IDENTIFICATION OF *Trypanosoma cruzi* ANTIGENS RECOGNIZED BY T CELLS AND IMMUNE SERA FROM CHAGASIC PATIENTS

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Infection with *Trypanosoma cruzi* (*T. cruzi*) causes a wide clinical spectrum ranging from indeterminate form, in which morbidity is apparently absent upon clinical examination and can only be observed by rather elaborated methods, to severe heart disease which causes sudden death or heart failure (1). The mechanism of pathogenesis of the cardiac lesions are not fully understood and the involvement of auto immune phenomena in chronic cardiopathy has been postulated to explain the occurrence at late lesions in the different sites where the parasite is not readly demonstrable by histological examination (2). Since humoral and cellular immune responses have been suggested to play a role in the pathogenesis of Chagas' disease through an effector (3-6), and/or immunoregulatory mechanism (7,8), we decided to investigate humoral and cellular responses of sera and peripheral blood mononuclear cells (PBMC) from chagasic patients with different clinical forms to specific antigens of *T. cruzi* epimastigotes (EPI).

To evaluate cellular and humoral responses of chagasic patients to specific fractions of *T. cruzi* antigens we have used Western immunoblot (9,10) and a T cell Western assays (11,12). By these methods we simultaneously analysed the humoral and cellular immune responses to fraccionated epimastigotes antigens.

One mg of EPI (13) antigens was submitted to SDS-PAGE electrophoresis (9) and the proteins transferred to nitrocellulose (NC) as described by TOWBIN and colleagues (10). NC was handled asseptically, washed in PBS with 100 µg/ml of Gentamycin for 4 hr and then placed on top of 96-well flat bottom tissue culture

plates and cut with 6mm tissue biopsy punch into each well and incubated with 250.000 PBMC in a CO_2 incubator at 37°C for 6 days. Cultures were then exposed to [^{3}H] TdR (0.5 µCi/well) during the last 18 hr of culture and processed for scintilation counting. Strips of the same NC 0.5 cm wide were used for Western immunoblot analysis (14) with human sera diluted at 1:80.

All patients in the current study (n = 15 patients) were chronically infected and presented positive sorology for Chagas' disease. These patients were divided into indeterminate and cardiac groups, according to a minimum clinical criteria based on physical examination, chest X-ray and electrocardiography.

The representative patterns of lymphocyte response among indeterminate (I) and cardiac (C) were heterogeneous (Fig. 1). However, PBMC from most patients of the indeterminate group presented a higher response to high molecular weight (M.W.) antigens (> 100 kDa) than those from cardiac group. In addition, in both groups intense peak proliferative response were consistently seen in fractions of the M.W. ranging from 43-60 kDa. Proteins on this molecular weight range are strongly recognized by sera from all patients tested and by a monoclonal antibody against GP57/51 (15) showing a broad difuse band in Western blotting analysis (data not shown). The contribution of GP57/51 to the overall response to EPI antigen was then investigated. Table I shows the PBMC responsiveness of 3 individuals with Chagas disease to either crude EPI antigen and purified GP57/51. The mean E-C values indicate that the proliferative response to GP57/51 accounted for at least 30% of the total response obtained by crude epimastigote antigen stimulation suggesting that the higher response to the 43-60 kDa peak in the T cell Western assay may be a reflexion of the presence of the GP57/51. In three out of 15 patients studied (one cardiac and two indeterminate) the fractionated antigen induced a very low lymphoproliferative response (Fig. 2) that did not differ from control levels. These

results are in agreement with the observation of MORATO et al.(13) that about 28% of chagasic patients do not respond to crude EPI antigens.

The recognition of GP57/51 by antibodies and T cells from almost all patients and the relationship of the magnitude of T cell response of chagasic patients to GP57/51 (Table I) suggest that this antigen may play an important role in the immunity of Chagas' disease. Although there was no dramatic differences in the magnitude of PBMC response to specific EPI antigens in relation with clinical forms, it is possible that preferential induction of different subsets of T-helper cells by specific antigens of an infectious agent (16), different stages of the disease or host genetic background (17), may be crucial in the immunoregulation through a production of different lymphokynes, with important consequences for both protection and immunopathology.

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TABLE I - Response of Peripheral Blood Mononuclear Cells from chagasic patients to epimastigotes antigens (EPI), GP51 and PHA

Patient code	Control*	EPI* 20 µg/ml	GP51* 5 µg/ml	PHA** 5 µl/ml
195	1234*	5000	1037	27000
196	530	34584	14844	45462
197	1080	12962	6925	38358

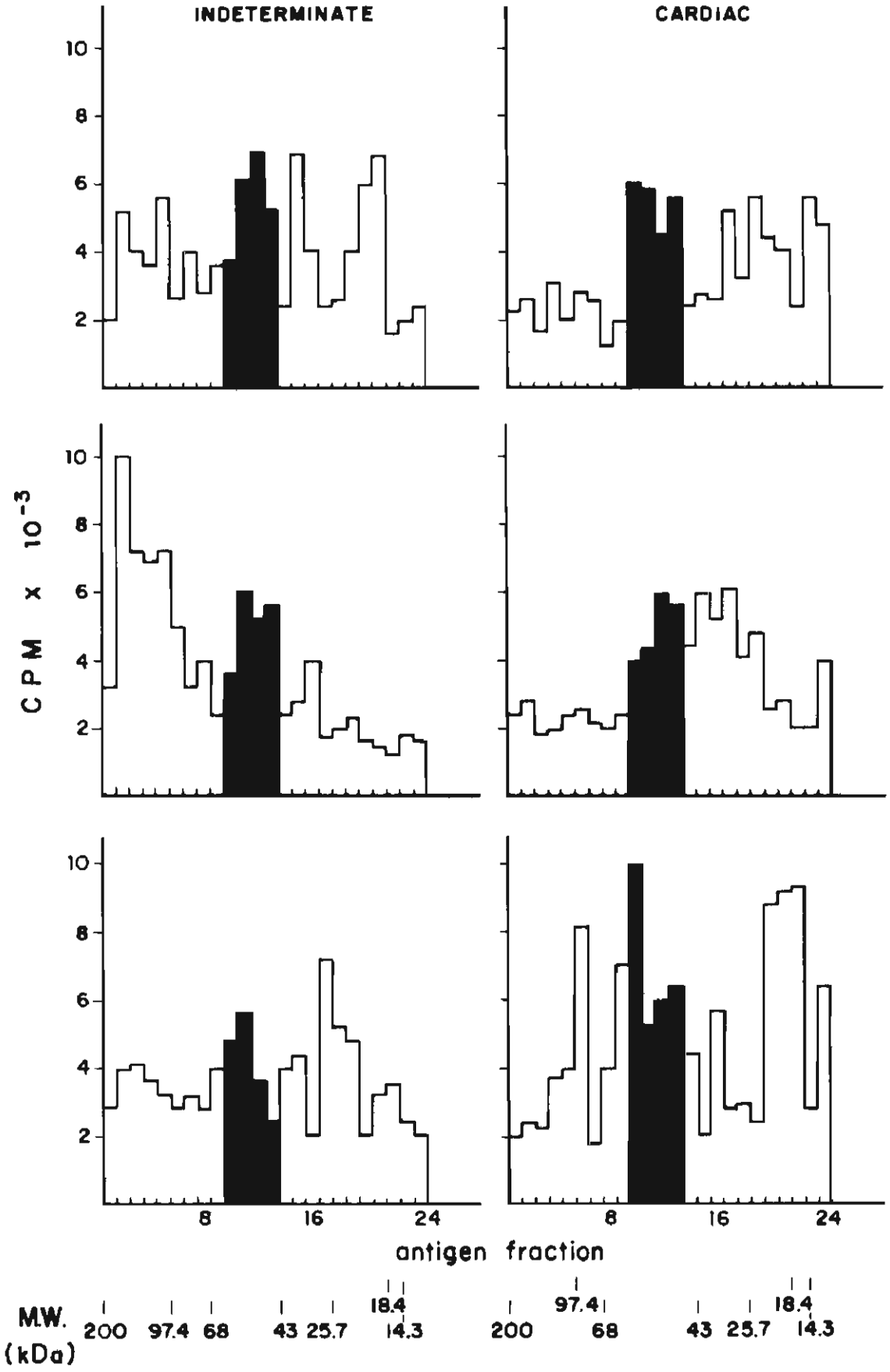
* 6 days of culture, 250000 cells/well

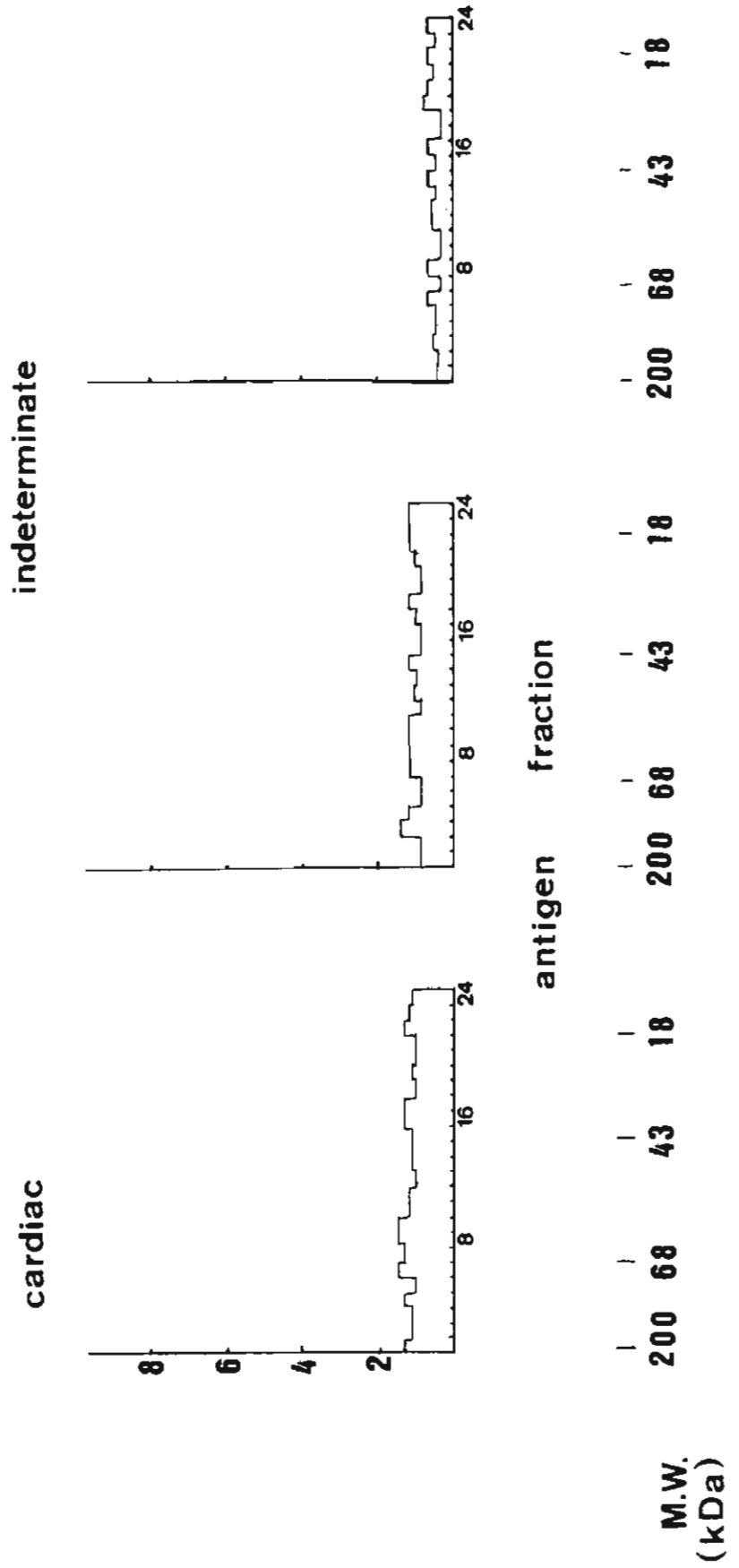
**3 days of culture, 150000 cells/well
Results expressed in Δcpm

LEGENDS FOR FIGURES

Fig. 1 - PBMC response to SDS-PAGE *Trypanosoma cruzi* antigens on immunoblots. PBMC from chagasic patients (250.000/well) were cultivated in presence of epimastigote antigens bound to nitrocellulose blots (24 fractions) at 37°C in a CO_2 incubator. After 5 days, the cultures were exposed for 18 hs to 0.5 µCi TdR and harvested and processed for radioactivity measurements. Controls of unstimulated cultures ranged from 653 to 998. The results are expressed in cpm (cpm experimental - cpm control).

Fig. 2 - Representative patterns of non-reactive PBMC from chagasic patients to SDS-PAGE *Trypanosoma cruzi* antigens on immunoblots. Culture conditions as in figure 1.


INDETERMINATE
CARDIAC
CPM x 10^{-3}
10
8
6
4
2
8
16
24
antigen fraction
MW. (kDa)
200
97.4
68
43
25.7
18.4
14.3


cardiac
indeterminate
8
6
4
2
8
16
24
200
68
43
18
antigen fraction
M.W.
(kDa)