Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. I Vol. 83, November, 1988/Page 427

IMMUNOBIOLOGICAL RESPONSES TO SHORT CARBOHYDRATE EPITOPES IN *Trypanosoma cruzi*

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We have detected in a previous work cross-reactions between Chagasic (Ch) or hyperimmune rabbit sera and β-D-galactofuranose-containing polysaccharides (1). Ch sera immunoprecipitated an alkali-extracted *Trypanosoma cruzi* (epimastigote) galactomannan and a *Dactylium dendroides* galactoglucomannan carrying similar epitope. This epitope, β-D-Galf-1,3-α-D-Manp predominates in a major lipopeptidophosphoglycan complex of *T. cruzi* epimastigotes although it may also be present in the other life-cycle forms. Antibodies raised in mice against *D. dendroides* gave positive immunofluorescence (IF) and *Staphylococcus* adherence tests with trypomastigotes but were not lytic or protective against experimental infections with virulent *T. cruzi*.

The role of lytic antibodies in complement-mediated reactions has been stressed as markers for monitoring the progress and perhaps eventual cure of the chronic stage of Chagas disease (2,3). Lytic antibodies were believed to be raised only in infected individuals rather than in those immunized by various cellular or subcellular preparations. Although this seems to be generally correct insofar as native unconcentrated serum Igs are concerned, antibodies (Abs) from Ch patients isolated on immobilized epimastigote or blood trypomastigote antigenic extracts lysed trypomastigotes (4,5). Also, we observed that antibodies eluted from immobilized glycoprotein 25 kDa from epimastigotes partly lysed trypomastigotes (6). In searching for other specificities that could effect lysis and protect against infection,results were obtained (7) which paralleled those reported for autologous antibodies reacting with human senescent red blood cells (8). Erythrocyte-bound antibodies could be eluted from cells with melibiose or α-Me Galpyranoside, less effectively with galactose. They agglutinated rabbit but not healthy young human erythrocytes apparently by reacting with a cell surface glycosphingolipid consisting of a ceramide pentahexoside of the following structure: α-Galp-1,3-β-Galp-1,4-β-GlcNAc-1,3-β-Galp-1,4-Glc-1,1-Cer (9). Towbin et al.(10) found that normal human serum (NHS) and sera from patients with leishmani-

asis and Chagas disease or from monkeys infected with T. cruzi or T. rhodesiense had IgG binding mouse laminin a reaction blocked by the disaccharide α-Galp-1,3-Galp.

In a series of communications, Milani et al. (11,12) found that: a) affinity-purified melibiose-binding NHS and Ch Abs were lytic to DEAE-cellulose purified metacyclic trypomastigotes (Y,CL strains) by the alternative complement pathway (ACP); melibiose-bound Abs of both IgM and IgG classes agglutinated trypomastigotes and gave positive IF with live trypomastigotes — the specificity clearly involved α-Galp terminal residues; b) a rabbit serum raised against group C Neisseria meningitidis agglutinated metacyclic trypomastigotes of G and Y strains at 1/65,000 and 1/8,200 respectively. Reactions were inhibited by purified N. meningitidis polysaccharide C; c) anti-N. meningitidis rabbit serum lysed G and Y strains (trypomastigotes) by ACP at 1/32 as did a rabbit serum raised against the α-2,9-polysialic polysaccharide (CL and Y strains); the latter also gave positive IF with fixed CL,G, and Y trypomastigotes; d) single protection experiment with mice challenged by 2×10^5 Triatoma infestans-derived metacyclic trypomastigotes using anti-polysialyl Abs was unsuccessful; combined with anti Galp (Ch) polysialyl Abs protected mice against a challenge by 2×10^4 virulent trypomastigotes; e) anti-Galp and anti-polysialyl Abs recognized predominantly 72K and 70K components, respectively, from trypomastigote (Y)-radiolabeled (^{125}I) lysates.

We now show (Milani and Travassos, 1988, present meeting) that the anti-Galp Abs reactive with T. cruzi have a specificity for the α-Galp-1,3-β-Galp-1,4-GlcNAc epitope. IF reactions with live or fixed trypomastigotes were obtained with affinity-purified Abs using the trisaccharide epitope immobilized on Synsorb columns. In accordance with this specificity IF reactions were also observed with Bandeiraea simplicifolia IB_4 lectin. Galili (personal communication) found percentages for affinity-purified anti-Galp Abs from total IgG of 0.4 in 6 NHS, of ≈ 1 in 5 Ch sera and of 1.74 in 5 leishmaniasis sera. Milani (7) also obtained increased anti-Galp concentration in Ch sera as compared with NHS. Although being isolated in the same affinity columns as the Ch sera Abs, anti-Galp Abs from NHS seem to react differently in ELISA tests with certain antigens, parasitic or not. Reactivity of gp25 with NHS anti-Galp Abs was much reduced in comparison with that with Ch Abs (7). The same difference in response seemed to happen with T. cruzi glycolipids and mouse laminin (unpublished results). However, similar concentrations of NHS and CH anti-Galp Abs were able to cause the same degree of lysis of trypomasti-

gotes. Although the anti-Galp Abs'concentration in NHS is high — 30 to 70 µg/ml (13) or even more (Galili, personal communication) — it is not sufficient for extensive lysis of T. cruzi trypomastigotes. A high proportion of lysis is attained by incubation of parasites with NHS anti-Galp Abs purified and concentrated by affinity chromatography as mentioned before.

One way of increasing the anti-Galp titers in humans, assuming that they may have a role in protection against T. cruzi infections, could be active immunization with bacteria sharing similar antigenic structure. By using direct immunostaining and ELISA, anti-Galp Abs were found to interact with several Escherichia coli, Klebsiella, and Salmonella strains, some of which were isolates of the normal intestinal flora (14). The anti-Galp-binding sites were present on the carbohydrate portion of bacterial lipopolysaccharides. It has still to be demonstrated whether the Abs raised by immunization with a particular bacterial strain, expressing the α-Galp-1,3-Galp epitope, will lyse trypomastigotes and whether the high anti-Galp titers associated with the infection by T. cruzi will promote an autoimmune disease. High titers are expected because there is no immune tolerance to the α-Gal-1,3-Gal carbohydrate structure in humans, Old World monkeys and anthropoid apes (15).

Oliveira et al. (16) in our laboratory isolated Ch Abs having an affinity for N. meningitidis polysaccharide C. Antibodies adsorbed to the insoluble form of the polysaccharide and eluted from it with KSCN, were lytic to trypomastigotes in a complement-mediated reaction (ACP) and gave positive ELISA tests with fixed trypomastigotes (Y strain). A further investigation (Oliveira et al., 1988, present meeting) revealed that the human anti-polysialyl Abs reacted in ELISA with the gp25 as well as with total epimastigote sialoglycolipids. The positive reaction with gp25 was inhibited by both acetylated and O-deacetylated polysaccharide C; that with sialoglycolipids was preferably inhibited by the deacetylated form. Previously, we have shown that Ch sera reacted in ELISA tests with a partially purified epimastigote sialoglycolipid (17). Reactivity of one Ch serum was inhibited by N-acetylneuraminic acid. Removal of sialyl terminal units by Clostridium perfringens (but not Vibrio cholerae) neuraminidase rendered the glycolipid much more reactive with this serum, probably a result of the increased exposure of subterminal galactose units. It is still unclear how the sialic acid units are linked on the cell surface of T. cruzi. Antibodies desorbed from the polysaccharide C were reisolated from a column with bound sialyllactose. Recovered Abs had reduced capacity to lyse trypomasti-

gotes in comparison with the total Abs bound to the polysaccharide C. Apparently this resulted from selection of cross-reacting antibodies with less affinity for trypomastigote surface components carrying sialyl residues.

The present results offer as perspectives the identification of surface antigens on T. cruzi trypomastigotes which may act as targets in complement-mediated reactions leading to cell lysis. Antigens with the α-Gal-1,3-Gal epitope might be those of choice concerning eventual immunotherapy of Chagas disease due to lack of tolerance in man to this immunogenic structure and the fact that this epitope is cryptic in human cells. The role of anti-sialic acid Abs is also an important aspect to be investigated in relation to protection. Participation of short carbohydrate epitopes in host cell penetration of parasites is another aspect to be elucidated.

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