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Correlation between isoenzyme patterns and biological behaviour of different strains of *Trypanosoma cruzi*

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Summary

Three strains of *Trypanosoma cruzi*, used previously as prototypes for a classification based on the host-parasite relationships, as well as several stocks isolated from different geographical areas in Brazil, were submitted to isoenzymic analysis. Their isoenzyme patterns revealed a clear correlation with the biological data. The patterns obtained with the enzymes PGM, GPI, ASAT and ALAT permitted discrimination between each of the described types. Only one type was found in each geographical area studied, indicating a possible relationship between regional patterns and clinical presentation of Chagas' disease.

Introduction

Clinical presentation of Chagas' disease, as well as the susceptibility of *Trypanosoma cruzi* to chemotherapy (CANÇADO & BRENER, 1979), may vary in different geographical areas (AMORIM *et al.*, 1979; REZENDE, 1979). It has been suggested that these aspects could be related to parasite strain differences (ANDRADE & ANDRADE, 1966; ANDRADE & FIGUEIRA, 1970). In a previous study involving infections in mice (ANDRADE, 1974) different *T. cruzi* stocks have been divided into three "Types", referring to a standard description based on the morphology of the parasite in the peripheral blood and on the behaviour in the mouse, i.e., parasitaemic curves, mortality rates and histopathological findings.

TYPE I—Strains which are reticulotropic and highly virulent, with maximum parasitaemia on the 7th to 12th days, and with predominance of slender blood forms.

TYPE II—Myotropic strains, especially involving the heart muscle, with late parasitaemic peak(20th day), when mortality reaches a maximum.

TYPE III—Myotropic strains (with preference for skeletal muscle) that cause low mortality and late parasitaemic peaks, and present a predominance of trypomastigote broad forms in peripheral blood.

The stability of the characteristics of the strains and the evidence that one type predominates in a given geographical area have apparently strengthened the usefulness of the concept of strain types (ANDRADE *et al.*, 1981). On the other hand, isoenzymic typing has been applied for strain differentiation (GODFREY, 1976), as well as the classification of the parasites based on their enzymic patterns on zymodemes (MILES *et al.*, 1978) or groups (ROMANHA *et al.*, 1979).

Some degree of geographical distribution of enzymic types has been observed (MILES *et al.*, 1981). The need to correlate enzyme patterns with other biological characteristics of the strains was stressed by GODFREY (1976) and MILES *et al.* (1981) suggested the differences in enzymic patterns between stocks from Venezuela and Brazil may be associated with the observed differences in clinical disease.

Although some variation may appear with different conditions of culture (ROMANHA *et al.*, 1979), this may be due to population selection in an original mixed stock, and isoenzymic patterns have proved to be a stable feature of the strains (MILES *et al.*, 1980). In the present investigation the isoenzymic patterns of different strains of *T. cruzi* were analysed and compared to the morphological and behavioural features presented by the same strains.

Materials and Methods

1. T. cruzi *strains*

21 stocks of *T. cruzi* previously characterized and grouped into Types I, II and III, were tested. Four of these strains were considered as prototypes. These were the Y (SILVA & NUSSENZWEIG, 1953) and Peruvian (NUSSENZWEIG & GOBLE, 1966) strains (Type I), the 12 SF strain (ANDRADE, 1974; Type II) and the Colombian strain (FEDERICI *et al.*, 1964; Type III). The remaining strains have been recently isolated through xenodiagnosis from patients living in three endemic areas of Chagas' disease in Brazil—five from São Felipe (Bahia), four from Mambaí (Goiás) and eight from Montalvania (Minas Gerais). The strains from the two first localities were classified as Type II, and those from Montalvania belonged to Type III (ANDRADE *et al.*, 1981). The parasites were maintained through serial passage in mice and were cultured in Warren's liquid medium for the preparation of enzymic extracts. Culture forms of *T. cruzi* were washed according to ROMANHA *et al.* (1979), submitted to lysis and enzymatic extraction, and stored in liquid nitrogen as small pearls until used (MILES *et al.*, 1977).

2. *Electrophoresis*

The following enzymes were investigated: Aspartate aminotransferase (E.C. 2.6.1.1. ASAT), alanine aminotransferase (E.C. 2.6.1.2. ALAT, phosphoglucomutase (E.C. 2.7.5.1. PGM), glucosephosphate isomerase (E.C. 5.3.1.9.GPI), malate dehydrogenase (oxaloacetate decarboxylating) (NADP+) (E.C. 1.1.1.40. ME) and glucose-6-

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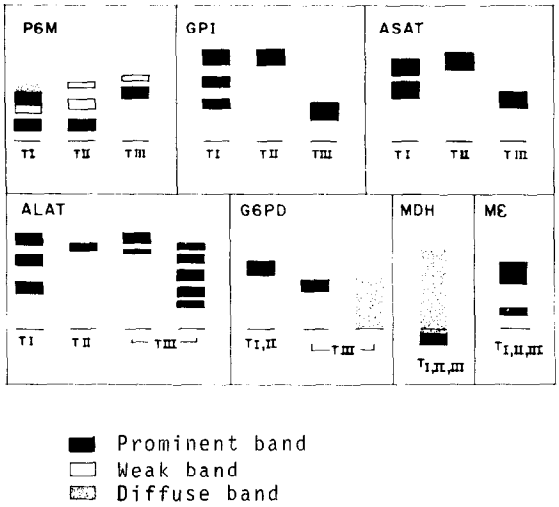

Fig. 1. Comparative electrophoretic patterns of *T. cruzi* stocks classified in three different types for the enzymes PGM, GPI, ASAT, ALAT, G6PD, MDH and ME. Stocks of *T. cruzi*: Type I (T I) —Peruvian and Y; Type II (T II)—5 isolates from São Felipe (BA), 4 isolates from Mambaí (GO); and Type III (T III)—Colombian strain and 8 isolates from Montalvania (MG).

phosphate dehydrogenase (E.C. 1.1.1.49, G6PD). For the malate dehydrogenase (E.C. 1.1.1.37, MDH) a citrate buffer (sodium citrate 75 mM plus HCl, pH 6·0) was used diluted 1:10 in the gel.

25 volts/cm were applied for two hours and a tetrazolium salt staining was used for the reading. This was done with a developing tris buffer/HCl, beta NAD and malate in a final concentration of 100·00 mM, 0·275 mM and 101·38 mM, respectively, besides the MTT (0·36 mM) and PMS (0·03 mM) (Romanha, personal communication). As a reference control, the prototypes of each of the three morphobiological patterns were included on each electrophoretic running.

Results

The patterns obtained for each enzyme studied are shown in Fig. 1 and some examples of enzymic types are shown in Fig 2. The patterns obtained were suitable for comparison and were reproducible with regard to relative band position. However, migration distances may vary because of differences in local voltage (MILES *et al.*, 1980). The enzymes PGM, GPI, ALAT and ASAT allowed good discrimination between the three strain-types. The ALAT displayed two distinct enzymic patterns specific for Type III stocks.

Usually, the bands obtained with G6PD were rather diffuse and weak and did not distinguish between Types I and II, although they allowed the separation of these two types from Type III. A diffuse pattern, without band individualization, occurred with two *T. cruzi* samples from Type III. A similar enzymic pattern for all the strains was obtained with MDH and ME. The distinctive patterns (obtained with these four enzymes) for each *T. cruzi* type and the distribution of the stocks from different regions among those types are shown on Table I.

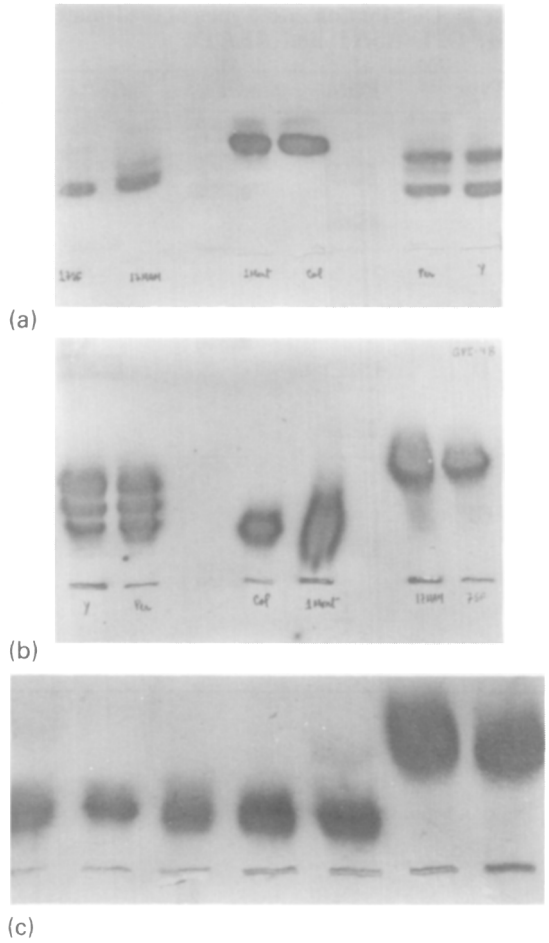

Fig. 2. Photograph of starch gel plates exemplifying the discrimination of different *T. cruzi* types by their electrophoretic patterns. The enzymes shown are: (a) PGM, (b) and (c) GPI. The *T. cruzi* stocks are (from left to right): (a) São Felipe and Mambí (Type II); Montalvania and Colombian (Type III); Peruvian and Y (Type I); (b) Peruvian and Y (Type I); Montalvania and Colombian (Type III); Mambaí and São Felipe (Type II); (c) five isolates from Montalvania (Type III) and two isolates from Mambaí (Type II).

Comments

Results obtained with the isoenzymes tested in this study allowed a positive correlation between *T. cruzi* classification of strain types, based on the behaviour and morphology of the parasite, and their respective isoenzymic patterns. Thus, the analysis of the patterns obtained with PGM, GPI, ASAT and ALAT enzymes distinguished between each of the three strain types of *T. cruzi* so far described. Furthermore, strains from the same geographical area, shown in previous studies to belong to only one strain type (ANDRADE 1974; ANDRADE *et al.*, 1981), also had the same isoenzymic pattern, with the exception of two strains from Montalvania, with regard to G6PD.

By comparing our results with those of MILES *et al.* (1980) for the patterns defined as zymodemes, we observed some similarities among the patterns shown by the strains included in Type III and those for

Table I—Distribution into Types of the isolates of *T. cruzi* studied and the corresponding enzymic patterns for PGM, GPI, ASAT and ALAT

Type	PGM	GPI	ASAT	ALAT	Identification	Origin	No. studied
I					Peruvian	Peru	1
					Y	São Paulo (Br.)	1
II					São Felipe	Bahia (Br.)	5
					Mambaí	Goiás (Br.)	4
III					Colombiana	Colombia	1
					Montalvania	Minas Gerais (Br.)	8

Fig. 3. States of Brazil in which the one *T. cruzi* isolate was obtained. São Felipe (BA) 5 isolates (Type II); Mambaí (GO) 4 isolates (Type II); Montalvania (MG) 8 isolates (Type III).

zymodeme I. It is interesting to note that all the strains isolated from Montalvania belong to Type III and that most of the human isolates from the nearby area of the São Francisco valley displayed the pattern of zymodeme I, as showed by BARRETT *et al.* (1980). Regarding the correlation of Type II and zymodeme II, it should be noted that these groups include *T. cruzi* strains from São Felipe (Bahia) area, which is the same area whence MILES *et al.* (1977) isolated the strains showing zymodeme II. Such correlation was not found for the Type I strains which differ from all zymodeme patterns proposed by MILES *et al.* (1980). DVORAK *et al.* (1980) found that the Tulahúen strain (a reticulotropic strain, probably belonging to Type 1) could not be classified into any of the three zymodemes. This finding agrees with our interpretation that the reticulotropic strains (like those of Type I) are not represented in the zymodemes previously described but the possibility of influence of long-term maintenance of these stocks in the laboratory on the enzymic pattern cannot be excluded. So far, we have not detected strains which could fit the pattern of zymodeme III. Since zymodeme III is found mostly in wild animals (MILES *et al.*, 1980) and our stocks are human isolates, this might explain the lack of this pattern among our samples.

In summary, the results in this study indicate once more that *T. cruzi* is not a homogeneous population. The approach of investigating the multiple parameters of this parasite, including isoenzymic typing is shown to be sound and to have clear biological implications. Thus, different strain types show diverse behaviour in experimental hosts (ANDRADE, 1974), different antigenic compositions (ANDRADE *et al.*, 1981), different susceptibilities to therapeutic drugs (ANDRADE, 1979) and probably different capacities for congenital transmission of infection (ANDRADE, 1982). Moreover, each geographical area seems to have only a single strain type. Further data on this subject is needed to enable exploration of its practical aspects.

References

Amorim, D. S., Manso, J. C., Gallo, Jr. L. & Marin Neto, J. S. (1979). Clinica: Forma crônica cardíaca. In:

Trypanosoma cruzi *e Doença de Chagas*. Brener, Z. & Andrade, Z. (Editors). Rio de Janeiro: Guanabara Koogan, pp. 266-311.

Andrade, S. G. (1974). Caracterização de cepas do *Trypanosoma cruzi* isoladas no Recôncavo Baiano (Contribuição ao estudo da Patologia geral da doença de Chagas em nosso meio). *Revista de Patologia Tropical*, **3**, 65-121.

Andrade, S. G. (1979). Patologia da Doença de Chagas experimental: o problema das cepas do *Trypanosoma cruzi* e sua relação com a resposta aos quimioterápicos. *Revista Médica da Bahia*, **25**, 256-261.

Andrade, S. G. (1982). The influence of the strain of *Trypanosoma cruzi* in placental infections in mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **76**, 123-128.

Andrade, S. G. & Andrade, Z. A. (1966). Estudo histopatológico comparativo das lesões produzidas por duas cepas do *Trypanosoma cruzi*. *O Hospital (Rio de Janeiro)*, **70**, 1268-1278.

Andrade, S. G., Andrade, V., Rocha Filho, F. D. & Barral Netto, M. (1981). Análise antigênica de diferentes cepas do *Trypanosoma cruzi*. *Revista do Instituto de Medicina Tropical de São Paulo*, **23**, 245-250.

Andrade, S. G. & Figueira, R. M. (1970). Caracterização morfobio lógica e histopatológica de diferentes cepas do *Trypanosoma cruzi*. *Gazeta Médica da Bahia*, **70**, 32-42.

Andrade, S. G., Magalhães, J. B., Pontes, A. L., Andrade, V. & Brodskyn, C. (1981). Tipagem de cepas do *Trypanosoma cruzi* procedentes de diferentes áreas endêmicas e investigação da resposta aos quimioterápicos. Pesquisa Básica em Doença de Chagas, VIII Reunião Anual. Program e Resumo de comunicações, Caxambu, Brasil, 66 pp.

Barrett, T. V., Hoff, R. H., Mott, K. E., Miles, M. A., Godfrey, D. G., Teixeira, R., Almeida de Souza, J. A. & Sherlock, I. A. (1980). Epidemiological aspects of three *Trypanosoma cruzi* zymodemes in Bahia State, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 84-90.

Cançado, J. R. & Brener, Z. (1979). Terapeutica. In: Trypanosoma cruzi *e Doença de Chagas*. Brener, Z. & Andrade, Z. (Editors). Rio de Janeiro: Guanabara Koogan, pp. 362-424.

Dvorak, J. A., Hartman, D. H. & Miles, M. A. (1980). *Trypanosoma cruzi:* correlation of growth kinetics to zymodeme type in clones derived from various sources. *Journal of Protozoology*, **27**, 472-474.

Federici, E. E., Abelmann, W. B. & Neva, F. A. (1964). Chronic and progesssive myocarditis and myositis in C_3H mice infected with *Trypanosoma cruzi*. *American Journal of Tropical Medicine and Hygiene*, **13**, 272-280.

Godfrey, D. G. (1976). Biochemical strain characterization of trypanosomes. American trypanosomiasis research, *PAHO Scientific Publication*, No. 318, pp. 91-97.

Miles, M. A., Laham, S. M., Souza, A. A. & Povoa, M. (1980). Further enzymic characters of *Trypanosoma cruzi* and their evaluation for strain identification. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 221-237.

Miles, M. A., Povoa, M. M., Prata, A., Cedillos, R. A. & Souza, A. A. & Macedo, V. (1981). Do radically dissimilar *Trypanosoma cruzi* strains (zymodemes) cause Venezuelan and Brazilian forms of Chagas Disease? *Lancet*, **20**, 1338-1340.

Miles, M. A., Souza, A., Povoa, M., Shaw, J. J., Lainson, R. & Toye, P. J. (1978). Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochthonus patients with Chagas' disease in Amazonian, Brazil. *Nature*, **272**, 819-821.

Miles, M. A., Toye, P. J., Oswald, S. C. & Godfrey, D. G. (1977). The identification by isoenzyme patterns of two distinct groups of *Trypanosoma cruzi* circulating independently in a rural area of Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 217-225.

Nussenzweig, V. & Goble, F. (1966). Further studies on the antigenic constitution of strains of *Trypanosoma* (*Schizotrypanum*) *cruzi*. *Experimental Parasitology*, **18**, 224-230.

Rezende, M. J. (1979). Clinica: manifestações digestive. In: Trypanosoma cruzi *e Doença de Chagas*. Brener, Z. & Andrade, Z. (Editors). Rio de Janeiro: Guanabara Koogan, pp. 312-361.

Romanha, S. J., Silva Pereira, A. A., Chiari, E. & Kilgour, V. (1979). Isoenzyme patterns of cultured *Trypanosoma cruzi* changes after prolonged subculture. *Comparative Biochemistry and Physiology*, **62**, 139-142.

Silva, L. H. P. & Nussenzweig, V. (1953). Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. *Folia Clinica y Biologica*, **20**, 191-208.

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