

Short Communication

**CHAGAS' DISEASE: DELAYED TYPE HYPERSENSITIVITY
TO PPD IN RELATION TO SEROREACTIVITY TO
TRYPANOSOMA CRUZI IN ECUADOR**

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Abstract: In a population from an area endemic for Chagas' disease in Ecuador, cell-mediated immune activity was assessed using delayed type hypersensitivity (DTH) reaction to PPD. Expression of DTH was significantly suppressed in individuals seropositive to *Trypanosoma cruzi*, and the suppression was markedly greater in the age group 10-39 years old than those over 40 years. The results show that cell-mediated immune response is apparently suppressed in patients with Chagas' disease, and tends to be restored in chronic stage. No difference was noted in the serum concentration of immunoglobulins between patients and controls.

INTRODUCTION

It has been well known that infections with several species of parasites are associated with modification of immune response to heterologous antigens. Delayed type hypersensitivity (DTH) skin reactions are used as an effective tool to assess cell-mediated immunity in humans, and have been found to be suppressed in helminthic and protozoal infection (Greenwood *et al.*, 1973; Grove and Frobos, 1979; Kawabata *et al.*, 1983). In patients with Chagas' disease, caused by *Trypanosoma cruzi*, the previous reports on DTH to unrelated heterologous antigens remain controversial. Expression of DTH reactions in acute chagasic patients was observed to be less than that in nonchagasic controls (Teixeira *et al.*, 1978a), whereas no such suppression was found in the chronic patients (Montufar *et al.*, 1977; Corsini *et al.*, 1981).

On the other hand, it has been suggested that *T. cruzi* isolated from various geographic areas may have distinct properties because clinical manifestations show great regional differences. In the present study the expression of DTH to purified protein derivatives (PPD) of tuberculin and the quantification of serum concentration of immunoglobulins (Igs) was performed in a population from an endemic area of Chagas' disease in an attempt to assess their immunological status in Ecuador.

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MATERIALS AND METHODS

Subjects: The study was conducted in 2 endemic foci for Chagas' disease, Zaruma and surrounding areas (Province of El Oro), and Pedro Carbo and surrounding areas (Province of Guayas). DTH reaction was examined in 255 individuals, while the Ig was quantified in 137 individuals. These subjects were examined for their serum antibodies to *T. cruzi* with a commercially available indirect hemagglutination (IHA)-kit (Hema Chagas, Polychaco, S. A. I. C., Argentina), and classified into 2 groups according to the seroreactivity. IHA titers of 16 or greater were interpreted as positive in this study.

DTH reaction: DTH reaction to intradermal injection of 0.1 ml PPD from *Mycobacterium tuberculosis* RT23, equivalent to 0.005 μ g of PPD-s, were measured 48 hours later. Induration of 5 mm in diameter or more was considered a positive reaction.

Quantification of serum Ig: The serum concentration of IgG, IgM and IgA were determined by single radial immunodiffusion (SRID) method in agar. The plates were purchased from Medical Biological Laboratories (MBL) Co. Ltd., Japan.

RESULTS AND DISCUSSION

Cell-mediated immunity was assessed by testing DTH reactions to PPD in individuals seropositive or seronegative to *T. cruzi*. Positive DTH were significantly less frequent in seropositive individuals than in seronegative individuals (Table 1). When the magnitude of the reaction was analyzed in relation to age among the seropositive group, the DTH was weaker in the 10-39 year age group ($p < 0.01$) than in the over 40 year age group ($p < 0.05$). The present results suggest that cell-mediated immunity was suppressed in seropositive individuals, but was restored in the older age group, although the difference between 2 groups was not significant. The normalization trend for cell-mediated immunity shows that the proliferative response of lymphocytes from *T. cruzi*-infected mice was suppressed during acute infection, and returned to normal levels during the chronic stage (Hayes and Kierszenbaum, 1981). Humoral immunity remain suppressed since the antibody response to typhoid vaccine or sheep erythrocytes in patients with chronic Chagas' disease remains low (Cunningham *et al.*, 1980; Corsini *et al.*, 1981).

No significant differences in the serum levels of IgG, IgM and IgA were noted between the

Table 1 Delayed type hypersensitivity to PPD in a population from an endemic area of Chagas' disease in Ecuador

Age group (years)	No. of positives ^{a)} /No. of Examined (%)		p ^{b)}
	Seropositives ^{c)}	Seronegatives	
10-39	9/34 (26.5)	64/122 (52.5)	<0.01
40-	22/59 (37.3)	23/40 (57.5)	<0.05

a) reactivity to PPD

b) p values with chi-square test

c) seroreactivity to *T. cruzi* by IHA

Table 2 Serum levels of immunoglobulins in a population from an endemic area of Chagas' disease in Ecuador

	Seropositives ^{a)} (n=96)	Seronegatives (n=41)
IgG	1555.2 (449.2) ^{b)}	1470.9 (433.5)
IgM	139.9 (67.3)	153.0 (73.3)
IgA	240.7 (87.6)	242.9 (100.4)

a) seroreactivity to *T. cruzi* by IHA

b) mean (S.D.), mg/dl

seropositive and seronegative groups (Table 2). The results of our study and previous ones (Lelchuk *et al.*, 1970; Corsini *et al.*, 1981) indicate a normal level of Ig in chagasic patients, but an elevated level of Ig in the acute phase of the infection (Vattuone *et al.*, 1973; Schuñis *et al.*, 1978). Great care, however, must be taken on the fact that the immunological status observed in this study might be restricted to Ecuadorian Chagas' disease caused by different geographical strains of parasites.

Our understanding on the immunopathological implication of immune suppression and its restoration on the clinical course of Chagas' disease is limited. Since DTH reaction mediated by *T. cruzi*-sensitized T-lymphocytes has been shown to be involved in the autoimmune destruction of heart cell *in vitro* (Teixeira *et al.*, 1978b), restoration of cell-mediated immunity might be associated with development of chronic heart lesions. In addition, transition from latent to chronic stage, defined by electrocardiographic abnormalities, was found in individuals over 40 years old that were seropositive to *T. cruzi* in Ecuador (Kawabata *et al.*, 1987). To know the precise mechanisms responsible for an evolution of chronic lesions in Chagas' disease, further investigations is needed.

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短 報

エクアドル国のシャーガス病流行地住民集団における遅延型皮内反応

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エクアドル国のシャーガス病患者の細胞性免疫機能を検索する目的で PPD に対する遅延型アレルギー反応を検討した。シャーガス病流行地の住民255名を対象に *T. cruzi* 抗体を IHA test (Hemo chagas, Polychaco SAIC) で検査すると陽性者は93名、陰性者は162名であった。ツベルクリン反応の陽性率は、IHA 陽性者の方が陰性者より有意に低く、特に10-39歳群でその傾向が強かった。この結果からシャーガス病患者では細胞性免疫機能の低下があり、10-39歳群では強い抑制があるが、慢性期になると回復傾向があると推測された。他方、免疫グロブリンを SRID で測定すると IgG, IgM, IgA とともに IHA 陽性者と陰性者の間に差はみられなかった。

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