The validity of serodiagnosis using a monoclonal antibody against *Trypanosoma cruzi*-specific M_r 25 000 antigen for chagasic patients without cardiomyopathy

The serodiagnosis of Chagas' disease must be specific because *Trypanosoma cruzi* shares a large number of antigens with other trypanosomatidae (Afchain *et al.*, 1979). We recently described a competitive enzyme-linked immunosorbent assay (c-ELISA) using a monoclonal antibody (MAb) against the *T. cruzi*-specific M_r 25 000 antigen, for the specific serodiagnosis of Chagas' disease (Tachibana *et al.*, 1988). However, Santos-Buch *et al.* (1985) demonstrated that the M_r 25 000 antigen was recognized only by sera from chagasic patients with cardiomyopathy, but not by sera from patients free of heart disease. Therefore, the present study was undertaken to determine whether or not the c-ELISA can be used for the diagnosis of Chagas' disease in patients without cardiomyopathy.

The 83 sera used were collected in endemic areas of Ecuador. An indirect haemaggluti-

nation test (IHA) was carried out with a commercial IHA-kit (Hemo Chagas, Polychaco S.A.I.C.). IHA titres of 1:16 or greater were judged as positive. The electrocardiograms (ECG) were recorded as previously described (Kawabata *et al.*, 1987) and classified as normal, borderline, or abnormal (Maguire *et al.*, 1983). C-ELISA was performed as described previously (Tachibana *et al.*, 1988). Briefly, each well of a microplate was coated with the epimastigote antigen of *T. cruzi* Tulahuen, then treated with equal volumes of a 1:10 serum dilution and horseradish peroxidase-labelled MAb TCF87 for three hours. After washing with phosphate buffered saline containing Tween 20, the reaction was developed by the addition of substrate. Optical density (o.d.) was read at 492 nm. In the present study, sera were scored as positive if their o.d. values were less than the mean o.d. of the negative controls minus three standard

TABLE
Comparison of the c-ELISA and IHA serologic tests for Chagas' disease, and correlation with ECG readings

ECG	No. of subjects	IHA			c-ELISA	
		No. of subjects	No. of subjects	Mean titre (log ₂)	No. positive	Mean inhibition (%)*
Normal	36	Negative	4		0/4	
		Positive	32	6.6	32/32	78.2
Borderline	11	Negative	0			
		Positive	11	7.4	11/11	81.3
Abnormal	36	Negative	2		1/2	56.9
		Positive	34	6.5	34/34	78.4

*Mean inhibition (%) = (1 - mean sample o.d./mean control o.d.) × 100.

deviations (24.9% inhibition). The mean o.d. was calculated from 36 control sera obtained from individuals living in non-endemic areas.

The results are summarized in the Table. Seropositivity determined by c-ELISA correlated well with the IHA data: i.e. c-ELISA recognized, with equal sensitivity, not only the IHA positives with abnormal ECGs (mean inhibition, 78.4%), but all those with normal ECGs as well (mean inhibition, 78.2%). This finding closely parallels the IHA data, where the mean titres of both groups were almost identical, 6.5 and 6.6 respectively. These results demonstrate that the c-ELISA, using a MAb against the M_r 25 000 antigen of *T. cruzi*, is applicable for the serodiagnosis of Chagas' disease in individuals without cardiomyopathy. Therefore, the M_r 25 000 antigen recognized by our MAb seems to be distinct from the antigen identified by Santos-Buch *et al.* (1985). In the c-ELISA test series, one of the IHA negatives with an abnormal ECG turned up positive. The same serum, when examined by an indirect immunofluorescent antibody test, again proved positive. We conclude, therefore, that the c-ELISA diagnosis was correct and that the IHA reading was a false-negative. In our hands, we feel the c-ELISA is somewhat more sensitive than the commercially available IHA test. Even though both tests gave equivalent results with the limited number of serum samples (83), the

use of the strictly-defined o.d. minus three standard deviations to determine the borderline is more rigid and more sensitive than the use of a 1:16 titre to indicate the minimum positive IHA reading.

H. TACHIBANA

Department of Parasitology,
Tokai University School of Medicine,
Bohseidai, Isehara, Kanagawa 259-11, Japan

M. KAWABATA

Department of Clinical Pathology,
Nihon University School of Medicine,
Itabashi, Tokyo 173, Japan

T. MIMORI

Department of Parasitic Diseases,
Kumamoto University School of Medicine,
Honjo, Kumamoto 860, Japan

Y. HASHIGUCHI

Department of Parasitology,
Kochi Medical School,
Nankoku, Kochi 783, Japan

K. NAGAKURA AND Y. KANEDA

Department of Parasitology,
Tokai University School of Medicine,
Bohseidai, Isehara, Kanagawa 259-11, Japan

Received 16 July 1990, Accepted 25 November 1990

REFERENCES

- AFCHAIN, D., LE RAY, D., FRUIT, J. & CAPRON, A. (1979). *Journal of Parasitology*, **65**, 507-514.
- KAWABATA, M., UCHIYAMA, T., MIMORI, T., HASHIGUCHI, Y. & DE CORONEL, V. V. (1987). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 7-10.
- MAGUIRE, J. H., MOTT, K. E., LEHMAN, J. S., HOFF, R., MUNIZ, T. M., GUIMARÃES, A. C., SHERLOCK, I. & MORROW, R. H. (1983). *American Heart Journal*, **105**, 287-294.
- SANTOS-BUCH, C. A., ACOSTA, A. M., ZWEERINK, H. J., SADIGURSKY, M., ANDERSEN, O. F., VON KREUTER, B. F., BRODSKY, C. I., SADIGURSKY, C. & CODY, R. J. (1985). *Clinical Immunology and Immunopathology*, **37**, 334-350.
- TACHIBANA, H., NAGAKURA, K. & KANEDA, Y. (1988). *Parasitology Research*, **74**, 409-414.