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Collaborative Research Programme

TERMINAL EVALUATION REPORT

UNIDO contract # 92/048

ICGEB ref. #: CRP/ ARG91-01

Project initiation: 02-04-92

Project termination: 31-03-95



Collaborative Research Programme

TERMINAL EVALUATION REPORT

Part 1

Title of Project	
IMPROVEMENT OF CHAGAS DISEASE DIAGNOSIS AND PROGNOSIS OF CARDIAC INVOLVEMENT.	
Keywords: Chagas disease; <i>Trypanosoma cruzi</i> ; Chagasic cardiomyopathy; serodiagnosis; recombinant antigens; B-cell epitope mapping; diagnostic kit	
UNIDO contract # 92/048	ICGEB ref #: CRP/ ARG91-01
Project initiation: 01-03-92	Project termination: 31-03-95
Principal Investigator's name: Dr. Mariano J. Levin	
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Abstract:	
<p>The C-terminal regions of the <i>T. cruzi</i> P proteins are strongly immunogenic in Chagas disease. All the forms of these proteins show the ability to generate autoimmune responses but their antigenic properties are not the same. The low molecular weight P proteins induce autoreactivity against the human counterparts while antibodies reacting with a pentapeptide from the C-terminal region of the P0 protein is able to induce a functional autoimmune response against the cardiovascular human β1-adrenergic receptor. Synthetic peptides derived from these proteins, as well as from other parasitic antigens and purified recombinant proteins, were used to test their ability to determine the serological profile of severe Chagas heart disease patients. When a serial statistical analysis of the results is made, it is shown that severe chagasic cardiopathies are characterized by high antibody levels against <i>T. cruzi</i>, JL7 and synthetic peptides. The results of this assay indicate that these reagents may be used in the construction of diagnostic kits for Chagas disease and its most severe symptoms.</p>	

OBJECTIVES/METHODOLOGY

(proposed at the time of the submission of the research proposal)

This study is designed to establish two types of serodiagnostic tests: one for the specific diagnosis of chronic *T. cruzi* infection (Chagas disease); the other one to diagnose the chagasic cardiomyopathy and to prognosticate the onset of the chagasic myocardial damage.

The feasibility of this proposal is based on recent results that allowed the identification of two types of recombinant *T. cruzi* antigens; those reacting with sera from chagasic individuals with different clinical forms of the disease (diagnostic recombinant JL7 and JL8), and those reacting predominantly with sera from Chagas heart disease (ChHD) patients (prognosis recombinants JL5, JL9 and T.31).

Differential B cell epitope mapping shall be performed in order to define more sensitive and specific reagents for prognosis of ChHD.

The resulting recombinant antigens and the synthetic peptides will constitute the basis of a new generation of diagnostic kits for Chagas disease.

1- Cloning and sequence analysis of the ribosomal P proteins of *T. cruzi*

Immunological screening of *T. cruzi* expression libraries with sera from chagasic patients with severe Chagas heart complaint led to the characterization of four different *T. cruzi* ribosomal P proteins: P_{JL5} (107 amino acids), P₁ (109 amino acids), P_{2b} (112 amino acids), and P₀ (321 amino acids). The proteins were compared with the corresponding proteins of yeast, rat and human, and renamed according to an unifying nomenclature (1, P_{2b}) and TcP_{2β} (107 aa, previous name JL5), each of which is encoded by multiple genes (1). Southern analysis of several *T. cruzi* strains such as Maracay, Tulahuen, RA, CL and Y revealed a complex genomic organization for the TcP_{2β} gene family (2). Genomic Lambda Zap II clones containing four TcP_{2β} loci, TcP_{2β}-H1.3, -H1.5, -H1.8 and -H6.4 were isolated and sequenced (2), while hybridization of TcP_{2β} probes with Southern blots of electrophoretic karyotypes revealed that TcP_{2β} genes were distributed in six chromosomal bands (3). Characterization of eighteen full length TcP_{2β} cDNA clones allowed identification of six TcP_{2β} mRNAs that differed in nucleotide substitutions. Sequence comparison of the cDNAs with the previously characterized genomic clones revealed that four transcript species derived from the TcP_{2β}-H1.3, -H1.5, -H1.8 and -H6.4 genes (2). One of the other cloned transcripts, named TcP_{2β}-H1.5 A, was identical to the first reported cDNA, P-JL5 (4); and the sixth variant corresponded to a novel sequence, that was designated TcP_{2β}-X. Comparison of the six TcP_{2β} cDNA variants indicated that TcP_{2β}-H1.5 A harbored the consensus nucleotide sequence for each polymorphic position, and was consequently designated as the TcP_{2β} prototype. TcP_{2β}-X differed from the prototype in ten nucleotide changes, being the most divergent sequence among the six cloned mRNAs. The identification of transcripts encoding multiple TcP_{2β} variants allowed assumption that in *T. cruzi* all the members of the family are actively transcribed, in contrast to the findings reported for higher eukaryotic organisms.

The distribution of different type of substitutions seemed to follow the structural organization of the TcP_{2β} protein domains, i) a N-terminal globular region with identical amino-acid sequences conserved due to synonymous changes, that included the selection of unusual codons, ii) a central globular and a hinge region that differed in conservative amino-acid substitutions, and iii) a C-terminus that was totally homologous at the nucleotide level, indicating that each P protein domain was under different selective pressures.

Phosphorylation of eukaryotic acidic ribosomal proteins has been implicated in the regulation of the rate of protein synthesis, through binding to the ribosomal body. Then, it is probable that, a) the conservation of putative phosphorylatable Serines in one domain, and b) the diversity in number of serines in other regions, are biologically relevant; they may be indicative of the existence of a Trypanosoma specific mechanism able to modulate the functional roles of the different members of the TcP_{2β} protein family. Furthermore it is certain that sequences with or without Ser, may be more, or less antigenic according to the surrounding motifs. In the case of the C-P₀ protein the Ser in the C-terminal region is important in the definition of a cross-reactive epitope (1,5, see below).

(Continues pages 3a-e)

**Work plan and time schedule
(originally envisaged)**

TASKS

- 1- Characterization of *T. cruzi* antigens that cross-react with human proteins.
- 2- Genetic and immunologic characterization of recombinants, DNA sequencing, and sequence comparisons.
- 3- Subcloning and production of cloned antigens in bacterial cultures to be used in diagnostic kits.
- 4- Epitope mapping of recombinants.
- 5- Assessment of anti-recombinant and synthetic peptide antibody profile of different sera (double-blind study).
- 6- Correlation of clinical findings with the serological tests.
- 7- Development of diagnostic kit

TASKS	1992a	1992b	1993a	1993b	1994a	1994b
1	X	X				
2	X	X	X	X		
3	X	X	X	X	X	X
4	X	X	X	X	X	X
5	X	X	X	X	X	X
6					X	X
7			X	X	X	X

a: first semester

b: second semester

Work plan and time schedule

(actual)

Project landmarks, duration of individual tasks (use bar charts); evaluation criteria (publications, patents, services, training)

TASKS

- 1- Characterization of *T. cruzi* antigens that cross-react with human proteins.
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TASKS	1992a	1992b	1993a	1993b	1994a	1994b
1	X	X	X	X	X	
2	X	X	X	X	X	X
3	X	X	X	X	X	X
4	X	X	X	X	X	X
5	X	X	X	X	X	X
6				X	X	X
7						X

a: first semester

b: second semester

PROJECT LANDMARKS

- 1- Characterization of the ribosomal P protein family in *T. cruzi* and *Leishmania* from the *braziliensis* complex. Based on these basic studies the amino acid sequences of the ribosomal P proteins were disclosed, allowing comparison with known human epitopes, such as those of the beta-receptors. Moreover, the genetic organization of the TcP2beta genes exposed the existence of SIRE, a repetitive sequence of the parasite genome that is currently used in the study of *T. cruzi* genomic organization. The TcP2beta genes, SIRE, and SIRE associated sites have been used in the characterization of the electrophoretic karyotype of the parasite. (Papers: Vazquez, MP *et al*, Nucl. Acids Res. 20, 2893, 1992; Vazquez, MP *et al*, Nucl. Acids Res. 20, 2599, 1992; Schijman, AG, Nucl. Acids Res. 20, 2894, 1992; Levin, MJ *et al*, Parasitol. Today 9, 381-384, 1993; Schijman, AG *et al*, Gene, submitted; Vazquez, MP *et al*, Mol. Biochem. Parasitol. 64, 327-336, 1994; Levin, MJ *et al*, Mem. Inst. Oswaldo Cruz 89, 17-18, 1994; Cano, MI *et al*, Mol. Biochem. Parasitol. 1995, in press; PhD Thesis: Schijman, A., 1992).

(continues 5a)

NETWORKING

Collaboration with other laboratories

Laboratories that were in contact with our group during the period covered by this report (1992-1995): M.S. FIOCRUZ, Centro de Pesquisas Rene Rachou, Belo Horizonte, Brazil; Instituto de Higiene, School of Medicine, Universidad de la Republica, Montevideo, Uruguay; Instituto de Medicina Tropical Alexander Von Humboldt, Lima, Peru; Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; Instituto de Patologia Tropical de Saude Publica, Universidad Federal de Goias, Goiania, Brazil; Escola Paulista de Medicina, Sao Paulo, Brazil.

Participants of this net were in permanent contact. They exchanged reactive reagents, serum samples, plasmids and plasmidic constructions, and shared information and evaluated in common experiences performed in different laboratories. These meetings were supported by the Iberoamerican Network of laboratories, CYTED (Spain, and Latin-American countries). CYTED also supported the visits of scientists from INGEBI to the mentioned latin american and spanish labs. At the level of plasmid construction and purification of recombinant proteins, the work was performed in collaboration with Dr. Wim Degraeve (Instituto Oswaldo Cruz, Rio de Janeiro, Brazil). Moreover, results obtained with recombinant JL7 were compared with those obtained by Dr. Franco da Silveira with a similar protein characterized in his laboratory and named H49. Laboratories in Sao Paulo, Goias and Buenos Aires shared a number of serum samples of normal and chagasic patients in an attempt to study differential reactivities observed with recombinants H49 and JL7, as well as with synthetic peptides.

The heads of the labs mentioned above agreed with INGEBI, to perform a double-blind assay of the recombinants and synthetic peptides with Dr. Luquetti (Institute of Tropical Medicine, Goiana, Brazil).

Contacts were made with the Instituto Boliviano de Biologia de la Altura (Dr. Mireille Hontebeyrie's laboratory, IBBA, La Paz, Bolivia). In fact, the work concerning the antibodies reacting with the anti β -1 receptors was performed in close collaboration with the IBBA, the Centro de Pesquisas Rene Rachou together with Dr. Johan Hoebeker from the University Francois Rabelais, Tours, France. The work performed on the antigenicity of HSP70 and P proteins in *Leishmaniasis* was carried out by INGEBI, the Instituto de Medicina Tropical Alexander Von Humboldt, and the laboratory of Dr Carlos Alonso (Centro de Biologia Molecular, Universidad Autonoma de Madrid, Spain).

PUBLICATIONS

SEE ATTACHED. (pages 6a-b).

STATEMENT OF EXPENDITURES

To be filled by ICGEB		To be filled by the Affiliated Centre	
Budgets as per original proposal		Summary of expenditures *	
1) Capital equipment	US\$	1) Capital equipment	US\$ 18,680
2) consumables	US\$	2) consumables	US\$ 27,900
3) training	US\$	3) training	US\$
4) literature	US\$	4) literature	US\$ 5,775
5) miscellaneous	US\$	5) miscellaneous	US\$ 7,200
TOTAL GRANT	US\$.....	TOTAL	US\$ 59,555

Please itemize the following budget categories (if applicable)

Capital equipment

- 1 environmental shaker (room temperature-60°C)
- 1 environmental shaker (0-60°C)
- 2 sequencing cells
- 1 PC 386
- 2 -20°C freezers Whirlpool

Training (provide names, duration of training, host laboratory)

Literature

Curr. Op. Immunol., Curr. Op. Gen. and Dev., Curr. Biol.,
 Curr. Infect. Dis., Paras. Tod., Immunol. Tod.
 (1992-1995)

Books: YAC Libraries-A User Guide, University of Wisconsin
 Biotechnology Center; Gene and Chromosome Analysis,
 Methods in Molecular Genetics, Academic Press;
 Biocomputing Informatics and Genome, Academic Press;
 Genes V, R. Lewin

* Please do not send invoices, receipts etc.; these should be kept by the Affiliated Centre for future reference and sent to ICGEB upon request.

2- Epitope mapping of ribosomal P proteins

Previous results have revealed that patients with chronic Chagas heart disease (cChHD) present a strong humoral response against the C-terminal portion of the three low molecular weight ribosomal P proteins of *T. cruzi* (1). In fact, the P epitope was mapped to the common C-terminal amino acid sequence of these proteins, a highly acidic, Glu/Asp-rich C-terminal domain, EEEDDDMGFGLFD (R-13). This peptide shares more than 90% homology with the C-terminal region of the human ribosomal P protein, EESDDDMGFGFLD (H-13), which is, in turn, the target of the auto anti-P-antibodies in systemic lupus erythematosus (SLE)(1,6). Chronic ChHD anti-*T. cruzi* ribosomal P, and SLE anti-P antibodies, share the same specificity, mapping to the 13-C terminal residues of the parasite and host p proteins (7,8). Furthermore, antibodies against the *T. cruzi* ribosomal P protein C-terminal region react with their human counterparts, defining a cross-reactive peptide epitope in cChHD (7,8).

Using nitrocellulose filter replica 1×10^5 phage of a blood stream trypomastigote λ gt11 cDNA library was screened independently with a 1:2000 dilution of a serum from a cChHD patient, and with a P+ SLE serum. The cChHD serum reacted with 8 recombinants whereas the SLE serum recognized 5. Three phage were recognized by both sera, and encoded C-terminal portions of the *T. cruzi* ribosomal P1 and P2 proteins. The other SLE + phage, encoded the C-terminal portion of the mouse ribosomal P1 protein, identical to the corresponding regions of the human P proteins. These phage reacted weakly with 1:100 dilutions of cChHD serum. Subsequently, the serum levels of antibodies against *T. cruzi* and human ribosomes, recombinant *T. cruzi*, mouse and human ribosomal P proteins were measured by ELISA. The analysis of 35 chagasic sera (N=35) showed that all presented a strong reaction to the parasite ribosomal proteins, and the majority reacted with the *T. cruzi* P recombinant. Eight out of 35 chagasic sera presented low, but positive, reactions against both mammal ribosomal P proteins. These results were confirmed measuring the antibody levels to the C-terminal peptides of the parasite and host P proteins, R-13 and H-13, respectively. The serologic profile of a chagasic serum presented high anti-R-13 and low H-13 levels, while the SLE P+ serum showed similar anti-R-13 and anti-H-13 levels.

The fine specificities of anti-P antibodies in cCh HD and SLE were also different when analyzed by a panel of synthetic peptides. The chagasic sera showed a marked preference for the R-11 peptide (EDDDMGFGLD) demonstrating the amazing specificity of this response, since the human and the parasite peptide differ by only one non conservative substitution. This type of anti-P response is similar to that induced in mice by immunization with *Artemia salina* ribosomes (they contain P proteins with a R-13 C-terminal sequence), but different from that observed in SLE (9). The pattern of peptide recognition of the anti-R-13 and anti-H-13 antibodies purified from total chagasic serum and their affinity constants suggested that the differential binding of the chagasic anti-P antibody to R-13 and H-13 is due to differences in the affinity of the antibody to the peptides.

The *T. cruzi* ribosomal P proteins present several features that may be important in the immunopathology of Chagas disease. Their exposed location on the ribosome and the amplification of their parasite specific, Ser free C-terminal domain, generate a strong anti-parasite P response, associated with anti-P autoimmunity.

It is proposed that the infection generates, among other pathological effects, a chronic ribosome immunizing state that contributes to the autoimmune pathogenesis of the disease (Kaplan, D *et al*, Anti-P autoantibodies elicited by *T. cruzi* P antigens differ from those found in Lupus patients, in preparation).

The anti-R13 response is a feature of the chagasic infection, since antibodies against this epitope are rarely found in *Leishmania sp.* infected individuals. To explain the different response to this highly conserved C-terminal region in both parasitosis, we undertook the cloning of the P proteins from the *Leishmania braziliensis* complex. Screening of a *L. (V)peruviana* expression library with radioactive cDNA probes of *Leishmania infantum* ribosomal P2 α and P2 β proteins resulted in the isolation of a 665 bp, and a 270 bp cDNA encoding the *L. (V)peruviana* P2 α and P2 β proteins, respectively. The amino acid sequence of the P2 α protein is almost complete, lacking only the 3 N-terminal residues, while the P2 β cDNA encodes the C-terminal half of the protein. They are 88% and 72% homologous to the P2 α and P2 β of *L. infantum*, respectively.

Interestingly, the 13 C-terminal amino acid sequence of the four *Leishmania* proteins is the same: EEADDDMGFGLFD (L-13). This sequence is almost identical to *T. cruzi* sequence: EEEDDDMGFGLFD (R-13), except for the non conservative amino acid substitution of the Ala by the Glu residue.

Synthetic peptides and recombinant proteins encompassing the antigenic portions of the P proteins of both parasites were used in ELISA and Western blots to analyze their antigenicity in sera from infected individuals. No reactivity against the P peptides was detected in 40 sera from Peruvian individuals with *Leishmaniasis*. The sera from chagasic patients reacted with the R-13 peptide but reacted weakly with the L-13 peptide. Sera from six Bolivian patients with the mixed infection show a chagasic pattern of anti-P reactivity, recognizing R-13, and weakly L-13. The results demonstrate the extreme importance of the Glu residue located 11 residues from the C-terminus in the generation of the anti-ribosomal P protein reactivity, and non antigenic nature of the C-terminal portion of the *Leishmania* P proteins in the human infection.

3- Characterization of a *T. cruzi* peptide sequence that cross reacts with the second extracellular loop of the β -1 adrenoreceptor

Sequence comparisons revealed that a five amino acid peptide of the C-terminal portion of the *T. cruzi* ribosomal P0 protein might have the property to induce a crossreactive, autoimmune response. This sequence AESEE, is homologous to the AESDE sequence present in the second extracellular loop of the β -1 adrenoreceptor (β -1 loop peptide: HWWRAESDEARRCY NDPKCCDFVTNR, also named H26R).

Since the second extracellular loop of the human β 1-adrenergic receptor has been shown to contain a functionally autoimmune B cell epitope (10), H26R recognition by sera of Chagasic patients was assayed. Table 1 summarizes the results. Thirteen out of 36 sera of chagasic patients were found to be positive compared to 0 out of 8 control sera ($p < 0.05$). This response was compared to that against the immunodominant ribosomal P0 protein from *T. cruzi* (C-P0) and the peptide derived from it and thought to cross-react with the receptor (P0- β). As already shown

elsewhere (5), more than 80% of the chagasic sera reacted with C-P0 while only 47% reacted with P0- β . While there was no correlation between the response against H26R and C-P0 ($p = .282$), a significant correlation was found between the response against H26R and P0- β 1 ($p = .0147$). The specificity of the response against H26R and C-P0 was assessed by assaying sera of 15 patients with leishmaniasis. None of them yielded a positive response (Ferrari, I *et al*, J. Exp. Med. 1995, in press).

Specific anti-H26R antibodies from Chagasic patients serum samples were affinity-purified to assay for functional recognition of the β 1-adrenergic using spontaneously beating neonatal rat heart myocytes. Since this system has already been used for the study of anti-receptor autoantibodies in idiopathic dilated cardiomyopathy (27) and the sequence of the second extracellular loop of rat and human β 1-adrenergic receptor are totally similar (12), it is suitable for cross-reactive studies. To increase the specificity of the functional response, assays were performed in the presence of 1 μ M atropine to avoid any interference with autoantibodies directed against the muscarinic acetylcholine receptor as those which are present in sera of chagasic patients (13).

The chagasic purified antibodies showed the same positive chronotropic response as those purified from patients with idiopathic dilated cardiomyopathy (14). After 60 minutes of preincubation with the antibodies, a significant increase in beating frequency of the heart myocytes was observed, that was completely blocked by the addition of the β 1-specific blocker, bisoprolol.

When the antibodies were preincubated with 14 μ g/ml P0- β before addition to the cardiomyocytes, the positive chronotropic effect of the purified antibodies was inhibited completely for the antibodies purified from a Chagasic patient (serum sample number 99) and partially those from another Chagasic patient (serum sample number 32). Peptides alone did not have any effect on the cardiomyocytes. To ascertain that the antibody-peptide complex had no toxic effects on the cells, the cardiomyocytes were treated with 10 μ M isoproterenol, a β -adrenergic agonist. The cardiomyocytes reacted in a normal way to this stimulant.

In contrast 20 μ g/ml of peptide did not inhibit the positive chronotropic effect of IgG fractions from patients with idiopathic dilated cardiomyopathy (Ferrari, I *et al*, J. Exp. Med. 1995, in press).

Table 1: Number of patients sera positive against the three antigens

Patients	H26R	C-P0	P0- β
Healthy controls (n = 8)	0	0	0
Chagasic patients (n = 36)	13	30	17
Leishmaniasis patients (n = 15)	0	0	N.D.

N.D. not determined

The results suggest a general mechanism in Chagas disease (molecular mimicry), by which antibodies generated against parasite intracellular proteins may have a direct effect on membrane proteins of the host cells, affecting their function and/or viability.

4- Evaluation of serological markers of human *T. cruzi* infection and putative markers of heart pathology

The antigens tested as possible markers of *T. cruzi* human infection and Chagasic heart pathology were the following: recombinant antigens JL7 (15, JL7 was the only recombinant antigen used for ELISA), JL5 (15, used in phage dot array immunoassay) and C-P0 (5, used in phage dot array immunoassay); synthetic peptides R-13 (EEEDDDMGFGLFD)(16), H-11 (SDDDMGFGLFD) (16), PO-13 (EDDDDDFGMGALF)(16), PO- β (AESEE)(16), JL9-18 (SAYRKALPQEEEDV GPR)(17), JL9-19(VDPDHFIRSTTQDAYRPVDP)(17), Tc HSP70 (ANGILNVSAEEKGTGK) (18), Hu HSP70(ANGILNVSAVDKSTGK)(18) and β 1 (HWWRAESDEARRCYNDPKCCDFV TNR)(19). A *T. cruzi* homogenate was used as ELISA reagent to determine the level of anti-*T. cruzi* antibodies.

The cDNA insert of phage JL7 was subcloned in an expression plasmid that allowed the production of an almost pure insert-encoded product fused to the first 10 residues of the *Escherichia coli* DNA polymerase I. The recombinant (R-JL7; MW 36 kDa), was produced in bacterial cultures and purified by HPLC. The purified product was used in ELISA to evaluate the levels of anti-JL7 antibodies in chagasic patients.

The network laboratories agreed that sera to be tested with the recombinantes should be collected by Dr. A.O. Luquetti from the Institute of Tropical Medicine, Goiana, Brazil. The assay was to be performed in a double-blind fashion. Accordingly, Dr. Luquetti sent to INGEBI four hundred and sixty (460) Latin-America coded serum samples to test the above mentioned reactive reagents. Three members of INGEBI performed 33.000 ELISA. Analysis of the results demonstrated that the combination of the *T. cruzi* homogenate and the JL7 recombinant is excellent as a specific reactive reagent of human *T. cruzi* infection. JL9-18 and JL9-19 as well as R-13, and the other peptides are good markers of severe chagasic cardiomyopathy.

The results of this assay have undergone exhaustive discussions with Dr. Luquetti that visited our laboratory twice in 1994 to check lists and confirm data about serum samples. Statistical analysis demonstrates that when a serial analysis of the results is made, the patients with severe chagasic heart complaint present a very specific serological profile. Severe chagasic cardiopathies are characterized by high antibody levels against *T. cruzi*, JL7 and to the synthetic peptides. A first analysis of the results was informed in our 1993-1994 annual report. The complex statistical analysis of these large number of data has delayed publication of the results, that will be published in 1995 (Kaplan *et al*, A combination of serological tests for diagnosis of severe Chagas cardiomyopathy, in preparation). The results of this assay indicate that the reagents used may be the basis of a new generation of diagnostic kits for Chagas disease serology.

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- 2- Epitope mapping of cross-reactive *T. cruzi* proteins. Serological anti-HSP70 response in patients with *Leishmania braziliensis braziliensis* infection (Papers: Levin, MJ *et al*, *Parasitol. Today* 9, 381-384, 1993; Kaplan, D *et al*, *Biol. Res.* 26, 273-275, 1993; Levin, MJ *et al*, *FEMS Microbiol. Medical Immunol.* 7, 205-210, 1993; Kaplan, D *et al*, *Mem. Inst. Oswaldo Cruz* 88, 51-52, 1993; Rosenbaum, MB *et al*, *J. Cardiovascular Electrophysiology* 5, 367-375, 1994; Ferrari, I *et al*, *J. Exp. Med.* 1995, in press; Kaplan, D *et al*, Anti-P autoantibodies elicited by *T. cruzi* P antigens differ from those found in Lupus patients, in preparation; Thesis: Levy-Yeyati, P, 1992).
- 3- Subcloning and production of cloned antigens in bacterial cultures to be used in diagnostic kits. The cDNA encoding for 4 antigens: the diagnostic antigen JL7, and the cross-reactive *T. cruzi* antigens JL9, TcP2beta (JL5), and TcP0 were subcloned in expression plasmids. The plasmids were used to transfect bacteria, that were used to produce the corresponding recombinant protein (Papers: Ferrari, I *et al*, *J. Exp. Med.* 1995, in press; Kaplan, D *et al*, A combination of serological tests for diagnosis of severe Chagas cardiomyopathy, in preparation).
- 4- Epitope mapping of recombinants and further definition of cross-reactive epitopes. Comparison of ribosomal P proteins sequences revealed homology with the corresponding humans counterparts, and with sequences found in the extracellular loops of the beta-1 adrenergic receptor and the muscarinic receptor, both present in the surface of cardiocytes. Functional studies demonstrate that peptides derived from the amino acid sequence of the P0 parasite protein inhibit the effect of anti-beta 1 receptor antibodies purified from chagasic sera (Papers: Kaplan, D *et al*, *Mem. Inst. Oswaldo Cruz* 88, 51-52, 1993; Rosenbaum, MB *et al*, *J. Cardiovascular Electrophysiology* 5, 367-375, 1994; Ferrari, I *et al*, *J. Exp. Med.* 1995, in press; Kaplan, D *et al*, Anti-P autoantibodies elicited by *T. cruzi* P antigens differ from those found in Lupus patients, in preparation).
- 5- Assessment of anti-recombinant and synthetic peptide antibody profile of different sera (double-blind study) and correlation of clinical findings with the serological tests (Papers: Levin, MJ *et al*, *FEMS Microbiol. Medical Immunol.* 7, 205-210, 1993; Rosenbaum, MB *et al*, *J. Cardiovascular Electrophysiology* 5, 367-375, 1994; Ferrari, I *et al*, *J. Exp. Med.* 1995, in press; Kaplan, D *et al*, A combination of serological tests for diagnosis of severe Chagas cardiomyopathy, in preparation; Kaplan, D *et al*, Anti-P autoantibodies elicited by *T. cruzi* P antigens differ from those found in Lupus patients, in preparation).
- 6- The development of a diagnostic kit is currently under way.

PUBLICATIONS

- 1) Vazquez, MP, Schijman, AG, Panebra, A, Levin, MJ. Nucleotide sequence of a cDNA encoding another *Trypanosoma cruzi* acidic ribosomal P2 type protein (TcP2b). *Nucl. Acids Res.* **20**, 2893, 1992.
- 2) Vazquez, MP, Schijman, AG, Levin, MJ. Nucleotide sequence of a cDNA encoding a *Trypanosoma cruzi* acidic ribosomal P1 type protein. *Nucl. Acids Res.* **20**, 2599, 1992.
- 3) Schijman, AG, Levin, MJ. Nucleotide sequence of the cDNA encoding a *Trypanosoma cruzi* acidic ribosomal P0 protein: a novel C-terminal domain in *T. cruzi* ribosomal P proteins. *Nucl. Acids Res.* **20**, 2894, 1992.
- 4) Levin, MJ, Vazquez, M, Kaplan, D, Schijman, AG. The *Trypanosoma cruzi* ribosomal P protein family: Classification and antigenicity. *Parasitol. Today* **9**, 381-384, 1993.
- 5) Kaplan, D, Vazquez, M, Lafon, S, Schijman, AG, Levitus, G, Levin, MJ. The chronic presence of the parasite and anti-P autoimmunity in Chagas disease: The *Trypanosoma cruzi* ribosomal P proteins, and their recognition by the host immune system. *Biol. Res.* **26**, 273-275, 1993.
- 6) Levin, MJ, Kaplan, D, Ferrari, I, Arteman, P, Vazquez, M, Panebra, A. Humoral autoimmune response in Chagas disease: *Trypanosoma cruzi* ribosomal antigens as immunizing agents. *FEMS Microbiol. Medical Immunol.* **7**, 205-210, 1993.
- 7) Kaplan, D, Vazquez, M, Saunal, H, Muller, S, Levitus, G, Van Regenmortel, MHV, Levin, MJ. Differences in the immune response of patients with Chagas disease or systemic lupus erythematosus to human ribosomal P peptides. *Mem. Inst. Oswaldo Cruz* **88**, 51-52, 1993.
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- 10) Levin, MJ, Ferrari, I, Vazquez, M, Franco da Silveira, J, Cano, I, Degrave, W, Requena, JM, Alonso, C, Zingales, B, Gonzalez, A, Hernandez, R, Ramirez, JL, Aldao, RR, Saumier, M, Billault, A, Le Paslier, D, Cohen, D. Toward the physical map of the *Trypanosoma cruzi* nuclear genome. *Mem. Inst. Oswaldo Cruz* **89**, 17-18, 1994.
- 11) Cano, MI, Gruber, A, Vazquez, M, Cortes, A, Levin, MJ, Gonzalez, A, Degrave, W, Rondinelli, E, Zingales, B, Ramirez, JL, Alonso, C, Requena, JM, Franco da Silveira, J. Molecular karyotype of *Trypanosoma cruzi* CL-Brener reference clone of the *T. cruzi* genome project. *Mol. Biochem. Parasitol.* 1995, in press.

- 12) Ferrari, I, Levin, MJ, Wallukat, G, Elies, R, Lebesgue, D, Chiale, P, Elizari, M, Rosenbaum, M, Hoebeke, J. Molecular mimicry between the immunodominant ribosomal protein P0 of *T. cruzi* and a functional epitope on the human beta1-adrenergic receptor. *J. Exp. Med.* 1995, in press.
- 13) Schijman, AG, Vazquez, MP, Ben Dov, C, Ghio, S, Lorenzi, H, Levin, MJ. Cloning and sequence analysis of the ribosomal P2beta protein cDNA variants of *Trypanosoma cruzi*. *Gene*, submitted.
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- 15) Kaplan, D, Saunal, H, Lopez Bergami, P, Van Regenmortel, MHV, Levin, MJ. Anti-P autoantibodies elicited by *T. cruzi* P antigens differ from those found in Lupus patients. In preparation.

PhD THESIS

Patricia Levy-Yeyati: "Antigenos del estadio trypomastigote sanguíneo de la cepa RAI de *Trypanosoma cruzi* de la cepa Boitron de *Leishmania braziliensis braziliensis*: Respuesta humoral, anti-HSP70 en la enfermedad de Chagas y Leishmaniasis". School of Sciences, University of Buenos Aires, 1992.

Schijman, A.: "Familia de proteínas ribosomales P en *Trypanosoma cruzi*. Identificación de las proteínas TcP0, TcP2a y TcP2b. Clonado de sus ARNm y caracterización genómica parcial". School of Sciences, University of Buenos Aires, 1992.