

Analysis of cloned Trypanosoma cruzi proteins that are antigenic during the acute and chronic phase of Chagas' disease.

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An expression DNA library from Trypanosoma cruzi was constructed in the lambda vector gt11. Ten different clones were isolated with the aid of a serum from a Chagasic patient. T. cruzi proteins identified with antibodies against each antigen ranged in apparent molecular weight from 85 to higher than 205kDa (Ibañez et al. Mol. Biochem. Parasitol. 25:175-184, 1987). Nucleotide sequence analysis of nine of the isolated clones showed that eight of them code for antigens which contained tandemly repeated amino acid motifs (Ibañez et al. Mol. Biochem. Parasitol. 30:27-34, 1988). The length of the repeats range in size from 5 to 68 amino acids among clones and are highly conserved within each clone. At least 3 of the cloned antigens were shown to be present at the surface of the parasite by immunofluorescence experiments with live trypanosomes.

We have now analyzed sera collected from patients in the acute and chronic phases of Chagas disease with the aim of detecting antibodies specifically generated during each disease phase. Fusion proteins from clones number 1, 2, 7, 10, 13, 19, 26, 30, 36, 49 and 54 were transfer to nitrocellulose filters and reacted with each of the sera tested and protein A labelled with 125I. Radiographic signals over the background observed with lambda gt11 phage without insert were recorded as positive. Twenty six of 28 sera from acute cases collected in the States of Minas Gerais and Bahia (Brazil) reacted preferentially with antigen number 7 and to a lesser extent with antigens number 13 (11 positive sera) and 36 (10 positive sera). Few of them reacted with antigens number 1 and 2. On the other hand, most of the 37 sera from chronic cases collected in the States of Goias and Bahia (Brazil) detected antigens number 1 (27 positive sera) and 2 (25 positive sera) and only 3 of them

detected antigen number 7. These results showed that clone number 7 codes for a protein that is highly antigenic during the acute period of the disease while clones 1 and 2 code for proteins which are antigenic during the chronic periods of Chagas' disease. Thus, antibodies against the above mentioned antigens may be useful for diagnosis since they do not react with control sera nor with sera from patients with Leishmaniasis (Ibañez et al. *Mol. Biochem. Parasitol.* 30:27-34, 1988). In addition, the presence of antigen 7 in plasma from infected animals suggest another diagnostic possibility, namely the use of antibodies against antigen 7 for the direct detection of parasite products.

The antigen encoded by clone 7 was analyzed in more detail. Antibodies obtained from rabbits immunized with clone 7 fusion protein detected five protein bands (165 to 205kDa) in Western blots of total trypomastigote proteins but failed to detect any band in the epimastigote stage of the parasite. Antigen 7 was found to be present in supernatants of trypomastigotes grown in Vero cell cultures. This reaction was not due to parasite contamination in the supernatants since antibodies against other parasite antigens failed to detect proteins in the above mention supernatants. Antigen 7 was also found in plasma from acutely infected mice but not in control animals. Since a serum from an acute human case mainly detected antigen 7 in supernatants of trypomastigotes, we conclude that this antigen is the main one shed by the parasite during the acute phase of Chagas' disease.

The 3' end of the gene contained in clone 7 was sequenced. Starting from the 5' end of the insert, there are 138bp of non repeated DNA, then 504bp containing 14 repeats of 36bp each and finally 129bp of non repeated DNA up to the first stop codon which is followed by two other stop codons also in the correct reading frame. The deduced amino acid sequence of the repeats is highly hydrophilic while that corresponding to the last 117bp is highly hydrophobic. These results suggest that antigen 7 is anchor in the surface membrane and that a cleavage step must take place in order to explain the presence of antigen 7 in parasite supernatants and plasma from infected mice.