2005 International Nuclear Atlantic Conference - INAC 2005
Santos, SP, Brazil, August 28 to September 2, 2005
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 85-99141-01-5

INFLUENCE OF IONIZING RADIATION ON *Trypanosoma cruzi*

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ABSTRACT

Chagas' disease is among the major health problems in South Americ impairing the wealth fare population. Since its discovery, in 1909, by Carlos Chagas, American Trypanosomiasis showed significant differences in the resistance of infected people. Such observations led to the hypothesis that the genetic background of the host could have an influence on the development of the disease and lifespan of infected people. Considering that ionizing radiation has been successfully employed to modify the immunological properties of biomolecules studies, this paper reports on results obtained by comparing infectivity and immunogenicity of native and irradiated *T. cruzi*. It was observed that radiation process causes inability of trypanosomes to infect and kill mice, however these results are different according to the strain mice studied. Different strains were immunized with native *T. cruzi* or 2 KGy irradiated parasite in a ^{60}Co source with 10^3 or 10^5 forms. The results obtained by ELISA method indicated that when immunized with native *T. cruzi*, all different strain mice have produced significant antibodies title levels. However, if irradiated parasite is employed, smaller antibodies title is observed. These results indicate that ionizing radiation is a good tool to modify *T. cruzi* in order to get a less infective parasite, considering that although susceptible mice strain have presented no significant immune response, they did not die. These data could help to understand the immune mechanisms involved in recognition, processing and presentation of both native and irradiated parasites.

1. INTRODUCTION

Chagas disease is caused by *Trypanosoma cruzi*, a flagellated protozoan parasite which is transmitted to humans mainly in two ways, either by a blood-sucking reduviid bug which deposits its infective faeces on the skin at the time of biting, or directly by transfusion of infected blood. Humans and a large number of species of domestic and wild animals constitute the reservoir, and the vector bugs infest poor housing and thatched roofs. The disease has a wide distribution in Central and South America, being found only in the American Hemisphere. It is endemic in 21 countries, with 16-18 million persons infected and 100 million people at risk. Currently, there is a continued decreasing trend in the prevalence of house infestation by the vector bug *(Triatoma infestans)* and the incidence of human infection in children and youngsters, in the countries of the Initiative of the Southern Cone. However, the implementation of treatment strategies is seriously limited due to the fact that the drugs are not available to most patients in many endemic countries. A broader issue is the lack of precise epidemiological information about the magnitude of the morbidity and mortality associated with *T. cruzi* infection. The difficulty of identifying individuals in the indeterminate phase of the infection, and the social "discrimination" imposed by some

national legislation, exacerbate the neglect of Chagas disease, resulting in low priority for support of Chagas disease research and control both at national and international levels [1]. Among the major problems and challenges are the definition of a long-term research agenda for the future, aiming at the development of new prevention and control tools, such as efficient vaccines and safer drugs. Many studies are ongoing on immune response and new tools are being employed in order to elucidate the different way used by *T. cruzi* during disease development. Studies using ionizing radiation to kill or sterilize protozoan parasites have been described as an efficient method [2]. Ionizing radiation consists of electromagnetic waves resulting from nuclear transitions. It can interact with biomolecules in two ways: directly, when the radiation hits the molecule, or indirectly when free radicals are generated and react with the target molecule. With proteins, radiation promotes changes in their enzymatic, pharmacological and immunological properties [3]. Radiation has been successfully employed to modify biomolecules, reducing or abolishing their biological activity without affecting their immunogenic properties [4]. This methodology could be used to produce toxoids and vaccines. The production of modified antigens with lower toxicity and preserved or improved immunogenicity would be useful. However, in order to develop such methodology, a good comprehension on the immunological behavior of irradiated antigens would be necessary. In the present work, we used ionizing radiation on *T. cruzi*, the protozoan that causes Chagas disease, in order to compare the IgG_T immune response developed among different mice strains.

2. MATERIALS AND METHODS

2.1 Reagents

All reagents were commercially acquired and had analytical grade. Tripomastigotes of *T. cruzi* strain was from Immunology Department – UNICAMP, where the parasites are maintained by weekly infections in mice. The culture form of parasites were from Tropical Medicine Institute IMT-FMUSP

2.2 Animals

Resistant C57BL6/UNI, intermediate BALB/c and susceptible A/J mice to *T.cruzi* were from CEMIB/UNICAMP. and they were maintained in sterilized mini isolators and absorbent media, with food and water *ad libitum*. The manipulation of these animals before or during the experiments was according to the “Principles of Laboratory Animal Care” (NIH publ. N^o 86-23, revised in 1985) and to the “Principles of Ethics in Animal Experimentation” (COBEA – Colégio Brasileiro de Experimentação Animal).

2.3. Development of recombinant mice (AG)

Programmed matting between susceptible (A/J) and resistant (C57BL6/UNI) strains were carried out in order to get a recombinant mice strain. The matting process followed the patterns protocols [5] with few modifications. In this particular case, the response to *T. cruzi* infection was employed to classify animals according to their resistance patterns. The selected resistant mice of each generation were backcrossed with susceptible parental strain [6].

2.4 *T. cruzi* irradiation

T cruzi forms (tripomastigotes from infected mice or epimastigotes from LIT medium were suspended in sterile saline to a final concentration of 10^4 or 10^6.parasites/ml These solutions were irradiated with a low dose of 100 Gy or 2000 Gy of gamma radiation,. All irradiation procedures were performed using gamma rays derived from a ^{60}Co source (Gamma Cell, Atomic Agency of Canada Ltd) at room temperature and in the presence of atmospheric O_2, with a 5170Gy/h dose rate.

2.5 Production of antibodies

Specific anti-native or anti-irradiated *T. cruzi* antibodies were obtained by immunizing those different mice strains with the parasite in its native or irradiated form, following a classical immunization protocol [7]. Blood samples were collected and after centrifugation, the plasma was separated and frozen.

2.6 Enzyme linked immunosorbent assay (ELISA)

96 wells microplates were coated with *T. cruzi* from cell culture (1,0µg/well/50µl) overnight. The plates were then blocked with 5% skim milk in phosphate buffered saline (PBS). The plasma samples were then incubated for one hour. Peroxydase labeled antibodies specific against mouse IgG_T were then allow to react individually with the bound antibodies. Finally, the reaction was developed adding a chromogenic solution containing 0.5 mg/ml orto phenyl diamine in 50 mM citrate buffer pH 5 in the presence of 1µl/ml hydrogen peroxide. After 20 minutes incubation, the reaction was interrupted by the addition of 50 µl 2 M citric acid and the plates were analyzed on a microplate reader at 450 nm.

3. RESULTS

A recombinant mice strain was produced after several backcrosses procedures between those resistant and susceptible mice, previously selected with *T.cruzi* injection. This process resulted in an almost 90% genetically susceptible animals which could resist a 10^3 parasites injection and it was named “AG” family.

The results observed when different strains of mice were immunized with irradiated and non irradiated (native) *T. cruzi* show that native *T. cruzi* induces slightly higher titles of IgG_T antibodies when compared with those that have received the irradiated form, as shown at figures 1 and 2, respectively of intermediate BALB/c mice and resistant C57BL6/UNI mice. In this response , it is clear that despite similar levels for the infection, the immunization with irradiated parasites induces a less evident antibody production in the resistant mice, when compared to the strong production observed in the intermediate BALB/c mice. On the other hand, susceptible strain (A/J) did not present a significant immune response to irradiated *T. cruzi* (Figure 3)

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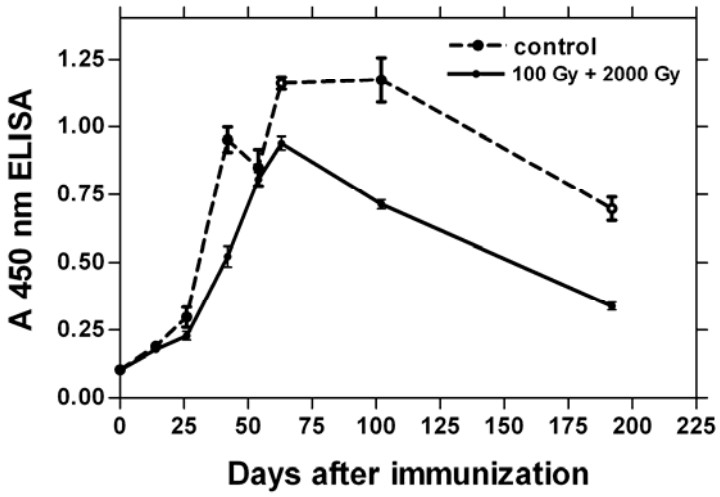

Figure 1 –Anti *T.cruzi* IgG production in intermediate BALB/c mice challenged with naive or irradiated parasites. Dashed line represents control infected animals and Solid line, mice challenged with irradiated parasites.

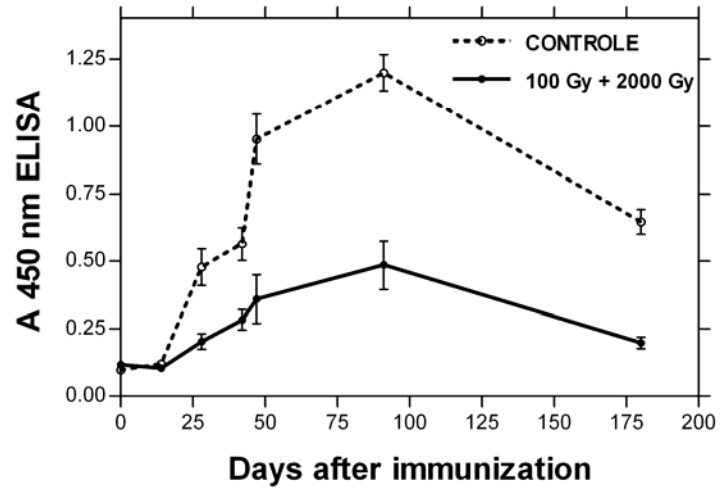

Figure 2. Anti *T.cruzi* IgG production in resistant C57BL6/UNI mice challenged with native or irradiated parasites. Dashed line represents control infected animals and Solid line, mice challenged with irradiated parasites. Bars represent SEM of 6 mice.

We also have tested the immune humoral response in the AG strain, which was produced through several backcrosses programmed matting, using susceptible A/J and resistant C57BL/6 as parental lines [8].

Irradiated *T. cruzi* induced different immune response among all tested strains mice, showed in Figure 3, analyzed by the immune response in the 6th week of infection or immunization.

Either resistant or intermediate mice strain showed significant levels of IgG_T antibodies production while (A/J) susceptible strain mice did not present a relevant immune response. In addition, despite of the recombinant mice (AG) present almost 90% of susceptible genome, they could survive when they received the same dose used to immunize a resistant strain

(C57BL/6-UNI) and both presented higher IgG response than those presented with susceptible strain.

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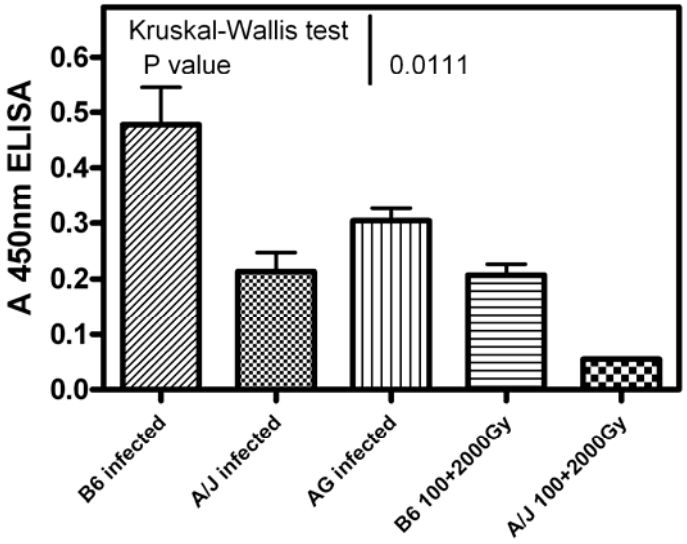

Figure 3. Humoral response measured as anti *T.cruzi* IgG in blood from mice with genetic susceptibility to *T.cruzi* infection.

4. DISCUSSION

Chagas disease exists only on the American Continent and although it had been first described in 1909 by the Brazilian physician Carlos Chagas, almost a hundred years later, the host-parasite interaction concerning to immune response is not completely understood despite of being the ones responsible for the parasite survival in the host cell.

In the present work we decided to study the influence of ionizing radiation on T. cruzi, since many authors have related some effects of gamma rays on protozoan. Some authors used a 550 Gy dose to irradiate *Toxoplasma gondii* tissue cists and showed the abolishment of their infection capacity [9]. Some authors showed that 2000 Gy irradiated *T. gondii* RH tachyzoites failed to reproduce either *in vitro* or *in vivo*, keeping a respiratory response and the ability to invade cells as well as the protein and nucleic acid synthesis power [10]. We have compared different strain of mice immunized with native or irradiated *T. cruzi* in order to study the immune response to this protozoan. Our results demonstrated that ionizing radiation causes some modifications on *T. cruzi* since they induced different infectivity and immune response in the tested strains. These findings are in agreement with others related earlier using also proteic antigens [11]. The results showed that gamma rays decrease the parasite infectivity rate without affecting its capacity to stimulate immune system, using pathways that could be not involved in the protection, since susceptible strain mice did not present a relevant immune response but could resist to an infection. This fact could be explained by the activation, during the infection, of immune response that involves only cellular components, as the CD8 response, or the failure of some innate mechanisms, without participation of antibodies, in the susceptible host.

An interesting point is the extent of the induced humoral response that was quite long, existing more than six months of infection or immunization, suggesting a good immune memory response in those animals

5. CONCLUSIONS

- All mice strain produced higher antibodies title when immunized with native *T. cruzi*
- The irradiated *T. cruzi* were able to stimulate the immune system and the resulting antibodies were able to react with the native form of them.
- Irradiated parasites induced smaller IgG_T response in mice when compared with those that had received a native form
- It is possible to get recombinant mice that resist to *T. cruzi* even presenting almost 90% of susceptible genome.
- Future works will be need because our results suggest that the use of irradiated antigens in susceptible host would be an elegant alternative to suppress such failures, inducing an adequate immune response that cannot be constructed during infection what could represent an alternative for vaccination in the susceptible host.

ACKNOWLEDGMENTS

The authors wish to thank CNPq and INSERN from Brazil.

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