Trypanosoma cruzi Mucins: Potential Functions of a Complex Structure

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The protozoan *Trypanosoma cruzi*, the etiological agent of Chagas disease (Chagas 1909), displays on its surface various glycoconjugates which appear to be involved in the recognition and invasion of mammalian host cells, as well as in establishing and sustaining the chronic infection (Travassos & Almeida 1993). The majority of these molecules are attached to the parasite via a post-translational modification of a glycosylphosphatidylinositol (GPI) anchor (Ferguson 1997). The two most abundant glycoconjugates present in all *T. cruzi* developmental stages are the glycoinositolphospholipids (GIPLs), of which the major constituent was formerly known as lipopeptidophosphoglycan (LPPG) (Lederkremer et al. 1976), and the mucin-like glycoproteins. The latter were first observed by Alves and Colli (1975) during the process of purification of epimastigote glycoproteins by gel chromatography. The mucin-like characteristics of these glycoconjugates was recognized by Schenkman et al. (1993) including their high content of hydrophilic amino acids (threonine, serine, lysine and glycine), *O*-linked oligosaccharides and sialic acids, typical features that define mammalian mucins. In *T. cruzi*, the mucins are the main acceptors of sialic acid via a *trans*-sialidase reaction (Previato et al. 1985, Zingales et al. 1987, Schenkman et al. 1991, 1993, 1994) which can use different α2,3-sialylated donors.

T. cruzi mucins migrate in SDS-polyacrylamide gels as double- or triple bands with apparent molecular mass of 35-43 kDa, in epimastigote, and 35-50 kDa, in metacyclic trypomastigote (Previato et al. 1985, Yoshida et al. 1989). In this insect-derived infective stage the 35-50 kDa mucin-like glycoproteins seem to be implicated in the modulation of the processes of host cell adhesion and invasion (Ruiz et al. 1993, 1998, Yoshida et al. 1997). In tissue culture-derived trypomastigotes, *T. cruzi* mucins have a polydisperse migration of 60-200 kDa (Schenkman et al. 1991), with better resolution under special electrophoresis conditions yielding broad bands at 74, 96, and 120-200 kDa (Almeida et al. 1993). In recent years, the composition and chemical structure of *T. cruzi* "mucins", particularly their *O*-linked oligosaccharides and GPI-anchors have been investigated (Schenkman et al. 1993, Previato et al. 1994, 1995, Almeida et al. 1994b, Serrano et al. 1995, Camargo et al. 1997). Epimastigote, tissue culture-derived trypomastigote and metacyclic trypomastigote mucins have oligosaccharide chains internally linked to *N*-acetylglucosamine (GlcNAc) units *O*-glycosidically-linked to threonine, representing approximately 60% of the total mass of the glycoprotein (Previato et al. 1994, Almeida et al. 1994b, Serrano et al. 1995). These oligosaccharides are mostly branched and contain Gal*p*, Gal*f*, and sialic acid (SA) units besides *O*-linked GlcNAc in their composition. More recently, Previato et al. (1998) have characterized the activity of the enzyme uridine diphospho-*N*-acetylglucosamine: polypeptide-α-*N*-acetylglucosaminyltransferase (*O*-alpha-GlcNAc-transferase), which is responsible for the transfer of GlcNAc to threonine residues of the mucin polypeptide chain during the biosynthesis of the *O*-linked oligosaccharides. This novel enzyme presents different catalytic properties when compared to the mammalian cell-derived *O*-beta-GlcNAc transferase (Haltiwanger et al. 1992), and may represent an important target for the development of more specific drugs for the treatment of Chagas disease. A striking feature of these *O*-linked oligosaccharides is the presence of α-galactopyranosyl residues exclusively in the oligosaccha-

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rides isolated from mucins of tissue culture-derived trypomastigotes. This clearly explains the strong recognition of mammalian cell-derived trypomastigotes by lytic anti-α-galactosyl antibodies from patients with chronic Chagas disease (Ch anti-Gal) (Almeida et al. 1991, 1993, 1994a,b). Metacyclic trypomastigotes are also recognized and destroyed by lytic Ch anti-Gal, but the target epitope is a 72 kDa glycoprotein, which has not been structurally characterized (Travassos et al. 1993).

One of the main differences among mucins from the various stages resides on the phosphatidylinositol (PI) moiety of the GPI anchor, isolated by nitrous deamination. While the epimastigote mucin GPI contains mainly 1-*O*-(C16:0)alkyl-2-*O*-(C16:0)acylglycerol-3-phospho-1-*myo*-inositol, the metacyclic mucin anchor is predominantly formed by different species of phosphoceramide-inositol (~70%), containing dihydrosphingosine (C18:0) and lignoceric (C24:0) or palmitic acid (C16:0) (Serrano et al. 1995). Conversely, the PI isolated from the GPI anchor of mammalian cell-derived trypomastigote mucins are constituted by an alkylacyl-glycerol chain, containing mainly unsaturated fatty acid (C18:1 or C18:2) (Camargo et al. 1997).

Regarding the native mucin polypeptide chain, little is known at present. Available data are derived from the cloning and expression of mucin genes. *T. cruzi* mucins are transcribed from families of up to five hundred genes (Di Noia et al. 1998). Several of these genes have recently been cloned from epimastigote and trypomastigote genomic and cDNA libraries and had their polypeptide sequence deduced (Reyes et al. 1994, Di Noia et al. 1995, 1996, 1998, Salazar et al. 1996, Freitas-Junior et al. 1998). From these studies, we can group the deduced polypeptide sequences into two major families. Family I polypeptides are formed by central domains rich in Thr, Lys and Pro, organized in blocks containing the repetitive motif $(Thr)_8Lys(Pro)_2$. These central blocks are flanked by a highly variable and short (7-12 amino acid) N-terminal region and a more conserved C-terminal region. Conversely, Family II polypeptides, despite showing N- and C-terminal regions similar to Family I, they have not the repetitive $(Thr)_8Lys(Pro)_2$ motifs. Furthermore, Family II has a much lower concentration of Thr residues than Family I (Di Noia et al. 1996). Recent unpublished observations from our group indicate that Family I sequences are expressed in epimastigotes and metacyclic mucins, whereas Family II are found in trypomastigote mucins and, possibly, amastigote mucins. This was assumed by comparing the amino acid composition of DNA-deduced sequences with that of native mucins purified from the four *T. cruzi* stages. We have also observed that epimastigote and metacyclic mucins, contrary to trypomastigote mucins, are almost completely resistant to trypsin digestion. This is compatible with the existence of central domains containing the motif $(Thr)_8Lys(Pro)_2$ in mucins from insect-derived parasite stages. It is well documented in the literature that a Pro residue, located at the carboxy terminus of a Lys residue, can completely block the action of trypsin.

Recent studies show that mucins from trypomastigotes, but not from epimastigotes and metacyclic forms, can potently induce the synthesis of proinflammatory cytokines (TNF-α, IL-12) and nitric oxide (NO) by IFN-γ-primed murine macrophages (Camargo et al. 1997a, 1997b, Gazzinelli et al. 1997). The strong inducing activity of the trypomastigote mucins is achieved at subpicomolar (0.01-0.1 pM) concentrations. Experimental evidence indicates that the mucin GPI contains the minimal structure responsible for its bioactivity. Chemical treatments, such as nitrous deamination and mild alkaline hydrolysis, under conditions that exclusively affect the GPI anchor structure can completely abolish the cytokine/NO inducing activity of trypomastigote mucins (Camargo et al. 1997). Recent observations clearly demonstrate that, indeed, a highly purified trypomastigote mucin GPI can strongly activate murine macrophages. The precise role of GPI-anchored trypomastigote mucins in the pathophysiology of experimental and human infection by *T. cruzi* is not known, but mucins induce several cytokines, such as IL-12 and TNF-α, which are thought to be involved in protection and pathophysiology of experimental Chagas disease (Brener & Gazzinelli 1997). We have also provided strong evidence that cyclic AMP modulates trypomastigote mucin-induced IL-12 production by macrophages indirectly, following the release of IL-10. In contrast, the effects of cyclic AMP regulation of TNF-α production are probably direct, and largely independent of IL-10 production (Procópio et al. 1999).

Another important development in the study of *T. cruzi* trypomastigote mucins has been that of diagnostic application. Based on the knowledge that trypanolytic antibodies found in sera from patients with chronic Chagas disease react preferentially with trypomastigote mucins, particularly with epitopes containing terminal α-galactopyranosyl units (Almeida et al. 1991, 1993, 1994a,b) a diagnostic method has been devised using chemiluminescent (CL)-ELISA of high sensitivity and specificity (Almeida et al. 1997) which can also be used in blood bank screening. Since lytic antibodies correlate with active infection

(Krettli & Brener 1982, Galvão et al. 1993) the CL-ELISA method has also been used to monitor successful chemotherapy of Chagas disease in children from an endemic area (Andrade et al. 1997) and adults (unpublished). In both cases the CL-ELISA using purified trypomastigote mucin antigens proved to be a powerful diagnostic procedure clearly correlating negative titers with parasitological cure.

The importance of the mucin glycoproteins in *T. cruzi* both as constitutive structural elements in all developmental stages of the parasite and as inducers of immunological responses which can deeply affect the progression of Chagas disease is now well documented, stimulating studies to further our knowledge of their chemical characterization and functional properties.

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