INFECTION AND IMMUNITY, May 1994, p. 1643–1651
0019-9567/94/$04.00+0

Vol. 62, No. 5

Antigens Shared by *Leishmania* Species and *Trypanosoma cruzi*: Immunological Comparison of the Acidic Ribosomal P0 Proteins

YASIR A. W. SKEIKY,[1] DARIN R. BENSON,[1] MOHAMED ELWASILA,[2,3] ROBERTO BADARO,[4] JAMES M. BURNS, JR.,[5] AND STEVEN G. REED[1,6]*

Infectious Disease Research Institute, Seattle, Washington 98109[1]; Department of Biology, Faculty of Science, Elain, United Arab Emirates[2]; Department of Zoology, Faculty of Science, University of Khartoum, Khartoum, Sudan[3]; Federal University of Bahia, Salvador, Bahia, 40.140, Brazil[4]; Meharry Medical College, Nashville, Tennessee 37208[5]; and Cornell University Medical College, New York, New York 10021[6]

Received 30 November 1993/Returned for modification 4 January 1994/Accepted 2 February 1994

Patients with visceral leishmaniasis produce high levels of immunoglobulin, but the specificities of antibodies produced are not well characterized. In an effort to identify leishmania antigens that are specific to *Leishmania* species or are cross-reactive with other parasitic protozoa, we have cloned and characterized full-length genomic and cDNA clones encoding a *Leishmania chagasi* acidic ribosomal antigen, LcP0, recognized during human infections. The protein is homologous to the *Trypanosoma cruzi* and human ribosomal proteins TcP0 and HuP0, respectively. Unlike most higher eukaryotes, but similar to TcP0, LcP0 has a C-terminal heptapeptide sequence resembling those of the archaebacterial acidic (P-like) proteins. The highly charged C-terminal acidic domain of LcP0 contains a serine residue typically found in most eukaryotes but lacking in all *T. cruzi* P proteins we have characterized thus far. *L. chagasi*-infected individuals as well as those with *T. cruzi* infections have antibodies cross-reactive with recombinant LcP0 and TcP0 as well as HuP0. However, the properties of anti-P0 antibodies in *T. cruzi* and *L. chagasi* infection sera are quite different. Through the use of synthetic peptides, we showed that while *T. cruzi* infection anti-TcP0 antibodies are exclusively directed against the C-terminal domain of TcP0, *L. chagasi* infection sera contain antibodies reactive with epitopes other than the C-terminal sequence of LcP0. Thus, anti-LcP0 antibodies in *L. chagasi* infection sera represent the first characterized deviation from the restricted immunodominant C-terminal epitope involved in the generation of anti-P0 antibodies following infection or autoimmune diseases.

Leishmania organisms are intracellular protozoan parasites of macrophages which cause clinical diseases ranging from self-limiting cutaneous lesions to highly destructive mucosal involvement and mild-to-fatal visceral infections. *Trypanosoma cruzi*, another intracellular protozoan parasite, is the causative agent of Chagas' disease and is a major cause of chronic heart disease throughout South America. Immunoblotting of parasite antigens as well as immunoscreening of expression libraries, enzyme-linked immunosorbent assays (ELISAs), and immunofluorescence studies, have been used to analyze the humoral immune response to parasite antigens for the identification of specific and cross-reactive antigens of *Leishmania chagasi* and *T. cruzi* (1, 2, 4–6, 10, 28, 30, 40, 43). In the case of specific antigens identified through the use of patient infection sera, this is usually interpreted as being species specific. Alternatively, this may simply reflect the biased antibody titers to shared antigens found in sera of individuals with *Leishmania* or *T. cruzi* infections.

We have been interested in identifying serological epitopes shared by *L. chagasi* and *T. cruzi* in order to compare their protein sequences, stage regulation, and immunodominant B-cell epitopes. To this end, we screened an *L. chagasi* expression library with sera from individuals with *T. cruzi* infection. This resulted in the cloning of an *L. chagasi* genomic sequence, LcP0, identified as the *Leishmania* homolog of the eukaryotic acidic ribosomal P-protein family.

* Corresponding author. Mailing address: Infectious Disease Research Institute, 41 Dravus St., Suite 75, Seattle, WA 98109. Phone: (206) 285-9080. Fax: (206) 285-9134.

The P proteins constitute a family of strongly acidic ribosomal proteins in an environment of mostly basic proteins located within the 60S ribosomal subunit (45, 46). Subunit depletion and antibody inhibition studies have suggested that the P proteins participate in protein synthesis (23, 34, 36, 42, 47). In eukaryotes, the P-protein family consists of three members, P0, P1, and P2, which form a pentameric complex in which P1/P2 homodimers are attached to a single P0 protein via their NH_2-terminal end (27, 47). The acidic proteins of eukaryotic and archaebacterial ribosomes, but not the homologous proteins from eubacteria, contain a highly conserved acidic carboxy domain followed by a conserved heptapeptide C-terminal sequence. These conserved C-terminal residues form the basis for the immunological cross-reactivity of the acidic ribosomal proteins both within and across species (14, 16, 33, 39).

We previously reported the cloning of the *T. cruzi* homolog, TcP0, following heterologous screening of a *T. cruzi* trypomastigote cDNA expression library (40). Of particular interest, although the P proteins are phylogenetically highly conserved, the majority of humans infected with *T. cruzi* have antibodies to *T. cruzi* as well as human P proteins. The *T. cruzi* P0 epitope was mapped to the C terminus, a sequence not shared by any other known P protein (40).

Herein, we report the cloning, expression, and biochemical characterization of full-length genomic and cDNA LcP0 sequences. The open reading frame of LcP0 encodes a ribosome-associated protein with a predicted molecular weight of 34,600 (322 amino acids). LcP0 is constitutively expressed at both the protein and mRNA levels. Evaluation of the human immune

response to LcP0 revealed that LcP0 is highly immunogenic in *L. chagasi*-infected individuals. However, unlike TcP0, the immunodominant B-cell epitope(s) of LcP0 is not restricted to the C-terminal amino acid residues.

MATERIALS AND METHODS

Parasites. *L. chagasi* (MHOM/BR/82/BA-2,C1 and MHOM/BR/84/Jonas), *L. amazonensis* (IFLA/BR/67/PH8), *L. brasiliensis* (MHOM/BR/75/M2903), *L. guyanensis* (MHOM/BR/75/M4147), *L. donovani* (MHOM/Et/67/HU3), *L. infantum* (IPT-1), *L. major* (LTM p-2), *L. tropica* (1063C), *T. cruzi* (MHOM/CH/00/Tulahuen C2), and *T. brucei* (TREU 667) were used and have been previously described (4). Promastigotes and epimastigotes were cultured in axenic media. *L. chagasi* and *L. amazonensis* amastigotes were obtained from spleens of Syrian hamsters and footpads of BALB/c ByJ mice, respectively, and purified as described before (3). K562 human erythroleukemic cells were a gift of M. Yagi, Seattle Biomedical Research Institute.

Library construction and isolation of genomic and cDNA clones. A genomic expression library was constructed with sheared DNA of *L. chagasi* (MHOM/BR/82/BA-2,C1) in bacteriophage lambda ZAPII (Stratagene, La Jolla, Calif.) (4). Poly(A)$^+$ RNA was purified from total *L. chagasi* (MHOM/BR/84/Jonas) promastigote RNA, using standard protocols (35). A cDNA expression library was constructed with the poly(A)$^+$ RNA, using the ZAP-cDNA unidirectional cloning kit (Stratagene). Construction and screening of the libraries and excision of pBSK(−) phagemid sequences were carried out according to the manufacturer's protocols. A pool of five *T. cruzi* infection serum samples was adsorbed to remove anti-*Escherichia coli* reactivity (5, 40) and used to screen the *L. chagasi* genomic expression library. This resulted in the identification of a clone containing a ~3.0-kb insert (pLcP0). The 3.0-kb insert of clone pLcP0 was used to screen an *L. chagasi* cDNA library to obtain clones containing the complete coding region of the gene as well as the 5′-spliced leader and 3′-flanking sequences. Overlapping clones were generated from both the coding (pBSK−) and noncoding (pBSK+) strands of the genomic insert by exonuclease III (19). Single-strand templates were isolated following infection with VCSM13 helper phage, as recommended by the manufacturer (Stratagene), and sequenced by the dideoxy chain terminator method (37) or by the *Taq* dye terminator system, using an Applied Biosystems Automated Sequencer, model 373A.

Northern (RNA) and Southern analyses. Total RNA was extracted by the acid guanidium isothiocyanate method (11), resolved on 1.5% formaldehyde-denaturing agarose gels (35), and transferred by capillary blotting onto Nytran membranes. Genomic DNA was prepared, digested with the restriction enzymes, separated on a 0.7% agarose gel, and blotted onto Nytran membranes (35). A radiolabelled full-length cDNA insert was prepared by the random priming method (15), and blots were hybridized overnight at 65°C, as described previously (40). Blots were washed twice at 65°C for 20 min each time with 2×, 0.5×, and 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.15 M sodium citrate) containing 0.1% sodium dodecyl sulfate (SDS).

Expression and purification of rLcP0 and rHuP0 antigens. The 43-kDa recombinant antigen (rLcP0) of the genomic clone pLcP0 was purified from 500 ml of isopropyl-β-D-thiogalactopyranoside (IPTG)-induced cultures (40). Full-length recombinant human P0 (rHuP0) was produced in *E. coli*, using the pET plasmid vector and a T7 polymerase expression system (Novagen, Madison, Wis.), as described before (39). The inclusion bodies were isolated and sequentially washed twice in 10 ml of TNE (50 mM Tris [pH 8.0], 100 mM NaCl, 10 mM EDTA) containing 2, 4, and 8 M urea. Fractions containing solubilized recombinant antigen (usually the 4 and 8 M urea supernatants) were pooled, dialyzed against phosphate-buffered saline, and concentrated by precipitation with 30% ammonium sulfate. Purification to homogeneity was accomplished by preparative SDS-polyacrylamide gel electrophoresis (PAGE), followed by excision and electroelution of the recombinant antigens as described previously (31).

Production of rabbit antiserum against rLcP0. An adult rabbit (New Zealand White; R & R Rabbitry, Stanwood, Wash.) was immunized by subcutaneous immunization with 200 μg of purified rLcP0 in incomplete Freund's adjuvant (GIBCO, Grand Island, N.Y.) together with 100 μg of muramyl dipeptide (adjuvant peptide; Calbiochem, San Diego, Calif.); this was followed by a boost 4 weeks later with 100 μg of rLcP0 in incomplete Freund's adjuvant alone. Three weeks later, the rabbit was boosted intravenously with 25 μg of rLcP0 in saline, and serum was collected 2 weeks later.

Antigens. Parasite and mammalian cell lysates were prepared by freeze-thaw lysis of pellets in SDS sample buffer without glycerol and β-mercaptoethanol. Insoluble material was separated from the supernatant by centrifugation at 10,000 rpm in a microcentrifuge. *L. chagasi* ribosomes were isolated as previously described for *T. cruzi* (40), and the final pellet was resuspended in SDS sample buffer. Protein concentrations were determined with the Pierce bicinchoninic acid protein assay kit.

In vitro translation and immunoprecipitation. Stage-specific *L. chagasi* and *T. cruzi* total RNA, 10 μg each, were translated in rabbit reticulocyte lysate in the presence of ^{35}S-methionine, as suggested by the supplier's protocol (Promega Corp.). Immunoprecipitations were performed with the indicated rabbit antiserum and *Staphylococcus aureus* (Cowan 1), as described before (40).

Patient sera. Leishmaniasis and *T. cruzi* infection serum samples were from well-characterized patients (confirmed by both parasitological and serological evaluation) from Brazil (4, 5, 40). Serum samples from uninfected individuals were from individuals from Seattle, Wash., and areas of Brazil where the disease is not endemic.

Immunoblot analysis. Five to 10 μg of parasite or cell extracts or 0.5 to 1.0 μg of recombinant antigens was separated by SDS–12.5% PAGE (22) and transferred electrophoretically to nitrocellulose membranes (44). Reactivities of the antisera were assessed as previously described (40), using ^{125}I-protein A followed by autoradiography.

ELISA. Microtiter plates (Probind; Falcon) were coated overnight with 250 ng of recombinant antigens or *L. chagasi* promastigote lysate (1 μg), and the reactivities of patient infection sera were analyzed with protein A-horseradish peroxidase (Zymed) as described previously (32, 40). The LcP0 C-terminal peptide contains 17 amino acids with the sequence EEPEESDEDDFGMGGLF. For peptide competition, diluted sera were preincubated with 5 μg of peptides for 1 h at room temperature prior to performance of the ELISA.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL Data Bank under accession number L29300.

RESULTS

Cloning and genomic organization of LcP0. Through heterologous screening, we sought to identify and characterize in *L. chagasi*- and *T. cruzi*-infected individuals some of the shared

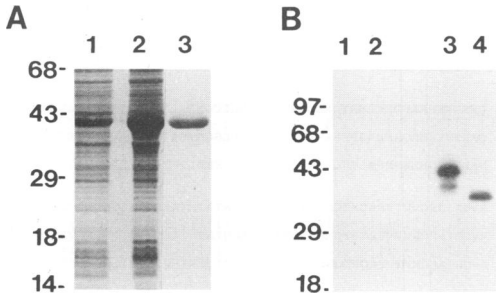

FIG. 1. (A) Expression and purification of rLcP0: Coomassie blue-stained SDS–12% PAGE of 10 μg (each) of *E. coli* lysates from uninduced (lane 1) and induced (lane 2) cultures of clone LcP0 and from purified rLcP0 (3 μg) (lane 3). (B) Immunoblot of purified rLcP0 (lanes 1 and 3, 20 ng) and *L. chagasi* promastigote lysates (lanes 2 and 4, 10 μg) probed with preimmune rabbit serum (lanes 1 and 2) or rabbit anti-rLcP0 serum (lanes 3 and 4).

antigens that may be immunogenic and contain cross-reactive epitopes. To this end, we screened an *L. chagasi* genomic expression library with pooled sera from individuals with *T. cruzi* infection. One clone, pLcP0, was isolated and characterized further. Expression of pLcP0 produced a recombinant fusion protein (rLcP0) of ~42 kDa (Fig. 1A), of which ~4 kDa represents a plasmid fusion sequence. Rabbit antisera raised against purified rLcP0 were used to probe immunoblots of *L. chagasi* promastigote lysate and purified rLcP0. The antiserum reacted specifically to a ~37-kDa antigen present in promastigotes as well as to the 42-kDa rLcP0 (Fig. 1B). These results suggested that rLcP0 contained the entire coding portion of LcP0.

The DNA and deduced amino acid sequences of pLcP0 revealed a single open reading frame (nucleotides 1 to 966, starting with the first Met residue) encoding 322 amino acids with a predicted molecular weight of 34,600 (Fig. 2) and an estimated pI of 4.9. The expressed fusion protein contains an additional 3 kDa (80 nucleotides of 5′-flanking sequences) and ~4 kDa of in-frame β-galactosidase sequence. To verify that the LcP0 genomic clone contained the full-length protein sequence, we screened an *L. chagasi* cDNA library with the LcP0 clone. A 1.2-kb cDNA clone was isolated, and partial sequence analysis of the 5′ and 3′ portions revealed that it encoded a full-length LcP0 insert. The sequence contained the last eight nucleotides of the *trans*-spliced leader sequence found on the 5′ end of all trypanosome nuclearly encoded transcripts (25), followed by a short (29-nucleotide) 5′-untranslated leader segment (Fig. 2). An open reading frame is predicted to initiate at the first AUG codon of the cDNA insert. Partial sequencing of the 3′ portion of the cDNA revealed an open reading frame and a stop codon (TAA)

```
                                                                CTGGCACACACCTTCGGCTTACAAACAACACCTTCACCGACCCTACGACAGATAGCCAAGGCTATTGCAAGTCTCACAAG  80
                                                                                                                CTTTATTG/
                                                                                                                SL
ATGCCGTCTATCACCACTGCCAAGCGCGAGTACGAGGAGCGCCTCGTCGACTGCCTGACCAAGTACAGCTGCGTGCTGTTCGTGGGCATGGACAACGTCC 100
 M  P  S  I  T  T  A  K  R  E  Y  E  E  R  L  V  D  C  L  T  K  Y  S  C  V  L  F  V  G  M  D  N  V   33

GCTCGCAGCAGGTGCACGATGTGCGCCGTGGCTGTCGCGGCAAGGCCGAGTTCATTATGGGCAAGAAGACGCTGCAGGCGAAGATCGTGGAGAAGCGCGC 200
R  S  Q  Q  V  H  D  V  R  R  G  C  R  G  K  A  E  F  I  M  G  K  K  T  L  Q  A  K  I  V  E  K  R  A 67

GCAAGCCAAGGACGCGAGCCCCGAGGCGAAGCCTTTCAACGATCAGTGTGAGGAGTACAACCTGCTGAGCGGCAACACCGGCCTCATCTTCACTAACAAC 300
  Q  A  K  D  A  S  P  E  A  K  P  F  N  D  Q  C  E  E  Y  N  L  L  S  G  N  T  G  L  I  F  T  N  N  100

GCTGTCCAGGAGATCACCTCTGTGCTTGACGGCCACCGCGTGAAGGCCCCGGCGCGTGTCGGAGCGATTCCGTGCGACGTGGTTGTGCCTGCTGGCAGCA 400
 A  V  Q  E  I  T  S  V  L  D  G  H  R  V  K  A  P  A  R  V  G  A  I  P  C  D  V  V  V  P  A  G  S   133

CCGGCATGGAGCCGACCCAGACGTCCTTCTTCCAGGCGCTGAACATTGCGACGAAGATTGCCAAGGGTATGGTGGAGATCGTGACGGAGAAGAAGGTGCT 500
T  G  M  E  P  T  Q  T  S  F  F  Q  A  L  N  I  A  T  K  I  A  K  G  M  V  E  I  V  T  E  K  K  V  L 167

GAGCGTCGGCGACAAGGTGGACAACTCGACGGCGACGCTGCTGCAAAAGCTGAACATCAGCCCGTTCTACTACCAGGTGAATGTGCTGTCCGTGTGGGAC 600
  S  V  G  D  K  V  D  N  S  T  A  T  L  L  Q  K  L  N  I  S  P  F  Y  Y  Q  V  N  V  L  S  V  W  D  200

CGCGGTGTGCTGTTCACCCGCGAGGACCTCATGATGACGGAGGACATGGTGGAGAAGATGCTGATGGAAGGCCTGAGCAACGTTGCGGCGATGGCGCTGG 700
 R  G  V  L  F  T  R  E  D  L  M  M  T  E  D  M  V  E  K  M  L  M  E  G  L  S  N  V  A  A  M  A  L   233

GTGCTGGCATCCCGACGTCTTCGACGATTGGCCCGATGCTGGTGGACGCCTTCAAGAACCTGCTGGCTGTCTCCGTGGCGACCTCGTACGAGTTCGAGGA 800
G  A  G  I  P  T  S  S  T  I  G  P  M  L  V  D  A  F  K  N  L  L  A  V  S  V  A  T  S  Y  E  F  E  E 267

GCACAACGGCAAGGAGCTGCGCGAGGCCGCGATCAACGGCCTGCTGGCCGGCTCTGGCTCGGCTGCTGCGGAGCCCGCCGCTGCCGCGCCGGCCGCCCCT 900
  H  N  G  K  E  L  R  E  A  A  I  N  G  L  L  A  G  S  G  S  A  A  A  E  P  A  A  A  A  P  A  A  P  300

AGCGCTGCTGCCAAGGAGGAGCCGGAGGAGAGCGACGAGGACGACTTCGGCATGGGCGGTCTCTTCTAAGCGACTCGCTATCCGCCACCCAGCACCGTCG 1000
 S  A  A  A  K  E  E  P  E  E  S  D  E  D  D  F  G  M  G  G  L  F  *                                    322

AGTGTTCGTGCGTTCGCATGGTGGACAGTGGCGAGCGTGTGATGCCCTTGGATCATCAGGAAGCAACTCTCTCCCTTTCTCTGGGTGTTCTTCGTTTCTT 1100

CTTTCATTTGTTTTTGATCGCCGTGGCGCTGCGGCGATCGCTCAGTTCTTATTTTCGATCAACCAACAACGAAGGAGACGACAGCAGACACGCGACACAT 1200
                                                                                                AAAAAAAAAAAAAAAAAAAA
                                                                                                Poly A
CTTCACGGAGGAAAGGGACGACGTACTAGCCGACATGGTGAGCAAACCATGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGACCTC          1293
```

FIG. 2. Nucleotide and predicted amino acid sequences of recombinant pLcP0 genomic DNA. Underlined residues indicate the sequenced portions (5′ and 3′) of the full-length LcP0 cDNA which are identical to the genomic sequences. The contiguous 5′-spliced leader (SL) and 3′ poly(A) tail of the mRNA are shown below the genomic sequence. Asterisk, termination codon.

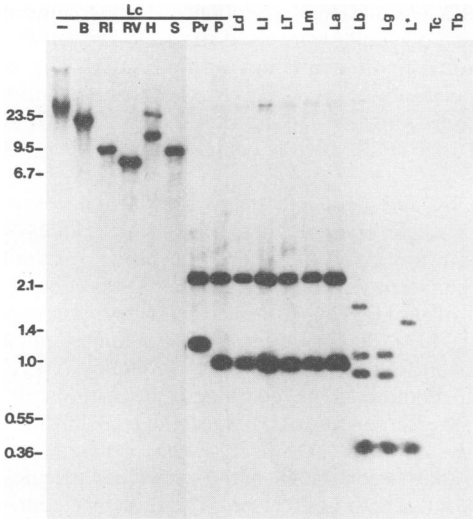

FIG. 3. Southern blot analysis of LcP0 genomic sequences. *L. chagasi* DNA (lanes Lc, 2.5 μg per lane) was digested with *Bam*HI (lane B), *Eco*RI (lane RI), *Eco*RV (lane RV), *Hin*dIII (lane H), *Sst*I (lane S), *Pvu*II (lane Pv), and *Pst*I (lane P). Lane — is control undigested DNA. DNA from *L. donovani* (lane Ld), *L. infantum* (lane Li), *L. tropica* (lane Lt), *L. major* (lane Lm), *L. amazonensis* (lane La), *L. brasiliensis* (lane Lb), *L. guyanensis* (lane Lg), an uncharacterized *Leishmania* species (lane L*), *T. cruzi* (lane Tc), or *T. brucei* (lane Tb) was digested with *Pst*I and analyzed by Southern blotting, using as a probe the ~1.0-kb internal *Pst*I DNA fragment of pLcP0 under stringent hybridization conditions. Values are the sizes, in kilobase pairs, of *Hin*dIII-*Hin*cII-digested lambda DNA.

```
T.CRUZI     MPSVS--EAKREYEERFNGCLTKIGRVLFCLMDNVRSQQVHDVRRDLRGLGELVMGKKTL 58
L.CHAGASI   MPSIT--TAKREYEERLVDCLTKYSCVLFVGMDNVRSQQVHDVRRGCRGKAEFIMGKKTL 58
HUMAN       MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGKAVVLMGKNTM 60

T.CRUZI     QKKIVERRAEDKKASAYDKLLYNTCIEKKLLCGNTALIFTNEEIPVITAVLDKHRVQAPA 118
L.CHAGASI   QAKIVEKRAQAKDASPEAKPFNDQCEEYNLLSGNTGLIFTNNAVQEITSVLDGHRVKAPA 118
HUMAN       MRKAIRGHLENNPALEKLLPHIRGNVGFVFTKEDLTEIRDMLLANKVPA--------AAR 112

T.CRUZI     RVGAIAPCDVIVPAGNTGMEPKATSFFQALNIATKIAKGTVEIVSDKKVLSVGDRVDNST 178
L.CHAGASI   RVGAI-PCDVVVPAGSTGMEPTQTSFFQALNIATKIAKGMVEIVTEKKVLSVGDKVDNST 177
HUMAN       A-GAIAPCEVTVPAQNTGLGPEKTSFFQALGITTKISRGTIEILSDVQLIKTGDKVGASE 172

T.CRUZI     ATLLQKLDISPFYYQVEVQSVWDRGMLFLREDLSITDDVVEKYLLEGISNVAALSLGAGI 238
L.CHAGASI   ATLLQKLNISPFYYQVNVLSVWDRGVLFTREDLMMTEDMVEKMLMEGLSNVAAMALGAGI 237
HUMAN       ATLLNMLNISPFSFGLVIQQVFDNGSIYNPEVLDITEETLHSRFLEGVRNVASVCLQIGY 232

T.CRUZI     PTAATLPHMIMDAFKTLLGASVATEYEFDEFDGKNLRKAALEGNLGGGVADAAAAADTGA 298
L.CHAGASI   PTSSTIGPMLVDAFKNLLAVSVATSYEFEEHNGKELREAAINGLLAGSGSAAAEPAAAAP 297
HUMAN       PTVASVPHSIINGYKRVLALSVETDYTFPLAEKVKAFLADPSAFVAAAPVAAATTAAPAA 292

T.CRUZI     AAAP--AAAAEPEEEDDDDD-FGMGALF- 323
L.CHAGASI   AAPS--AAAKEEPEESDEDD-FGMGGLF- 322
HUMAN       AAAPAKVEAKEESEESDED--MGFG-LFD 317
```

FIG. 4. Homology between the deduced amino acid sequences of *L. chagasi* P0 (LcP0) and those of *T. cruzi* (TcP0 [40]) and human (HuP0 [33]) P0 proteins. Gaps (–) have been introduced to maximize the homology. Vertical lines indicate identity, and double dots represent conservative changes. The hydrophobic domain (underlined) is followed by the highly charged segment (+) and a hydrophobic terminus (asterisks). Serine residues (underlined) within the charged (acidic) segment of the leishmania and human sequences are lacking in *T. cruzi*.

followed by a 203-nucleotide 3′-untranslated portion terminating in a stretch of poly(A) residues. The nucleotide sequences of the cDNA insert in the regions of overlap are identical to those of the genomic LcP0 clone. Therefore, sequences 5′ and 3′ of the AUG translation initiation and TAA termination codons represent flanking sequences. The junction of the splice-leader sequence conforms with the splice donor-acceptor site.

Southern blot analysis of LcP0 gene sequences. Southern analysis of *L. chagasi* genomic DNA digested with enzymes which cut both within and outside LcP0 revealed, at most, two hybridizing bands when probed with a ~1.0-kb internal *Pst*I fragment derived from the genomic pLcP0 clone (Fig. 3). The *Pst*I digest produced two hybridizing bands of ~1.0 and 2.2 kb. The 1.0-kb hybridizing species is in agreement with the presence of two internal *Pst*I restriction sites separated by ~1.0 kb of sequence. With genomic DNA digested with *Hin*dIII (which does not cut within LcP0), two hybridizing bands were detected. A virtually identical hybridization pattern was observed when the same blot was probed with the full-length cDNA insert of LcP0 (not shown). Taken together, the results of the Southern hybridization argue in favor of the presence of at least two copies of LcP0 organized in tandem and contained within a ~6.7-kb *Eco*RV locus (Fig. 3). Figure 3 also illustrates the cross-species conservation between P0 of *L. chagasi* and other kinetoplastid protozoa. Homologous sequences were detected in all *Leishmania* species tested. The hybridizing *Pst*I restriction fragments were identical for all *Leishmania* species except the *L. braziliensis* complex. Only under reduced stringency could cross-hybridizing species be detected in the more distantly related parasites *T. cruzi* and *T. brucei* (not shown).

LcP0 is the *L. chagasi* homolog of the eukaryotic ribosomal acidic phosphoproteins. Figure 4 shows alignment of the deduced primary sequence of *L. chagasi* P0 with protein sequences of *T. cruzi* TcP0 (40) and HuP0 (33). LcP0 has an overall homology of 84% (68.3% identity, 15.5% conservative substitution) with TcP0 and 59% (35% identity, 24% conservative substitution) with HuP0. The lengths (322, 323, and 317 amino acids), molecular weights (34,600, 34,900, and 35,300), and isoelectric points (4.9, 4.8, and 5.8) of LcP0, TcP0, and HuP0, respectively, are very similar.

The homology includes a clustering of residues which are evolutionarily conserved in higher eukaryotes: an Ala-rich region adjacent to a highly charged C terminus. This domain is the basis of the serological cross-reactivity among the eukaryotic P proteins (12, 13, 40, 45). Like other P0 proteins, LcP0 has an arginine-lysine-rich region (located at an equivalent position, residues 42 to 70) which is hypothesized to be involved in the binding to rRNA (26). The C-terminal hydrophobic domain of LcP0 is identical to that of TcP0 except for a single conservative substitution (alanine to glycine). However, unlike TcP0, the adjacent acidic domain of LcP0 is interrupted by a serine residue, a feature characteristic of higher eukaryotic P proteins.

Expression and developmental regulation of LcP0. To estimate the size(s) of LcP0 transcripts and determine their expression patterns during parasite development, Northern blots were probed with the full-length cDNA insert of LcP0. Two hybridizing species of ~1.4 and 1.3 kb with a relative abundance of 1:4, respectively, were detected in *L. chagasi* RNA preparations from both the insect stage (promastigote) and the intracellular amastigote forms (Fig. 5A). The same Northern blot also shows that the LcP0 probe cross-hybridizes with identically sized transcripts present in both promastigotes and amastigotes of *L. amazonensis.*

The protein synthetic profile of LcP0 was determined by

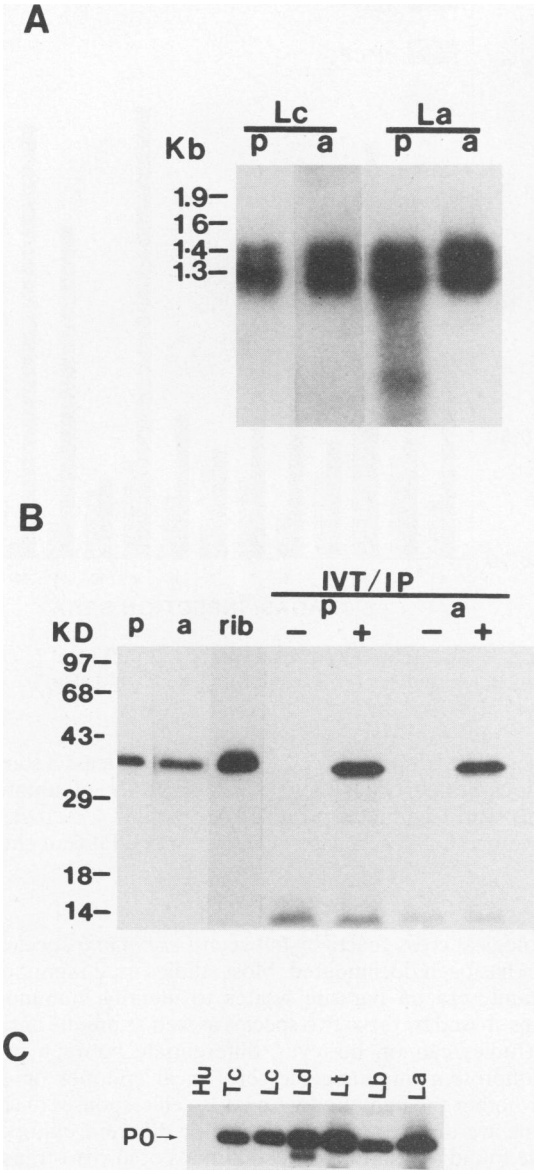

FIG. 5. (A) Northern blot analysis. Ten micrograms each of the indicated total RNA from the promastigote (lanes p) and amastigote (lanes a) stages of *L. chagasi* (Lc) and *L. amazonensis* (La) was hybridized with radiolabelled full-length LcP0 insert. Mobilities and sizes of single-stranded RNA are indicated. (B) Western blot (immunoblot) and immunoprecipitation of in vitro-translated RNA. Western blots of *L. chagasi* promastigote (lane p) and amastigote (lane a) lysates reacted with rabbit anti-rLcP0 antisera. Lane rib represents a purified polysomal fraction from the promastigote stage. Immunoprecipitations of translation reactions from 5 μg each of the indicated *L. chagasi* RNA, using rabbit anti-LcP0 antisera (IVT/IP), are shown. Lanes – and + are with preimmune and immune sera, respectively. (C) Immunoblot of 10 μg each of lysate prepared from K562 cells, a human erythroleukemic cell line (lane Hu), *T. cruzi* epimastigotes (lane Tc), or *L. chagasi* (lane Lc), *L. donovani* (lane Ld), *L. tropica* (lane Lt), *L. braziliensis* (lane Lb), and *L. amazonensis* (lane La) promastigote lysates and probed with rabbit anti-rLcP0 antisera.

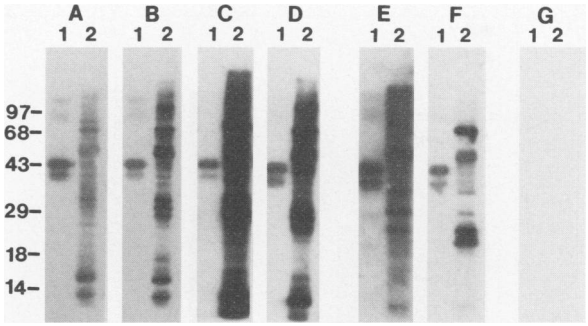

FIG. 6. Reactivity of patient sera with rLcP0. Blots containing 100 ng of purified rLcP0 (lanes 1) and 10 μg of *L. chagasi* promastigote lysates (lanes 2) were probed with sera from individual patients infected with *L. chagasi* (A to D) or *T. cruzi* (E and F). A control using pooled uninfected human sera (n = 5) is shown in part G. Molecular mass markers are shown in kilodaltons.

immunoblot analysis of *L. chagasi* lysates, using rabbit anti-rLcP0 serum. As shown in Fig. 5B, the antiserum detected a single protein of ~37 kDa in both the promastigote and the amastigote stages. The serum did not cross-react with proteins of sizes expected for the *L. chagasi* equivalent of P1 and P2 (10 to 14 kDa). The same blot also shows that purified *L. chagasi* ribosomes (lane rib) contain LcP0 (Fig. 5B). Immunoprecipitations of LcP0 from in vitro translations of total *L. chagasi* promastigote and amastigote RNA (Fig. 5B; lanes IVT/IP) were also performed. The sizes of the precipitated bands were indistinguishable from those of cell lysates. Figure 5C shows that rabbit anti-rLcP0 antisera cross-reacted with similar P0 proteins present in *L. donavani*, *L. tropica*, *L. braziliensis*, *L. amazonensis*, and *T. cruzi* but not with human P proteins. In addition, the rabbit anti-LcP0 serum bound to the C-terminal peptide (see below) of LcP0 but not to a P1/P2 peptide derived from the sequenced *T. cruzi* genes (39). The reactivity of the rabbit antiserum on TcP0 was not affected by an excess (25 μg) of the P1/P2 peptide, although 1 μg of the homologous P0 peptide abrogated the reactivity. Therefore, rabbit anti-rLcP0 serum may be used to detect P proteins with C-terminal residues similar to those found in LcP0 and TcP0.

Reactivity of *L. chagasi* patient sera with rLcP0. To determine whether *L. chagasi*-infected individuals make antibodies against LcP0, serum samples from *L. chagasi* patients were tested on immunoblots of rLcP0 and parasite lysate. Figure 6 shows that *L. chagasi* infection sera contain anti-LcP0 antibodies. Pooled sera from uninfected individuals showed no reactivity to LcP0 or to *L. chagasi* lysate. The reactivities of 42 additional *L. chagasi* infection serum samples on rLcP0 were evaluated by ELISA in the absence or presence of an LcP0 C-terminal amino acid peptide competitor. We first performed titration experiments with increasing amounts of the peptide competitor, using a range from 1 to 25 μg, and found no significant differences in the residual reactivities of *L. chagasi* infection sera on rLcP0 with 5 to 25 μg of peptide competitor (not shown). Subsequently, all competition studies were performed with 5 μg of LcP0 C-terminal peptide. Thirty-eight of the 42 (90.5%) infection serum samples were positive on rLcP0 (Fig. 7), with absorbance values of 0.09 to 4.4 ($\bar{x}$ = 0.56; standard deviation = 0.802). In most cases, preincubation of the same patient sera with the C-terminal peptide of LcP0 resulted in only partial reduction of patient serum reactivity on rLcP0 (decreasing antibody binding by ~35%). The reactivity of only 3 of the 38 *L. chagasi* patient serum samples was effectively

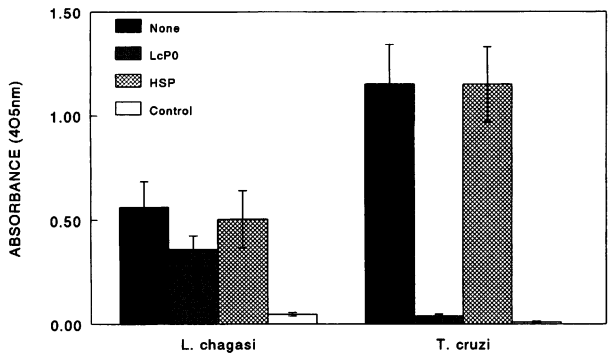

FIG. 7. ELISA evaluation showing the reactivities (absorbance values [mean ± standard error of the mean]) of *L. chagasi* (n = 42) or *T. cruzi* (n = 15) infection sera on rLcP0 in the absence or presence of 5 μg of LcP0 C-terminal peptide competitor. Control competition studies with a synthetic peptide derived from a *Leishmania* heat shock protein sequence (HSP) were performed on adjacent wells containing the LcP0 peptide. The reactivities of uninfected human sera (n = 10; control) are also shown.

inhibited by the LcP0 peptide. Similar competition studies using a peptide derived from a *Leishmania* heat shock protein sequence decreased the average absorbance value by only 11%.

We previously demonstrated that the immunological reactivity of sera from *T. cruzi*-infected individuals on rTcP0 was abolished following deletion of the carboxy-terminal six hydrophobic residues of TcP0 (40) and that 75% of *T. cruzi* infection sera had antibodies reactive with the TcP0 C-terminal peptide (39). In the present study, we extended our previous studies to determine the cross-reactivity and epitope analysis of *T. cruzi* infection sera on rLcP0. All 15 anti-TcP0-positive *T. cruzi* infection serum samples tested were also positive on rLcP0 (Fig. 7). In addition, the anti-P0 antibody titers in *T. cruzi* infection sera were about threefold higher than those found in *L. chagasi*-infected individuals. In sharp contrast to *L. chagasi* infection sera, the reactivities of all *T. cruzi* infection sera with rLcP0 were abrogated in the presence of LcP0 peptide but not with a similar excess of the heat shock peptide (Fig. 7). In fact, 1 μg of LcP0 peptide was as effective as 5 μg in abrogating the reactivities of the same *T. cruzi* infection sera (not shown). These results confirm our previous reports (39, 40) that *T. cruzi* infection anti-TcP0 antibodies are exclusively directed against the C-terminal residues of TcP0. The results also indicate that the conservative (alanine to glycine) as well as additional substitutions in the terminal residues of the LcP0 peptide do not affect binding and epitope restriction of *T. cruzi* patient sera on rLcP0. In this regard, LcP0 peptide is as effective as the TcP0 C-terminal peptide in inhibiting the binding of anti-TcP0 antibodies in *T. cruzi* infection sera to rLcP0 or rTcP0 (not shown). In addition, *L. chagasi* infection sera reacted strongly to rTcP0 (not shown).

Reactivity of *L. chagasi* patient sera with rHuP0. We previously showed that *T. cruzi* infection sera with anti-rTcP0 antibodies can cross-react with HuP0 (39). In order to determine whether patients positive for anti-LcP0 antibodies in *L. chagasi*-infected individuals cross-reacted with the human P0 homolog, we compared the reactivities of *L. chagasi* infection sera on rLcP0 and rHuP0. All infection sera used were strongly positive on *L. chagasi* lysate (not shown). Of the 13 patient serum samples that were positive on rLcP0 ($\bar{x}$ = 1.0), 11 samples were also positive on rHuP0, but with absorbance values ($\bar{x}$ = 0.3) lower than those observed with rLcP0 (Fig. 8).

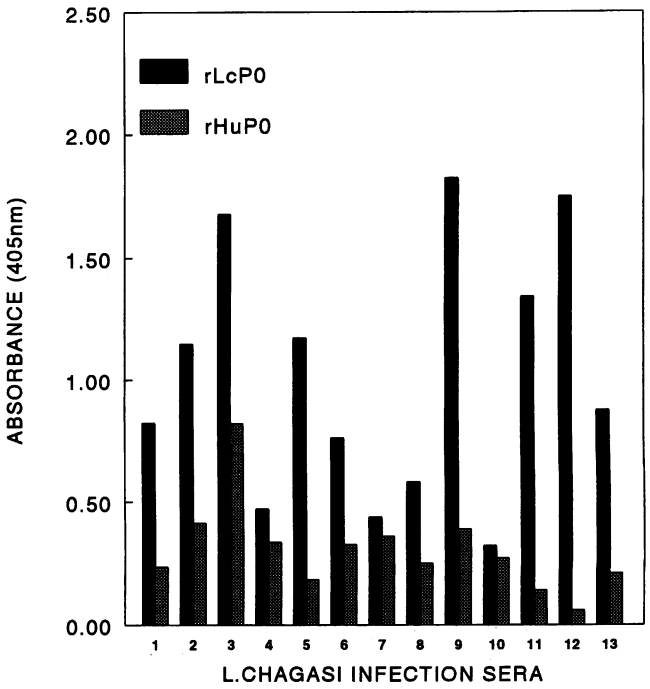

FIG. 8. ELISA showing the reactivities of 13 individual *L. chagasi* infection serum samples (1:50 dilution) on rLcP0 and rHuP0.

Only sera with absorbance values that were at least 3 standard deviations greater than the mean absorbance of uninfected controls were scored as positive. All patient sera that were negative on rLcP0 were also negative on rHuP0 (not shown).

DISCUSSION

Serological cross-reactivity between *Leishmania* species and *T. cruzi* has been documented. Most studies used immunoblots of patient sera on parasite lysates to identify immunogenic antigens shared by these two species as well as unique antigens. Such studies cannot, however, differentiate between protein and nonprotein immunogenic serological epitopes or determine whether the immunodominant B-cell responses of shared antigens are directed against similar or different epitopes. In fact, we found that immunoprecipitations of in vitro-translated *L. chagasi* and *T. cruzi* poly(A)$^+$ RNA with a rabbit serum against *T. cruzi* epimastigotes revealed very similar patterns of protein antigens shared by these two species (38a). It would be expected that many of the shared antigens identified by this method have conserved sequences and perform "housekeeping" functions during the life cycle of the parasites.

We sought to identify antigens shared by *L. chagasi* and *T. cruzi* through heterologous screening of an *L. chagasi* expression library with *T. cruzi* infection sera and report herein on the cloning and characterization of a highly immunogenic leishmanial antigen. LcP0 was identified as the *Leishmania* equivalent of the eukaryotic ribosomal phosphoprotein P0. The conserved C-terminal residues of the eukaryotic P-protein family form the basis for their immunological cross-reactivities both within and across species. However, unlike most higher eukaryotes, LcP0 has a C-terminal sequence that is similar to those of the archaebacterial acidic proteins and TcP0. A rabbit antiserum raised against rTcP0 or rLcP0 reacted exclusively with native P0 (and with peptides making up their terminal amino acid

sequences) but not with P1/P2 proteins (or their C-terminal sequences) of several trypanosomatid species, including *T. cruzi* and *T. brucei* (39, 40), and all five *Leishmania* species (*L. chagasi*, *L. donovani*, *L. tropica*, *L. braziliensis*, and *L. amazonensis*) analyzed in the present study. Therefore, the expression of a ribosomal P0 protein with archaebacterial type C-terminal sequences (FGMGA/GLF) different from those found in the P1/P2 proteins (eukaryotic type, MGFGLFD) of the same species appears to be a general feature of the trypanosomatid P-protein family. All five *Leishmania* species were also found to contain the lower-molecular-weight members (P1/P2) of the P-protein family with C-terminal sequences that are immunologically different from LcP0 and TcP0 but related to other eukaryotic P proteins (unpublished results).

Another feature of the P-protein family of eukaryotes is that their C-terminal acidic domain contains at least one phosphorylation site (usually serine), hence, their designation as phosphoproteins. The serine residue(s) has been shown to represent a phosphorylation site for casein kinase II (18, 21, 24). Phosphorylation of these residues may influence the antigenicity of the P proteins or affect the ribosome function (18, 23, 29, 49). However, none of the members of the *T. cruzi* P-protein family we have characterized thus far contain a phosphorylation site within their C-terminal acidic domain (39, 40). The only exception is a *T. cruzi* clone, C-P0 (38), derived from a PCR reaction which encodes the last 78 amino acids of TcP0. Therefore, it was unexpected to find that the acidic C-terminal domains of both the genomic and cDNA sequences of LcP0 contain a potential phosphorylation site.

LcP0 is transcribed as two mRNA species of ~1.3 and ~1.4 kb. The variance in lengths of the two LcP0 mRNA species does not appear to be due to size differences within their protein coding domains since only one translation product was detected. Therefore, the difference in transcript sizes may reflect transcription products from two different gene copies with variable 5′- and/or 3′-untranslated sequences or the utilization of multiple polyadenylation sites. We showed that the P0 gene(s) of *L. amazonenis* is also transcribed as two mRNA species with sizes identical to those for *L. chagasi*. In *T. cruzi* and *T. brucei*, P0 is transcribed as a single 1.3-kb mRNA species (40). The presence of two P0 transcripts appears to be a general feature of *Leishmania* P0. Homologous P0 sequences were detected in the genomes of all *Leishmania* species tested. The hybridizing *Pst*I restriction fragments were identical for all *Leishmania* species except the *L. braziliensis* complex, suggesting a high degree of conservation in both the nucleotide sequence and the genomic organization of *Leishmania* P0 genes. Similar sequence conservation was found at the protein level, as demonstrated by immunoblot assays with anti-P antibodies.

During the preparation of this report, the P0 gene from another *Leishmania* species, *L. infantum* (LiP0), was cloned and characterized (41). LcP0 shares 98.5% homology with LiP0 (96% identity and 2.5% conservative substitution) and their *Pst*I hybridization patterns were identical, as was that of *L. donovani* P0. By comparison, LcP0 shows 68% homology with TcP0. The characterization of the P0 genes from *L. chagasi* and *L. infantum* represents the first comparison of an antigen gene shared by these two species and further demonstrates their close relationship. LcP0 shares several features with LiP0 which may represent a general characteristic of *Leishmania* P0. These include (i) the presence of two P0 mRNA species of different sizes and (ii) a conservative substitution of alanine to glycine within the C-terminal hydrophobic domain. Also (iii), like the P proteins of higher eukaryotes, LcP0 and LiP0 have a serine residue at identical positions within their highly charged terminal acidic domains. In fact, the amino acid sequences of the C-terminal domain characteristic for all eukaryotic ribosomal P proteins are identical for LcP0 and LiP0. In the present work, we expanded our studies beyond the molecular characterization to present a detailed antigenic comparison of human, *T. cruzi*, and *L. chagasi* P0 proteins.

Unlike *T. cruzi* infection sera, the serological reactivity of *L. chagasi* infection sera with rLcP0 was not restricted to the C terminus. Acute visceral leishmaniasis is associated with marked depression of T-cell proliferative responses (9), serum suppressor factors, circulating immune complexes, and marked hypergammaglobulinemia (8, 17). The increased immunoglobulin levels do not represent only parasite-specific antibodies but rather appear to be a consequence of polyclonal B-cell activation (7, 17, 50). We found that patient anti-LcP0 antibodies also react with rHuP0. This provides evidence for infection-induced, cross-reactive antibodies against a defined antigen shared by the parasite and the host in human visceral leishmaniasis. Similar to the reactivities of *L. chagasi* infection sera on rLcP0, we expect that the cross-reactive serologic epitope on HuP0 is not restricted to the C terminus. Therefore, while patient serological cross-reactivity exists between the P0 proteins of *T. cruzi* and *L. chagasi*, the selection of anti-P0 B-cell epitopes is radically different in patients infected with *T. cruzi* and *L. chagasi*.

The target P0 epitope(s) of *L. chagasi* patient sera represents the first characterized deviation from the restricted immunodominant C-terminal epitope in the generation of anti-P0 antibodies either following infection (*T. cruzi* [39, 40]) or as a spontaneous process in human and mouse systemic lupus erythematosus (14, 16, 20). Although the use of rLcP0 or TcP0 cannot alone distinguish between protozoan infection caused by *T. cruzi* or *Leishmania* species, these antigens could, in combination with the C-terminal peptide competition assays, be used to distinguish between infection caused by the two different parasites. The differences in the anti-P0 epitope selection of TcP0 and LcP0 most likely reflect the outcome of infection with two different protozoan species causing different diseases rather than differences in the immunogenicity of TcP0 and LcP0. Chagas' disease and systemic lupus erythematosus are both chronic diseases characterized by autoimmune pathology, unlike *L. chagasi* infection. In this regard, it is interesting that the restricted C-terminal epitope selection of TcP0 and HuP0 in both Chagas' disease and systemic lupus erythematosus and high anti-P-protein antibodies are associated with two unrelated chronic diseases having significant autoimmune components.

ACKNOWLEDGMENTS

We thank Jeffrey Guderian for help in the expression and purification of rLcP0 and rHuP0. We also thank Marcia Piuvezam and Dimas T. Covas, University of Sao Paulo, Ribeirao Preto, Brazil, Jose Mauro Peralta, University of Rio de Janeiro, Rio de Janeiro, Brazil, and Jose Borges, Fiocruz, Rio de Janeiro, Brazil, for patient sera; Benjamin Rich, Harvard Medical School, Boston, Mass., for providing us with the human P0 construct; and Paul R. Sleath, Immunex Corp., Seattle, Wash., for synthesizing the P0 peptides. We thank Marilyn Parsons for helpful discussions and critical review of the manuscript and Karen Kinch for assistance with manuscript preparation.

This work was supported by grants AI-25038, AI-22726, AI-30639, and AI-16282 from the National Institutes of Health. Yasir A. W. Skeiky is a fellow of the Medical Research Council of Canada.

REFERENCES

1. **Araujo, F. G.** 1986. Analysis of *Trypanosoma cruzi* antigen bound by specific antibodies and by antibodies to related trypanosomatids. Infect. Immun. **53**:179–185.

2. **Badaro, R., S. G. Reed, A. Barral, G. Orge, and T. C. Jones.** 1986. Evaluation of the micro enzyme-linked immunosorbent assay (ELISA) for antibodies in American visceral leishmaniasis: antigen selection for detection of infection specific responses. Am. J. Trop. Med. Hyg. **35:**72–78.
3. **Burns, J. M., J. M. Scott, D. M. Russo, E. M. Carvalho, C. J. March, K. Van Ness, and S. G. Reed.** 1991. Characterization of a membrane antigen of *Leishmania amazonensis* that stimulates human immune responses. J. Immunol. **146:**742–748.
4. **Burns, J. M., Jr., W. G. Shreffler, D. R. Benson, H. W. Ghalib, R. Badaro, and S. G. Reed.** 1993. Molecular characterization of a kinesin-related antigen of *Leishmania chagasi* that detects specific antibody in African and American visceral leishmaniasis. Proc. Natl. Acad. Sci. USA **90:**775–779.
5. **Burns, J. M., Jr., W. G. Shreffler, D. E. Rosman, P. R. Sleath, C. J. March, and S. G. Reed.** 1992. Identification and synthesis of a major conserved antigenic epitope of *Trypanosoma cruzi*. Proc. Natl. Acad. Sci. USA **89:**1239–1243.
6. **Camargo, M. E., and C. Rebonato.** 1969. Cross-reactivity in fluorescence tests for *Trypanosoma* and *Leishmania* antibodies. Am. J. Trop. Med. Hyg. **18:**500–505.
7. **Campos-Neto, A., and M. M. Bunn-Morrno.** 1982. Polyclonal B-cell activation in hamsters infected with parasites of the genus *Leishmania*. Infect. Immun. **38:**871–876.
8. **Carvalho, E. M., B. S. Andrews, R. Martinelli, M. Dutra, and H. Rocha.** 1983. Circulating immune complexes and rheumatoid factor in visceral leishmaniasis and schistosomiasis. Am. J. Trop. Med. Hyg. **32:**61–68.
9. **Carvalho, E. M., R. Badaro, S. G. Reed, W. D. Johnson, Jr., and T. C. Jones.** 1985. Absence of gamma interferon and interleukin-2 production during active visceral leishmaniasis. J. Clin. Invest. **76:**2066–2069.
10. **Chiller, T. M., M. A. Samudio, and G. Zoulek.** 1990. IgG antibody reactivity with *Trypanosoma cruzi* and *Leishmania* antigens in sera of patients with Chagas' disease and Leishmaniasis. Am. J. Trop. Med. Hyg. **43:**650–656.
11. **Chomczynski, P., and N. Sacchi.** 1987. Single step method of RNA isolation by acid guanidinum thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
12. **Elkon, K., E. Bonfa, R. Llovet, W. Danho, H. Weissbach, and N. Brot.** 1988. Properties of the ribosomal P2 protein autoantigen are similar to those of foreign protein antigens. Proc. Natl. Acad. Sci. USA **85:**5186–5189.
13. **Elkon, K., S. Skelly, A. Parnassa, W. Moller, W. Danho, H. Weissbach, and N. Brot.** 1986. Identification and chemical synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus. Proc. Natl. Acad. Sci. USA **83:**7419–7423.
14. **Elkon, K. B., A. P. Parnassa, and C. L. Foster.** 1985. Lupus autoantibodies target ribosomal P proteins. J. Exp. Med. **162:**459–471.
15. **Feinberg, A. P., and B. Vogelstein.** 1984. A technique for radiolabelling DNA restricted endonuclease fragments to high specific activity. Anal. Biochem. **137:**266–268.
16. **Francoeur, A.-M., C. L. Peebles, K. J. Heckman, J. C. Lee, and E. M. Tan.** 1985. Identification of ribosomal protein autoantigens. J. Immunol. **135:**2378–2384.
17. **Galvao-Castro, B., J. A. Sa Ferreira, K. F. Marzochi, M. C. Marzochi, S. G. Coutinho, and P. H. Lambert.** 1984. Polyclonal B cell activation, circulating immune complexes and autoimmunity in human American visceral leishmaniasis. Clin. Exp. Immunol. **56:**58–66.
18. **Hasler, P., N. Brot, H. Weissbach, A. P. Parnassa, and K. B. Elkon.** 1991. Ribosomal proteins P0, P1, and P2 are phosphorylated by casein kinase II at their conserved carboxyl termini. J. Biochem. **266:**13815–13820.
19. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III in DNA sequence analysis. Gene **28:**351–359.
20. **Hines, J. J., H. Weissbach, N. Brot, and K. Elkon.** 1991. Anti-P autoantibody production requires P1/P2 as immunogens but is not driven by exogenous self-antigen in MRL mice. J. Immunol. **146:**3386–3395.
21. **Kuenzel, E., J. Mulligan, J. Sommercorn, and E. Krebs.** 1987. Substrate specificity determinants for casein kinase II as deduced from studies with synthetic peptides. J. Biol. Chem. **262:**9136–9140.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
23. **MacConnel, W. P., and N. O. Kaplan.** 1982. The activity of the acidic phosphoproteins from the 80s rat liver ribosome. J. Biol. Chem. **257:**5359–5366.
24. **Marchiori, F., F. Meggio, O. Marin, G. Borin, A. Calderan, P. Ruzza, and L. A. Pinna.** 1988. Synthetic peptide substrates for casein kinase. 2. Assessment of minimum structural requirements for phosphorylation. Biochim. Biophys. Acta **971:**332–338.
25. **Milhausen, M., R. G. Nelson, S. Sather, M. Selkirk, and N. Agabian.** 1984. Identification of a small RNA containing the Trypanosoma spliced leader: a donor of shared 5′ sequences of trypanosomatid mRNAs? Cell **38:**721–729.
26. **Mitsui, K., T. Nakagawa, and K. Tsurugi.** 1989. The gene and the primary structure of acidic ribosomal protein A0 from yeast *Saccharomyces cerevisiae* which shows partial homology to bacterial ribosomal protein L10. J. Biochem. **106:**223–227.
27. **Moller, W., and J. A. Maassen.** 1985. On the structure and dynamics of L7/L12 from *E. coli* ribosome, p. 310. *In* B. Hardesty and G. Kramer (ed.), Structure, function and genetics of ribosomes. Springer-Verlag, New York.
28. **Nafziger, D. A., R. F. Recinos, C. A. Hunter, and J. E. Donelson.** 1991. Patients infected with *Leishmania donovani chagasi* can have antibodies that recognize heat shock and acidic ribosomal proteins of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. **49:**325–328.
29. **Nairn, A. C., J. A. Detre, J. E. Casnellie, and P. Greengard.** 1982. Serum antibodies that distinguish between the phospho- and dephospho-forms of a phosphoprotein. Nature (London) **299:**734–736.
30. **Reed, S. G., R. Badaro, and R. M. Llyod.** 1987. Identification of specific and cross-reactive antigens of *Leishmania donovani chagasi* by human infection sera. J. Immunol. **138:**1596–1601.
31. **Reed, S. G., E. M. Carvalho, C. H. Sherbert, D. Pedral Sampaio, D. M. Russo, O. Bacelar, D. L. Pihl, J. M. Scott, A. Barral, K. H. Grabstein, and W. D. Johnson, Jr.** 1990. In vitro responses to Leishmania antigens by lymphocytes from patients with leishmaniasis or Chagas' disease. J. Clin. Invest. **85:**690–696.
32. **Reed, S. G., W. G. Shreffler, J. M. Burns, Jr., J. M. Scott, M. de G. Orge, H. W. Ghalib, M. Siddig, and R. Badaro.** 1990. An improved serodiagnostic procedure for visceral leishmaniasis. Am. J. Trop. Med. Hyg. **43:**632–639.
33. **Rich, B. E., and J. A. Steitz.** 1987. Human acidic ribosomal phosphoproteins P0, P1, and P2: analysis of cDNA clones, in vitro synthesis, and assembly. Mol. Cell. Biol. **7:**4065–4074.
34. **Richter, D.** 1973. Competition between the elongation factors 1 and 2, and phenylalanyl transfer ribonucleic acid for the ribosomal binding sites in a polypeptide-synthesizing system from brain. J. Biol. Chem. **248:**2853–2857.
35. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
36. **Sanchez-Madrid, F., R. Reyes, P. Conde, and J. P. G. Ballesta.** 1979. Acidic ribosomal proteins from eukaryotic cells: effect on ribosomal functions. Eur. J. Biochem. **98:**409–416.
37. **Sanger, F., S. Nicklen, and A. R. Coulsen.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
38. **Schijman, A. G., G. Levitus, and M. J. Levin.** 1992. Characterization of the C terminal region of a *Trypanosoma cruzi* 38-kDa ribosomal P0 protein that does not react with lupus anti-P autoantibodies. Immunol. Lett. **33:**15–20.

38a. **Skeiky, Y. A. W., et al.** Unpublished data.

39. **Skeiky, Y. A. W., D. R. Benson, J. A. Guderian, P. R. Sleath, M. Parsons, and S. G. Reed.** 1993. *Trypanosoma cruzi* acidic ribosomal P protein gene family: novel P proteins encoding unusual cross-reactive epitopes. J. Immunol. **151:**5504–5515.
40. **Skeiky, Y. A. W., D. R. Benson, M. Parsons, K. B. Elkon, and S. G. Reed.** 1992. Cloning and expression of *Trypanosoma cruzi* ribosomal protein P0 and epitope analysis of anti-P0 autoantibodies in Chagas' disease patients. J. Exp. Med. **176:**201–211.

41. **Soto, M., J. M. Requena, and C. Alonso.** 1993. Isolation, characterization and analysis of the expression of the Leishmania ribosomal P0 protein genes. Mol. Biochem. Parasitol. **61:**265–274.
42. **Stacey, D. W., S. Skelly, T. Watson, K. Elkon, H. Weissbach, and N. Brot.** 1988. Systemic lupus erythematosus antiribosome P autoantibodies inhibit protein synthesis. Arch. Biochem. Biophys. **267:**398–403.
43. **Tachibana, H., K. Nagakura, and Y. Kaneda.** 1988. Serodiagnosis of Chagas' disease using monoclonal antibody against *T. cruzi*-specific Mr 25,000 antigen. Parasitol. Res. **74:**409–414.
44. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
45. **Towbin, H., H. P. Ramjoue, H. Kuster, D. Liverani, and J. Gordon.** 1982. Monoclonal antibodies against eukaryotic ribosomes: use to characterize a ribosomal protein not previously identified and antigenically related to the acidic phosphoproteins P1/P2. J. Biol. Chem. **257:**12709–12715.
46. **Tsurugi, K., E. Collatz, K. Todokoro, N. Ulbrich, H. N. Lightfoot, and I. G. Wool.** 1978. Isolation of eukaryotic ribosomal proteins: purification and characterization of the 60S ribosomal subunit proteins La, Lb, Lf, P1, P2, L13′, L14, L18′, L20, and L38. J. Biol. Chem. **253:**946–955.
47. **Uchimi, T., R. Traut, and R. Kominami.** 1990. Monoclonal antibodies against acidic phosphoproteins P0, P1, and P2 of eukaryotic ribosomes as functional probes. J. Biol. Chem. **265:**89–95.
48. **Uchiumi, T., A. J. Wahba, and R. R. Trout.** 1987. Topography and stoichiometry of acidic proteins in large ribosomal subunits from Artemia Salina as determined by crosslinking. Proc. Natl. Acad. Sci. USA **84:**5580–5584.
49. **Vidales, F. J., M. T. Saenz-Robles, and J. P. G. Ballesta.** 1984. Acidic proteins of the large ribosomal subunit in Saccharomyces cerevisiae. Effect of phosphorylation. Biochemistry **23:**390–396.
50. **Weintraub, J., M. Gottlieb, and F. I. Weintbaum.** 1982. *Leishmania tropica*: association of a B-cell mitogen with hypergammaglobulinemia in mice. Exp. Parasitol. **53:**87–96.