CH-87-11 HUMAN CHAGAS' DISEASE: EVOLUTION OF THE ANTIBODY RESPONSE AND RECOGNITION OF ANTIGENS OF Trypanosoma cruzi.

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Although Chagas' disease is a major public health problem in large areas of Latin America few studies have been conducted in humans to examine the evolution of the antibody response, of lytic antibodies and of antigens of the causative agent, Trypanosoma cruzi, recognized by these antibodies. Because infection with T. cruzi occurs predominantly in rural areas, detailed clinical and serological observations have been limited by the fact that physicians are usually not available and by the difficulty of diagnosing the initial infection and of obtaining specimens from the onset. In endemic areas, the occurrence of inapparent infection is more frequent than the flagrant, apparent acute disease. Overt, and often grave, acute T. cruzi infections usually occurs during infancy and childhood (1,2).

Systematic serologic evaluation of serum samples collected at different periods of time following infection are of great value in the diagnosis of the infection (3) , as well as to evaluate the effects of specific therapy and to establish cure (4). In addition, the examination of the antigens of T. cruzi recognized by antibodies formed at different periods of time during the infection is of importance because of the demonstration that, of the large array of antibodies produced in response to infection with T. cruzi, apparently, only those antibodies with the ability to lyse circulating trypanosomes may participate in the antibody-mediated resistance (5).

We considered of interest, therefore, to analyse the evolution of the antibody response of an individual who acquired a T. cruzi infection through a laboratory accident and to examine the antigens of the three stages of the life cycle of T. cruzi recognized by antibodies in serum collected at various periods of time during the infection. The IgM and subclasses of IgG antibody responses were examined by using an enzyme-linked immunosorbent assay (ELISA) (6) and the indirect immunofluorescent antibody test (IFA) (7). Antibodies with capacity to lyse trypanosomes were examined by using the complement-mediated cell lysis (CML) assay (8). Antigen recognition was determined by the western blot technique (9) and immunoprecipitation of radioactively labeled antigens (10). Because of the demonstration that bloodstream forms of T. cruzi display neuraminidase (Nase) activity, we also examined serial serum samples from the infected individual for Nase and anti-Nase activity by using the technique of agglutination of

erythrocytes by peanut agglutinin (Arachis hypogaea agglutinin, PNA) as previously described (11).

Our approach highlighted differences in the expression of immunoglobulin isotypes by the infected host, corroborated the possible clinical utility of the CML assay to determine cure of the infection as previously suggested and revealed striking differences in the recognition of antigens of each of the three stages of T. cruzi. In addition, we demonstrated the development of seric Nase and anti-Nase activity early in the infection.

Thus, specific IgM and IgG antibodies to T. cruzi, were detected by both ELISA and IFA tests only 35 days after the infection. Of interest was that IgG antibodies were detected at the same time as IgM antibodies. Peak titers of both immunoglobulins were reached five days after they were first detected. Thereafter the titers in both serological tests decreased steadily and were negative one year after the infection.

The concentration of T. cruzi-specific antibodies of each one of the subclasses of IgG increased following infection. Peak titers for all subclassess was noted around day 50 of infection. By day 80, the levels of IgG1, IgG3 and IgG4 antibodies had returned to levels in serum collected before the infection. The levels of IgG2, however, remained elevated up to 300 days after infection.

Circulating trypanosomes were never demonstrated by direct microscopy of large quantities of concentrated blood. Mouse inoculation, however, indicated that circulating parasites were present one week before development of a positive serology. Thus, 2 of 5 mice inoculated with blood collected on day 27 of infection revealed patent parasitemia when examined one week later (day 35 of infection). Of the mice inoculated with blood collected on day 27, 35 and 40 after infection 40,100,and 40 %, respectively, developed parasitemia. Mice inoculated after day 40 did not develop either parasitemia and positive serology. This was possibly due to the treatment with nifurtimox (10 mg/kg/day for 120 days) initiated on day 35 when infection with T. cruzi was proven.

Repeated CML assays with serum samples collected before infection (day -60) and up to 20 days after the infection never revealed any lysis of parasites. Percent lysis of 11 to 27 % were noted with serum samples collected 20, 25 and 30 days after the infection when serological tests were still negative. At day 35 when both the ELISA and the IFA tests detected specific antibodies to T. cruzi the percent lysis was 89 %. Thereafter the percent lysis remained elevated in each one of the serum samples collected up to day 300 after infection. Subsequent samples collected after this period of time (360, 400 days after infection) did not reveal any lysis.

Western blots were performed with antigens of epimastigotes, spleen amastigotes

and blood trypomastigotes to compare the antigens of these stages of the life cycle of T. cruzi recognized by antibodies and to determine differences in antigen recognition at different periods of time during the infection with the organism. A large number of polypeptides of epimastigotes were bound by IgM antibodies that were not specifically directed against T. cruzi. Thus, numerous faintly and strongly stained bands distributed over the entire range of the separated polypeptides in the nitrocellulose paper were noted in a blot treated with serum collected 60 days before the infection. A blot with serum collected at 35 days of infection, when serological tests became positive, revealed bands indicative of antigens recognized by specific IgM antibodies in the area above the 92.5 K molecular weight (MW) marker, in the area of the 66 K and below the 45 K and 31 K MW markers. In addition, bands which were weakly stained with serum collected prior to the infection became noticeably darker. After day 80 of infection the binding of IgM antibodies to specific antigens became less and less pronounced as the infection and the treatment progressed. By day 300 of infection the pattern of antigens recognized by IgM antibodies was similar to the pattern noted with serum collected before the infection.

IgG antibodies also bound nonspecificaly to epimastigote polypeptides. The bound antigens, however, were different from those bound by IgM antibodies. Most noticeable in this respect were the low MW antigens (below the 21.5 MW marker) which were recognized by IgM but not by IgG antibodies. As it was noted in the IgM blot, in the blot developed for IgG antibodies some antigens yielding faint bands with serum collected before and during the first 30 days of infection became darkly stained after the serologic conversion. In addition, bands not observed before serologic conversion later became visible. In contrast to what was noted with IgM antibodies, specific antigens were strongly bound by IgG antibodies in serum collected up to 300 days after infection when ELISA and IFA serologic titers were quite low (1:20). Blots performed with serum collected after negativation (<1:10) of the ELISA and IFA tests had a pattern of recognized antigens similar to that noted serum collected before the infection.

The pattern of the western blots of antigens of amastigotes and blood trypomastigotes recognized by IgG antibodies was considerable simpler, particularly for trypomastigotes, than the pattern of epimastigote antigens recognized by the same antibodies. A group of antigens of MW higher than 92.5 K were strongly bound by antibodies in the amastigote preparation but not in the trypomastigote preparation. Antigens in the area of the 66 K and between 66 K and 45 K MW markers were recognized in both amastigote and trypomastigote antigen preparations and an antigen in the area of the 31 K MW marker was strongly recognized in the amastigote preparation but faintly recognized in the trypomastigote preparation.

Western blots with antigens of epimastigotes from different strains of T. cruzi

were quite similar suggesting that the major antigens may be represented in all strains of the organism. Minor antigens may have quantitative differences which may result in bands stained with different intensities.

Immunoprecipitation of ^{125}I-labeled epimastigote antigens revealed a group of antigens (12 to 15) over the entire range of the 5 to 15% gradient gel. A wide band just below the area of the 97 K MW marker and a well defined and deeply stained band above the 66 K MW marker probably represented the 90 K and the 72 K glycoproteins of the cell membrane surface of T. cruzi which have been shown to be immunoprecipitated by antibodies in serum of individuals infected with the organism. In addition to these bands, other bands representing recognized antigens were noted.

Nase activity was detected in serum 12 days after the accidental infection, peaked at the time when clinical symptoms appeared, and dropped to undetectable values by the day antibodies specific for T. cruzi were first demonstrated. A seric factor blocking Nase activity was demonstrated when antibodies were first detected and persisted with high titers for at least 12 weeks thereafter. Erythrocyte and white blood cell counts as well as hemoglobin and hematocrit were below the lower limit of normality when seric neuraminidase activity was at its peak.

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