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NOTES

Assay for Detection of *Trypanosoma cruzi* Antibodies in Human Sera Based on Reaction with Synthetic Peptides

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Synthetic peptides modelled according to the amino acid sequences derived from the repeated domains of five *Trypanosoma cruzi* antigens were used in an immunoradiometric assay to detect antibodies appearing after natural human infections. An enzyme-linked immunosorbent assay and an indirect immunofluorescence assay performed with a complex antigenic mixture from parasites were used as controls. The results indicate that the synthetic peptides were recognized by a large proportion of serum samples collected from 34 patients with Chagas' disease in Chile and point to their possible use in diagnosis.

Chagas' disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a chronic illness affecting about 24 million people in Central and South America. In most cases, after an asymptomatic acute phase with parasitemia, parasite growth is controlled by the host immune response. The infection remains quiescent for many years before entering into a chronic phase during which parasites are hardly detectable in the blood of patients (3, 9). Consequently, detection of specific antibodies in the patients' serum is important for diagnosis of the disease (10). Selection of specific antigens is vital to achieve an accurate diagnosis. However, most if not all of the currently available antigen preparations for serology represent a highly complex mixture of molecules extracted from whole parasites or parasite fractions (3). Thus, there is an evident need for better-characterized reagents.

Recombinant DNA technology has been used recently to overcome the problem of preparing purified parasite antigens (6, 7). A collection of recombinant DNA clones expressing parasite antigens has been isolated from a genomic *T. cruzi* library with the aid of a chronic chagasic serum (6). Some of the isolated antigens (clones 1, 2, 13, 30, and 36) reacted with a large fraction of serum samples from chronic chagasic patients (5, 7). One of the recombinant antigens, the shed acute-phase antigen (SAPA), detected antibodies in a large proportion of serum samples from patients in the acute phase of the infection (1, 12). The primary structure, deduced from the nucleotide sequence of the cloned DNA fragments encoding the parasite antigens, revealed that *T. cruzi*, like plasmodia (8), possesses antigens made up of arrays of tandemly repeated amino acid sequences (7). In this report, we show that synthetic peptides whose sequences correspond to these repeated domains of *T. cruzi* antigens are indeed recognized by a large proportion of serum samples collected from patients in Chile with chronic Chagas' disease.

Thirty-four serum samples were collected in Santiago, Chile, from individuals attending a health clinic. The age of the patients ranged from 8 to 64 years. Five patients had cardiac disease, 10 had gastrointestinal lesions, 12 had the combined syndrome, and 17 were in the indeterminate phase of Chagas' disease. Serum samples from five healthy donors ranging from 17 to 37 years of age were obtained from Hospital San Juan de Dios blood bank. Five different peptides representing the repeat units of cloned *T. cruzi* antigens (1, 2, 13, 36, and SAPA) were used (Fig. 1). The amino acid sequence was deduced from the DNA sequence described previously (1, 7). The peptides were synthesized by using t-Boc (*tert*-Butoxy-carbonyl) chemistry on a solid phase (11) in a 430 A Automatic Peptide Synthesizer (Applied Biosystems). After cleavage and deprotection of protective groups with hydrogen fluoride, the purity of the peptides was examined by reversed-phase chromatography and plasma desorption mass spectrometry (13). An indirect immunoflu-

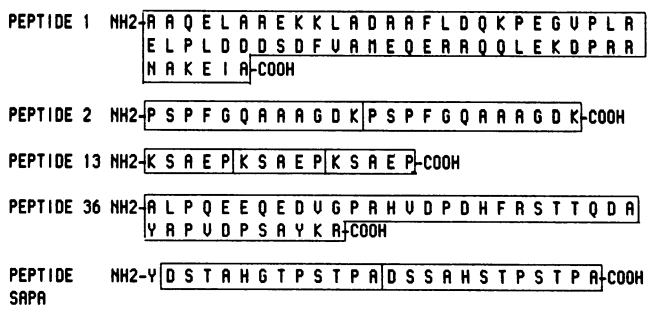

FIG. 1. Sequences of the synthetic peptides used in IRMAs. Peptides 1 and 36 were synthesized as monomers of the repeat units of 60 and 38 amino acids, respectively (7). Peptide 2 is 24 amino acids long and represents a dimer of the corresponding repeat unit (7). Peptide SAPA is 25 amino acids long and is a dimer representing the two most frequent variants of the repeat unit (1). Peptide 13 was synthesized as a trimer of the repeated pentapeptide unit (7).

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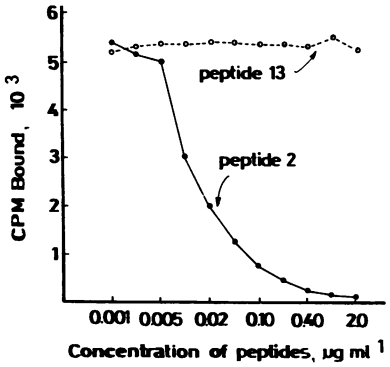

FIG. 2. IRMA to determine the presence of specific antibodies to synthetic peptide 2. Serum from one chagasic patient (90-69, Table 1) was reacted with peptide 2 immobilized in microtiter plates. The specific antibodies were detected with radiolabeled affinity-purified goat antibodies to human IgG and counted. Two reactions were performed, one in the presence of different dilutions of the homologous peptide (peptide 2) and the other in the presence of serial dilutions of a heterologous peptide (peptide 13) as indicated.

orescence assay (IFA) was performed as described by Camargo (2) with fixed cultured epimastigotes as the antigen and anti-human immunoglobulin G (IgG) conjugated with fluorescein isothiocyanate (Biomerieux). The enzyme-linked immunosorbent assay (ELISA) was performed by the method of Voller (15) with soluble extract from epimastigotes diluted to 10 µg/ml in 100 mM $NaHCO_3$ (pH 9.6) and goat anti-human IgG conjugated with peroxidase (Sigma). An immunoradiometric assay (IRMA) was used to evaluate the synthetic peptides and was performed as previously described (14). Briefly, wells of polyvinyl chloride flexible microtiter plates (Falcon Labware) were coated with 50 µl of synthetic peptides diluted to 20 µg/ml in 100 mM $NaHCO_3$ (pH 9.6), and after reaction with serial dilutions of human sera, the bound antibodies were detected by ^{125}I-labeled affinity-purified goat anti-human IgG (Sigma). The specificity of the reaction was evaluated by inhibition assays using homologous and nonrelated peptides. Affinity-purified goat anti-human IgG was radiolabeled with $Na^{125}I$ to a specific activity of 2.5×10^9 cpm/µg of protein by using the iodogen method according to the manufacturer (Pierce Chemical Co.). Uncoupled iodide was removed by passing the radiolabeled antibody through a Sephadex G-25 column.

Control experiments were first performed to check the specificity of the assay with synthetic peptides. Specificity of the antibody binding was evaluated by performing competitive inhibition assays with homologous and heterologous peptides. Figure 2 shows, as an example, that the specific recognition of the synthetic peptide from antigen 2 was inhibited by the simultaneous addition of the homologous peptide but not by the addition of the synthetic peptide corresponding to antigen 13. Assays performed with serial dilutions of serum from a chagasic patient and peptides SAPA and 36 as antigens also showed that binding of antibodies was inhibited by the homologous but not by the heterologous peptide (Fig. 3).

A panel of serum samples obtained from 34 patients with chronic Chagas' disease was then analyzed. Thirty-two of 34 serum samples had detectable antibodies against *T. cruzi* epimastigotes as determined by IFA (cutoff = 1/20). The other two serum samples had a low titer of 1:20 (Table 1). Most serum samples were also positive by ELISA (cutoff = 1/100) except for four samples which were negative. Xenodiagnosis was positive in 11 of the samples tested. Altogether, these results show that that 30 of the serum samples tested were undoubtedly from chagasic patients and that 4 of them might be considered borderline since they were negative by ELISA and had low titers by IFA. Dilutions of each serum sample were then tested against peptides containing the repeats from antigens 1, 2, SAPA, 13, and 36 (Fig. 1). The results obtained with peptides correlated very well with IFA and ELISA. All 30 serum samples that were positive by both IFA and ELISA were reactive with one or more of the synthetic peptides (Table 1). Of the four serum samples that had low titers by IFA and were negative by ELISA, one was positive with synthetic peptides (number 8 in Table 1), two had low titers with only one peptide each (numbers 15 and 22 in Table 1), and the last one was negative. This latter serum sample was collected from a patient having no clinical

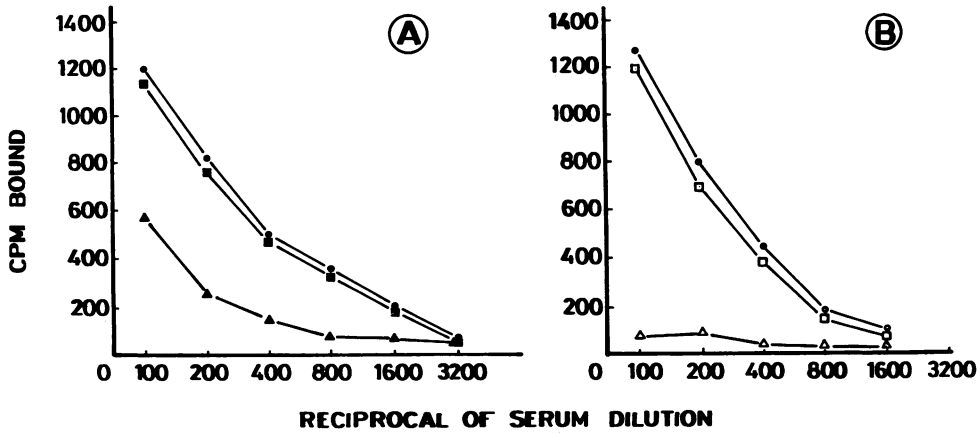

FIG. 3. IRMAs to determine the presence of specific antibodies to peptides SAPA and 36 in serum from a chagasic patient. Different dilutions of serum 89-92 (Table 1) were reacted with peptides immobilized in microtiter plates, and the specific antibodies were detected as described in the legend to Fig. 2. (A) SAPA peptide was used as the antigen, and the reaction was performed in the absence of competing peptides (●) or in the presence of heterologous peptide 36 (■) or homologous SAPA peptide (▲). (B) Peptide 36 was used as the antigen, and the reaction was performed in the absence of competing peptides (○) or in the presence of heterologous SAPA peptide (□) or homologous peptide 36 (△). Inhibition experiments were performed in the presence of 10 µg of homologous or heterologous peptides per ml as indicated.

TABLE 1. Comparative results obtained by IFA and ELISA performed with conventional antigens and by IRMA with synthetic peptides in serum samples from chagasic patients

Patient	Age (yrs)	Serum no.	IgG titer		XEN[a]	IRMA IgG titer of peptide[d]:				
			IFA	ELISA		1	2	SAPA	13	36
1	52	89-248	320	3,200	+	—	3,200	—	—	—
2	NG[b]	90-669	640	3,200	ND[c]	3,200	3,200	—	—	100
3	NG	90-670	320	3,200	ND	—	—	—	—	800
4	NG	90-715	640	3,200	ND	—	640	—	—	—
5	21	89-1595	640	3,200	−	—	3,200	—	—	—
6	30	90-716	1,280	3,200	−	800	1,280	800	—	800
7	42	90-712	1,280	3,200	ND	800	1,280	800	160	800
8	37	90-711	80		ND	—	800	800	800	—
9	40	90-525	640	3,200	+	3,200	1,280	—	—	800
10	64	88-2480	160	3,200	+	1,600	3,200	3,200	1,600	3,200
11	37	89-97	640	3,200	−	1,600	3,200	—	—	3,200
12	48	89-42	320	3,200	−	—	1,600	400	—	3,200
13	29	88-2244	40	3,200	ND	—	3,200	1,600	—	3,200
14	41	89-92	160	3,200	−	800	3,200	800	100	200
15	31	88-2279	20		−	—	—	—	40	—
16	47	88-2345	20		−	—	—	—	—	—
17	52	88-2238	160	3,200	+	—	3,200	—	—	—
18	29	88-2370	1,280	3,200	−	—	3,200	—	40	3,200
19	52	88-2457	160	3,200	+	—	3,200	800	—	—
20	24	88-2321	640	3,200	+	3,200	3,200	—	160	800
21	19	88-2426	640	3,200	+	3,200	3,200	—	160	800
22	27	89-365	40		−	—	200	—	—	—
23	20	89-218	1,280	3,200	−	800	3,200	400	160	3,200
24	57	89-250	1,280	3,200	−		3,200	—	80	3,200
25	30	89-749	320	3,200	−	400	3,200	—	—	200
26	39	89-364	640	3,200	+	800	3,200	200	160	3,200
27	39	89-524	160	3,200	+	—	3,200	—	80	800
28	42	90-69	320	3,200	ND	—	1,600	—	—	—
29	49	89-513	320	3,200	−	1,600	800	400	—	800
30	16	89-110	160	3,200	−	1,600	1,600	—	—	400
31	36	89-99	320	3,200	−	—	3,200	—	160	800
32	12	89-168	160	3,200	−	—	3,200	—	—	800
33	12	89-874	80	3,200	+	—	800	—	80	—
34	8	88-2453	40	3,200	+	400	3,200	—	160	—

[a] XEN, xenodiagnosis.
[b] NG, not given.
[c] ND, not determined.
[d] —, titer was 0.

symptoms (patient no. 16 and Table 1). Five serum samples from healthy donors were used as controls. None of them was positive by IFA, ELISA, or the peptide-IRMA with any of the synthetic peptides used as antigens (data not shown). In addition, serum samples from five patients with distomatosis, two patients with tuberculosis, seven patients with hydatidosis, five patients with toxoplasmosis, five patients with AIDS, and two patients with leishmaniasis were tested against the synthetic peptides, and all were negative in the IRMA.

Differences in reactivity to various synthetic peptides were observed (Table 1). Antibodies against peptides 2 and 36 were found at high levels in most serum samples, whereas low antibody levels were found with peptide 13. The percentage of serum samples having antibodies against each of the five peptides was also different. Peptide 2 was by far the one that reacted with the most serum samples (91%). Peptide 36 reacted with 65%, while peptides 1 and 13 both reacted with 44% of the serum samples tested. As expected, SAPA, which detects mainly sera from acute infections, was the antigen reacting with the fewest chronic human chagasic serum samples (32%). From these results, it is likely that a combination of three synthetic peptides (2, 13, and 36) would be enough to detect most if not all of the sera from infected patients (97% of serum samples in Table 1).

These experiments show that the repeat units represent epitopes present in the corresponding native proteins of *T. cruzi*. A remaining question is whether all polyclonal antibodies to the cloned antigens are, as in plasmodia (4, 16), mainly directed against the repeat domains. Further studies will be needed to determine whether such antipeptide antibodies of a T-cell-associated response play a role in controlling parasite growth after a natural infection. In summary, synthetic peptides modelled on the repeat units of *T. cruzi* antigens might become a powerful tool to be used in studies of the immune status toward *T. cruzi*.

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