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Research Article

Experimental Chagas disease: Therapeutic Vaccination with *Trypanosoma rangeli* Modulates the Antibody Response and Helps to Control *Trypanosoma cruzi* infection

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Abstract

Chagas' disease, produced by *Trypanosoma cruzi*, affects millions of people in Latin-America and is now being globalized through congenital, transfusional and transplantation transmission. In our laboratory we have developed an experimental mouse model of vaccination with non pathogenic *Trypanosoma rangeli*, that stimulates both innate and adaptive immunity, modulates the pattern of cytokines and soluble mediators, reduces parasite charge and mortality, with absence of histological and autoimmune lesions. This vaccine also protects guinea pigs and dogs, domestic reservoirs of *T. cruzi*. In this work we study the therapeutic effect of the vaccine. BALB/c albino mice were infected with trypomastigotes of *T. cruzi* and then vaccinated with fixed epimastigotes of *T. rangeli*, at different times post infection during acute period. The control group was only infected and inoculated with PBS. The course of infection and the pattern of specific immunoglobulin response (IgM, IgG, IgG1, IgG2 and IgG3) were analysed in both groups. The results showed that vaccinated mice had a better outcome of infection than controls, with significantly lower parasitemia and mortality rates. The level of specific IgG antibodies, measured by immunoenzymatic assay, was significantly higher in vaccinated animals from 15th to 40th post infection days (p= 0.02 – 0.003). IgG1 showed the same pattern of response (p= 0.02 - 0.005) whereas IgG2a and IgG2b levels were similar in both groups. In turn, IgG3 was significantly higher in vaccinated mice at the same period. In the chronic period (80th-120th days post infection) all measured isotypes did not show between-group differences. Otherwise, IgM was similar in both groups. In conclusion, as observed in preventive vaccination, this therapeutic approach of *T. rangeli* vaccination triggers a high production of *T. cruzi* reactive antibodies, favouring the clearance of circulating parasites.

ABBREVIATIONS

T. cruzi: Trypanosoma cruzi; T. rangeli: Trypanosoma rangeli; OD: Optical Density; PBS: Phosphate Buffer Saline; PI: Post Infection; ELISA: Enzyme Linked Immnosorbent Assay; Ig: Immunoglobulin

INTRODUCTION

Chagas' disease, produced by *Trypanosoma cruzi*, is one of the main endemic diseases in Latin-America, with nearly 16 millions of people infected and 90 millions at risk [1]. *Trypanosoma rangeli* also infects mammals, including humans, through the same triatomines in various areas of Latin-America, but does not produce the disease in humans [2]. In Chagas' disease, as in other parasitic diseases, a fully effective vaccine is not yet available, despite attempts of different research groups using different *T. cruzi* antigens, recombinant antigens, and also the administration of plasmid DNA encoding several genes [3-5]. The results of these assays vary from no disease protection to the partial reduction of short-term mortality and morbidity rates. A model for vaccinating

mice with *T. rangeli* against *T. cruzi* infection has been developed in our laboratory [6]. The strategy of vaccinating with *T. rangeli* is based on the argument that if a vaccine for humans is developed using this parasite in future, the auto-aggression phenomenon triggered by employing *T. cruzi* could be avoided. In fact, the role played by autoimmune mechanisms in Chagas' disease pathology has been proposed by numerous research groups [7,8]. *T. rangeli* shares areas of geographical distribution, epidemiological characteristics and antigenic and immunogenic components with *T. cruzi* [9,10]. In our previous studies, mice vaccinated with different strains of fixed *T. rangeli* epimastigotes showed high titres of specific antibodies against *T. cruzi* associated with protection of mice from lethal *T. cruzi* infection, with the absence of histopathological and autoimmune type lesions [11] along with a particular pattern of cytokines [12]. Additional work performed in our laboratory demonstrated that immunisation with *T. rangeli* significantly reduced parasite burden in *T. cruzi* experimentally infected guinea pigs [13] or dogs, in captivity under controlled conditions [14], and in rural areas [15].

An alternative approach for vaccination strategy is the use of therapeutic vaccines, in a previously infected host. These immunological interventions are developed with the objective to enhance immunity of the infected host and, in case of chronic infection, to redirect immunity to a protective status [16,17]

The aim of the present work was to study the efficacy of therapeutic vaccination in mice infected with virulent trypomastigotes of *T. cruzi* and subsequently vaccinated with *T. rangeli*, by evaluating the course of infection, and to analyze the immunoglobulin isotype pattern in response to vaccination.

MATERIALS AND METHODS

Parasites

T. cruzi: Trypomastigotes of the Tulahuén strain were maintained by weekly intraperitoneal sub inoculations in Balb/c mice. Blood samples of these animals were obtained by cardiac puncture.

T. rangeli: The Colombian strain 2378 was cultured in monophasic medium [18]. Epimastigotes were harvested in the exponential phase of growth, washed with phosphate buffer saline (PBS) and fixed with glutaraldehyde 0.1%. They were washed with PBS again and resuspended in PBS at a concentration of 1 x 10^9/mL [6].

Mice: Balb/c mice were maintained under standard conditions in the animal colony of our Laboratory.

Infection and vaccination schedule

Groups of three to four-week old mice (n = 10 in each experiment) were infected with 500 trypomastigotes of *T. cruzi* Tulahuén strain by the intraperitoneal route. Then, they were vaccinated with three doses, on days 5th, 9th and 14th post infection, with 0.1 mL of fixed *T. rangeli* epimastigotes containing 1 x 10^8 parasites, by intradermal injections, emulsified with the same volume of aluminum hydroxide, containing 8.5 mg/mL (Sigma). Control mice only received PBS.

Parastemia evaluation

The levels of parasitemia were evaluated according to the Pizzi method modified by Brener [19] using 5 µL of blood collected from the tail vein on days 15th, 20th and 30th p.i.

Enzyme linked immunosorbent assay (ELISA) for immunoglobulin isotypes: *T. cruzi*-specific IgM, total IgG and IgG1, IgG2 and IgG3 isotypes were determined by ELISA (Sigma). ELISA tests were performed with microplates coated with *T. cruzi* lysate (Wiener Lab, Argentina) and rabbit peroxidase conjugate anti isotypes (Sigma-Aldrich). Optical density (OD) was measured at 450 nm in an ELISA plate reader. In both tests, the serum of three uninfected mice was used as a negative control. The cut-off value was calculated through the mean of negative controls + 100. The performance of this serological test was previously described [12].

Statistic: Comparisons were carried by the non- parametric Mann Whitney test (Graph Pad program). The level of statistical significance was set at $p < 0.05$.

Ethical standards: All experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996). Mice were cared according to the ethical standards for animal testing and experimentation.

RESULTS AND DISCUSSION

As depicted in Figure (1), vaccinated mice had a better infection outcome than controls, with significantly lower parasitemia, which became undetectable around 30 days p.i. These results were similar to the ones seen in our earlier works preventive vaccination [6,11,20]. In fact, both schedules of vaccination with *T. rangeli* protect mice against challenge with *T. cruzi.* The present dose of 500 trypomastigotes of *T. cruzi* yielded a 30% mortality in non-vaccinated mice.

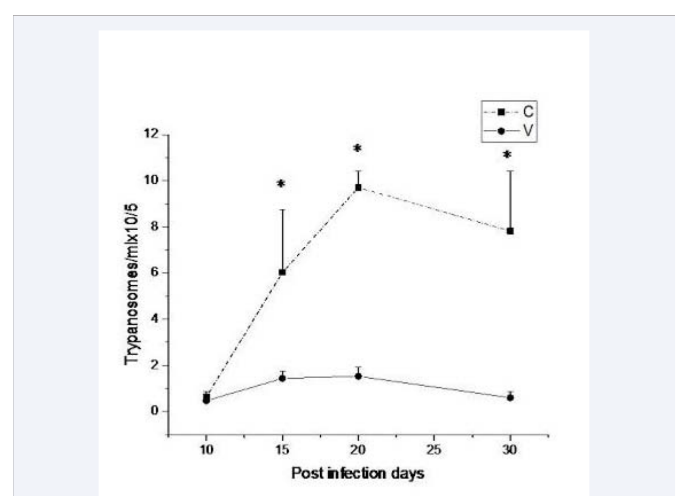

Figure 1 Parasitemia levels (arithmetic mean + standard error) in mice infected with *T. cruzi* (--■--) or infected with *T. cruzi* and vaccinated with fixed epimastigotes of *T. rangeli* (-●-). (*): significant differences between both groups evaluated by the Mann Whitney U test (p 0.0005 – 0.00001).

Figure (2) shows the level of specific IgM and IgG anti *T. cruzi* antibodies, measured by ELISA. As seen, IgM had similar pattern in both groups (Figure 2A). On the contrary, IgG was significantly higher in vaccinated animals from 15 to 40 days p.i. The mean values of vaccinated and non-vaccinated mice at day 15 p.i were 633 ± 79 (range 508-778), and 274 ± 27 (range 221-310, p=0.03) respectively. From days 20 to 29 p.i. levels in vaccinated mice were 1486 ± 108 (range 1054-1818) whereas in the non-vaccinated ones 827 ± 53 (range 723-892, p=0.02). Results from days 30-40 p.i were as follows: vaccinated mice 2281 ± 67 (range 1054-1818) non-vaccinated mice 1793 ± 81 (range 1348-2038, p=0.003). In the chronic period, levels in both groups remained similar.

Further comparisons revealed that IgG1 and IgG3 isotypes mostly contributed to such increased IgG amounts, since these isotypes showed the same pattern of response respect total IgG (Figure 3A,3D). Analysis of IgG1 within vaccinated mice yielded the following results, day 15 p.i.:1288 ± 138 (range 1124 -1564), 20-29 days p.i., 2400 ± 128 (range 1867-2820) and 2824 ± 134 (range 2075-3172) during the 30-40 days p.i. period. As regards non-vaccinated mice values in the same time-point evaluations were as follows: 342 (range 260-402, p=0.02); 525 ± 24 (range 489-570, p=0.02) and 1820 ± 133 (range 1423-2288, p=0.005). Again, values during the chronic period remained similar in both groups.

Unlike this, IgG2a and IgG2b levels were similar in vaccinated and control groups throughout the studied period (Figure 3B,3C).

Regarding IgG3, the values in vaccinated mice were as follows: day 15p.i. 569 ± 102 (range 425-767), 20-29 days p.i., 910 ± 96 (range 709-1264), 30-40 days p.i., 1609 ± 94 (range 1234 - 2038). Non-vaccinated mice yielded the following results: day 15 p.i., 357 ± 20 (range 326 - 395), 20-29 days p.i., 589 ± 68 (range 454-775, p=0.03), 30-40 days p.i., 1166 ± 79 (range 934-1530, p=0.005). Again, in the chronic period IgG3 levels were similar in vaccinated and non-vaccinated mice (Figure 3D).

The *in vivo* biological activities of IgG antibodies are known to result from their functional nature, in which antigen recognition by the Fab is coupled to the effector and immunomodulatory diversity found in the Fc domain [21]. Subclasses of IgG display substantial differences in their ability to mediate effector responses, contributing to variable activity of antibodies against microbes and tumors [22].

The biological characteristics of IgG1 and IgG3, at least in humans, are mainly facilitation of opsonisation, sensitization to NK cells and strong activation of complement, all mechanisms involved in protection to pathogens, i.e., *T. cruzi,* favouring the clearance of circulating parasites.

Although the therapeutic vaccination triggers a high production of *T. cruzi* reactive antibodies, the immunoglobulin isotype pattern is different from the one seen with prophylactic vaccination. In fact, the latter induced a significant increase of total IgG, IgG1, IgG2a and IgG2b [20], whereas IgG3 was similar in vaccinated and control mice. In the therapeutic vaccine assayed in this work, specific increases of total IgG and IgG1 were also observed, as did IgG3 immunoglobulins, whereas IgG2a and IgG2b levels were indistinguishable between both infected groups, regardless of whether they were vaccinated or not. Taken together, these results showed that the effector response involved in the protection is different according to the vaccination schedule, even if both of them are protective.

The IgM response also displayed differences. In the prophylactic approach IgM values were more augmented in vaccinated mice, whereas in therapeutic vaccination their levels remained similar in both groups, throughout the study period. This may be due to the fact that IgM antibodies arise early in the infection [23], for which subsequent vaccination would not further raise their already increased levels.

As expected, infected mice developed all isotypes of anti- *T. cruzi* antibodies, although IgG1 and IgG3 subclasses remained below the amounts seen in vaccinated mice. This difference may be relevant for the course of the infection, being associated of with a better resistant state.

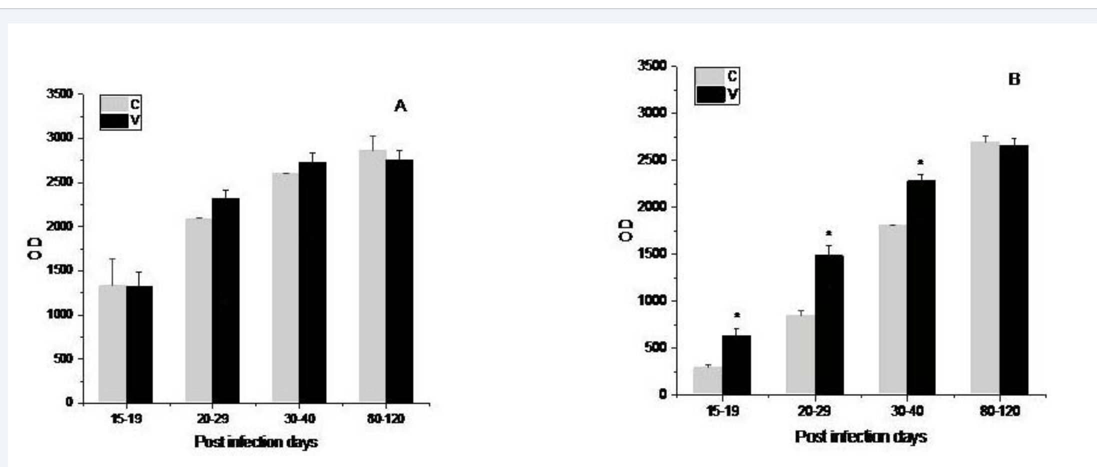

Figure 2 Specific antibodies levels, measured by ELISA of IgM (A) and IgG (B) isotypes, (arithmetic mean + standard error, of OD) in sera of mice infected with *T. cruzi* (grey bars) or infected with *T. cruzi* and vaccinated with fixed epimastigotes of *Trypanosoma rangeli* (black bars). (*): significant differences between both groups evaluated by the Mann Whitney Utest (p 0.02 – 0.003).

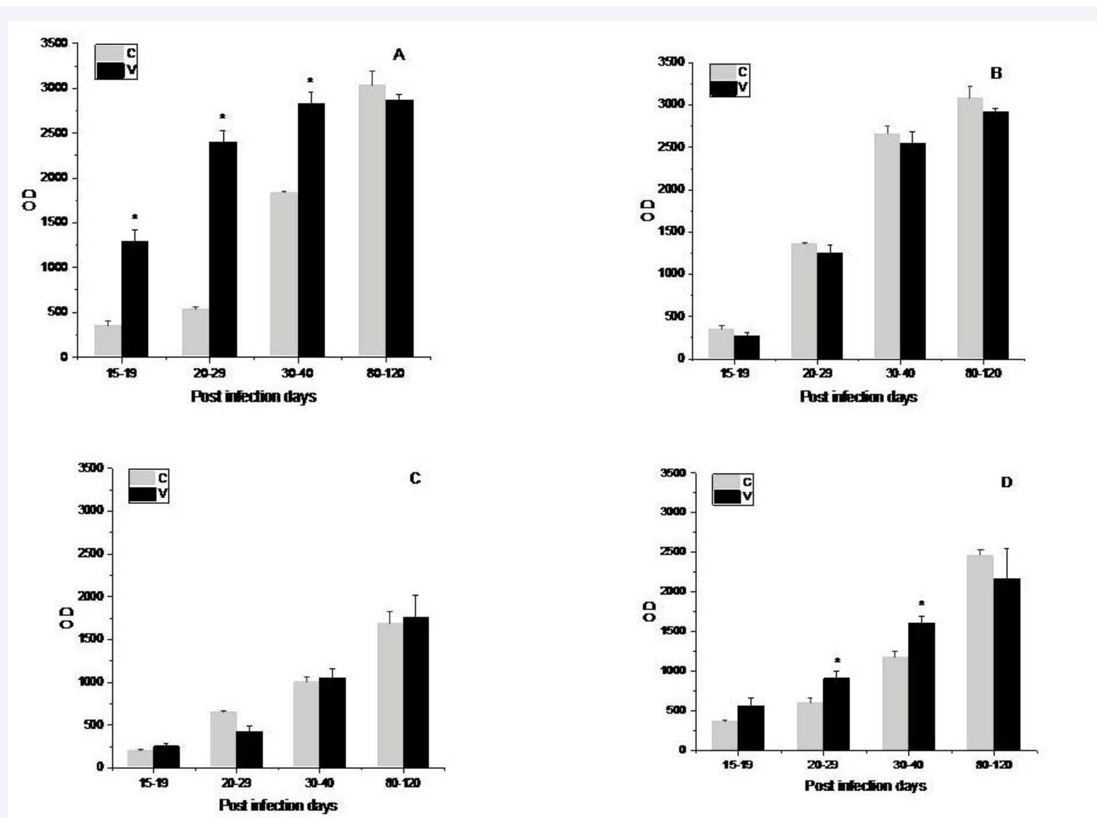

Figure 3 Specific antibodies levels measured by ELISA, of IgG1 (A), IgG2a (B), IgG2b (C) and IgG3 (D) isotypes. (Arithmetic mean + standard error of DO) in sera of mice infected with *T. cruzi* (grey bars) or infected with *T. cruzi* and vaccinated with fixed epimastigotes of *T. rangeli* (black bars). (*): significant differences between both groups evaluated bythe Mann Whitney Utest (p 0.02 – 0.005).

The use of *T rangeli* instead of *T. cruzi* as immunogen, in our prophylactic and therapeutic vaccination studies, was decided to discard the possibility of some autoimmune reaction, since such mechanism may partly account for the tissue damage occurring in chronic Chagas' disease [7,8,24]. In this sense, Dumonteil et al., stated that one potential concern is the possibility of inducing autoimmunity as a result of therapeutic vaccination. Therefore, it will be essential to consider and monitor autoimmune sequels as a part of the clinical development plan of the Chagas Vaccine Initiative [16]. In this sense it is important to emphasize that in our hands that vaccination with *T. rangeli* did not induce autoimmune lesions [11].

Arce Fonseca et al. [25], highlight the importance of developing a vaccine in the veterinary field. Rodriguez Morales et al. [26], also state on a scientific basis for the use of immunization in humans and domestic reservoirs in endemic areas for prevention and control of Chagas' disease as well as that *T. rangeli*–mediated immunoprotection may lead to possible preventive tools aimed at reducing the risk of *T. cruzi* infection. Finally, in agreement to our proposal [14,15], Aparicio Burgos et al. [27], also suggest that vaccination of dogs, via blocking the parasite transmission to triatomines, will potentially be useful in preventing human infection. This provides a stimulating background for further improving vaccine efficacy to interrupt the domestic cycle of parasite transmission.

CONCLUSIONS

Several experimental works performed in our laboratory have demonstrated that different mechanisms are involved in the resistance to *T.cruzi* infection induced trough vaccination with *T. rangeli*, including either innate or adaptive immune response [12,20,28]. Couple to present results the bulk of evidence points out that prophylactic and therapeutic vaccination triggers some different but equally effective mechanisms when challenging mice with *T. cruzi.*

To our knowledge, this is the first demonstration of a protective effect of *T. rangeli* vaccination on experimental Chagas' disease given in a therapeutic fashion.

Taken as a whole, the results of prophylactic and therapeutic vaccination with *T. rangeli* would make unnecessary to know whether domestic animals are infected with *T. cruzi* or not, since both approaches are effective for the elimination of circulating *T. cruzi* and, therefore, for their efficacy as reservoirs.

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REFERENCES

1. Coura JR. Chagas disease: what is known and what is needed - A background article. Mem Inst Oswaldo Cruz. 2007; 102: 113-122.

2. Añez N, Velandia J, Rodríguez A M. Estudios sobre *Trypanosoma*

rangeli Tejera, 1920. VIII. Respuesta a las reinfecciones en dos mamíferos. Mem Inst Oswaldo Cruz. 1985; 80: 149-153.

3. Camargo EP. Perspectives of vaccination in Chagas disease revisited. Mem Inst Oswaldo Cruz. 2009; 104: 275-280.

4. Cazorla SI, Frank FM, Malchiodi EL. Vaccination approaches against *Trypanosoma cruzi* infection. Expert Rev Vaccines. 2009; 8: 921-935.

5. Arce-Fonseca M, Ballinas-Verdugo MA, Zenteno ER, Suárez-Flores D, Carrillo-Sánchez SC, Alejandre-Aguilar R, et al. "Specific humoral and cellular immunity induced by *Trypanosoma cruzi* DNA immunization in a canine model. Vet Res. 2013; 44: 15.

6. Basso B, Moretti E, Vottero-Cima E. Immune response and *Trypanosoma cruzi* infection in *Trypanosoma rangeli* immunized mice. Am J Trop Med Hyg. 1991; 44: 413- 419.

7. Gironès N, Cuervo H, Fresno M. *Trypanosoma cruzi*-induced molecular mimicryn and Chagas' disease. Curr Top Microbiol Immunol. 2005; 296: 89-123.

8. Bonney KM, Taylor JM, Daniels MD, Epting CL, Engman DM. Heat-killed *Trypanosoma cruzi* induces acute cardiac damage and polyantigenic autoimmunity. PLoS ONE. 2011; 6: e14571.

9. Basso B, Moretti E, Vottero-Cima E. Antigenic relationships between *Trypanosoma cruzi* and *Trypanosoma rangeli*. Rev Iber Parasitol. 1987; 47: 15-21.

10. Saldaña A, Souza OE. *Trypanosoma rangeli* and *Trypanosoma cruzi*: cross-reaction among their immunogenic components. Mem Inst Osvaldo Cruz. 1996; 91: 81-82.

11. Basso B, Moretti E, Fretes R. Vaccination with fixed epimastigotes of different strains of *Trypanosoma rangeli* protects mice against *Trypanosoma cruzi* infection. Mem Inst Osvaldo Cruz. 2008; 103: 370-374.

12. Basso B, Cervetta L, Moretti E, Carlier Y, Truyens C. Acute *Trypanosoma cruzi* infection: IL-12, IL-18, TNF, sTNFR and NO in *Trypanosoma rangeli*-vaccinated mice. Vaccine. 2004; 22: 1868-1872.

13. Basso B, Moretti E, FretesR. Vaccination with *Trypanosoma rangeli* induces resistance of guinea pigs to virulent *Trypanosoma cruzi*. Vet Immunol Immunopathol. 2014; 157: 119-123.

14. Basso B, Castro I, Introini V, Gil P, Truyens C, Moretti E. Vaccination with *Trypanosoma rangeli* reduces the parasite burden of dogs infected with *Trypanosoma cruzi,* leading to lower infection rate of the vector *Triatomainfestans.* Vaccine. 2007; 25: 3855-3858.

15. Basso B, Marini V, Gauna D, Frías M. Vaccination of dogs with *Trypanosoma rangeli* induces antibodies against *Trypanosoma cruzi* in a rural area of Córdoba, Argentina. Mem Inst Oswaldo Cruz. 2016; 111: 271-274.

16. Dumonteil E, Bottazzi ME, Zhan B, Heffernan MJ, Jones K, Valenzuela JG, et al. Accelerating the development of a therapeutic vaccine for human Chagas disease: rationale and prospects. Expert Rev Vaccines. 2012; 11: 1043-1055.

17. Resende Pereira I, Vilar-Pereira G, Marques V, da Silva AA, Caetano B, Moreira OC, et al. A Human Type 5 Adenovirus-Based *Trypanosoma cruzi* Therapeutic Vaccine Reprograms Immune Response and Reverses Chronic Cardiomyopathy. PLoS Pathog. 2015; 11: e1004594.

18. Basso B, Moretti E, Dominguez M. Cultivo de *Trypanosoma cruzi* en medio mmonofásico con aireación para obtención de antígenos. Medicina (Buenos Aires). 1980; 40: 428-432.

19. Brener Z. Therapeutic activity and criterion of cure on mice experimentally infected with *Trypanosoma cruzi*. Rev Inst Med Trop Sao Paulo. 1962; 4: 389-396.

20. Marini V, Moretti E, Bermejo D, Basso B. Vaccination with *Trypanosoma rangeli* modulates the profiles of immunoglobulins and IL-6 at local and systemic levels in the early phase of *Trypanosoma cruzi* experimental infection. Mem Inst Oswaldo Cruz. 2011; 106: 32-37.

21. Smith P, DiLillo DJ, Bournazos S, Li F, Ravetc JV. Mouse model recapitulating human Fcγ receptor structural and functional diversity. Proc Natl Acad Sci U S A. 2012; 109: 6181-6186.

22. Nimmerjahn F, Ravetch JV. Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science. 2005; 310: 1510-1512.

23. el Bouhdidi A, Truyens C, Rivera MT, Bazin H, Carlier Y. *Trypanosoma cruzi* infection in mice induces a polyisotypic hypergammaglobulinaemia and parasite-specific response involving high IgG2a concentrations and highly avid IgG1 antibodies. Parasite Immunology. 1994; 16: 69-76.

24. Marin-Neto JA, Cunha-Neto E, Maciel BC, Simões MV. Pathogenesis of chronic Chagas heart disease. Circulation. 2007; 115: 1109-1123.

25. Arce-Fonseca M, Rios-Castro M, Carrillo-Sánchez SC, Martínez-Cruz M, Rodríguez-Morales O. Prophylactic and therapeutic DNA vaccines against Chagas disease. Parasit Vectors. 2015; 8: 121.

26. Rodríguez-Morales O, Monteón-Padilla V, Carrillo-Sánchez SC, Rios-Castro M, Martínez-Cruz M, Carabarin-Lima A, et al. Experimental Vaccines against Chagas Disease: A Journey through History. J Immunol Res. 2015; 2015.

27. Aparicio-Burgos JE, Zepeda-Escobar JA, Montes de Oca-Jimenez R et al. Immune Protection against *Trypanosoma cruzi* Induced by TcVac4 in a Canine Model. PLoS Negl Trop Dis. 2015; 9: e0003625.

28. Basso B, Marini V. Experimental Chagas disease. Innate immune response in Balb/c mice previously vaccinated with *Trypanosoma rangeli*. I. The macrophage shows immunological memory: Reality or fiction. Immunobiology. 2014; 219: 275-284.

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