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 arc reticuloeroplastic 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 course.

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 (FERREIRA 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 Sao Felipe (Bahia), four from Mambei (Goias) 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-
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 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 58 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 morphological patterns were included on each electrophoretic running.

Results

The patterns obtained for each enzyme studied are shown in Fig. 1 and some examples of enzyme 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 enzymatic 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 1.

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 Montalvarina, 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 enzymatic patterns for PGM, GPI, ASAT and ALAT

<table>
<thead>
<tr>
<th>Type</th>
<th>PGM</th>
<th>GPI</th>
<th>ASAT</th>
<th>ALAT</th>
<th>Identification</th>
<th>Origin</th>
<th>No. studied</th>
</tr>
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<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
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<td>Peru</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
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<td>1</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>São Felipe</td>
<td>Bahia (Br.)</td>
<td>5</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Mambai</td>
<td>Goiás (Br.)</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Colombiana</td>
<td>Colombia</td>
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<td></td>
<td>Montalvania</td>
<td>Minas Gerais (Br.)</td>
<td>8</td>
</tr>
</tbody>
</table>

zynodeme 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 zynodeme I, as showed by Barrett *et al.* (1980).

Regarding the correlation of Type II and zynodeme 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 zynodeme II. Such correlation was not found for the Type I strains which differ from all zynodeme patterns proposed by Miles *et al.* (1980).

Dvořák *et al.* (1980) found that the Tulahuen strain (a reticulotropic strain, probably belonging to Type I) could not be classified into any of the three zynodemes. This finding agrees with our interpretation that the reticulotropic strains (like those of Type I) are not represented in the zynodemes previously described but the possibility of influence of long-term maintenance of these stocks in the laboratory on the enzymatic pattern cannot be excluded. So far, we have not detected strains which could fit the pattern of zynodeme III. Since zynodeme 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 isoenzyme 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


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