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Major *Trypanosoma cruzi* Antigenic Determinant in Chagas' Heart Disease Shares Homology with the Systemic Lupus Erythematosus Ribosomal P Protein Epitope

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A *Trypanosoma cruzi* λgt11 cDNA clone, JL5, expressed a recombinant protein which was found to react predominantly with chronic Chagas' heart disease sera. The cloned 35-residue-long peptide was identified as the carboxyl-terminal portion of a *T. cruzi* ribosomal P protein. The JL5 13 carboxyl-terminal residues shared a high degree of homology with the systemic lupus erythematosus (SLE) ribosomal P protein epitope. Synthetic peptides comprising the 13 (R-13), 10 (R-10), and 7 (R-7) carboxyl-terminal residues of the JL5 protein were used to study, by enzyme-linked immunosorbent assay, the specificity of the Chagas' disease anti-JL5 and SLE anti-P antibodies. The R-13 peptide defined a linear antigenic determinant of the JL5 recombinant protein. As was proved for JL5, R-13 defined antibody specificities which were significantly increased in chronic Chagas' heart disease patients. Only SLE anti-P positive sera were found to react with JL5 and R-13. Fine epitope mapping showed that Chagas' disease anti-JL5 and SLE anti-P antibodies define similar epitopes within the R-13 peptide. The binding of the SLE sera to JL5 was completely blocked by the R-13 peptide, indicating that the shared specificity between anti-JL5 and anti-P autoantibodies was exclusively limited to the conserved linear epitope(s) within the R-13 peptide. The prevalence of high anti-R-13 antibody titers in Chagas' heart disease patients supports the hypothesis that postulates the existence of autoimmune disorders in Chagas' heart disease.

Chagas' disease, caused by the hemoflagellate *Trypanosoma cruzi*, is a widespread tropical disease affecting most Latin American countries. The acute period of the infection lasts 1 or 2 months and is usually symptomless. It can be estimated that more than 90% of the infected individuals enter the chronic period of infection and can develop the illness without warning (8).

Most of the chronically infected individuals never show discernible evidence of the disease, in spite of the fact that the infection persists throughout life. A variable percentage, up to 30%, with strong regional differences, develops symptoms of visceral damage. The ability to diagnose these symptoms early is lacking at present.

The most frequent clinical manifestation of the disease is the development of a panmyocarditis, which is anatomically expressed by myocardial damage associated to mononuclear inflammatory foci scattered throughout the heart (8). The coexistence of areas of myocytic degeneration, inflammatory infiltration, and fibrosis suggests a chronically evolving process. Since the intracellular forms of the parasite are rarely found in chagasic myocarditis, the hypothesis of an autoimmune process has been proposed (8, 16, 23, 27).

In terms of the human humoral response, several authors have described the existence of circulating antibodies against different self antigens (23, 26, 27, 29, 32). However, no link between these antibodies and chagasic pathology has been firmly established (17, 27).

In a recent attempt to identify parasite antigens defined by sera from subjects with chronic Chagas' heart disease (cChHD), a *T. cruzi* λgt11 recombinant antigen, JL5, was characterized that reacted predominantly with sera from this group of patients (20). The antigenic determinant of the JL5 recombinant was a small 35-amino-acid peptide. The nucleotide and the deduced amino acid sequence, together with other experimental data, allowed identification of this peptide as the carboxyl-terminal portion of a *T. cruzi* ribosomal P protein. The carboxyl-terminal undecapeptide in JL5, EDDDMGFGLFD, was highly homologous to the same region of the human P proteins, SD(E/D)DMGFGLFD (24). The latter sequence has been identified as the P protein epitope in systemic lupus erythematosus (SLE) (10, 11).

In this report, we have attempted to map the main antigenic region of the *T. cruzi* ribosomal P protein encoded by the JL5 recombinant and to compare it with the known SLE P protein autoepitope. Synthetic peptides comprising the 13, 10, and 7 carboxyl-terminal residues of JL5 protein were used to study, by enzyme-linked immunosorbent assay (ELISA), the specificity of the Chagas' disease anti-JL5 and SLE anti-P antibodies and to map the epitopes defined by them. In addition, the titer of circulating antibodies to the JL5 β-galactosidase (β-gal) fusion protein and to the synthetic peptides was measured both in cChHD sera and in sera from chronically infected subjects presenting no clinical evidence of heart damage. The results of this study were expected to provide information as to whether different clinical forms of Chagas' disease could be serologically differentiated and could give new insights on the origin of autoantibodies elicited during *T. cruzi* infection.

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MATERIALS AND METHODS

Hydrophilicity plot. Each residue of JL5 was assigned a numerical value related to the free energy of water-vapor transfer and the interior and exterior distribution of amino acid side chains (18); the average value of nine consecutive amino acids was calculated by using a HYDRPHIL program (31).

Human sera. Sera were obtained from chronically infected chagasic individuals from the same endemic region, located in the northwest of Argentina. These individuals were evaluated at the Servicio de Cardiología, Ramos Mejía Hospital (Buenos Aires, Argentina) by clinical examination, resting electrocardiography, ajmaline tests, and M- and B-mode echocardiography. Two groups, which are representative of distinct evolutive forms of chronic chagasic infection, were selected; one group was composed of 20- to 35-year-old patients with overt cChHD ($n = 15$), and the other group was composed of 41- to 68-year-old individuals chronically infected with *T. cruzi* without any clinical evidence of heart complaint (myocardial damage) (infected without heart complaint [IwHC]) ($n = 8$). The latter were representative of either a slowly evolving form of the disease or a chronic infection without apparent myocardial damage. The *T. cruzi* infection was assessed by complement fixation, passive hemagglutination, and immunofluorescence. All chagasic serum samples ($n = 23$) were negative for antinuclear antibodies when tested by indirect immunofluorescence at low serum dilutions (1:50 to 1:100). A group of healthy subjects ($n = 23$) and a group composed of patients with nonchagasic idiopathic dilated cardiomyopathy ($n = 7$) were included. SLE serum samples were from European patients ($n = 12$). All SLE sera were positive for antinuclear antibodies since they were detected by indirect immunofluorescence at a 1:1,000 serum dilution. Furthermore, all SLE sera showed anti-double-strand and anti-single-strand DNA antibody titers that exceeded 10 times the mean titer of normal sera; six of them presented anti-P protein antibodies, as determined by Western blot (immunoblot) analysis of ribosomal proteins (30).

Synthetic peptides. Peptides were prepared by the solid-phase method of Merrifield as described by Muller et al. (22), with a semiautomatic Multisynthesizer NPS 4000 (Neosystem, Strasbourg, France). Cleavage from the resin was done by the high hydrogen fluoride method. Crude peptides were purified by preparative medium-pressure liquid chromatography. The purified products were assessed by analytical high-pressure liquid chromatography, amino acid analysis (6 N HCl, 110°C, 20 h), and fast atom bombardment mass spectrometry measurements.

Purification of β-gal fusion proteins. *Escherichia coli* RY 1089 bacteria lysogenized with JL5 λgt11 phage or with nonrecombinant λgt11 were cultured and induced as described previously (14); the bacteria were pelleted and lysed, and β-gal fusion proteins were purified with a ProtoSorb *lacZ* column (Promega Biotec, Madison, Wis.) following the instructions of the manufacturer. The purity was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot.

Coupling the peptides to protein carriers. Peptides were coupled at a molar ratio of 1:50 to bovine thyroglobulin or bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) with 0.05% glutaraldehyde (Serva, Heidelberg, Federal Republic of Germany) as described previously (7).

Measurement of the antigenic reactivity of synthetic peptides and fusion proteins. ELISA was carried out as follows. Wells of polystyrene plates (CBA, Paris, France) were coated overnight at 4°C with 3 μM conjugated peptide in carbonate buffer (0.1 M; pH 9.6) or with 20 μg of protein per ml (*T. cruzi* total homogenate of β-gal fusion proteins) in phosphate-buffered saline (pH 7.4) (PBS). After five washes with PBS containing 0.1% Tween 20, the plates were incubated with the sera diluted in PBS–Tween 20–0.5% gelatin for 2 h at 37°C. After five washes in PBS-Tween 20, the plates were incubated for 2 h at 37°C with goat anti-human immunoglobulin conjugated with horseradish peroxidase (Biosys, Compiègne, France). After five washes with PBS-Tween 20, plates were developed by ortho-phenylenediamine (Sigma Chemical Co.) substrate. The A_{490} was read. For inhibition experiments, sera at a 1:1,000 dilution were first incubated for 2 h at 37°C with the indicated amount of bovine serum albumin-conjugated peptide or fusion protein. In these experiments, a 13-amino-acid peptide with the sequence AEAALVKMALMKV coupled to bovine serum albumin was employed as a negative control. In all cases, serial dilutions of sera from patients were made to ensure that, at the dilution tested, reactivity of the peptides or the proteins was on the linear portion of the titration curve. All of the optical density values represent binding of immunoglobulin to the peptide or recombinant protein after subtraction of binding of the same serum immunoglobulin to the carrier protein or to β-gal obtained from nonrecombinant λgt11. In each set of experiments, the same marker serum, H2, and a normal reference serum were always included as controls.

Statistical analysis. The mean titers from cChHD patients were compared with those from infected individuals without heart complaint by Student's *t* test. Correlation between anti-JL5 and anti-R13 antibody titers was performed by linear regression analysis. Values of $P < 0.05$ were considered significant.

RESULTS

The carboxyl-terminal portion of the *T. cruzi* ribosomal P protein encoded by the JL5 recombinant peptide is represented in Fig. 1. The hydrophilicity plot reveals an alanine-rich hydrophobic region, ranging from residues 1 to 21, and a hydrophilic carboxyl-terminal peptide, ranging from residue 22 to the final aspartic acid residue. The cloned *T. cruzi* ribosomal P protein sequence is compared with the corresponding sequences from *Saccharomyces cerevisiae* (15), *Artemia salina* eL12 and eL12′ (1, 2), rat (21), and human ribosomal P proteins P1, P2, and P0 (24) (Fig. 2). The *T. cruzi* 13-carboxyl-terminal-amino-acid sequence shares more than 90% homology with the human ones. To verify whether this conserved, hydrophilic portion of the parasite ribosomal P protein was involved in the immunological reaction with chagasic sera, a 13-amino-acid peptide was synthesized (R-13; Fig. 2B). This peptide was compared with the JL5 recombinant fusion protein for their ability to react with sera from clinically characterized *T. cruzi*-infected individuals. Twelve SLE serum samples (among them, six anti-P positive serum samples) were included in the assays to allow comparisons between P positive sera from parasite-infected and noninfected subjects.

As shown in Tables 1 and 2 and Fig. 3 both mean antibody titers against the R-13 peptide and the JL5 recombinant protein were significantly higher for patients with cChHD when compared with the mean titers measured for sera from IwHC individuals (Table 2). Furthermore, although two cChHD serum samples with high anti-JL5 antibody titers

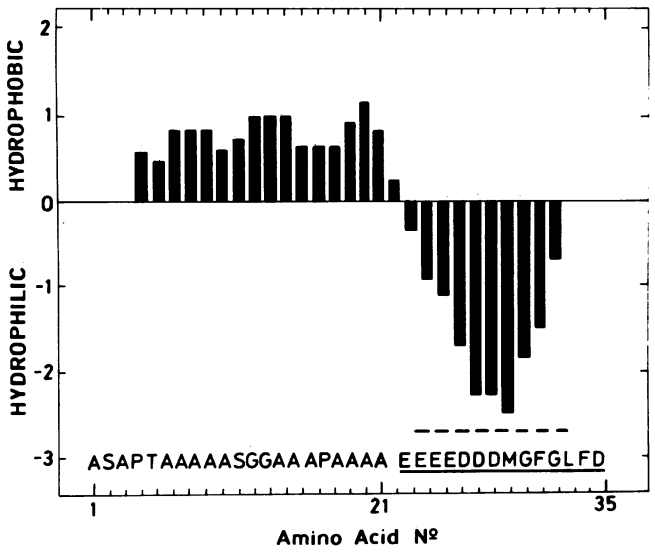

FIG. 1. Amino acid sequence and hydrophilicity plot of the carboxyl-terminal portion of the *T. cruzi* ribosomal P protein encoded by recombinant JL5, as described by Kyte and Doolittle (18). The calculation was done by using the HYDRPHIL program (31). The dashed line corresponds to the carboxyl-terminal hydrophilic region (underscored residues).

failed to react with the R-13 peptide, a significant correlation between anti-R-13 and anti-JL5 reactivities was found (r = 0.7; $P < 0.05$), indicating that the R-13 peptide defines a linear antigenic region of the JL5 recombinant protein involved in the immunoreactivity with cChHD sera. Sera from normal, noninfected individuals and from patients with nonchagasic idiopathic dilated cardiomyopathy failed to react with R-13 and JL5 (Table 1). Only the six anti-P positive SLE serum samples were found to react with R-13 and JL5, indicating that both *T. cruzi* peptides contained sequences recognized by anti-human P protein antibodies.

To determine the fine specificity of the anti-R-13 antibodies from cChHD sera and to compare it with that of SLE anti-P antibodies, the binding of the antibodies to the synthetic peptides R-7 and R-10 was analyzed by ELISA (Fig. 2B). Most cChHD sera presented a similar reactivity to the R-7 and the R-10 peptides (Table 1; Fig. 4). These reactivities were always lower than the anti-R-13 reactivities. The results suggested that the three glutamic residues were critical in determining the immunological reactivity of the R-13 epitope. The reactivity of 6 out of 13 cChHD serum samples (Table 1) with the R-10 and R-7 peptides (H1 and H4 in Fig. 4A) revealed the existence of another epitope located within the seven carboxyl-terminal residues.

Three out of six anti-P positive SLE serum samples showed similar antibody titers to the three terminal peptides (Table 1; Fig. 4, serum S1). The other three showed an anti-R-13, anti-R-10, and anti-R-7 profile reminiscent of that observed for cChHD sera (Table 1; Fig. 4C, serum S4). The specificity of the anti-R-13 reaction was verified by ELISA inhibition experiments in which R-7, R-10, and R-13 peptides were used to inhibit the binding to R-13. The inhibition experiments are in agreement with the direct binding assays (Fig. 5).

To define the specificity of cChHD and SLE antibodies to the cloned portion of the *T. cruzi* P protein, the inhibition of the ELISA reaction between recombinant JL5 and anti-P antibodies by the three carboxyl-terminal peptides was studied. Results in Fig. 6A and B showed that only a partial inhibition was obtained for cChHD sera. On the contrary, the two SLE serum samples studied were almost completely inhibited by the R-13 peptide (Fig. 6C and D), indicating that this anti-P protein reactivity was only directed to the conserved carboxyl-terminal epitope of *T. cruzi* JL5.

DISCUSSION

The ELISA measurements allowed us to quantify the immunological response of chagasic sera to the carboxyl-terminal portion of the *T. cruzi* ribosomal P protein encoded

A.

JL5	ASAPTAAAAASGGAAAPAAAA.....EEEEDDDMGFGLFD
YPA1	+G*S+**+P*+++*+**+G+D**....EEKEEEAK**S**********
ASRP12'	+GVGA*P**+G++**TE*P**KEEKKEEKKEES*****+*********
ASRP12	PT+GAP****G*+******E*KEAKKEEKKEES*****+*********
RLRP2	P++GAV*V+**+P*******++APAAAEE.KEES**KK*+********
P2	V+VSA+P+++*+P+*++AP***.EEKKDEKKEES**S**********
P1	*P*AG**P**+GP+P+TA**V*EEKKVEAKKEES**S**********
P0	V+*APV***T+++P**A**P*....KVEAKEES**S*+*********

B.

eL12/12'	*A. salina*	E E E D E D M G F G L F D
P1/P2	*H. sapiens*	E E S D D D M G F G L F D
P0	*H. sapiens*	E E S D E D M G F G L F D
R-13	*T. cruzi*	E E E D D D M G F G L F D
R-10	*T. cruzi*	D D D M G F G L F D
R-7	*T. cruzi*	M G F G L F D

FIG. 2. (A) Amino acid sequence comparisons of *T. cruzi* JL5 and the ribosomal P protein family. Gaps were introduced to allow comparison between homologous sequences (dots). YPA1, *S. cerevisiae* YPA1 ribosomal protein partial sequence from residue 69 to the carboxyl-terminal residue (15); ASR12, *A. salina* eL12 ribosomal protein sequence from residue 67 to the carboxyl-terminal residue (2); ASR12', *A. salina* eL12' ribosomal protein partial sequence from residue 64 to the carboxyl-terminal residue (1); RLRP2, rat liver ribosomal P2 protein partial sequence from residue 68 to the carboxyl-terminal residue (21); P2, human P2 protein sequence from residue 78 to the carboxyl-terminal residue (24); P1, human P1 protein sequence from residue 74 to the carboxyl-terminal residue (24); P0, human P0 protein sequence from residue 275 to the carboxyl-terminal residue (24). Sequence homology with respect to JL5 is indicated with asterisks and plus signs indicate conservative amino acid changes, so that T and S, D and E, A and S, and A and G are considered to be homologous pairs. (B) Amino acid sequence comparison of carboxyl-terminal synthetic peptides of *T. cruzi* JL5 and the human (*H. sapiens*) and *A. salina* 13 terminal residues; underscored residues define the reported SLE ribosomal P epitope (10).

TABLE 1. Binding in ELISA of serum antibodies to synthetic peptides R-13, R-10, and R-7, JL5 β-gal fusion protein, and *T. cruzi* extracts

Sera[a]	Binding[b] of antibodies to:				
	JL5	R-13	R-10	R-7	*T. cruzi*
NI (mean)	22	10	7	9	27
NIDC (mean)	57	30	28	25	69
cChHD					
H1	752	357	144 (40)	120 (36)	412
H2	930	256	67 (27)	70 (28)	485
H3	1,047	461	64 (14)	60 (13)	380
H4	604	307	205 (70)	169 (56)	372
H5	702	247	25 (10)	18 (7)	321
H6	498	237	25 (11)	24 (10)	357
H7	789	351	ND (ND)	ND (ND)	388
H8	746	408	189 (46)	112 (25)	361
H9	531	192	21 (11)	18 (9)	345
H10	786	95	70 (50)	66 (46)	369
H11	1,080	45	27 (40)	31 (46)	417
H12	1,049	204	55 (27)	50 (25)	421
H13	330	240	118 (49)	108 (47)	270
H14	1,683	526	93 (17)	94 (17)	403
H15	1,394	540	ND (ND)	ND (ND)	342
IwHC					
I1	294	63	48 (77)	54 (86)	344
I2	171	112	55 (50)	46 (40)	180
I3	137	36	27 (77)	21 (60)	173
I4	215	109	48 (45)	43 (40)	208
I5	88	73	21 (28)	ND (ND)	220
I6	265	91	73 (80)	75 (83)	180
I7	449	180	13 (8)	15 (9)	345
I8	686	405	109 (27)	84 (20)	390
SLE					
S1	650	600	570 (95)	588 (98)	62
S2	300	160	135 (85)	115 (85)	75
S3	220	105	108 (91)	63 (60)	70
S4	550	260	163 (63)	156 (60)	35
S5	417	130	91 (70)	84 (67)	100
S6	370	180	135 (77)	90 (50)	68

[a] All serum samples were diluted 1:500. NI, Normal individuals (n = 23); NIDC, patients with non-chagasic idiopathic dilated cardiomyopathy (n = 7); SLE, Anti-P positive SLE sera.

[b] Expressed as optical density. Values in parentheses indicate percentages which represent (binding to peptide/binding to R-13) × 100. ND, Not determined.

by the JL5 recombinant. All *T. cruzi*-infected individuals had a positive anti-JL5 antibody titer, exceeding several times the absorbance values observed for normal controls. A significant correlation between high anti-JL5 antibody titers and cChHD was found, confirming previous results (20).

The linear antigenic determinant of JL5 was predicted to lie in the carboxyl-terminal end, as was the case for the SLE

TABLE 2. Levels of antibodies against JL5 and R-13 in serum from patients with Chagas' disease

Antigen	A_{490}[a]		P
	cChHD (n = 15)	IwHC (n = 8)	
JL5	861.4 ± 340.0	288.4 ± 182.6	<0.01
R-13	297.7 ± 149.6	133.6 ± 110.0	<0.05

[a] Mean ± standard deviation of A_{490} (10^3) after subtraction of binding to nonrecombinant λgt11 (JL5) or bovine thyroglobulin (R-13).

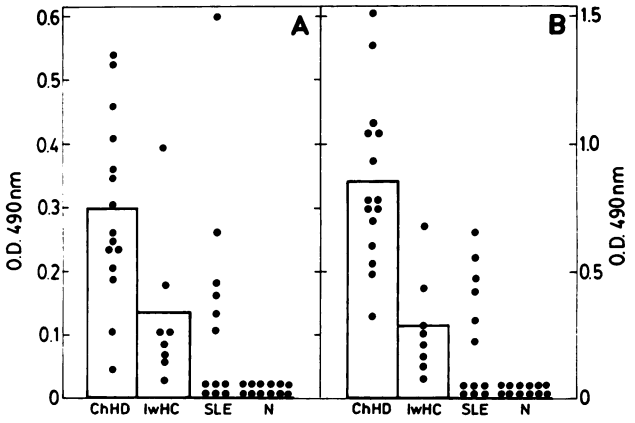

FIG. 3. Binding in ELISA of antibodies to R-13 (A) and JL5 β-gal fusion protein (B). Serum samples are from cChHD patients (n = 15), IwHC patients (n = 8), SLE patients (n = 13), and normal, noninfected individuals (N) (n = 23). These serum samples were all diluted 1:500. The optical density reading at 490 nm (O.D. 490 nm) represents binding to the peptide after subtraction of binding to peptide-free thyroglobulin. Bars represent mean values.

ribosomal P protein autoepitope (10). The pattern of binding of chagasic sera to R-13 and to the JL5 recombinant protein demonstrated that the R-13 peptide constituted a linear epitope of the carboxyl-terminal portion of the *T. cruzi* P protein. As previously stated for JL5, R-13 defined antibody specificities which were significantly increased in cChHD patients. However, the number of clinically characterized subjects studied should be increased to determine the diagnostic and prognostic value of anti-R-13 antibodies. The ELISA used herein represents a considerable advance in this direction, since it simplifies the procedure and renders it suitable for routine analysis.

cChHD serum epitope mapping with R-13, R-10, and R-7 suggested the existence of at least two epitopes, one contained within the seven carboxyl-terminal residues and the other one present in R-13 but not in R-10. These two epitopes were also mapped by SLE anti-P antibodies.

The ability of the R-13 peptide to completely inhibit the reactivity of SLE anti-P antibodies to the JL5 recombinant protein proved that the specificity shared between SLE anti-P antibodies and cChHD anti-JL5 antibodies was con-

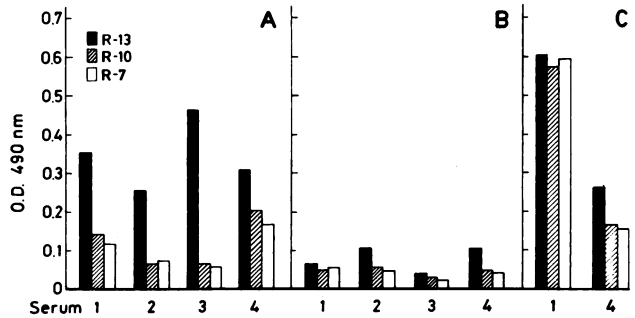

FIG. 4. Pattern of binding in ELISA of antibodies to carboxyl-terminal synthetic peptides R-13, R-10, and R-7. (A) Sera from cChHD patients (H1, H2, H3, and H4); (B) sera from IwHC patients (I1, I2, I3, and I4); (C) sera from SLE patients (S1 and S4). These sera were diluted 1:500. The optical density reading at 490 nm (O.D. 490 nm) represents binding to the peptide after subtraction of binding to peptide-free thyroglobulin.

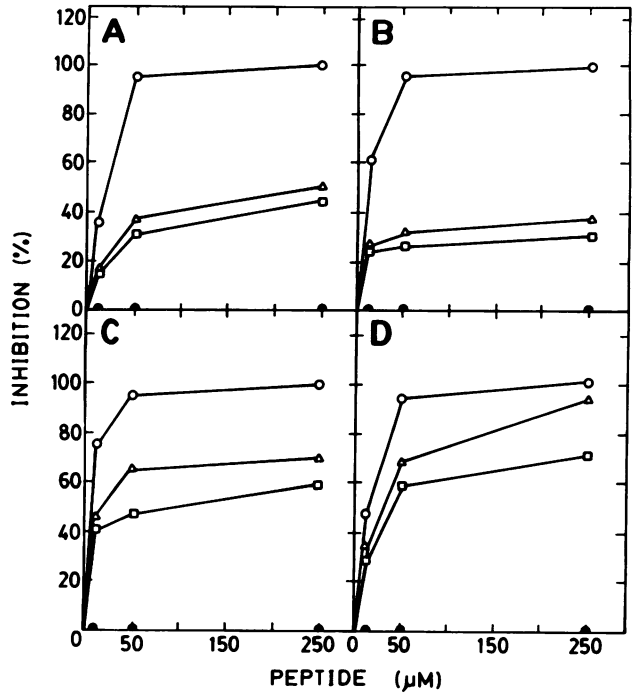

FIG. 5. Inhibition of the ELISA reaction between anti-P antibodies and R-13 by the synthetic carboxyl-terminal peptides. (A and B) cChHD sera H1 and H2, respectively; (C and D) SLE sera S4 and S1, respectively. Serum samples diluted 1:1,000 were incubated with increasing concentrations of conjugated peptide R-13 (○), R-10 (△), R-7 (□), or AEAELVKMALMKV (●) and were assayed for binding to R-13.

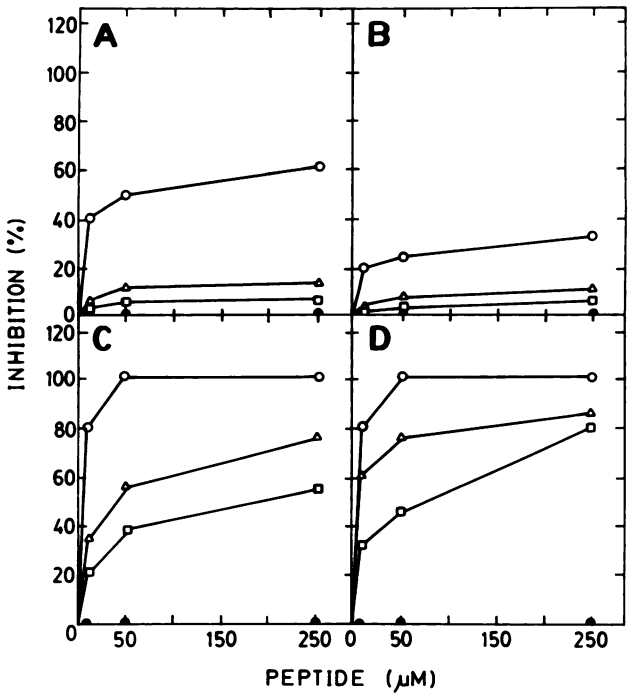

FIG. 6. Inhibition of the ELISA reaction between anti-P antibodies and the JL5 recombinant by synthetic carboxyl-terminal peptides. (A and B) cChHD sera H1 and H4, respectively; (C and D) SLE sera S4 and S1, respectively. Serum samples were diluted 1:1,000, incubated with increasing concentrations of conjugated peptide R-13 (○), R-10 (△), R-7 (□), or AEAELVKMALMKV (●), and assayed for binding to JL5 fusion protein.

tained in the R-13 peptide. Its amino acid sequence defined the Chagas and SLE anti-P antibody specificity.

In addition to the identification of this shared sequential determinant, the fact that synthetic peptides did not completely inhibit the reaction between the JL5 and cChHD sera revealed the existence of either conformational or sequential epitopes in the JL5 recombinant protein that are specific for the *T. cruzi* infection (Fig. 6A and B). These observations suggested that only a variable percentage of the total cChHD anti-JL5 reactivities (from 20 to 40%, depending on the serum sample) would be able to cross-react with the ribosomal P proteins of the host. As predicted and in accordance with the statements above, when anti-JL5 antibodies, immunoselected from different cChHD sera, were tested for antihuman ribosome reactivity by immunoblotting, the intensity of the reaction with the three ribosomal P proteins (P0, P1, and P2) varied depending on each serum anti-R13 antibody titer (G. Levitus, M. Hontebeyrie-Joskowicz, and M. J. Levin, submitted for publication).

Anti-P antibodies in Chagas' disease may be induced by the presence of cross-reactive epitopes in the parasite (molecular mimicry). However, it is intriguing that in other chronic parasitic infections such as malaria, anti-P antibodies could not be detected (6), although *Plasmodium falciparum* has been shown to possess ribosomal P proteins that react with SLE anti-P antibodies (12). While our findings suggest the possible role of molecular mimicry in the induction of anti-P antibodies, other autoimmune mechanisms known to occur during chronic *T. cruzi* infection, such as polyclonal B- and T-cell activation (9, 16, 23) and development of cell-mediated immunity against host antigens (13, 16, 19, 23), may operate alone or in association and may contribute to generate the chagasic anti-P response.

As described for autoimmune diseases, various parasitic and bacterial infections are associated with the induction of autoantibodies to intracellular antigenic determinants (3, 6, 28). Antinuclear antibodies, for example, a hallmark of SLE, have been reported to be present in malaria and leprosy (6). On the contrary, they were not detected in the chagasic sera used in this study (see Materials and Methods). Nevertheless, our results show that autoantibodies with a similar specificity to those found in SLE do indeed exist in Chagas' disease. The anti-P antibody specificity is the first autoantibody common to SLE and Chagas' disease that has been characterized. Anti-P antibodies have been particularly associated with lupus psychosis (5) and were also found in sera from SLE patients with cardiovascular and muscular involvement (4). It is thus remarkable that, for *T. cruzi*-infected subjects, the highest anti-P antibody titers correspond to cChHD patients.

So far there is no evidence about the role, if any, of anti-P antibodies in the pathology of Chagas' disease. Of interest, however, is the observation that immunization of mice with ribosomal *T. cruzi* antigens induced an intense myocarditis compatible with an anti-self, organ-specific, immunologic response (25).

The results herein support the hypothesis that postulates the existence of autoimmune disorders in cChHD. We further suggest that the study of anti-P antibody induction during *T. cruzi* infection may provide some clues as to the origin of anti-P antibodies in SLE.

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LITERATURE CITED

1. **Amons, R., W. Pluijns, J. Kriek, and W. Möller.** 1982. The primary structure of protein eL12′/eL12′-P from the large subunit of *Artemia salina* ribosomes. FEBS Lett. **146**:143–147.
2. **Amons, R., W. Pluijns, and W. Moller.** 1979. The primary structure of ribosomal protein eL12/eL12-P from *Artemia salina* 80S ribosomes. FEBS Lett. **104**:85–89.
3. **Argov, S., C. L. Jaffe, M. Krupp, H. Slor, and Y. Shoenfeld.** 1989. Autoantibody production by patients infected with *Leishmania*. Clin. Exp. Immunol. **76**:190–197.
4. **Bonfa, E., and K. B. Elkon.** 1986. Clinical and serological associations of the antiribosomal P protein antibody. Arthritis Rheum. **29**:981–985.
5. **Bonfa, E., S. J. Golombek, L. D. Kaufman, S. Skelly, H. Weissbach, N. Brot, and K. B. Elkon.** 1987. Association between lupus psychosis and anti-ribosomal P protein antibodies. N. Engl. J. Med. **617**:265–271.
6. **Bonfa, E., R. Llovet, M. Scheinberg, J. M. De Souza, and K. B. Elkon.** 1987. Comparison between autoantibodies in malaria and leprosy with lupus. Clin. Exp. Immunol. **70**:529–537.
7. **Briand, J. P., S. Muller, and M. H. V. Van Regenmortel.** 1985. Synthetic peptides as antigens: pitfalls of conjugation methods. J. Immunol. Methods **78**:59–69.
8. **Chiale, P., and M. B. Rosembaum.** 1989. Clinical and pharmacological characterization and treatment of potentially malignant arrythmias of chronic chagasic cardiomyopathy, p. 601–620. *In* E. M. Vaughan Williams and T. J. Campbell (ed.), Handbook of experimental pharmacology. Springer-Verlag KG, Berlin.
9. **D'Imperio Lima, M. R., H. Eisen, P. Minoprio, M. Joskowicz, and A. Coutinho.** 1986. Persistence of polyclonal B cell activation with undetectable parasitemia in late stages of experimental Chagas disease. J. Immunol. **137**:353–356.
10. **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.
11. **Elkon, K. B., S. Skelly, A. P. 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.
12. **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.
13. **Hontebeyrie-Joskowicz, M., G. Said, G. Milon, G. Marchal, and H. Eisen.** 1987. L3T4+ T cells able to mediate parasite-specific delayed type hypersensivity play a role in the pathology of experimental Chagas disease. Eur. J. Immunol. **17**:1027–1033.
14. **Huynh, T. V., R. A. Young, and R. W. Davis.** 1985. Constructing and screening cDNA libraries in gt10 and λgt11, p. 49–78. *In* D. M. Glover (ed.), DNA cloning techniques: a practical approach, vol. 2. IRL Press, Oxford.
15. **Itoh, T.** 1981. Primary structure of an acidic ribosomal protein YPA1 from *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **671**:16–24.
16. **Joskowicz, M.** 1985. Maladie de Chagas et autoimmunité. Med. Sci. **1**:412–418.
17. **Kierzembaum, F.** 1986. Autoimmunity in Chagas' disease. J. Parasitol. **72**:201–211.
18. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
19. **Laguens, R. P., P. Cabeza Meckert, and J. G. Chambó.** 1988. Antiheart antibody-dependent cytotoxicity in the sera of mice chronically infected with *Trypanosoma cruzi*. Infect. Immun. **56**:993–997.
20. **Levin, M. J., E. A. Mesri, R. Benarous, G. Levitus, A. Schijman, P. Levy-Yeyati, P. Chiale, A. Ruiz, A. Kahn, M. B. Rosembaum, H. N. Torres, and E. L. Segura.** 1989. Identification of major *Trypanosoma cruzi* antigenic determinants in chronic Chagas heart disease. Am. J. Trop. Med. Hyg. **41**:530–539.
21. **Lin, A., B. Wittman-Liebold, J. McNally, and I. G. Wool.** 1982. The primary structure of the acidic phosphoprotein P2 from rat liver 60 S ribosomal subunits. J. Biol. Chem. **257**:9189–9197.
22. **Muller, S., M. Couppez, J. P. Briand, J. Gordon, P. Sautiére, and M. H. V. Van Regenmortel.** 1985. Antigenic structure of histone H2B. Biochim. Biophys. Acta **827**:235–246.
23. **Petry, K., and H. Eisen.** 1989. Chagas disease: a model for the study of autoimmune diseases. Parasitol. Today **5**:111–116.
24. **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.
25. **Ruiz, A., M. Esteva, P. Cabeza Meckert, R. P. Laguens, and E. L. Segura.** 1985. Protective immunity and pathology induced by inoculation of mice with different subcellular fractions of *Trypanosoma cruzi*. Acta Trop. **42**:299–309.
26. **Sadigursky, M., A. M. Acosta, and C. A. Santos Buch.** 1982. Muscle sarcoplasmic reticulum antigen shared by a *Trypanosoma cruzi* clone. Am. J. Trop. Med. Hyg. **31**:934–941.
27. **Schmuñis, G.** 1987. Autoimmunity in Chagas' disease. Mem. Inst. Oswaldo Cruz Rio J. **82**(Suppl. I):287–310.
28. **Shoenfeld, Y., and D. A. Isenberg.** 1988. Mycobacteria and autoimmunity. Immunol. Today **9**:178–182.
29. **Szarfman, A., V. P. Terranova, S. L. Renard, J. M. Foidart, F. M. Lima, J. I. Scheinman, and G. R. Martin.** 1982. Antibodies to laminin in Chagas' disease. J. Exp. Med. **155**:1161–1171.
30. **Towbin, H., H.-P. Ramjoué, H. Kuster, D. Liverani, and J. Gordon.** 1982. Monoclonal antibodies against eucaryotic ribosomes. J. Biol. Chem. **257**:12709–12715.
31. **Van Regenmortel, M. H. V., and G. Daney de Marcillac.** 1988. An assessment of prediction methods for locating continuous epitopes in proteins. Immunol. Lett. **17**:95–108.
32. **Van Vooris, W. C., and H. Eisen.** 1989. A surface antigen of *Trypanosoma cruzi* that mimics mammalian nervous tissue. J. Exp. Med. **169**:641–652.