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Purification and Characterization of an 80-Kilodalton *Trypanosoma cruzi* Urinary Antigen

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A *Trypanosoma cruzi* antigen eliminated in the urine of experimentally infected dogs was detected by enzyme-linked immunosorbent assay between 9 and 28 days after infection. The parasite urinary antigen (UAg) was purified by affinity chromatography with polyclonal antibodies to *T. cruzi*. The eluate of the antibody column was subjected to high-performance liquid chromatography and showed a single peak of A_{280}. This antigen was the only parasite component found in the urine of infected dogs during the course of acute *T. cruzi* infection. Antigen characterization was performed by two-dimensional gel electrophoresis, lectin affinity chromatography, proteolytic digestion, and Western blotting (immunoblotting). The isolated UAg exhibited a relative molecular size of 80 kilodaltons (kDa), an isoelectric point of 6.2 to 6.8, binding to concanavalin A, and sensitivity to trypsin. The parasite antigen was electroeluted from polyacrylamide gels and subjected to acid hydrolysis and amino acid analysis by reverse-phase high-performance liquid chromatography. The 80-kDa glycoprotein was recognized by serum antibodies from a wide variety of *T. cruzi*-infected hosts. The UAg proved to be a highly antigenic component present in different strains of *T. cruzi*. This 80-kDa polypeptide resembles one of the parasite antigens previously found in the urine of patients with acute Chagas' disease.

A major characteristic of parasitic infections is the presence of a variety of antigens in the circulation of humans and other infected hosts. In American trypanosomiasis, the etiologic agent of which is the protozoan flagellate *Trypanosoma cruzi*, parasite-derived antigens have been detected in biological fluids in human and experimental infections (2, 3, 12). Recent *T. cruzi* infection is often associated with antigenemia, both as free antigens and as antigens within immune complexes (10, 21, 24). Increasing attention has also been directed toward demonstration of soluble trypanosome antigens in the urine of infected hosts. Bongertz et al. (7) have detected *T. cruzi* antigens eliminated in the urine of acutely infected mice and dogs. We have reported the occurrence of antigenuria in infants with acute and congenital Chagas' disease (17). In that series (17), detection of parasite antigens in the urine by enzyme-linked immunosorbent assay (ELISA) proved to be the most effective procedure for achieving early and precise proof of infection. Two different glycoproteins with relative molecular weights (M_rs) of 55,000 and 80,000 were recognized in urine samples from two patients with acute infection. Katzin et al. (25) were able to identify several parasite urinary antigens (UAgs) with a monoclonal antibody in patients with chronic Chagas' disease.

As urine specimens obtained in our laboratory are mainly from children and therefore not abundant, we decided to use the dog model for experimental *T. cruzi* infection as a source of parasite UAg. In this report, we describe the isolation and characterization of an 80-kilodalton (kDa) trypanosome antigen present in the urine of recently infected dogs.

MATERIALS AND METHODS

Experimental acute *T. cruzi* infection. Two mongrel dogs between the ages of 3 and 5 years, weighing approximately 10 kg each, were inoculated by the intramuscular route with 2.5×10^7 trypomastigote forms of *T. cruzi* RA (19) purified from the blood of Rockland mice at the peak of parasitemia (7 days postinfection). Before infection, the dogs were free of detectable antibodies in serum against *T. cruzi* by direct agglutination (35) and indirect hemagglutination (9) assays (Laboratorios Polychaco, Buenos Aires, Argentina). Two other age- and sex-matched mongrel dogs without serologic and parasitologic evidence of *T. cruzi* infection were used as noninfected controls. Urine specimens from both infected and control animals were collected daily from day 1 of infection for 28 days. Total voided volumes ranged between 200 and 900 ml/day. For urine collection, metabolic cages were adapted with a system of metallic and paper filters to avoid contamination with feces. Sodium azide (0.2%, wt/vol) was used for urine preservation. Whole trypanosomes were not observed in these urine specimens. Samples were filtered through a membrane (0.45-μm pore size; Millipore Corp., Bedford, Mass.) and concentrated by precipitation with 75% (vol/vol) cold ethanol for 48 h at −20°C. Pellets were obtained after centrifugation at 10,000 × *g* for 30 min at 4°C and suspended in 0.01 M phosphate-buffered saline (PBS), pH 7.2. Blood samples were also collected weekly for specific antibody serology tests (direct agglutination and indirect hemagglutination) and determination of circulating parasites by the microhematocrit procedure (15).

Sera. A rabbit hyperimmune antiserum to *T. cruzi* was obtained after infection of rabbits by the intramuscular route with 7.5×10^6 bloodstream RA strain parasites. The immunoglobulin fraction was separated by precipitation with ammonium sulfate and immunoadsorbed with both *T. cruzi* and human tissue antigens as described previously (17). Specific antibody titers ≥1:256 were demonstrated in this preparation by direct agglutination and indirect hemagglutination. Serum samples (one from each individual) were also obtained from (i) three chronic patients whose sera are used routinely as positive controls for the serodiagnosis of Chagas' disease at our laboratory; (ii) three congenitally

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infected patients with trypanosomes in the blood visualized by the microhematocrit test who were born in a nonendemic area and whose mothers were serologically positive for Chagas' disease; and (iii) three acutely infected patients from an endemic area who had a history of symptoms of <30 days, parasitemia detectable by the microhematocrit test, and a known portal of entry. Sera were collected from mice chronically infected with either the RA (50 parasites per animal) or CAI (5×10^5 parasites per animal) (20) strain of *T. cruzi*, capable of inducing (RA) or unable to induce (CAI) a lytic antibody response in the host as determined by a complement-mediated lysis test (29). Serum specimens from noninfected individuals, rabbits, and mice, none of them with detectable antibodies against *T. cruzi* by conventional serology, were used as controls for nonspecific reactivity in the Western blotting (immunoblotting) technique (see below).

ELISA for antigen detection. A double-sandwich ELISA (17) was used to demonstrate antigen in urine and in the eluted fractions obtained by affinity chromatography (AC) and high-performance liquid chromatography (HPLC). Microdilution polystyrene plates (Immulon 2; Dynatech Laboratories, Inc., Alexandria, Va.) with 96 flat-bottomed wells were coated with 100 μl of rabbit anti-*T. cruzi* immunoglobulin (30 μg of total protein per ml) in 0.1 M carbonate buffer (pH 9.6) and incubated overnight at 4°C. The plates were washed three times in 0.01 M PBS (pH 7.4) containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, Mo.). Wells were postcoated with 1% bovine serum albumin (Sigma) in PBS-Tween 20 and left at 37°C for 45 min. After the plates were washed, 100 μl of each sample was added to the wells and incubated for 2 h at 37°C. The plates were washed again, and 100 μl of human anti-*T. cruzi* immunoglobulin was added and incubated at 37°C for 60 min. Plates were washed three times, and 100 μl of goat anti-human immunoglobulin G (IgG) labeled with alkaline phosphatase (Miles Laboratories, Inc., Elkhart, Ind.), diluted 1:1,000 in PBS-Tween 20, was dispensed into each well. After 60 min at 37°C and thorough washing, 100 μl of substrate (0.1% *p*-nitrophenyl phosphate [Sigma] in diethanolamine buffer with 0.5 mM magnesium chloride) was added, followed by 50 μl of 3 M sodium hydroxide 30 min later. ELISA results were read with a Dynatech microELISA reader (model MR 590) at a wavelength of 410 nm.

AC. Immunoadsorbents were prepared with a CNBr-activated Sepharose-4B matrix (Pharmacia, Uppsala, Sweden), coupled either to specific antibodies to *T. cruzi* or to lectin, in accordance with the procedures suggested by the manufacturer. CNBr-Sepharose-4B gel was swollen and washed in 1 mM hydrochloride and coupling buffer (0.2 M $NaHCO_3$, 0.5 M NaCl, pH 8.5). Rabbit hyperimmune anti-*T. cruzi* IgG, previously dialyzed overnight against coupling buffer, was allowed to react with the gel (21 mg of protein per ml of swollen gel) and gently mixed end-over-end overnight at 4°C (22). Unbound antibody was washed away with coupling buffer, and the gel was suspended in blocking buffer (1 M ethanolamine [pH 8.0] containing 0.5 M NaCl) and kept at room temperature for 2 h. Finally, the gel was alternately washed five times with high-pH (coupling buffer) and low-pH (0.1 M sodium acetate, 0.5 M NaCl, pH 4.0) buffer solutions. The efficiency of the coupling procedure was 95%. The gel was then poured into a 1.2-cm-diameter glass column to a height of 9 cm. Every ELISA-positive urine sample was used for antigen isolation by AC. Urine from noninfected animals was also affinity chromatographed and considered as control for nonspecific binding to the immunoadsorbent. A 10-ml portion of a concentrated urine sample was applied to the column at a very low flow rate (200 μl/min). Monitoring of protein elution was started immediately after sample application by measuring the A_{280} of the column effluent with a UV-1 single-path monitor connected to a two-channel recorder (Uvicord I 4700; LKB Produkter AB, Bromma, Sweden). Fractions of 1 ml were collected with an automatic fraction collector (LKB). After unbound material was washed from the column with 0.01 M PBS (pH 7.2), reaching an A_{280} of 0, the retained protein was eluted with citrate-phosphate buffer (0.1 M citric acid, 0.2 M Na_2HPO_4, pH 3.0). The antigen-containing fractions were pooled and concentrated with a concentrator (Minicon B-15; Amicon Corp., Lexington, Mass.). The concentrated pool was divided in two aliquots for lectin AC and HPLC analysis.

Lectin AC (26) was performed with a concanavalin A (ConA)-Sepharose (Pharmacia) column (volume, 0.6 ml; height, 5 cm) equilibrated in 140 mM NaCl–10 mM Tris hydrochloride (pH 8.6) containing 2% Nonidet P-40 before adsorption of the sample. The antigenic material was dialyzed against this buffer and applied directly to the column. Nonadsorbed material was eluted with Tris hydrochloride buffer at a constant flux of 0.2 ml/min. Bound material was eluted with the same buffer containing a mixture of 100 mM each α-methyl-D-mannoside and α-methyl-D-glucoside. The carbohydrate-eluted material was precipitated for 48 h by the addition of 3 volumes of cold ethanol at −20°C. The pellet was suspended in electrophoresis sample buffer (see below) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

HPLC. The affinity-purified material was dialyzed against 100 mM sodium phosphate–100 mM KCl (pH 6.3) and subjected to HPLC. The antigenic preparation was applied to a size-exclusion HPLC column (Ultropac TSK-G 3000 SW, 600 by 75 mm; LKB) equilibrated with the above-mentioned buffer at a flow rate of 1 ml/min at 22°C. Protein elution was monitored spectrophotometrically at 280 nm. Fractions of 1 ml were collected. The column was calibrated with a mixture of molecular size standards (thyroglobulin, 670 kDa; human IgG, 160 kDa; bovine serum albumin, 68 kDa; ovalbumin, 49 kDa; myoglobin, 17 kDa). The protein-containing fractions were concentrated in Minicon B-15 filters (Amicon) and tested for antigenic reactivity by ELISA. The ELISA-reactive fractions from the HPLC column containing the antigen of interest were pooled and analyzed by gel electrophoresis.

Isoelectric focusing. Electrophoresis was performed under equilibrium conditions by the method of O'Farrell (31). Protein was analyzed in a pH gradient, determined by a mixture of Ampholines (1% pH 3.5 to 10.0; 0.7% pH 5.0 to 7.0), in the presence of urea. Gels were prerun for 15 min at 200 V, 30 min at 300 V, and 30 min at 400 V. The sample was loaded on the gel, overlaid with urea, and electrofocused at 400 V for 16 h. Marker proteins at 5 μg each were used as internal isoelectric point (pI) standards (phosphorylase *b*, M_r 92,500, pI 6.9; bovine serum albumin, M_r 68,000, pI 5.8; and ovalbumin, M_r 45,000, pI 5.4). After focusing, the gels were removed and equilibrated in electrophoresis sample buffer for 30 min at room temperature and loaded immediately onto a second-dimension gel (SDS-PAGE).

SDS-PAGE. The discontinuous buffer system described by Laemmli (27) was used with 10% acrylamide slab gels. The stacking gel was 5% acrylamide, and the acrylamide/bisacrylamide ratio was 30:1. Molecular weight standards (Low Molecular Weight Electrophoresis Calibration Kit; Pharmacia) were included in each gel. Samples were dissolved in 62

mM Tris hydrochloride (pH 6.8) containing 2.3% (wt/vol) SDS, 10% (wt/vol) glycerol, and 0.01% bromophenol blue (electrophoresis sample buffer) and placed in a boiling water bath for 3 min before being loaded on gels. Reduction, when required, was performed by the addition of 5% (vol/vol) 2-mercaptoethanol in sample buffer. Electrophoresis was done overnight at constant current (6 mA). Gels were fixed and stained in 0.2% Coomassie blue R-250 for visualization of protein bands. Glycoproteins were identified by staining with periodic acid-Schiff base reagent (PAS) (14). Protein in the gel was either immunoblotted or electroeluted for amino acid analysis (see below).

Western blotting. The separated polypeptides in the gels (SDS-PAGE) were transferred to nitrocellulose paper sheets (BA-85, 0.45-μm pore size; Schleicher and Schuell, Inc., Keene, N.H.) as previously described (23). Protein transfer was performed at 200 mA for 2 h in a semidry electroblotter (model A; Ancos, Copenhagen, Denmark). The transfer buffer was 25 mM Tris–192 mM glycine–20% (vol/vol) methanol. Thereafter, the paper was placed in a sealed plastic bag with blocking buffer (3% [wt/vol] defatted milk, 2% [wt/vol] glycine, 1 mM phenylmethylsulfonyl fluoride, 0.01% [wt/vol] sodium azide) and incubated overnight at 4°C on a rocking platform to block the remaining protein-binding sites. The sheets were rinsed with 50 mM Tris-buffered saline (pH 6.8) and cut lengthwise into strips which were individually treated with different serum samples (see above) diluted 1:50 in blocking buffer and incubated for 2 h at room temperature with constant agitation. After being washed with 50 mM Tris-buffered saline containing 0.01% (vol/vol) Tween 20, the strips were immersed in a 1:500 conjugate solution (alkaline phosphatase-labeled antibodies) in 0.01 M PBS (pH 7.4)–0.05% Tween 20 for 1 h at 37°C with gentle rocking. The following conjugates were used to detect the corresponding human and animal antibodies: swine anti-rabbit IgG (Dakopatts, Glostrup, Denmark), rabbit anti-human IgG and anti-human IgM (Dakopatts), rabbit anti-mouse IgG (Dakopatts), and rabbit anti-dog IgG (Sigma).

Another washing step was followed by the addition of substrate (1.47% [vol/vol] 5-bromo-4-chloro-3-indolyl phosphate, 4 mg/ml in methanol-acetone [2:1]; 0.01% [wt/vol] Nitro Blue Tetrazolium in 100 mM ethanolamine with 0.2 mM magnesium chloride). Color was allowed to develop for 20 min, and the reaction was stopped by rinsing with distilled water.

Enzymatic treatment. Protease digestion of the purified antigenic material was performed by the addition of 1% (wt/wt) trypsin (tolylsulfonyl phenylalanyl chloromethyl ketone [TPCK] treated; Worthington Diagnostics, Freehold, N.J.) to approximately 50 μg of protein in 0.1 M NH_4CO_3, pH 8.2 (33). Protein determinations were done by the protein-dye binding assay (8) with bovine serum albumin as the standard. After 4 h at 37°C, the reaction was allowed to proceed overnight at room temperature with constant agitation; then it was stopped with 2 N acetic acid, and the digest was filtered through a Millex-HA membrane (Millipore) and subjected to SDS-PAGE.

Amino acid analysis. Subsequent to SDS-PAGE, isolated protein bands were detected by immersion of the gel in 1 M aqueous KCl for 5 min. After visualization, bands were individually excised from the gel and electroeluted in an electrophoretic concentrator (model 1750; Instrumentation Specialties Co., Lincoln, Nebr.) as described previously (T. Bergman and H. Jörnvall, Eur. J. Biochem., in press), except that no glass fiber filter was used. Eluted samples were recovered in small volumes of 10 mM ammonium bicarbonate buffer (pH 8) and were dried under vacuum for subsequent hydrolysis in 6 M hydrochloride and amino acid analysis by reverse-phase HPLC as described previously (5).

RESULTS

***T. cruzi* infection in dogs.** Acute *T. cruzi* infection was confirmed in both infected animals by a positive microhematocrit test showing parasitemia detectable at day 14 postinfection (p.i.). Serologic tests also yielded a positive outcome (titers ≥1:256 by direct agglutination and indirect hemagglutination) in serum samples from these two dogs collected on days 14, 21, and 28 after the inoculation of trypomastigotes.

Antigen detection by ELISA. Antigenuria was demonstrated by ELISA in recently infected dogs. The presence of *T. cruzi* antigen was confirmed from day 9 p.i. through day 28 p.i. The highest absorbance values were observed in those samples corresponding to week 2 of infection. Nonspecific reactivity was not found in samples from the two control animals.

Antigen purification. AC with specific anti-*T. cruzi* antibodies served as the first step in the purification of the UAg from the ELISA-positive samples. Components of these preparations that did not bind to the antibody column were eluted with PBS and showed no reactivity in the ELISA. Urinary specimens from noninfected dogs were not retained by the immunoadsorbent. The antigen-containing fractions were pooled and subjected to HPLC analysis. A typical HPLC elution profile is seen in Fig. 1 along with the corresponding ELISA reactivity associated with the different fractions. The greatest activity was found in fractions 15 to 17, coinciding with the single peak at A_{280} which appeared at an elution time corresponding to an M_r of approximately 80,000. To determine whether the antigenuria pattern changed with the course of infection, we analyzed affinity-purified samples collected for 28 days p.i. by size-exclusion HPLC. No variations were observed when preparations corresponding to days 9 to 28 p.i. were individually applied to the column (data not shown). The ELISA-reactive fractions were pooled, concentrated in a Minicon B-15 concentrator, and used for antigen characterization.

Antigen characterization and amino acid analysis. The antigenic material isolated from urinary specimens was subjected to two-dimensional gel electrophoresis. This analysis revealed the presence of a single protein band of M_r 80,000 with a pI between 6.2 and 6.8 (Fig. 2). Determination of M_r by SDS-PAGE with or without prior reduction of the sample showed that the reduced protein migrated just slightly slower than the nonreduced protein (data not shown). Identical bands were identified by electrophoretic analysis of materials obtained at different times (days 9 to 28) p.i. AC on ConA-Sepharose demonstrated the glycoprotein nature of UAg. The lectin-Sepharose column retained a component of 80 kDa which bound strongly to ConA, as visualized by subsequent SDS-PAGE and PAS staining (Fig. 3). This UAg also proved to be trypsin sensitive. Cleavage fragments were not detected by SDS-PAGE after proteolytic digestion of the glycoprotein (Fig. 3). To establish the antigenicity of the isolated polypeptide, we characterized the UAg by Western blotting with different specific antisera as primary antibodies (Fig. 4). The 80-kDa protein was recognized by anti-*T. cruzi* IgG antibodies present in rabbit hyperimmune serum and in sera from human patients with acute, congenital, or chronic Chagas' disease, from chronically infected mice with or without detectable lytic antibodies to *T. cruzi*, and from dogs during the acute phase of

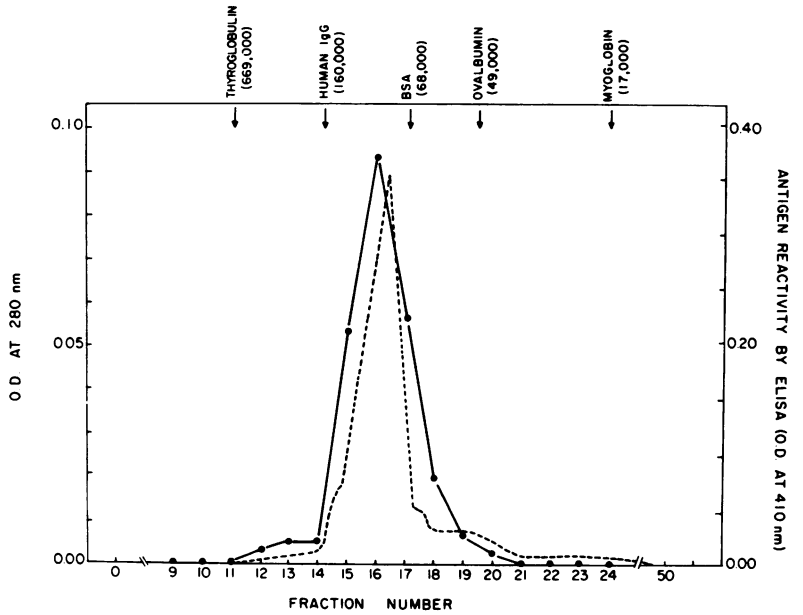

FIG. 1. HPLC analysis of *T. cruzi* UAg eliminated by infected dogs. Concentrated urine was chromatographed on antibody-Sepharose immunoadsorbent, and the eluate was applied to a size-exclusion column of TSK-G 3000 SW (7.5 by 60 cm) at a constant flow rate of 1 ml/min. Symbols: ●, A_{280}; ----, antigen reactivity by ELISA. The positions at which molecular weight standards leave the column are indicated by arrows. BSA, Bovine serum albumin; O.D., optical density.

infection (14 days p.i.). Serum from one of the three acutely infected patients containing specific IgM antibodies reacted with the single 80,000-M_r protein band. Normal sera from humans, mice, and rabbits as well as dog sera collected prior to infection were unable to detect the *T. cruzi* UAg by the immunoblotting procedure. Analysis of the amino acid composition of the parasite antigen present in urine was performed after separation by SDS-PAGE. Following elution and acid hydrolysis of the protein, the phenylthiocarbamyl derivatives of the amino acids were analyzed (Table 1).

DISCUSSION

The results presented above show that we detected, purified, and characterized a *T. cruzi* UAg eliminated by dogs during the acute stage of infection. Detection was performed by ELISA as previously described for UAg in human acute and congenital Chagas' disease (17). Apparently, major levels of antigenuria occur during week 2 of the first month of infection as evidenced by high absorbance values in ELISA corresponding to samples obtained at that time.

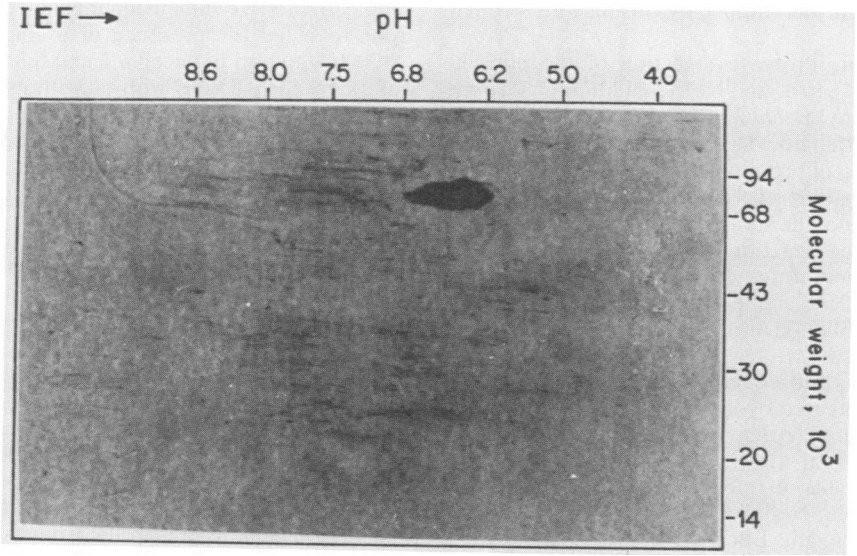

FIG. 2. Two-dimensional analysis of antigenic material purified from urinary specimens from *T. cruzi*-infected dogs. The antigen was isolated by AC and HPLC and subjected to isoelectric focusing (IEF) followed by PAGE.

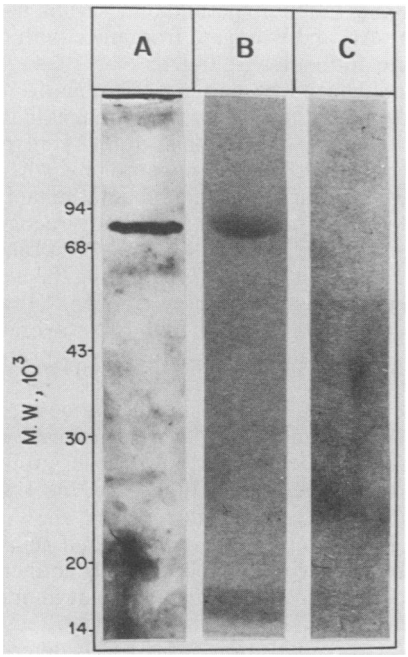

FIG. 3. SDS-PAGE characterization of *T. cruzi* UAg. Lane A, Coomassie blue-stained protein with a molecular weight (M.W.) of 80,000, purified by using an antibody-Sepharose column, from the urine of dogs with acute *T. cruzi* infection. Lane B, PAS-stained parasite glycoprotein, isolated by AC on ConA-Sepharose. Lane C, Trypsin digestion of the affinity-purified *T. cruzi* UAg. Numbers on the left represent molecular weight markers.

TABLE 1. Amino acid composition of the 80-kDa *T. cruzi* antigen found in the urine of infected dogs[a]

Amino acid	Residues/100 amino acids
Cys	ND[b]
Asx	7.2
Thr	5.7
Ser	14.8
Glx	11.8
Pro	4.6
Gly	ND[c]
Ala	9.4
Val	6.2
Met	0.0
Ile	3.0
Leu	7.5
Tyr	5.4
Phe	3.5
Trp	ND[d]
Lys	8.8
His	2.7
Arg	9.4

[a] The polypeptide was separated by PAGE and subsequent electroelution, hydrolyzed, and analyzed by HPLC.
[b] ND, Not determined. Cys was not detected because the sample was nonoxidized and noncarboxymethylated.
[c] Gly was not possible to quantitate because electrophoresis buffer contained free glycine.
[d] Trp was not detected because the sample was hydrolyzed with hydrochloride.

To our knowledge, our attempt constitutes the first reported purification of a *T. cruzi* antigen from urinary specimens performed by a polyclonal specific antiserum attached to a solid phase. The method proved to be effective for isolation of antigen from the urine without further contaminants. A single parasite component was retained by the antibody column, and the results of HPLC analysis attest to the purity of the antigen-containing eluate. No changes were observed in the antigenuria pattern during the course of acute infection.

We used two-dimensional gel electrophoresis, lectin AC, trypsinization, and Western blotting for characterization of the trapped antigen. By isoelectric focusing and SDS-PAGE we could identify an 80-kDa polypeptide with a pI of 6.2 to 6.8. No significant differences were visualized in the migration of the protein by SDS-PAGE under either reducing or nonreducing conditions, indicating that the UAg is not a disulfide-cross-linked multimer. The isolated antigen is a

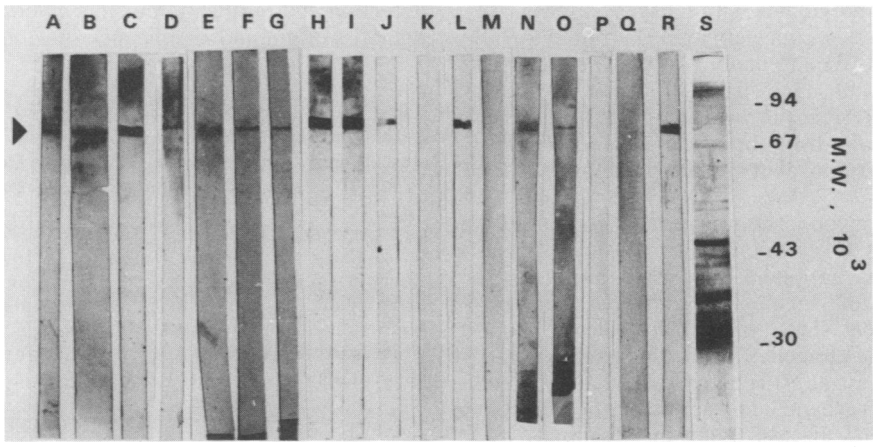

FIG. 4. Immunoblotting analysis of *T. cruzi* UAg. The affinity-isolated antigen was run on polyacrylamide gels and blotted onto nitrocellulose paper. Strips were incubated with sera from acutely infected patients (lanes A to D), congenitally infected patients (lanes E to G), chronically infected patients (lanes H to J), a normal human (lane K), a dog acutely infected with *T. cruzi* (lane L), a dog before experimental infection with *T. cruzi* (lane M), a mouse infected with *T. cruzi* RA (lane N), a mouse infected with *T. cruzi* CAI (lane O), a normal mouse (lane P), a normal rabbit (lane Q), and a rabbit hyperimmunized with *T. cruzi* (lane R). Lane S shows a control reaction represented by blotting of a *T. cruzi* lysate (10^4 parasites) recognized by rabbit hyperimmune serum. Antibody binding was detected with alkaline phosphatase-conjugated anti-IgG from humans, dogs, mice, or rabbits, except that alkaline phosphatase-labeled anti-human IgM was used in lane D. The arrow indicates the position of the antigenic band (80 kDa). M.W., Molecular weight.

glycoprotein containing mannose or glucose or both as demonstrated by binding to ConA and subsequent SDS-PAGE with PAS staining. The UAg also exhibited sensitivity to trypsin.

Several researchers have identified a *T. cruzi* surface antigen with characteristics similar to those described for UAg in the present work. A glycoprotein with affinity for ConA was found to be common to both infective (trypomastigote) and noninfective (epimastigote) forms of *T. cruzi*. It had a mass of 80 kDa, a pI of 5.3 to 6.3, and charge heterogeneity and was susceptible to elimination by trypsin digestion (1). Lanar and Manning (28) reported that culture-form trypomastigotes possess a cell surface protein of 82 kDa and pI 6.6, whereas the staphylomastigote, a form which arises by transformation of either bloodstream or culture-form trypomastigotes in acellular culture conditions to an amastigotelike form capable of cellular division, exhibits an 80-kDa surface polypeptide with a pI of 6.0 to 6.5. Katzin and Colli (26), using AC of surface-labeled parasites from epimastigote, trypomastigote, and amastigote stages on ConA-Sepharose, demonstrated the common existence of an 80,000-M_r glycoprotein. A carbohydrate-containing antigen from *T. cruzi* which stained positive by PAS and was precipitable by ConA has been detected in the blood of acutely infected mice (21).

The parasite component described here could be released as free antigen in the bloodstream of the infected host and then eliminated in the urine, conserving intact its antigenic properties. Even if this UAg has a molecular weight close to the normally accepted limits for glomerular filtration mechanisms, this function depends not only on the size but also on the three-dimensional conformation of the moieties (6). Existence of parasites in the urinary tract cannot be ruled out either (34).

The *T. cruzi* UAg eliminated by acutely infected dogs appears to be identical to one of the two antigens found in the urine of patients with acute Chagas' disease (16). That glycoprotein, detected in recently infected infants, had an M_r of 80,000, showed affinity for ConA, and had a pI between 6.0 and 6.5. Small discrepancies in pI could be attributed to direct measurement of pH on urea gels, which may not give precise values (31). In contrast to what has been demonstrated in individuals with Chagas' disease, we failed to detect the other glycoprotein (55 kDa, pI 6.5 to 7.0) in dog urine specimens. We are unable to produce a consistent explanation of this absence. It may be speculated that the number and characteristics of parasite antigens excreted in the urine depend on both the infecting strain of *T. cruzi* and the mechanisms or removal of circulating antigens in the different infected hosts.

The 80-kDa urinary glycoprotein proved to be markedly antigenic as revealed by Western blotting analysis. UAg was recognized by polyclonal antibodies present in the sera of *T. cruzi*-infected animals and humans. Serum IgG antibodies from patients at different stages of Chagas' disease bound specifically to this single antigen. These results are in agreement with reports showing an 80-kDa trypomastigote surface polypeptide which was recognized by serum antibodies from individuals with Chagas' disease living in Argentina (18), Mexico (13), and Brazil (36) and also by mouse (13) and rabbit (36) specific antisera. IgM antibodies from one acutely infected patient were also able to detect the 80,000-M_r antigenic band. We tested the specificity of the anti-IgG and anti-IgM conjugates used in the present study and found no evidence of cross-reactivity. Therefore, it is most likely that both antibodies recognize a common UAg. This antigen also reacted with the rabbit hyperimmune serum used for its purification by AC and with sera from mice with or without detectable lytic antibodies to *T. cruzi*.

UAg was recognized by serum antibodies from infected dogs which eliminated this *T. cruzi* component in their urine. We could not verify whether this antibody response was mainly elicited by the 80-kDa antigen or by other trypanosome polypeptides sharing a common determinant. Beard et al. (4) showed that a monoclonal antibody recognized surface antigens of 80, 76, 70, 42, and 38 kDa in tissue culture-derived trypomastigotes, suggesting the existence of a common epitope in these polypeptides. However, an 80-kDa trypomastigote protein found in several *T. cruzi* strains could participate in the induction of protective immunity in vivo (32).

The UAg is probably located in the surface membrane of the parasite. As noted above, a *T. cruzi* surface glycoprotein akin to the UAg has been reported by other groups. Moreover, the strong antigenicity exhibited by the UAg, recognized by a wide variety of specific antisera, shows that UAg is expressed in several *T. cruzi* strains and reinforces the hypothesis about a membrane site for this antigen. Surface components are considered as the dominant immunogens in *T. cruzi* infection (30).

The amino acid composition of UAg was determined and showed relatively high proportions of serine and glutamate. The absence of methionine was also observed, as has been described for the lipopeptidophosphoglycan complex of *T. cruzi* epimastigotes (15). The existence of an antigenic relationship between this complex and the 80-kDa UAg is considered unlikely in view of important biochemical and structural differences reported for these moieties (11). Details of the chemical structure of the carbohydrate portion of UAg, together with its interaction with the host immune system, remain to be elucidated.

To define the occurrence of antigenuria with a characteristic antigenic pattern as a phenomenon usually demonstrable in recent *T. cruzi* infection, further studies will be done in other animal models experimentally infected with several strains of the parasite. Isolation of UAg will allow us to prepare antibodies directed to this antigen. With a monospecific antiserum against UAg, we plan to achieve a better detection and purification of this glycopeptide and to investigate its origin and significance in *T. cruzi* infection.

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