J.B., Fernandes, N.C., Junqueira, A.C., Naiff, R.D., Barret, T.B., Degrave, W., Zingales, B., Campbell, D.A., Coura, J.R., 1998. Brazilian isolates of *Trypanosoma cruzi* from humans and triatomines classified into two lineages using mini-exons and ribosomal RNA sequence. American Journal of Tropical Medicine and Hygiene 58, 807–811.

Ferreira, A.W., de Avila, S.D., 1995. Laboratory diagnosis of Chagas' heart disease. Revista Paulista de Medicina 113, 767–771.

Filardi, L.S., Brener, Z., 1987. Susceptibility and natural resistance of *Trypanosoma cruzi* strains to drugs used clinically in Chagas disease. Transactions of the Royal Society of Tropical Medicine and Hygiene 81, 755–759.

Giordanengo, L., Maldonado, C., Rivarola, H.W., Iosa, D., Girones, N., Fresno, M., Gea, S., 2000. Induction of antibodies reactive to cardiac myosin and development of heart alterations in cruzipain-immunized mice and their offspring. European Journal of Immunology 30, 3181–3189.

Gomes, M.L., Galvão, L.M., Macedo, A.M., Pena, S.D., Chiari, E., 1999. Chagas' disease diagnosis: comparative analysis of parasitologic, molecular and serologic methods. American Journal of Tropical Medicine and Hygiene 60, 205–210.

Gomes, Y.M., Pereira, V.R., Nakazawa, M., Rosa, D.S., Barros, M.D., Ferreira, A.G., Silva, E.D., Ogatta, S.F., Krieger, M.A., Goldenberg, S., 2001. Serodiagnosis of chronic Chagas infection by using EIE-recombinant Chagas-Biomanguinhos kit. Memórias do Instituto Oswaldo Cruz 96, 497–501.

Guedes, P.M.M., Veloso, V.M.V., Tafuri, W.L., Galvão, L.M., Carneiro, C.M., Lana, M., Chiari, E., Ataide-Soares, K., Bahia, M.T., 2002. The dog as a model for chemotherapy of the Chagas' disease. Acta Tropica 84, 9–17.

Guedes, P.M., Veloso, V.M., Gollob, K.J., Afonso, L.C., Caldas, I.S., Vianna, P., de Lana, M., Chiari, E., Bahia, M.T., Galvão, L.M., 2008. IgG isotype profile is correlated with cardiomegaly in Beagle dogs infected with distinct *Trypanosoma cruzi* strains. Veterinary Immunology and Immunopathology 124, 163–168.

Hernández-Becerril, N., Nava, A., Reyes, P.A., Monteon, V.M., 2001. IgG subclass reactivity to *Trypanosoma cruzi* in chronic chagasic patients. Archivos de Cardiologia de Mexico 71, 199–205.

Jones, E.M., Colley, D.G., Tostes, S.J., Lopes, E.R., Vnencak-Jones, C.L., MacCurley, T.L., 1993. Amplification of a *Trypanosoma cruzi* DNA sequence from human inflammatory lesions in human chagasic cardiomyopathy. American Journal of Tropical Medicine and Hygiene 41, 348–357.

Malan, A.K., Avelar, E., Litwin, S.E., Hill, H.R., 2006. Serological diagnosis of *Trypanosoma cruzi*: evaluation of three enzyme immunoassays and an indirect immunofluorescent assay. Journal of Medical Microbiology 55, 171–178.

Marcon, G.E., Andrade, P.D., de Albuquerque, D.M., Wanderley, J.S., de Almeida, E.A., Guariento, M.E., Costa, S.C., 2002. Use of a nested polymerase chain reaction (N-PCR) to detect *Trypanosoma cruzi* in blood samples from chronic chagasic patients and patients with doubtful serologies. Diagnostic Microbiology and Infectious Disease 43, 39–43.

Mejia, A.M., Triana, O., 2005. Genetic variability of *Trypanosoma cruzi* in blood and organs of infected mice determined by LSSP-PCR. Biomedica 25, 76–86.

Michailowsky, V., Luhrs, K., Rocha, M.O., Fouts, D., Gazzinelli, R.T., Manning, J.E., 2003. Humoral and cellular immune responses to *Trypanosoma cruzi*-derived paraflagellar rod proteins in patients with Chagas's disease. Infection and Immunity 71, 3165–3171.

Ministério da Saúde, 1998. Normas técnicas para coleta, processamento e transfusão de sangue, componentes e derivados. Portaria no. 721, Diário Oficial da União, Brasília.

Morgan, J., Dias, J.C., Gontijo, E.D., Bahia-Oliveira, L., Correa-Oliveira, R., Colley, D.G., Powell, M.R., 1996. Anti-*Trypanosoma cruzi* antibody isotype profiles in patients with different manifestations of Chagas disease. American Journal of Tropical Medicine and Hygiene 55, 355–559.

Myamoto, C.T., Gomes, M.L., Marangon, A.V., Araújo, S.M., Bahia, M.T., Lana, M., Toledo, M.J.O., 2006. *Trypanosoma cruzi*: sensitivity of the polymerase chain reaction for detecting the parasite in the blood of mice infected with different clonal genotypes. Experimental Parasitology 112, 198–201.

Ostermayer, A.L., Castro, A.M., 1997. Diagnóstico sorológico da doença de Chagas. In: Dias, J.C., Coura, J.R. (Eds.), Clínica e Terapêutica da Doença de Chagas. Editora Fiocruz, Rio de Janeiro.

Revelli, S., Le Page, C., Piaggio, E., Wietzerbin, J., Botasso, O., 1999. Benznidazole, a drug employed in the treatment of Chagas' disease, down-regulates the synthesis of nitrite and cytokines by murine stimulated macrophages. Clinical and Experimental Immunology 118, 271–277.

Rocha, M.O., Teixeira, M.M., Ribeiro, A.L., 2007. An update on the management of Chagas cardiomyopathy. Expert Review of Anti-Infective Therapy 5, 727–743.

Rowland, E.C., Mikhail, K.S., McCormick, T.S., 1992. Isotype determination of anti-*Trypanosoma cruzi* antibody in murine Chagás disease. Journal of Parasitology 78, 557–561.

Spinella, S., Liegeard, P., Hontebeyrie-Joskowicz, M., 1992. *Trypanosoma cruzi*: predominance of IgG2a in nonspecific humoral response during experimental Chagas's disease. Experimental Parasitology 74, 46–56.

Teixeira, M.M., Gazzinelli, R.T., Silva, J.S., 2002. Chemokines, inflammation and *Trypanosoma cruzi* infection. Trends in Parasitology 18, 262–263.

Tibayrenc, M., Ward, P., Moya, A., Ayala, F.J., 1986. Natural populations of *Trypanosoma cruzi*, the agent of Chagas disease, have a complex multiclonal structure. Proceedings of the National Academy of Sciences of the USA 83, 115–119.

Tibayrenc, M., Neubauer, K., Barnabé, C., Guenine, F., Skarecky, D., Ayala, F.J., 1993. Genetic characterization of six parasitic protozoa: parity between random-primer DNA typing and multilocus enzyme electrophoresis. Proceedings of the National Academy of Sciences 90, 1335–1339.

Toledo, M.J.O., Lana, M., Carneiro, C.M., Bahia, M.T., Machado-Coelho, G.L., Veloso, V.M., Barnabé, C., Tibayrenc, M., Tafuri, W.L., 2002. Impact of *Trypanosoma cruzi* clonal evolution on its biological properties in mice. Experimental Parasitology 100, 61–172.

Toledo, M.J., Bahia, M.T., Carneiro, C.M., Martins-Filho, O.A., Tibayrenc, M., Barnabé, C., Tafuri, W.L., de Lana, M., 2003. Chemotherapy with benznidazole and itraconazole for mice infected with different *Trypanosoma cruzi* clonal genotypes. Antimicrobial Agents and Chemotherapy 47, 223–230.

Volter, A., Bidwell, D.E., Bartlett, A., 1976. Enzyme immunoassays in diagnostic medicine - theory and practice. Bulletin of the Word Health Organization 53, 55–65.

World Health Organization, 1991. Control of Chagas Disease. WHO Technical Report Series 81:38-47.

World Health Organization, 2005. Tropical Disease Research: progress 2003-2004 Seventeenth Programme Report of the UNICEF/UNDP/World Bank/WHO. Special Programme for Research and Training in Tropical Diseases. Programme Report no. 17.

Zauza, P.L., Borges-Pereira, J., 2001. Sera levels of anti-*Trypanosoma cruzi* IgG in the course of chagasic cardiopathy in 10 years. Revista da Sociedade Brasileira de Medicina Tropical 34, 399–405.