

Do Lymphocytes from Chagasic Patients Respond to Heart Antigens?

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Lymphocyte transformation studies of nonadherent lymphocytes from chronic Chagasic and uninfected persons demonstrated that responses of all individuals to a mouse heart homogenate showed a correlation with responses to streptococcal antigens. Considering the known cross-reactions between streptococcal and cardiac antigens and the high reactivity of Chagasic patients to streptococcal antigens, it is possible that positive lymphocyte transformation to unfractionated heart antigen preparations may not represent specific reactivity to heart antigens.

It has been suggested that the pathological lesions of the heart and intestinal tract observed in Chagas' disease are the result of autoimmune sensitization induced by *Trypanosoma cruzi* (1, 10, 12, 13). Antibodies against human cardiac tissue were demonstrated in chronic Chagasic patients but in only half of the patients with asymptomatic disease and not in uninfected controls (1). Cytotoxic lymphocytes capable of killing both *T. cruzi*-infected and uninfected allogeneic heart cells have been detected in both rabbits infected with *T. cruzi* (10) and Chagasic patients (11). This in vitro killing of human heart cells was inhibited by microsomal fractions of *T. cruzi* and heart cells, implying a cross-reaction between *T. cruzi* and cardiac tissue. Wood et al. (15) have raised a monoclonal antibody against rat dorsal root ganglia which reacts with mammalian neurones, cardiac muscle, and *T. cruzi*. The role (if any) of these cross-reacting antigens in the pathogenesis of the disease is not yet known. Recently, Mosca and Plaja used in vitro lymphocyte transformation to demonstrate that nonadherent mononuclear cells from 27% of (chronic and asymptomatic) Chagasic patients gave positive responses after stimulation with a rat heart antigenic preparation (7). Further studies are reported here in which mouse and human heart homogenates were used to stimulate lymphocyte transformation.

Fourteen Chagasic patients (mean age \pm standard deviation, 44.5 \pm 12.9 years) and 11 seronegative persons (34.1 \pm 14.6 years) were studied. The Chagasic patients were seropositive to *T. cruzi* by enzyme-linked immunosorbent assay (14) and immunofluorescence (M. A. Miles, Ph.D. thesis, University of London, London, England, 1975), and all had chronic Chagasic

myocarditis (9). All except one of the seronegative controls were of racial and socioeconomic background similar to that of the Chagasic population. An epimastigote supernatant antigenic preparation (ES) was prepared from *T. cruzi* Y strain epimastigotes as previously described (Todd, Hoff, and Guimaraes, submitted for publication) and used in cultures at a concentration of 0.5 μ g/ml (4). Two cardiac extracts were prepared. The human donor was a 59-year-old female who had blood type O Rh positive and was seronegative for Chagas' disease and died from cancer of the colon. The myocardial tissue, which showed no gross or microscopic pathology, was taken 3 h after death. Washed tissue was mechanically homogenized on ice in Hanks balanced salt solution for 10 min and the homogenate was used in cultures at 60 μ g/ml (4). Washed myocardial tissue from healthy outbred Swiss white mice was mechanically homogenized as described above, frozen and thawed three times, centrifuged at 1,000 \times g for 15 min, and used at a concentration of 15 μ g/ml (4). Both cardiac preparations were sterile upon culture in thioglycolate medium. Three commercially prepared antigens were used at the concentrations indicated: streptokinase (SK)-streptodornase (SD), (25 U of SK and 6.3 U of SD per ml; Lederle Laboratories), *Candida albicans* extract (40 μ l/ml; Hollistier-Stier Laboratories), and purified protein derivative of tuberculin (25 μ g/ml; Ministry of Agriculture, Fisheries and Food, United Kingdom). Peripheral blood mononuclear cells (PBMN) were isolated over a Ficoll-diatrizoate mixture (LSM; Litton Bionetics), washed in minimal essential medium-S (GIBCO Laboratories), and suspended in RPMI 1640 medium (GIBCO Laboratories). Adherent cells

were removed by incubating 15×10^6 to 25×10^6 PBMN in a plastic petri dish (type 1007, 60 by 15 mm; Falcon Plastics, Inc.) in 10% normal human serum at 37°C for 1 h. After incubation, nonadherent cells were aspirated and washed in RPMI 1640 medium. These nonadherent mononuclear cells (NA-PBMN) were cultured in triplicate in 96-well plates (Falcon 3040) as previously described (Todd et al., submitted for publication). Blastogenic responses were evaluated by tritiated thymidine (^3H Tdr; New England Nuclear) incorporation on day 6 of culture as determined by liquid scintillation counting. The data are expressed as \log_{10} geometric mean counts per minute of triplicate cultures. Stimulation indices (SIs) were also calculated from the formula: $\text{SI} = \text{anti-log}(\log \text{ experimental cpm} - \log \text{ control cpm})$.

As expected, lymphocytes from Chagasic patients responded with significantly greater ^3H Tdr incorporation after stimulation with the *T. cruzi*-derived ES than did uninfected controls (Table 1 and Fig. 1). Lymphocytes from all of the 14 Chagasic patients displayed SIs of 2.5 or greater, up to the maximum of 40.0. These observations agree with those of earlier studies (5-7). The low but significant level of proliferation by cells from uninfected persons to ES (Table 1 and Fig. 1) is due to the slight mitogenic effect of the preparation at the concentration used (Todd et al., submitted for publication). Although the human heart antigenic preparation was undoubtedly allogeneic to all lymphocytes tested and did, indeed, cause SIs of >2.0 in three individuals, it was ineffective overall in inducing lymphocyte transformation (Table 1 and Fig. 1). In contrast, the mouse heart antigen significantly stimulated ^3H Tdr uptake by cells from both groups (Table 1 and Fig. 1). Responses were variable, with SIs ranging from 1.1 to 9.9 for Chagasic patients and 1.4 to 47.0 for controls. Except for one (Bahian) control, cells from both Chagasic and uninfected persons responded strongly to SK-SD, with SIs of >5.0 (Table 1 and Fig. 1). The finding of widespread reactivity to mouse heart by cells from both Chagasic patients and uninfected controls was unexpected. However, it was noted that a high responsiveness by cells from a given individual to the mouse heart antigen was often associated with high responsiveness to SK-SD. Figure 2A illustrates a regression analysis of SK-SD and mouse heart responsiveness for the 11 uninfected controls. The data indicate a strong and significant correlation between responses to the two antigenic preparations. The correlation in response to these two antigens is less strong, but still significant, for Chagasic patients (Fig. 2B). There was no significant correlation between responses to mouse heart and responses to the ES prepara-

TABLE 1. Mean blastogenic responses of NA PBMN from Chagasic patients and uninfected controls to various antigenic preparations

Antigens	\log_{10} geometric mean [^3H]Tdr incorporation \pm SD in ^a :	
	Chagasic patients (n = 14)	Uninfected controls (n = 11)
None	3.1088 \pm 0.27 (1,285)	2.7934 \pm 0.26 (621) ⁺
ES	4.0344 \pm 0.25 (10,824)**	3.0810 \pm 0.27 (1,205)** ⁺
Human heart	3.1071 \pm 0.28 (1,280)	2.9044 \pm 0.32 (802)
Mouse heart	3.5359 \pm 0.34 (3,434)**	3.5243 \pm 0.45 (3,344)**
SK-SD	4.2918 \pm 0.25 (19,578)**	4.0048 \pm 0.54 (10,111)** ⁺

^a Numbers in parentheses represent anti-logs of the means. Asterisks indicate a response significantly different from the corresponding control without antigen (*, $P < 0.01$; **, $P < 0.001$). Plus signs indicate that the responses of Chagasic and uninfected groups are significantly different for a given antigen (⁺, $P < 0.01$; ⁺⁺, $P < 0.001$).

tion, *Candida albicans* extract, or purified protein derivative for either Chagasic or uninfected groups (data not shown).

The inability of human heart antigens to stimulate significant blastogenesis in either group (Table 1) implies that these antigens did not induce the expression of any overt (or suppressed) reactivity to either cardiac or alloantigens which may have been present in the lymphocyte populations. In contrast, the murine cardiac antigens induced significant ^3H Tdr incorporation by lymphocytes from both Chagasic and normal individuals (Table 1). Additionally, these responses to mouse heart showed a correlation with blastogenesis stimulated by the streptococcal enzymes SK and SD but no correlation with responses to ES, *C. albicans*, or purified protein derivative (Fig. 2; see above).

The latter data suggest a similarity of antigenic determinants in the mouse heart homogenate and streptococcal antigens. The mouse heart homogenate was not contaminated with living streptococci since it was sterile; however, the presence of dead streptococci or streptococcal antigens per se, though unlikely, cannot be excluded. Immunological cross-reactions have been described between cardiac muscle tissue antigens and streptococcal antigen (3) and between streptococcal group A carbohydrate and heart valve structural glycoprotein (2). Moreover, it is considered that rheumatic fever develops in patients who have had previous contact with *Streptococcus* sp., especially those who developed high-titer streptococcal antibodies (8). There is, therefore, evidence to link immune

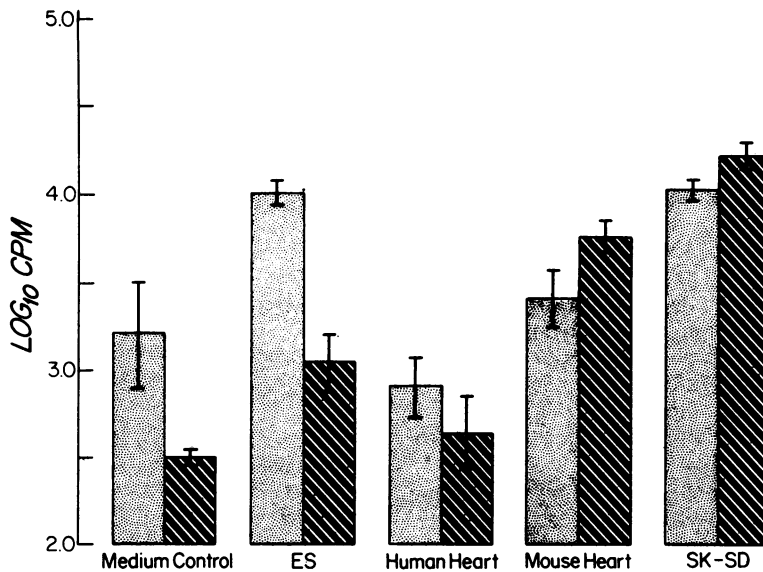


FIG. 1. Antigen-induced [^3H]Tdr incorporation by NA cells from one Chagasic patient (stippled bars) and one uninfected person (striped bars). The mean response of triplicate cultures \pm standard deviation is shown for each antigen.

reactivity induced by streptococcal antigens to reactivity with heart antigens. However, in the current context, the correlation noted is between responses to streptococcal antigens and mouse heart but not human heart. It is not known if streptococci have other antigens which cross-react with mouse heart but not human heart.

The lymphocytes from Chagasic patients may have been sensitized to human heart antigens (10–13), but the current lymphocyte transformation assay in which a human heart preparation was used did not detect expression of such reactivity (Table 1). Chagasic persons generally live (or have lived) in conditions where standards of hygiene are low (16) and, as indicated by blastogenic responses to SK-SD (Table 1 and Fig. 2B), they have had extensive contact with *Streptococcus* sp. The cross-reactions between certain streptococcal antigens and cardiac antigens are known (2, 3, 8), and an in vitro correlation is indicated (Fig. 2) between responses to SK-SD and mouse heart for both Chagasic and normal individuals. In view of these findings, it is suggested that positive lymphocyte transformation by Chagasic lymphocytes in response to either rodent or human heart antigen preparations may not be a true indication of prior sensitization against specific cardiac antigens. Clearly, further work is needed to clarify the interactions of *T. cruzi*, streptococci, and autoantigens with the immune system.

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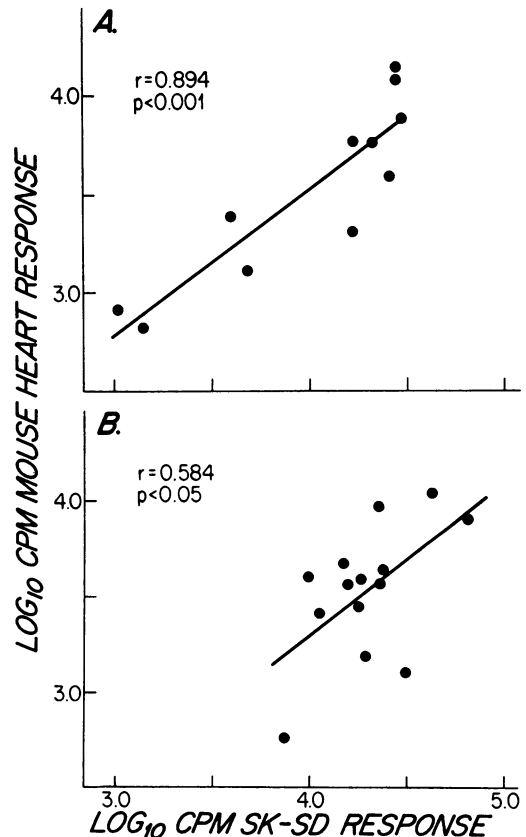


FIG. 2. Relationship between [^3H]Tdr incorporation induced by SK-SD and mouse heart antigen preparations for uninfected persons (A) and Chagasic patients (B).

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