

Isoenzyme characterization of *Trypanosoma cruzi* from congenital cases of Chagas' disease

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The isoenzyme profiles of a group of five *Trypanosoma cruzi* stocks obtained from congenital cases and of a second group of seven stocks obtained from chagasic mothers who did not transmit their infection congenitally were studied by electrophoresis of nine enzymes. All the mothers became infected in Bahia State (Brazil). With the exception of a triple 'heterozygous' GPI pattern, all *T. cruzi* stocks were identified as zymodeme 2 (Z2). The heterozygous pattern has not been recorded previously in Bahia.

This study shows that enzymically similar *T. cruzi* stocks can show different behaviour with respect to transplacental transmission, and also with respect to clinicopathological presentation and therapeutic response of the congenital cases.

In Salvador, Bahia State (Brazil), among chagasic mothers, transmission of congenital Chagas' disease occurs in 10.5% of births when the newborn weigh less than 2000 g, and in 1.6% of births when the birth weight is more than 2000 g (Bittencourt *et al.*, 1972; Bittencourt *et al.*, unpublished observations). In Santa Cruz, Bolivia, the frequency of congenital transmission is higher (Azougue, personal communication). In contrast, in Bambui, Minas Gerais State (Brazil), congenital transmission of Chagas' disease is a very rare occurrence (Dias, 1979).

Regional differences in the clinical presentation of Chagas' disease, and different chemotherapeutic responses, may be related to different strains of *Trypanosoma cruzi* (Andrade and Andrade, 1979; Cançado and Brener, 1979). Recently Andrade (1982) has shown that the Colombian, Honorina and Peruvian strains of *T. cruzi* have different tropisms for the placenta in experimentally infected mice. The incidence of placental parasitism with these strains was 98, 18 and 13%, respectively.

To see whether there is any correlation between congenital Chagas' disease and the strains of *T. cruzi* transmitted, we studied the isoenzyme profiles of a group of seven *T. cruzi* stocks obtained from chagasic mothers who did not transmit their infection to their offspring, and of a second group of five stocks obtained from congenital cases.

MATERIALS AND METHODS

Trypanosoma cruzi was isolated from eight women on the first day *post partum* and from four children with congenitally acquired infections.* With one exception, all the mothers became infected in the area of 'Reconcavo Baiano'. One mother had lived since birth in Salvador.

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*Only one of these women (Honorina—Case 5) transmitted the infection to the fetus.

Primary isolation of *T. cruzi* from two mothers was made by xenodiagnosis using fifth-instar nymphs of laboratory-bred *Triatoma infestans*. Faeces from xenodiagnosis bugs dissected 30 days after feeding were inoculated intraperitoneally into outbred white Swiss mice, and cardiac blood was cultured when the infection became patent. Primary isolation from six mothers and four children was by culture of 3 ml of sterile heparinized venous blood. The blood was separated over ficoll-hypaque and the mononuclear cell layer, where trypomastigotes were concentrated, was suspended in 2.0 ml of F-29 medium and overlaid on NNN medium using methods previously described (Pan, 1968; Hoff *et al.*, 1979). To obtain larger numbers of organisms, stocks were later transferred to LIT medium. The *T. cruzi* stock of one mother (Honorina—Case 5), after cultivation, had been passaged through experimental animals and had been deep-frozen since 1976. This sample was previously submitted to morpho-biologic characterization and was considered as type II (Andrade, 1976).

The production of lysates, electrophoretic methods and staining for nine enzymes were as previously described (Miles *et al.*, 1977, 1980; Barrett *et al.*, 1980). The enzymes used to examine the *T. cruzi* stocks were as follows: aspartate aminotransferase (ASAT, E.C.2.6.1.1.), alanine aminotransferase (ALAT, E.C.2.6.1.2.), phosphoglucumutase (PGM, E.C.2.7.5.1.), glucosephosphate isomerase (GPI, E.C.5.3.1.9.), aconitate hydratase (ACON, E.C.4.2.1.3.), aminopeptidase (PEP, E.C.3.4.11.1.), malic enzyme (ME, E.C.1.1.1.40.), glucose-6-phosphate dehydrogenase (G6PD, E.C.1.1.1.4.9.) and isocitrate dehydrogenase (ICD, E.C.1.1.1.42.). The *T. cruzi* stocks WA 250 (Z1), Esmeraldo (Z2) and CAN III (Z3), representing three principal Brazilian zymodemes, were used as controls (Miles *et al.*, 1980).

Transmission was considered not to have occurred when newborn offspring of seropositive mothers did not present circulating forms of *T. cruzi* as verified by both xenodiagnosis and direct blood examination. Direct blood examination was performed after concentration of venous blood, using the microhaematocrit heparinized tube method. In five of the cases where transmission was not detected, the placenta and placental adnexae were studied histologically and did not show either inflammation or parasitism.

All the mothers who did not transmit the infection congenitally, and two who did transmit the infection, were asymptomatic. Of the three remaining symptomatic mothers, one had chronic digestive Chagas' disease (megacolon) and another had chronic cardiac Chagas' disease; the third mother had oedema and dysphagia.

RESULTS

With one exception, the *T. cruzi* stocks from the seven mothers who did not transmit the infection showed the same isoenzyme profiles as stocks from the cases in which transmission of congenital Chagas' disease did occur. The exception was the *T. cruzi* stock from Case 5, in which a triple 'heterozygous' GPI pattern was seen similar to those described from Bolivia and Chile (Miles, 1983). All other stocks were identified as Z2.

The clinical features of the congenital disease attributable to these *T. cruzi* stocks are summarized in the Table.

DISCUSSION

In 1977, Miles *et al.* demonstrated differences in the electrophoretic profiles between *T. cruzi* from sylvatic habitats and from domestic hosts. These authors isolated stocks from an endemic area of eastern Bahia State (São Felipe). They named the sylvatic *T. cruzi* strain Z1 and the domestic strain Z2. Barrett *et al.* (1980), in another endemic area of eastern Bahia (Castro Alves), demonstrated three enzymically distinct groups of *T. cruzi* stocks, Z1, Z2 and Z3. Both Z1 and Z2 were infective to man in Bahia; Z3 was only found in a sylvatic habitat but has been reported elsewhere from man (Miles, 1983).

TABLE
Clinical features of the cases with congenital transmission of Trypanosoma cruzi

	<i>Clinical manifestations at birth</i>	<i>Evolution</i>
Case 1	Full-term. Asymptomatic. Placenta with focal villitis and perivillitis, without parasites	At 25 months no symptoms. Xenodiagnosis +ve. No response to Benzonidazole
Case 2	Full-term. Regurgitation and dysphagia	At 5 months—radiological diagnosis of megaesophagus. DBE +ve. Death at 6 months. Autopsy revealed megaesophagus and parasites in digestive tube and bladder. There was a marked reduction in the neuronal population of the Auerbach plexus
Case 3	Low birth weight. Arreflexy, athetotic movements, HE, tremors and respiratory distress	At 20 months diagnosis of cerebral palsy. DBE +ve despite treatment (Nifurtimox and Benzonidazole)
Case 4	Premature. Anaemia, jaundice, dyspnea and HE associated with PHD. After exchange transfusion symptomatology improved but disappeared only after specific treatment (Benzonidazole): placenta showed focal villitis and perivillitis without parasites	At 12 months asymptomatic. Xenodiagnosis and serology became negative after treatment
Case 5	Premature (twins). Both had anaemia and HE. Both placentae showed diffuse villitis and perivillitis with heavy parasitism	They died in the neonatal period. Autopsy revealed generalized inflammation with parasitized giant cells with huge hyperchromatic nuclei

PHD, Perinatal hemolytic disease.

HE, Hepatosplenomegaly.

DBE, Direct blood examination.

With the exception of the heterozygous pattern, all our *T. cruzi* stocks were identified as Z2, and all the mothers came from endemic areas in eastern Bahia. These findings are in agreement with the observations previously reported that Z2 *T. cruzi* is prevalent in the human cases in that area (Miles, 1983). The triple-banded GPI pattern has not been recorded previously from this region of Brazil (Miles, 1983). In this case the patient was infected in the area of 'Reconcavo Baiano'.

This study shows that stocks of *T. cruzi* that are enzymically indistinguishable may have different behaviour with respect to transplacental transmission. It was also observed that the congenital cases had different clinico-pathological presentations and responses to chemotherapy (Table). One child was asymptomatic since birth and did not respond to chemotherapy, one had an early megaesophagus and another, Case 3, had meningoencephalitis and developed cerebral palsy (Vieira *et al.*, 1983; Bittencourt *et al.*, 1984).

Two of these cases were treated but presented persistent parasitemia after treatment. Another showed a good response to chemotherapy, with negative results of xenodiagnosis and serology after treatment. In three cases (including the twins) autopsy was performed (Table). In the twins, giant parasitized cells with huge and hyperchromatic nuclei were seen in several organs and also in the placentae. These cells have been detected only in congenital human cases and in experimental material (Bittencourt, 1976). They were detected in Chile and in 50% of the congenital cases in Bahia (Rubio and Howard, 1963; Bittencourt, 1976). According to Rubio and Howard (1963), these cells might result from a characteristic

reaction of immature connective tissue to the presence of *T. cruzi*. As these cells appear only in autopsy cases with prominent parasitism of the macrophage system, Bittencourt (1976) suggested that the presence of these cells might be related to the strain of *T. cruzi*. The association of these cells with the heterozygous pattern may be only coincidence; but these giant cells have been described in congenital cases in Chile and Bolivia where *T. cruzi* isolates with a triple-banded GPI pattern have also been observed.

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