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

Daniela Maria dos Santos a, André Talvani a,b, Paulo Marcos da Mata Guedes a, George Luiz Lins Machado-Coelho a,c, Marta de Lana a,c, Maria Terezinha Bahia a,b,*

a Núcleo de Pesquisas em Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, MG 35400-000, Brazil
b Departamento de Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, MG 35400-000, Brazil
c Departamento de Farmácia, Universidade Federal de Ouro Preto, Ouro Preto, MG 35400-000, Brazil

* Corresponding author. Address: Laboratório de doença de Chagas, sala 40, Instituto de Ciências Biológicas, ICB II, Universidade Federal de Ouro Preto, Ouro Preto, MG 35400-000, Brazil. Fax: +55 21 3131 3559 16 80. E-mail address: mtbahia@nupeb.ufop.br (M.T. Bahia).

1. Introduction

The hemoflagellate parasite Trypanosoma cruzi is the causative agent of Chagas disease that is endemic to Central and South America (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; Mejía and Triana, 2005). The concept of genetic diversity in T. cruzi is generally supported by studies on different strains and stocks isolated from vertebrate...
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, zymodesmes, natural clones or clones 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 16.8% 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), IVc 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 genotypes 32 (classified as T. cruzi II) and genotype 39 (classified as T. cruzi III) (Anonymous, 1999) are closely related to each other but distinctly 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 peroxidase 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 χ² test with 2 × 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
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* I lineage).

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 *T. cruzi* genotypes 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

<table>
<thead>
<tr>
<th>T. cruzi genotype</th>
<th>IgG</th>
<th>IgG1</th>
<th>IgG2a</th>
<th>IgG2b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>T&lt;sup&gt;a&lt;/sup&gt;</td>
<td>T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>T&lt;sup&gt;a&lt;/sup&gt;</td>
<td>T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>T&lt;sup&gt;a&lt;/sup&gt;</td>
<td>T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acute phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0.41 ± 0.17</td>
<td>0.18 ± 0.05</td>
<td>0.41 ± 0.22</td>
<td>0.14 ± 0.07</td>
</tr>
<tr>
<td>20</td>
<td>0.65 ± 0.06</td>
<td>0.25 ± 0.08</td>
<td>0.83 ± 0.20</td>
<td>0.21 ± 0.07</td>
</tr>
<tr>
<td>32</td>
<td>0.25 ± 0.05</td>
<td>0.14 ± 0.01</td>
<td>0.22 ± 0.12</td>
<td>0.24 ± 0.15</td>
</tr>
<tr>
<td>39</td>
<td>0.36 ± 0.11</td>
<td>0.25 ± 0.07</td>
<td>0.28 ± 0.21</td>
<td>0.24 ± 0.18</td>
</tr>
<tr>
<td>Chronic phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0.47 ± 0.12</td>
<td>0.43 ± 0.15</td>
<td>0.61 ± 0.33</td>
<td>0.35 ± 0.16</td>
</tr>
<tr>
<td>20</td>
<td>0.55 ± 0.14</td>
<td>0.54 ± 0.06</td>
<td>0.54 ± 0.21</td>
<td>0.55 ± 0.19</td>
</tr>
<tr>
<td>32</td>
<td>0.32 ± 0.10</td>
<td>0.24 ± 0.05</td>
<td>0.25 ± 0.13</td>
<td>0.26 ± 0.16</td>
</tr>
<tr>
<td>39</td>
<td>0.47 ± 0.15</td>
<td>0.34 ± 0.12</td>
<td>0.36 ± 0.19</td>
<td>0.46 ± 0.31</td>
</tr>
</tbody>
</table>

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.

<sup>a</sup> NT non-treated animals.
<sup>b</sup> 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 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 II lineage present higher levels of blood parasites in comparison with those of T. cruzi I (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; Michailewsky 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.

Acknowledgments

This work was supported by grants from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Rede Mineira de Bioterismo (FAPEMIG), Universidade Federal de Ouro Preto (UFOP) and research fellowships (Maria Terezinha Bahia) from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We are grateful to Dr Michel Tibayrenc, Laboratoire de Génétique des Maladies Infectieuses, Unité Mixte de Recherche CNRS/IRD, Montpellier, France, for supplying T. cruzi clonal stocks.

References

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.
Dasos Santos et al. / Experimental Parasitology 121 (2009) 8–14


