SHORT COMMUNICATION

Antibody Isotype Responses in Balb/c Mice Immunized with the Cytoplasmic Repetitive Antigen and Flagellar Repetitive Antigen of Trypanosoma cruzi

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In the present report we analyzed the levels of IgG1, IgG2a, IgG2b and IgG3 isotypes from Balb/c mice immunized with cytoplasmic repetitive antigen (CRA), and flagellar repetitive antigen (FRA) of Trypanosoma cruzi. The immunization was done by subcutaneous route three times (20 days apart) and the analysis was performed 14 days after each treatment. CRA-immunized mice produced high levels of all IgG isotypes, mainly IgG3 and IgG1. FRA-immunization elicited only high levels of IgG1.

Key words: Trypanosoma cruzi - recombinant antigens - immunization - isotypes

Trypanosoma cruzi infection in both humans and mice develop a strong and heterogeneous humoral immune response as shown by circulating specific antibodies. These antibodies are involved in resistance to parasite and studies on the immunoglobulins isotypes present in host surviving acute infection has indicated the importance of these responses (Brodskyn et al. 1989, Bouhdidi et al. 1994). In infected mice, the major anti-parasite response consist of the IgG1 and IgG2 isotypes as demonstrated by indirect immunofluorescence (Araújo et al. 1984), western blot (Rowland et al. 1992), lytic activity (Krettli et al. 1979), T. cruzi bloodstream trypomastigotes (Brodskyn et al. 1989) and passive transfer protection (Takéhara et al. 1981).

CRA (cytoplasmic repetitive antigen) and FRA (flagellar repetitive antigen) recombinant antigens (Rec-Ag) of T. cruzi were obtained from Bio-Manguinhos-Fiocruz according to Krieger et al. (1992) and used as antigens. Groups of 6 to 8-week-old male Balb/c mice were injected by subcutaneous route with three doses of purified Rec-Ag of T. cruzi, in order to further use them in immunotherapy assays.

Male Balb/c mice (6 to 8 week-old) were used following the guidelines of the Ethical Committee to use of Experimental Animals from the Oswaldo Cruz Foundation (Ministry of Health, Brazil). CRA and FRA Rec-Ag were obtained from Bio-Manguinhos-Fiocruz according to Krieger et al. (1992) and used as antigens. Groups of 6 to 8-week-old male Balb/c mice were injected by subcutaneous route with three doses of purified Rec-Ag CRA (20 µg), and FRA (12 µg) at 20 days of intervals. The first dose was emulsified in complete Freund’s adjuvant and the following in incomplete Freund’s adjuvant. The same schedule was used for control mice that received only adjuvant.

Before and 14 days after each dose, sera from individual mice were tested for IgG1, IgG2a, IgG2b, and IgG3 isotypes by ELISA. Briefly, microtiter plates (Nunc-Immuno Plates, MaxiSorp, 96 wells, Nalgen Nunc International Corporation) were coated with 1 µg/ml of CRA or FRA (100 µl/well) diluted in 0.05 M Na2CO3 buffer, pH 9.6 and incubated overnight at 4°C. The plates were blocked with PBS-Tween 20 (0.05%) (PBS-Tw) containing 5% fat free milk (Nestlé), prior to incubation with 100 µl of sera diluted (1:100) in PBS-Tw (overnight, 4°C). The bound antibodies were detected by incubation with horseradish peroxidase-conjugated isotype specific rabbit anti-sheep immunoglobulin (Caltag). The reaction was detected by the addition of orthophenyldiamine-OPD plus H2O2 and stopped with H2SO4 2.5 N. Quantification of the reaction

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was determined by optical density using an automated ELISA reader at 490 nm (Bio-Rad 3550). Statistical analysis was performed using the Mann-Whitney *U*-test for non-parametric distributions.

The kinetics of *T. cruzi* specific antibody levels for each IgG isotype are shown in the Figs 1 and 2. We found a marked difference in the pattern of antibody response elicited by immunization with CRA Rec-Ag when compared to the FRA Rec-Ag. The levels of all IgG isotypes in CRA-immunized mice were significantly increased (*p* < 0.05) after the 2nd and 3rd immunizations when compared to the values observed in control mice and preimmune sera. Predominant levels of IgG3 and IgG1 characterized CRA immunization. In contrast, immunization with FRA elicited an antibody response mediated by IgG1 (*p* < 0.05). The isotypes IgG2a, IgG2b, and IgG3 were not detectable in the sera of FRA-immunized mice.

The immune response against *T. cruzi* experimental infection involves many factors. This parasite in the extracellular phase is susceptible for the destruction by immunological reactions which involve humoral factors or combined action of specifics antibodies and its receptors in leukocytes surface (Kierszenbaum & Lima 1983). Krettli and Brener (1976) have demonstrated that partial protection could be obtained by the transference of antibodies anti-tripomastigote proceeding from infected mice. Serum of animals infected with Y and Colombian strains have been capable to confer partial protection against Y strain, once the pre-incubation of the parasites with immune sera decreased the infectivity of tripomastigote. The ability of the antibodies in conferring protection depends on the class and subclass of immunoglobulin to which they belong, and only the transference of IgG2a and IgG2b antibodies could protect the animals, decreasing the parasitemia and mortality rates (Takehara et al. 1981, Brener & Krettli 1990). The detection of the different immunoglobulin isotypes induced by CRA demonstrate that this antigen is able to induce humoral immune response. Although this antigen was more potent to induce IgG1 and IgG3 isotypes, it also increased IgG2a and IgG2b levels. On the other hand, FRA was more potent...
to induce only IgG1 isotype. Previous studies have demonstrated that IgG2a, IgG2b and IgG1 are important isotypes involved in the elimination of blood forms of the parasite (Brodskyn et al. 1989). However, our results also suggest the possibility of IgG3 having a significant role in protection against infection. Thus, the antibodies detected in CRA and FRA-immunized mice could help to control of the parasitemia in a later infection. In conclusion, preliminary evidence was provided that both CRA and FRA Rec-Ag activate immune mechanisms involved in parasite elimination. These approaches are currently being evaluated in immunized and challenged mice.

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REFERENCES


