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DEVELOPMENT OF CHAGASIC AUTOIMMUNE MYOCARDITIS ASSOCIATED WITH ANTI-IDIOTYPE REACTION

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Chronic Chagas myocarditis is the most frequent cause of congestive heart failure and sudden death in endemic areas of the New World. The etiologic agent of Chagas disease is the hemoflagellate, Trypanosoma cruzi, an obligatory intracellular protozoan. The presence of anti-heart immune reactions in Chagas'disease has led many investigators to postulate that autoimmune phenomena play a role in the pathogenesis of chronic Chagas'heart disease. In previous works we have demonstrated that autoimmunity may be produced by cross - reacting antigens of host and T.cruzi (1,2). Recent investigation has shown that myotropic host-cell recognition is promoted by chemoaffinity of complementary parasite attachment molecules to receptors on the muscle fiber, and that these attachment sites are antigenic (3). Thus, when monospecific F(ab')2 anti-T.cruzi surface antigen is allowed to react with monospecific F(ab')2 anti-muscle surface antigen, immune complexes are formed in a concentration dependent reaction regardless of whether antibodies are experimentally derived or obtained from naturally infected subjects. As a result of this experimental observation, we suggested that it is possible that IgG anti-idiotype antibody against the protective primary anti-T.cruzi antibody may also be reactive to complementary receptors of the sarcolemma thereby inflicting myocarditis in susceptible subjects.

We sought to investigate the possible association of IgG anti-idiotype antibody activity (anti-Id) and severe chronic Chagas myocarditis. To accomplish this we compared serum levels of anti-Id and anti-Id immune complexes in ninety-two seropositive but asymptomatic

subjects of the Bahia State village of Moniz Ferreira, which is endemic for T.cruzi infection, with twenty-three seropositive patients with severe chronic myocarditis and congestive heart failure hospitalized in the Hospital Prof. Edgard Santos, Salvador. A group of eighty-four seronegative healthy village subjects served for control.

The antigens used were Acute T.cruzi infection F (ab')2 and Non-relevant F(ab')2. The F(ab')2 (acute infection) fragment was prepared from pepsin digests of IgG from 25 infants sera with acute parasitemia. Nonrelevant human F(ab')2 fragments were purchased from Calbiochem (La Jolla, California).

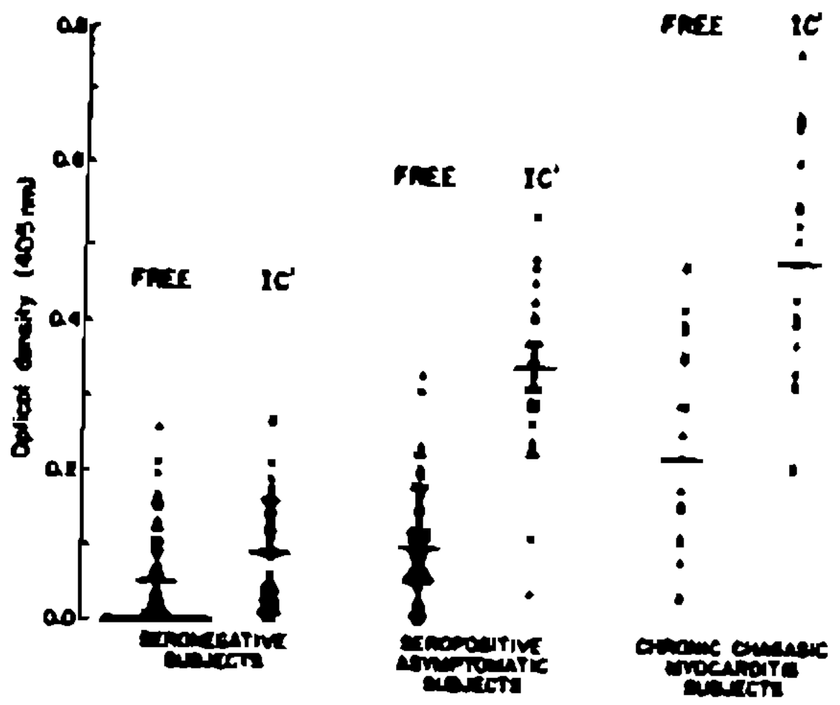
Total specific IgG anti-acute T.cruzi infection F(ab')2 - All tests were done in duplicate without prior knowledge of their origin. An enzyme linked immunosorbent assay (ELISA) was used. The sera were previously acidified with 0.15M NaCl in 0.01 M glycine - HCl buffer (pH 2.5) in a final 1:40 dilution to dissolve immune complexes. The acidified (pH 3) test sera were next diluted in microtiter wells previously coated with F(ab')2 antigens with an equal volume (100 ul) of 0.15 M NaCl in 0.05M phosphate buffer pH 7.4 with 5% FBS and 0.05% Tween 20. This resulted in conversion of the pH of the reaction mixture to 7.3 and allowed the nascent antibodies to react with the ex-cess solid.phase F(ab')2 antigens. Bound IgG was indexed with alkalin phosphatase conjugated affinity purified rabbit F(ab')2 anti human Fc fragment at an appropriate dilution. The plates were read in a Titertek Multiscan Plus spectrophotometer at an OD of 405 nm. The corrected specific total antibody activity was tabulated after subtraction of the OD obtained with non relevant human F(ab')2 in duplicate parallel tests.

Free IgG anti-idiotype activity and calculation of anti-idiotype immune complexes - This was done in a similar procedure at physiologic pH (7.4) by omitting the acid treatment. The OD obtained with non relevant F(ab')2 was used to correct for nonspecific reactons. Levels of anti-idiotype immune complexes were indexed by difference between the optical density of total and free specific IgG anti-T.cruzi infection F(ab')2.

The conditions for assay of anti-idiotype antibody activity described in this report allowed for the indexing of total and free IgG anti-acute T.cruzi infection F(ab')2 activity of the test sera . Assays done after dissolution of immune complexes at pH 3 indexed for total IgG anti-Id activity whereas assays at pH 7.4 indexed for free reactive antibody. The specificity of the assay was established by showing that treatment of the positive sera with live, tissue infective, metacyclic trypomastigotes (5x 10^6 per ml) failed to inhibit the reaction of IgG anti-acute T.cruzi F(ab')2 by less than 3-5%.

When the cut-off normal value was calculated from the median optical density plus two times the standard error of the mean of seronegative Moniz Ferreira subjects, 20 of 23 (87%) patients with severe chronic Chagas myocarditis showed abnormal elevated levels of IgG anti-acute T.cruzi infection F(ab')2. In contrast, 4 of 84 (4,8%) of apparently healthy, seronegative Moniz Ferreira subjects had values above normal calculated cut-off and this difference was statistically significant. Thirty of 92 (32,6%) seropositive but asymptomatic subjects showed abnormal elevated IgG anti-acute T.cruzi infection F(ab')2 activity. It is possible that subclinical myocarditis cases are included in the latter group because no sophisticated cardiology was done for these subjects in the seroepidemiologic survey. Nevertheless, when the Fisher exact test was used, statistically significant differences were again found between the group with congestive heart failure and severe chronic myocarditis and the asymptomatic group of seropositive petients.

The distribution of free and complexed IgG anti-acute T.cruzi infection F(ab')2 was compared among seronegative and seropositive asymptomatic Moniz Ferreira subjects and chronic Chagas myocarditis patients with congestive heart failure. Figure 1 plots the free IgG anti-Id and immune complexed IgG anti-Id for each subject.



When the t-distribution of IC' of each of the three groups is compared, each is statistically significantly different from the other (P 0.001). It can be appreciated from Figure 1 that the median values of free and complexed IgG anti-Id measured for the patients with chronic Chagas myocarditis are markedly elevated above seronegative subjects. It is particulary noteworthy that, even though values overlap among the seropositive subjects, IgG anti-Id immune complexes were highest among some of the patients with profound myocarditis.

The hypotesis that anti-Id reaction may be pathogenic has been raised in connection with several observations suggesting a link between viral infection and autoimmune disease, including postviral myocarditis (2,4). We propose a working hypothesis to explain the interrelationship between the formation of anti-idiotypic reaction and the development of chronic Chagas myocarditis. In the initial infection parasite attachment molecules interact through molded tertiary structures of epitopes with complementary receptor sites on the muscle sarcolemma to initiate recognition and parasitosis. Later in the infection anti-idiotype reaction down regulate the primary response against the parasite. In a subset of susceptible subjects anti-idiotypic reactions are raised and some are directed to the mirror-imaged internal structures of the sarcolemma receptors thereby inducing myocarditis.