

CO-2 CARBOHYDRATE EPITOPES (GALF → MANP) RECOGNIZED BY ANTIBODIES THAT INHIBIT TRYPOMASTIGOTE INTERIORIZATION INTO MAMMALIAN CELLS

B. Zingales, M.V. Arruda & W. Colli

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, C.P. 20780, São Paulo, Brasil

The trypomastigote form of *T. cruzi* must necessarily penetrate the mammalian cell in order to perpetuate its cycle in the vertebrate host. Data from our group (cf. Zingales, B. & Colli, W. Curr. Topics in Microbiol. and Immunol. 117: 129, 1985) showed that surface glycoproteins, specific of the trypomastigote are involved in parasite interiorization, since treatment of tissue culture-derived trypomastigotes (tct) of the Y strain with trypsin or tunicamycin promoted a drastic inhibition of the infection index in cultured LLCMK2 cells (monkey kidney epithelial cells). The glycoproteins were characterized by affinity chromatography in lectin-Sepharose columns, determining that the trypomastigote presents several high molecular weight (up to 200 kDa) glycoproteins that bind to Con A and a specific component of 85 kDa (Tc-85) that binds to WGA.

It was also observed that incubation of the parasites with human chagasic sera or with hyperimmune sera against trypomastigotes (but not against epimastigotes) inhibited parasite interiorization. This observation lead us to obtain specific antibodies in order to try to characterize the trypomastigote-specific antigens involved in the infection process.

Alves et al (Mol. Biochem. Parasitol. 21: 75, 1986) obtained three monoclonal antibodies that recognize Tc-85 and that inhibit parasite interiorization.

We obtained polyclonal antisera immunizing rabbits with trypomastigote polypeptides isolated by preparative SDS-PAGE. Antisera obtained against the bands of 175-150 kDa and 130-120kDa did not inhibit to an appreciable extent parasite interiorization.

On the other hand, antisera obtained against polypeptides of 90-80 kDa (serum A) and 60-50 kDa (serum B) inhibited the interiorization of tct of the Y strain up to 70% and that of metacyclic trypomastigotes (mt) of a clone derived from the CL strain (CL-14 clone) up to 50%. The inhibition promoted by the antisera was associated to the IgG fraction and a dose-response was found. Concomitant addition of the IgG fractions obtained from serum A and B did not increase the inhibition promoted by either fraction added independently.

It was also observed that antisera obtained against the 90-80 kDa and the 60-50 kDa polypeptides of the epimastigote form did not show a significant inhibitory effect on trypomastigote interiorization. This suggested that either the polypeptides comprised in these regions of molecular weight had structural differences between trypomastigotes and epimastigotes or that the obtained antisera presented differences in the specificity of the elicited antibodies.

The surface polypeptides recognized by serum A and B were identified by immunoprecipitation of radioiodinated trypomastigotes, followed by analysis by unidimensional and two dimensional gel electrophoresis. Surprisingly, it was observed that both serum A and B recognized surface polypeptides of 90 kDa, 80 kDa, 72 kDa and 58 kDa in tct (Y strain) and of 80 kDa, 77 kDa and 74 kDa in mt (CL-14 clone). These antigens presented acidic pI and, most probably, common epitopes.

Affinity chromatography of tct and mt lysates by Con A-Sepharose, followed by immunoprecipitation with the antisera, showed that the antigens are glycoproteins that bind to this lectin. The presence of Tc-85 among the recognized polypeptides was ruled out.

The following evidence suggested that the antibodies of serum A and B were mainly directed against carbohydrate epitopes: (a) the antisera do not immunoprecipitate tct antigens translated in cell free systems; (b) the antisera do not recognize (³⁵S)-methionine labeled polypeptides of tunicamycin-treated tct; (c) in pulse-chase experiments, it was observed that the epitopes identified

by the antisera only appear after a 3 h chase; (d) the reactivity of tct polypeptides to serum A and B is impaired after treatment with m-periodate/borohydride (Western blot analysis); (e) treatment of the glycoproteins with endoglycosidase H strongly reduces their reactivity.

As an attempt to characterize the nature of the recognized epitopes we performed radioimmunoassays (RIA) with tct lysates using monosaccharides or glycoconjugates as competitors. It was observed that D-mannose, D-galactose and LPPG (a glycoconjugate from *T. cruzi*) presented a clear inhibitory effect on the binding of both serum A and B. Treatment of LPPG with m-periodate/borohydride, under condition that affect galactose in the furanose configuration (Lederkremer et al. FEBS Lett. 116: 25, 1980), abolished its competitive activity.

The recognition of galactose-furanose units by serum A and B was verified employing as competitor in RIA the synthetic disaccharide Gal β 1 \rightarrow 3 Manp. In this case, it was observed that concentrations as low as 20 μ g/ml caused an inhibition of 47% and 25% respectively of the binding of serum A and B to tct antigens.

These observations indicate that the antibodies present in serum A and B are mainly directed against these epitopes. In order to verify the real participation of these carbohydrate epitopes in the infection of tct we are studying their effect in *in vivo* and *in vitro* systems, employing either synthetic oligosaccharides or natural related glycoconjugates.