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AUTOANTIBODIES IN CHAGAS' HEART DISEASE: POSSIBLE MARKERS OF SEVERE CHAGAS' HEART COMPLAINT

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The most severe manifestation of chronic Chagas' heart disease (cChHd) is the development of a panmyocarditis, anatomically expressed by myocardial tissue damage without detection of intracellular parasite forms (Rosenbaum, 1964). Accordingly, the hypothesis of an autoimmune process has been proposed to explain the pathogenesis of the disease (Rosenbaum, 1964; Schmuñis, 1987). Patients sera have been characterized by positive anti-*Trypanosoma cruzi* antibody titers, accompanied, in some cases, by detectable levels of anti-self antibodies (Sadigursky et al., 1982; Schmuñis, 1987; Petry & Eisen 1989). However, neither a clear correlation between the clinical status of the patients and the presence of circulating autoantibodies, nor the nature of the autoantibody triggering stimuli have been established.

We attempted to identify parasite antigens defined by sera from chagasic subjects with overt Chagas' heart disease (Levin et al., 1989). A λgt11 cloning strategy was designed that allowed the identification of two types of *T. cruzi* recombinant antigens: those reacting with sera from chagasic individuals with different clinical forms of the disease, recombinants JL7 (Levin et al., 1989) and JL8 (Levin et al., 1989), and those reacting predominantly with sera from Chagas' heart disease patients, such as recombinant JL5 (Levin et al., 1989), JL9 (Levin et al., 1989) and T. 31, a recombinant encoding the C-terminal region of the *T. cruzi* HSP70 (Levy-Yeyati et al., 1990).

JL7 – Our findings demonstrated that JL7 encoded a repetitive 68 amino acid sequence similar to the one present in the *T. cruzi* Miranda 76 antigen 1 (Ibañez et al., 1988), to the FRA antigen (Lafaille et al., 1989) and to the H-49 recombinant peptide (Cotrim et al., 1990). JL7 reacted with 68 out of 70 chronic chagasic sera from Argentina, tested in our laboratory.

Moreover, when this antigen was tested using a phage dot array immunoassay in the WHO/TDR Multicenter study, JL7 proved to be an excellent diagnostic antigen since it showed a Kappa index greater than 0.80. In another Multicenter study, the analysis of 226 sera (167 chagasic sera) showed that this recombinant maintained an excellent Kappa index (Ensayo de Recombinantes con Fines Diagnósticos, Red Iberoamericana de Laboratorios, INGEBI, Buenos Aires, September 1990; Análisis de Datos, EPM, São Paulo, 11-12 October 1990).

JL8 – This recombinant presented 7 repetitions of a 14 amino acid motif, similar to those present in the *T. cruzi* Miranda 76 antigen 30 (Ibañez et al., 1988), and in the CRA antigen (Lafaille et al., 1989). However, analysis of the repetitions showed the existence of punctual amino acid changes in relation to the other reported sequences, fact that may explain the low reactivity of JL8. It reacted with only 60% of the 70 Argentinian chagasic sera analyzed in our laboratory and did not even rank in the WHO/TDR Multicenter study.

Of great interest was the observation that this repetition shared more than 60% homology with a *Plasmodium falciparum* S antigen (Mesri, 1990). The variable region of the S antigen from *P. falciparum* NF-7 strain presents a repetitive 8 amino acid motif (Cowman et al., 1985). When an 8 amino acid dimer (16 amino acids) is compared with the *T. cruzi* 14 amino

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acid motif, a high degree of homology is evident:

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1: A E A T K V A E A E K Q K A
     * * *   *     * * *     * +

2: E A E A L K S D E A E A R K S D
   1           8              16
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1: JL8 (14 aa); 2: NF7-S (8 aa dimer)

Thus, JL8 might show reactivity with sera from malaria patients (Cowman et al., 1985; Mesri, 1990).

JL5 and R-13 – The JL5 recombinant protein reacted with the sera from patients with overt Chagas' heart disease, but reacted weakly or not at all, with the sera from infected adults without evidence of myocardial involvement (Levin et al., 1989). The cloned peptide was identified as the C-terminal portion of a *T. cruzi* ribosomal P protein: ASAPTAAAAASG GAAAPAAAAEEEEDDDMGFGLFD (Levin et al., 1989). Experimental evidences indicated that JL5 could bare epitopes crossreactive with the host ribosomal P proteins: 1) Sequence analysis and comparison revealed that the JL5 cDNA derived amino acid sequence was homologous to the C-terminal portion of the human ribosomal P proteins; notably, the C-terminal undecapeptide in JL5 was >90% homologous to the sequence that defines the ribosomal P protein epitope in systemic lupus erythematosus (SLE): SDDMGFGLFD (Elkon et al., 1988); 2) Phage-dot array immunoassays showed that sera from cChHD and SLE patients with positive anti-P reactivity reacted both with the JL5 recombinant (Levin et al., 1989) and with a JL5-derived recombinant containing the 15 C-terminal residues of JL5 (A1 recombinant, Levin et al., 1990); 3) Simultaneously, ELISA measurements with a synthetic peptide representing the 13 C-terminal residues of the JL5 recombinant (R-13) defined the specificity shared between cChHD anti-JL5 antibodies and SLE anti-P antibodies (Mesri et al., 1990).

Experiments were designed to confirm the autoimmune nature of the antibodies directed against the C-terminal portion of the parasite ribosomal P protein. Sera from cChHD patients reacted with several *T. cruzi* ribosomal antigens. Notably, anti-JL5 antibodies immunoselected from cChHD sera and an anti-P positive SLE serum defined the same group of parasite ribosomal proteins, and revealed that the P protein family in *T. cruzi* is composed by three low molecular weight components of 19 kDa, a doublet of 15 and 14 kDa, and a 38 kDa polypeptide (Levitus, G; Hontebeyrie-Joskowicz M. and Levin, M. J. submitted for publication).

Further immunoblotting analysis showed that anti-JL5 immunoselected antibodies reacted with HeLa ribosomal P proteins. This reaction was blocked when the anti-JL5 antibody preparation was preincubated with the A1 recombinant, a JL5-derived recombinant encoding the 15 C-terminal amino acids of JL5 (A1:AEEEEDDDMGFGLFD) (Levitus, G; Hontebeyrie-Joskowicz, M. and Levin M. J. submitted for publication).

Moreover, the anti-JL5 antibodies reacted with purified fusion proteins from bacteria lysogenized by λgt11 recombinants coding for the human ribosomal P1 and P2 proteins. These, together with other results and sequence comparisons, showed that the ribosomal P epitope shared by parasite and host was limited to the highly conserved 13 C-terminal residues, EEEDDDMGFGLFD (Rich & Steiz, 1987; Mesri et al., 1990; Schijman et al., 1990).

A synthetic peptide comprising the 13 C-terminal residues of the *T. cruzi* ribosomal P protein was used as antigen in an ELISA performed to assess the prevalence of the anti-R-13 autoantibody response in sera from 110 patients with various protozoan parasitosis, 50 SLE sera and 35 normal control sera. Positive anti-R-13 antibody levels were detected in 57% of the 44 cChHD patients studied. In this group, the anti-R-13 values were compared with the corresponding anti-*T. cruzi* titers measured by ELISA. A weak but significant correlation ($r = 0.6$; $P < 0.001$) was observed between the two assays. Eight percent of the sera from patients with leishmaniasis (24 sera obtained in Africa), and 20% of the sera from patients with African trypanosomiasis (20 sera) were positive. In contrast, all malaria serum samples were negative (20 sera obtained in Africa). The frequency of anti-R-13 positives among the SLE sera was 12% confirming previous reports on the frequency of anti-P antibodies in SLE (Elkon et al., 1988) (Levitus, G.; Hontebeyrie-Joskowicz, M. and Levin, M. J. submitted for publication).

The IgG subclasses displayed by anti-R-13

and anti-*T. cruzi* antibodies of 25 anti-R-13 positive chagasic sera were examined.

For 21 sera (84%) tested, the IgG1 and IgG3 isotypes accounted for the total anti-parasite activity, whereas for the remaining 4 (16%), the anti-*T. cruzi* reactivity was mainly IgG1. The IgG1 isotype accounted for the anti-R-13 activity of 23 sera (92%). Only 2 out of 25 individuals showed a mixed IgG1 and IgG3 anti-R-13 isotype distribution. The anti-R-13 response of SLE anti-P positive sera was equally represented by the IgG1 and IgG2 subclasses (Bonfa et al., 1988).

This IgG1 restricted anti-R-13 isotype profile indicated that the regulatory mechanisms involved in the Chagas' anti-R-13 and in the SLE anti-P autoantibody response are different, and suggests that the anti-R-13 response may be a component of the humoral anti-*T. cruzi* response. (Levitus, G.; Hontebeyrie-Joskowicz, M. and Levin M. J. submitted for publication).

The correlation of anti-R-13 antibody levels with different clinical forms of chronic Chagas' cardiomyopathy was studied in 29 patients. Patients were classified according to their clinical symptoms in three groups. Group 1: severe chronic chagasic cardiomyopathy (N = 14); Group 2: mild or moderate chronic chagasic cardiomyopathy (N = 6); and Group 3: preclinic chronic chagasic cardiomyopathy (N = 9).

The statistical analysis of the anti-R-13 ELISA measurements showed that the anti-R-13 antibody levels were significantly higher in sera from patients from Group 1 compared to the anti-R-13 antibody levels in sera from patients of Groups 2 and 3 taken together. Moreover, the levels of anti-R-13 antibodies in sera from chronic chagasic patients that presented histologic evidences of active myocardytis were significantly higher than those measured in sera from patients without myocardytis.

In view of the fact that high anti-R-13 antibody titers were only detected in chagasic patients with severe heart complaint, it may be relevant to point out that autoantibodies to the Ro antigen, markers of congenital heart block in SLE, presented an IgG1 restricted isotype profile (Christian & Elkon, 1986; Bonfa et al., 1988), as well as the anti-Jo-1 autoantibodies characteristic of patients with idiopathic inflammatory myopathy (Miller et al., 1990).

The C-terminal regions of the *T. cruzi* ribosomal P proteins are more than 90% homologous to the corresponding host cell proteins; therefore their immune recognition during *T. cruzi* infection implies a rupture of the host "self-nonself" discrimination system. This effect might be provoked by recurrent parasite lysis and the consequent release of parasite ribosomal antigens, being this permanent stimulation supported by exposure of the host ribosomes after tissue damage.

JL9 – This recombinant presented more than five repetitions of a 38 amino acid motif:

SAYRKALPQEEEEDVGPRH

VDPDHFRSTTQDAYRPVDP

This repetition showed certain homology to the microtubule associated protein MAP1B from mouse brain (Noble et al., 1989). In fact, the region comprised between residues 20, 21 and 22 (VDP) and residues 36, 37 and 38 (VDP) is homologous to the imperfect repeats in the C-terminal sequence of MAP1B (Noble et al., 1989).

D H F R S T T Q D A Y R P

MAP1B						S		R		R S		E	E	
Imperfect repeats		Y	S	Y	E	T		R		T T		P		
Consensus sequence						T		K		K T		D	D	

Interestingly, antibodies to this repetition were only detected in sera from cChHD patients. Furthermore, anti-JL9 antibodies were detected in *T. cruzi* infected mice (Kerner, N.; Levin, M. J. & Hontebeyrie-Joskowicz, M. submitted for publication). In a series of elegant experiments the crossreactivity of this repetition with self proteins has been demonstrated (Kerner et al., 1990; Kerner, N., Levin, M. J. and Hontebeyrie-Joskowicz, M. submitted for publication). The hydrophilicity pattern of the repetition predicts that the antigenic portion lies between residues 9 and 14, an E-rich region; several such E-rich regions are also found in MAP1B (Noble et al., 1989).

HSP70 – The C-terminal region of the *T.*

cruzi HSP70 (RA strain) has been cloned (Levy-Yeyati et al., 1990). Low or moderate anti-HSP70 antibody titers were measured by ELISA using the cloned HSP70 peptide as antigen in sera from cChHD patients. In these assays, anti-JL5 and anti-JL7 antibody levels were also measured. Interestingly, anti-JL7 and anti-JL5 antibody levels were always higher than those of HSP70. The cloned peptide contains a HSP70 amino acid sequence that has been found to be crossreactive with the mammalian HSP70 (Mattei et al., 1989), namely:

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1: A N G I L N V S A E E K G T G K
     * * * * * * * * *  + *  * * *

2: A N G I L N V S A V D K S T G K
     * * * * * * * * + *      * * *

3: A N G I L N V T A T K D S T G K
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1: *T. cruzi* HSP70; 2: HSC70 (Dworniczak & Mirault, 1987); 3: HSP70 (Hunt & Morimoto, 1985)

Based on sequence comparisons it may be suggested that during chronic *T. cruzi* infection, HSP70 regions shared by parasite and host could induce autoreactive antibodies.

This report indicates that antibodies against intracellular parasite proteins are present in sera from cChHD patients. Particularly, antibodies to the *T. cruzi* ribosomal P proteins (JL5, R-13) and to the *T. cruzi* MAP1B-like protein (JL9) have been found to crossreact with their host counterparts and are, hence, true autoantibodies.

Up-to-date, only anti-JL5 and fundamentally anti-R-13 antibody levels have been found to correlate significantly with the presence of severe chronic Chagas' heart complaint.

Finally, the data concerning the anti-ribosomal P protein response constitute a firm evidence of molecular mimicry involvement in the generation of autoantibodies during Chagas' disease. This was substantiated by the following facts: a) cChHD anti-JL5 antibodies react with the parasite and host ribosomal P proteins; b) the epitopes by them defined have been characterized in both, parasite and host, and share more than 90% homology; c) the significant correlation between anti-R-13 antibody levels and anti-*T. cruzi* antibody titers, as well as the prevalence of the IgG1 isotype in the IgG subclass distribution of the anti-R-13 and anti-*T. cruzi* antibody response; and d) the Chagas specificity of the anti-JL5 and anti-R-13 response.

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