SIALYLATION PROTECTS TRYPANOSOMA CRUZI TRYPOMASTIGOTES AGAINST LYtic ANTIBODIES IN HUMAN CHAGAS' DISEASE

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We and others have reported that lytic anti-anti-α galactosyl antibodies (anti-α-Gal) purified from chronic Chagas' disease patients recognize anti-α-galactosyl epitopes of O-linked oligosaccharide chains of mucin-like glycoproteins of trypomastigotes. These antibodies strongly agglutinate and destroy trypomastigotes independent of complement. By scanning electron microscopy we found that parasite membrane was dramatically affected by the presence of anti-α-Gal. We have also found previously that most of Chagasic patients present antibodies that
therapy, therapeutic failure occurred in 21% and side effects were observed in 28% of the cases. In the group undergoing the intermittent regimen only one patient spontaneously interrupted the treatment (3%), there was 9% of therapeutic failure and side effects were observed in 15.2% of the subjects. There was a statistically significant difference between the groups with regard to adherence to therapy (p<0.05, Fisher’s exact test). The mean time for the complete healing of lesions was similar: 97.4 ± 94.2 days for the continuous and 110.8 ± 61.1 days for the intermittent schedule. Our results show that, in our experience, the adherence to therapy is better with the intermittent schedule than with the continuous schedule of antimonial therapy. Financial support: CNPq.

EXPERIMENTAL INFECTIONS OF LEISHMANIA (LEISHMANIA) AMAZONENSIS IN CEBUS APPELLA (PRIMATES: CEBIDAE) : EVALUATION OF HUMORAL RESPONSES BY THE ELISA TECHNIQUE USING HOMOLOGOUS AND HETEROLOGOUS ANTIGENS.

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The present study is part of a project aimed at determining the immunogenicity and effectiveness of candidate leishmanial vaccines in a non-human primate model. The principal objectives of the present work were to confirm the infectivity of a single inoculum of 1.6 \times 10^6 Leishmania (Leishmania) amazonensis promastigotes in 7 laboratory bred monkeys (Cebus apella) and to determine their humoral response during different phases of the infection by the enzyme linked immune assay (ELISA) using heterologous and homologous leishmanial antigens. Promastigotes from 12 day old cultures, that represented the 2nd sub-passage from an infected hamster, were washed and suspended in a saline/glucose solution to give a final concentration of 1.6 \times 10^6 organisms per 0.1 ml. Each of the 7 animals was inoculated intradermally in a shaved area of the upper surface of the tail with 0.1 ml this solution. Heparanised blood samples were taken at the time of the inoculation and thereafter at 15 day intervals. The plasma samples thus obtained from each animal were then frozen. ELISA antigens of L. (L.) amazonensis and L. (Viannia) braziliensis were prepared by freezing (-182°C) and thawing (37°C) washed cultures 10 times. Optimum antigen concentrations were determined in a block tritration using different antigen and antibody concentrations. A human anti-IgG/peroxidase conjugate was used with an OPD substrate and reactions were read at 492nm. The different titers were transformed into neperian log values and analysed using the analysis of variance. Significant titers were found at 60 days (p > 0.5) and between 82 - 91 days (p > 0.5). The titers for these two periods were used to look for differences between the two antigens. All the animals developed eryhematous nodules 15-20 days post inoculation (p.i.) which persisted for approximately 3 months. The mean diameter of the lesions was 8.7 mm with maximum and minimum diameters of 13mm to 3 mm at 2 months p.i. Parasites were seen in the stained smears of 4 animals. The titers during these primary infections varied from 40 to 320, reaching their maximums 2 months p.i., with the homologous antigen. Titers with the L. (V) braziliensis heterologous antigen varied from 40 to 80, peaking 3 months p.i. The differences between the two antigens were significant and indicate that the homologous antigen should be used to follow the humoral response of C. apella to experimental infections of L. (L.) amazonensis. It was also confirmed that an inoculum of
1.6 x 10^6 promastigotes is infective and provokes an antibody responses that is comparable to that seen in man naturally infected with the same parasite indicating that an inoculum of this concentration is suitable for testing the immune state of monkeys that have been vaccinated with candidate vaccines.

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Key words: L. (L.) amazonensis; Cebus apella; humoral response

IMMUNOGENIC STUDY OF MICE STRAIN INFECTED WITH LEISHMANIA AMAZONENSIS.


In New World cutaneous leishmaniasis the delayed-hypersensitivity (DTH) level present a strong correlation with the different stages of the spectrum of the disease. Exarcebated specific DTH reaction occur chiefly in mucocutaneous form of the disease in which antibody levels is lower than those observed in patients with localized lesions. This is correlated with the results reported in experiments employing artificial immunizations using sheep red blood cell (Lagrange & Mackaness, 1975; J. Exp. Med. 141: 82-96). Diffuse cutaneous leishmaniasis on the other polar position of the spectrum, shows a limited expression of cell-mediated immunity (CMI) including protection and DTH to specific antigen. The present data deal with the induction of DTH to leishmania antigens in different inbred strains of mice, which can reproduce a similar spectrum of leishmaniasis.

When 1x10^4 amastigotes were used in C57BL/6 (intermediated), Balb/c (susceptible) and C3H.He/N relatively resistance mice. A significant increase in footpad swelling was elicited in BCG-pretreated mice by Riboleish; in only Balb/c mice.

In a second protocol were investigated the kinetics of DTH reaction to virulent amastigotes cells of L. amazonensis in outbred mice. Comparative kinetics between Riboleish vaccine and Montenegro antigen in footpad skin test were made through out the course of infection. Doses of 4x10^5 /ml, 4x 10^6/ml and 4x10^7/ml of Montenegro were inoculated in groups of OF1 mice on days 20, 30, 60 and 90 days after infection the 1x10^4 of L.amazonensis amastigotes (H21 strain). Simultaneously, groups of mice were inoculated with 10 mg of Riboleish (microsomal fraction) for comparison.

The present data shows that the microsomal fraction induce stronger DTH when compared with Montenegro reaction. Levels of DTH developed in response to different schedules of Riboleish vaccine, by normal mice, mice sensitized with 10mg of Riboleish and mice inoculated subcutaneously with 10^6 BCG 21 days before sensitization. Footpad tests for DTH were performed 6 days after sensitization. Means of 6± SEM.

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