ARTÍCULO ORIGINAL

Trypanosoma rangeli infected mouse sera reactivity with *Trypanosoma cruzi* synthetic peptides

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ABSTRACT

In man, differential diagnosis of Trypanosoma cruzi and Trypanosoma rangeli infections represents a serious problem, not only because both parasites present similar geographical distribution, the same hosts and sometimes the same insect vector, but also because they have common antigen determinants. In this work IgM and IgG humoral responses to T. cruzi synthetic peptides in mice infected with T. cruzi and with T. rangeli were analysed. In a immunoradiometric assay (IRMA) 6 synthetic peptides were used, denominated as clones 1, 2, SAPA, 13, 30 and 36. The results showed that sera from infected mice with T. rangeli recognized all peptides derived from T. cruzi proteins, at IgM as well as IgG level. Reactivity with peptide SAPA is discussed as previous work indicated that SAPA is not codified in the T. rangeli genome. Our results support the suggestion that crossed reactions are due to the fact that both parasites present common antigens.

Key words: *Trypanosoma cruzi*, *Trypanosoma rangeli*, cross-reaction.

INTRODUCTION

Two trypanosomes exist in Central and Southern America which are capable of infecting man, *Trypanosoma cruzi* and *Trypanosoma rangeli*. While *T. cruzi* causes Chagas' disease, a complicated and devastating disease that affects around 20 million people in America^{1,2}, *T. rangeli* may be pathogenic for invertebrate host but not pathogenic for vertebrate hosts³. Even though *T. rangeli* infection, up to now, has not been able to be associated with a specific pathology it does present a serious problem in the diagnosis of

Chagas' disease, because of the appearance of crossed reactions in serological test,^{4,5}. Both parasites have a similar geographical distributions, the same vertebrate host and in some regions they share the same insect vector⁶. Some authors have reported antigenic cross-reaction between both parasites in different immunological assays^{7,8}.

In this work, the humoral response of mice infected with *T. rangeli* and *T. cruzi* blood trypomastigotes was analysed, thereby determining IgM and IgG reactivity to *T. cruzi* synthetic peptides.

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MATERIALS AND METHODS

Mice: Males mice of the Swiss ICO NMRI were used for each one of the infection models (Parasitology Laboratory, Universidad Autónoma de Madrid, Spain).

Parasites stocks: The *T. rangeli* C23⁹ and the *T. cruzi* Y (Parasitology Laboratory, U.A.M., Spain) strains were used.

Infection models: One month old mice were infected with 10^4 *T.rangeli* blood trypomastigotes and another group with 10^4 *T. cruzi* parasites. Sera from this infected animals were used in this study.

Immunoradiometric assay (IRMA) with synthetic peptides: Six synthetic peptides were used modelled according to antigen repeated units' aminoacid sequences having been previously cloned from *T. cruzi*¹⁰ (clones 1, 2, SAPA, 13, 30 and 36). This peptides were kindly provided by Dr. A. Engstrom from the Immunology Department of Upsala University. Sweden.

The immunoradiometric method was used following the previously described technique¹¹. In summary, polyvinylchloride microplates (Falcon Labware, U.S.A.), were incubated for a whole night at 4° C with 50 µl peptide diluted in a optimum concentration with pH 9.6, 0.1 M sodium bicarbonate. Later excess peptide was

eliminated in three washes with Tween 20 buffer (0.9% NaCl, 0.05% Tween 20, 0.01% Sodium Azide) and the microplates were saturated with 200 µl 3% nonfat milk for 90 minutes. After washing, they were incubated for 90 minutes with 30 µl of serial dilutions from the distinct infected mouse sera samples. After washing in Tween 20 buffer the wells were incubated with 5×10^4 c.p.m. in 30 µl of ¹²⁵I radiolabelled affinity purified goat anti-mouse IgG or goat anti-mouse IgM (Sigma, U.S.A.) diluted with 3% nonfat milk. After next being incubated for 90 minutes at room temperature, the weels were washed five times in buffer to eliminate excess radioactive material, finally the weels were cut and counted in a gamma counter (Isodata).

RESULTS AND DISCUSSION

All animals infected with *T. rangeli* survived however with *T. cruzi* infection all mice died by day 23 post-infection.

T. rangeli and *T. cruzi* infected mouse sera's IgM reactivity is showed in Figure 1 and Figure 2, respectively. There is reaction with all synthetic peptides, with maximum reaction level being around day 15 and staying the reactivity until day 40, after infection with *T.rangeli*. In both

Figure 1. IgM reactivity in NMRI mice infected with *T. rangeli* C23 strain.

Figure 2. IgM reactivity in NMRI mice infected with *T. cruzi* Y strain.

Figure 3. IgG reactivity in NMRI mice infected with *T. rangeli* strain.

Figure 4. IgG reactivity in NMRI mice infected with *T. cruzi* Y strain.

situations the greater reactivity is with peptide 1.

There was also IgG response to all the peptides and again a greater reactivity with the peptide 1 is observed. IgG reactivity with *T. rangeli* started to climb notably on day 26 and continued until day 40 post-infection (Figure 3). Reactivity of sera from infected mice with Y strain shows a maximum level with all the peptides at the day 19 except for the peptide 1 that shows a peak in the day 15 pos-infection (Figure 4).

This results indicate that sera from infected mice by *T. rangeli* C23 strain recognized, at both IgM and IgG antibody level, all peptides derived from *T. cruzi* proteins, which were known to be localized in defined regions of the parasite¹². Furthermore, no significant differences were shown with the reactivity of *T. cruzi* infected mouse sera. The high reactivity with peptide 1 is interesting given this antigen's flagellar localization¹². Previous observations have shown that *T. cruzi* flagellar proteins are highly antigenic and can be effective in protection against the parasite's infectious forms^{13,14}.

Reactivity with SAPA was not in agreement with previous studies which indicates that SAPA is not codified in the *T. rangeli* genome¹⁵. However, this results could be explained by the fact that SAPA represents a family of distinct proteins and/or is due to genetic differences between the *T. rangeli* strains used. In previous works, infection with *T. rangeli* C23 strain

showed that it had a protector effect when faced with further infection by the *T. cruzi* Y strain¹⁶, therefore it would be interesting to study each one of the antigens' specific participation in the protection phenomena. Our results with synthetic peptides support the suggestion that crossed reactions are due to the fact that both parasites present common antigens.

REFERENCES

- 1.- SCHMUNIS G. American trypanosomiasis as a public health problem. In: Chagas' disease and the nervous system. Scientific Publication N° 547 1994. Ed. PAHO, Washington, U.S.A.: 3-28.
- 2.- DIAS J C P, SILVEIRA A C, SCHOFIELD C J. The impact of Chagas disease control in Latin America - A review. Mem Inst Oswaldo Cruz 2002; 5: 603-12.
- 3.- STEVENS J R, NOYES H, SCHOFIELD C J, GIBSON W. The molecular evolution of Trypanosomatidae. Adv Parasitol 2001; 48: 1-56.
- 4.- GULH F, HUDSON L, MARINKELLE C, et al. Antibody response to experimental *Trypanosoma rangeli* infections and its implications for immunodiagnosis of South American trypanosomiasis. Acta Trop 1985; 42: 311-8.
- 5.- SALDAÑA A, SOUSA O. *Trypanosoma rangeli*: Epimastigote immunogenicity and cross-reaction with *Trypanosoma cruzi*. J Parasitol 1996; 82: 363-6.
- 6.- BÓTERO D, RESTREPO P. Parasitosis Humanas. Ed. Corporación para Investigaciones Biológicas. Medellín, Colombia 1992: 191-206.
- 7.- ACOSTA L, ROMANHA A J, COSENZA H, et al. Trypanosomatid isolated from Honduras: Differen-

- tiation between *Trypanosoma cruzi* and *Trypanosoma rangeli*. Am J Trop Med Hyg 1991; 44: 676- 83.
- 8.- SALDAÑA A, ORN A, HENRIKSSON J, et al. Evaluación de cuatro métodos inmunobioquímicos/ moleculares en la identificación de cepas de *Trypanosoma cruzi* y *Trypanosoma rangeli*. Rev Med (Panamá) 1993; 18: 41-52.
- 9.- ZÚÑIGA C, PALAU M T, PENIN P, et al. Characterization of a *Trypanosoma rangeli* strain of a Colombian origin. Mem Inst Oswaldo Cruz 1997; 92: 523-30.
- 10.- VERGARA U, VELOSO C, GONZÁLEZ A, et al. Evaluation of an enzyme-linked immunosorbent assay for the diagnosis of Chagas' disease using synthetic peptides. Am J Trop Med Hyg 1992; 46: 39-43.
- 11.- VERGARA U, LORCA M, VELOSO C, et al. Assay for detection of *Trypanosoma cruzi* antibodies in human sera based on reaction with synthetic peptides. J Clin Microbiol 1991; 29: 2034-7.
- 12.- SOUTO-PADRÓN T, REYES M, LEGUIZAMÓN S, et al. *Trypanosoma cruzi* proteins which are antigenic during human infections are located in defined regions of the parasite. Eur J Cell Biol 1989; 50: 272-8.
- 13.- PEREIRA N, DE SOUZA W, MACHADO R, et al. Isolation and properties of flagella of trypanosomatids. J Protozool 1977; 24: 511-4.
- 14.- SEGURA E, BUA J, DE CAMPANINI A, et al. Monoclonal antibodies against the flagellar fraction of epimastigotes and passive immunoprotection in mice. Immunol Lett 1986; 13: 165-71.
- 15.- PONTES DE CARVALHO L, TOMLINSON S, NUSSENZWEIG V. *Trypanosoma rangeli* sialidase lacks trans-sialidase activity. Mol Biochem Parasitol 1993; 62: 19-22.
- 16.- ZÚÑIGA C, PALAU M T, PENIN P, et al. Protective effect of *Trypanosoma rangeli* against infection with highly virulent strain of *Trypanosoma cruzi*. Trop Med Int Health 1997; 5: 482-7.

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