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Biological behaviour of three strains of Trypanosoma cruzi *from Yucatan, Mexico.*

Original Article

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SUMMARY.

Introduction. *Trypanosoma cruzi* infects a large variety of hosts, and produces variable clinical manifestations in humans. The differences in pathogenicity are thought to be directly related to the genetic heterogeneity of the parasite, which implies that strains from distinct regional areas should be characterised to understand the epidemiology and clinical outcome of the infection. The *in vivo* behaviour of three strains from Yucatan, Mexico (H4, H5, H10), is thus reported here for the first time.

Material and Methods. Strains H4 and H5 were isolated from patients with severe pathologies, whereas strain H10 was avirulent

Results. Experimental infection of NIH mice with strain H4 resulted in high parasitemia (1.04×10^7 circulating parasite/mL) and tissue invasion, leading to a 50% survival. Strains H5 and H10 produced lower parasitemia (6.10×10^6 and 1.29×10^6 circulating parasite/mL), very limited tissue invasion, and 92% of infected mice survived.

Discussion. We conclude that these strains show a virulence comparable to strains from other regions, but that their biological behaviour failed to directly correlate with known genetic markers. ***(Rev Biomed 2001; 12:224-230)***

Key words: *Trypanosoma cruzi*, virulence, biological diversity, Chagas' disease, Mexico.

RESUMEN.

Comportamiento biológico de tres cepas de *Trypanosoma cruzi* de Yucatán, México.

Introducción. El protozoario *Trypanosoma cruzi*, infecta gran variedad de huéspedes, y produce una alta variabilidad en las manifestaciones clínicas en el humano. Las diferencias en la patogenicidad, están directamente relacionadas con la

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heterogenicadad genética de los parásitos, lo cual implica que cepas de diferentes áreas deben ser caracterizadas para entender el comportamiento epidemiológico y clínico de la infección. En este trabajo se reporta el comportamiento en vivo de tres cepas de Yucatán, México (H4, H5, y H10).
Material y Métodos. Las cepas H4 y H5, fueron aisladas de pacientes con patología severa, y la H10, de un paciente asintomático. Se infectaron ratones NIH, con las diferentes cepas de *Trypanosoma cruzi* .
Resultados. La infección experimental de ratones NIH con la cepa H4, resultó con alta parasitemia (1.04×10^7 parásitos circulantes/mL e invasión tisular, con 50% de sobrevivencia. Las cepas H5 y 10 produjeron baja parasitemia (6.10×10^6 and 1.29×10^6 parásitos circulantes/mL) y muy limitada invasión tisular y 92% de los ratones infectados sobrevivieron.
Discusión. Nosotros concluimos que estas cepas, tienen una virulencia comparable con cepas de otras regiones, pero que su comportamiento biológico falta relacionarlo directamente con sus marcadores genéticos. ***(Rev Biomed 2001; 12:224-230)***

Palabras clave: *Trypanosoma cruzi*, virulencia, diversidad biológica, Enfermedad de Chagas, México.

INTRODUCTION.

Chagas' disease represents an important public health problem in large part of Latin America with an estimated 16 million people infected (1), and the Yucatan peninsula is considered as an endemic region (2-5). The disease is caused by the protozoan parasite *Trypanosoma cruzi*, which infects a large variety of mammalian reservoirs and triatomine vector species, and produces highly variable clinical manifestations in humans. This variability in pathogenicity may be due to vector, which may modulate parasite virulence and behaviour (6), and human host characteristics, with immunological, genetic, nutritional, socio-economical factors that may influence the risks and the response to infection (7). However, the main factor involved in the variable pathology of Chagas' disease is thought to be the extensive genetic heterogeneity within *T. cruzi* species.

A large number of distinct strains of *T. cruzi* have been isolated from various hosts, vectors and geographical regions, and their genetic heterogeneity has been well documented by isoenzymes (8-14), RFLP analysis of kinetoplast DNA (15,16) or of rRNA gene spacers (16,17), RAPD profiles (11,13), and typing assays of mini-exon and rRNA sequences (18). These genetic markers allowed the classification of *T. cruzi* strains into distinct zymodemes, schizodemes, or lineages, respectively. In addition, in *vivo* and *in vitro* studies pointed out a similarly extensive variability in biological behaviour between these strains, ranging from strains of very low virulence that are quickly eliminated from their vertebrate host to highly virulent ones with high parasitemias, high tissue invasion and high mortality (9,19-25). Although various studies examined the relationship between biological behaviour and genetic markers from given zymodemes or schizodemes, a direct correlation has been difficult to establish (8,9,20,25,26), even though a few authors observed that some zymodemes had a higher proportion of virulent or avirulent strains (10,12,27,28).

Taken together, these studies underlined the need for a detailed characterisation of local *T. cruzi* strains (29,30) so that we may gain a better understanding of the relationship between molecular markers and pathogenicity of *T. cruzi* strains. Genetic analysis of strains isolated from chagasic patients in the Yucatan peninsula, Mexico, by RFLP (16) and isoenzyme (14) showed that these strains clustered together, appart from other strains from Central and South America. In the present work, we thus investigated the *in vivo* biological behaviour of three strains of *T. cruzi* from the Yucatan peninsula, Mexico, to complete their characterisation.

MATERIALS AND METHODS.

T. cruzi parasites were isolated from patients from the state of Yucatan, Mexico, and a complete clinical study of the patients was performed to characterise the clinical characteristics of the isolates. These isolates were maintained in the laboratory by successive passages in native NIH mice, and no significant variation of their virulence in mice has been observed since their isolation. Molecular analysis of these isolates by k-DNA and total DNA RFLP analysis (16) as well as by isoenzymes (14) has been reported previously, and showed that these isolates represented distinct strains, that were labelled H4, H5 and H10, respectively.

Biological behaviour was determined by experimental infection in mice. Eight week old female NIH mice were divided into groups of 12 and inoculated intraperitoneally with 5×10^5 blood trypomastigotes of the strains H4, H5 and H10, or saline solution, respectively. Blood samples were collected weekly and the parasitemia monitored with a Neubauer cell, until the disappearance of circulating parasites. Spontaneous mortality was recorded. Tissue tropism and damage was assessed for each strain by sacrifying mice at 20, 60 and 90 days post-infection. The heart, spleen, liver, oesophagus, intestine and muscle were sized and weighed, then fixed in formaldehyde 10%, included in paraffin, and sectioned.

RESULTS.

Clinical examination of each patient indicated that each *T. cruzi* strain induced a different type and severity of pathology (table I). The patient infected with the strain H4 showed a severe pathology that included adenomegaly, hepatomegaly, and myocarditis. Infection with the strain H5 also led to a severe pathology that affected the heart and the central nervous system. ECG alterations were observed with both strains. T. *cruzi* strain H10 on the other hand was of little virulence and the infected patient only had fever and the typical Romaña sign. From these clinical data, the strains H4 and H5 may be classified as virulent, whereas the strain H10 was clearly distinct, and almost avirulent.

Table 1
Clinical evaluation of patients infected with the different *T. cruzi* strains.

	T. cruzi strain		
	H4	H5	H10
Patient sex	Female	Female	Female
Patient age (years)	43	24	8
Bipalpebral edema	+	+	+
Fever	+	+	+
Adenomegaly	+	+	+
Cephalea	-	+	-
Exantema	+	-	-
Hepatomegaly	+	-	-
Tachycardy	+	+	-
E.C.G. alterations	+	+	-
Myocarditis	+	-	-
Disnea	-	+	-
Mialgy	-	+	-
Neck rigidity	-	+	-
Evolution	30th days	40th days	15th days
Overall severity	Severe	Severe	Benign

Biological behaviour was studied in more details by experimental infection in NIH mice. As shown in figure 1, a detectable parasitemia initiated between days 8 and 12 post-infection for all three *T. cruzi* strains, and lasted 48 days for the strains H4 and H10 and 76 days for the strain H5. Strain H4 gave the highest parasitemia, with two maximums of 1.04×10^7 and 6.10×10^6 circulating parasite/mL at days 20 and 28, respectively. Mice infected with strain H5 had a much lower parasitemia, with at least two maximums of 3.61×10^6 and 1.52×10^6 circulating parasites/mL, at days 20-24 and 40, respectively. Strain H10 induced an even lower parasitemia than both other strains, withonly a single maximum parasitemia of 1.29×10^6 circulating parasites/mL, at day 20.

Examination of various organs of the infected mice indicated that all three strains induced a small

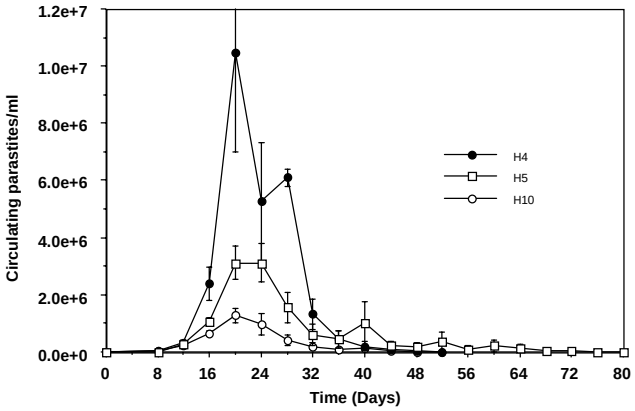

Fig. 1.- Circulating parasites in infected mice as a function of time. Mice were infected intraperitoneally with 5 x 10^5 blood trypomastigotes of the strains H4 (-●-), H5, (-❒-), and H10 (-O-). Each data point represents the mean ± SE of the parasitemia.

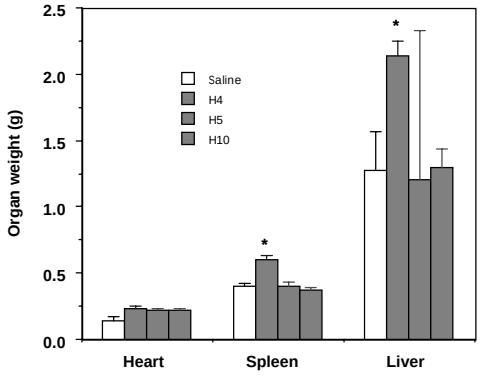

Fig. 2.- Organ weight in mice infected with strains H4, H5 and H10. Heart, spleen and liver were removed from mice inoculated with saline (□), or strains H4 (■), H5 (■), and H10 (■), and weighted. Data are presented as mean ± SE, and ***** indicates a significant difference ($p < 0.001$) with control mice (saline).

but not significant (F = 0.143, $p = 0.87$) increase in heart weight (fig. 2). Strain H4 induced a significant increase in spleen and liver weight (F = 17.49, p < 0.001, and F = 17.30, $p < 0.001$, respectively), whereas strains H5 and H10 did not cause significant changes in the weight of these organs (fig. 2). No changes were observed in oesophagus and intestine weight (data not shown). Histopathological study of these organs revealed strain-specific variations in tissue invasion and damage (fig. 3). In cardiac tissue, we observed 245 ± 54, 6 ± 4 and 4 ± 2 amastigote nest in mice infected with the stains H4, H5, and H10, respectively. Mice infected with all three strains presented disseminated inflammatory infiltration, and rabdomyolysis. In skeletal muscle, 54 ± 22 , 9 ± 5, and 1 ± 1 amastigotes nests in mice infected with the strains H4, H5, and H10, respectively (fig. 3), with also disseminated inflammatory infiltration, and rabdomyolysis. In the oesophagus, we observed 2 ± 1 amastigote nests only in 3/12 mice infected with the strain H4, with limited lymphocytic infiltrates.

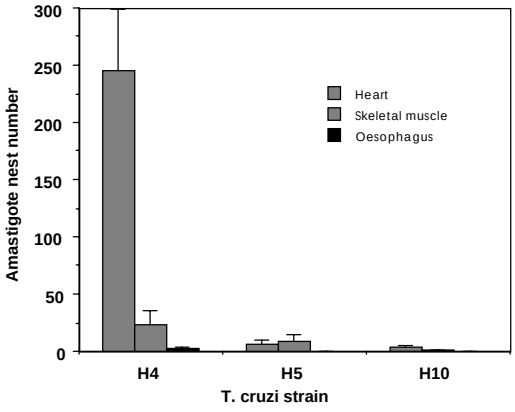

Fig. 3.- Amastigote nest number in mice infected with strains H4, H5 and H10. The number of nests was determined in heart, skeletal muscle and esophagus. Data are presented as mean ± SE.

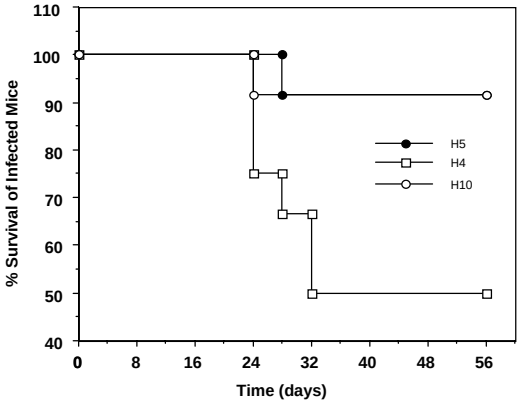

Fig. 4.- Survival to infection as a function of time. Mice were infected with strains H4 (-●-), H5, (-❒-), and H10 (-O-) and data are presented as percentage of surviving mice.

Mortality also showed differences between the strains, as shown in figure 4. In mice infected with the strain H4, 6/12 (50%) died spontaneously between days 24 and 30 post-infection, whereas only 1/12 (8.33%) died on day 28 and day 25 in mice infected by the strain H5 and H10, respectively.

DISCUSSION.

We report here the first data on the *in vivo* biological behaviour of *T. cruzi* strains from the Yucatan state, Mexico, considered an endemic area for Chagas' disease. Genetic analysis of Mexican *T. cruzi* strains by isoenzyme (14) and RFPL of ribosomal RNA gene spacer and kDNA (16) showed a clustering of the strains according to their geographical origin and apart from South American strains, with a general agreement between the different methods. Both studies found that strains H4 and H5 were genetically closely related, and that strain H10 was clearly distinct from them.

Clinical manifestations of both strains H4 and H5 appeared rather severe, and compared with disease descriptions from other endemic regions, could be classified as strains of medium virulence. Strain H10 gave almost no pathology, and could be classified as avirulent. Further analysis of the biological behaviour of the strains by experimental infection in NIH mice confirmed that the strains H4 and H10 were virulent and weakly virulent, respectively. The high parasitemia caused by strain H4 suggests that this strain has higher differentiation and invasion capacities than the other strains. The virulence of this strain was confirmed by the high number of amastigote nest observed and the significant mortality it induced. It had a strong tropism for cardiac muscle, but also showed capacity for invading skeletal and smooth muscle. Invasion of the digestive system has been reported for some other virulent strains in Mexico and South America, but an important observation is that the most virulent strains are usually isolated from vectors rather than patients (13). Strain H10 induced a low parasitemia, and very limited tissue invasion and damage in mice, in agreement with the benign clinical manifestations in the infected patient. Strain H5, that induced a relatively severe disease in the human patient, appeared of weak virulence in the mouse model. It had an acute phase somewhat longer than strains H4 or H10, but with a very low parasitemia, suggesting that it may be best adapted for natural transmission by remaining for long periods in its natural reservoir. Interestingly, this strain is closely related to three other strains isolated from opossums, the main reservoir of *T. cruzi*, as determined by RFLP of ribosomal RNA gene spacer and kDNA (16). Limited invasion of skeletal and cardiac muscle was observed with this strain, in contrast to the tropism and severe alterations observed in cardiac and central nervous system of the patient. This discrepancy between clinical and experimental infection data for strain H5 may be due to significant differences between mouse models and human disease. However, further discrepancies in the relationships between genetic markers and biological behaviour of these strains can be found in their *in vitro* behaviour (14) which indicated that growth and transformation was identical for the strains H4 and H10, and significantly different for the strain H5. Thus, taken together, these data suggest that neither *in vitro* behaviour, nor *in vivo* pathogenicity of the strains H4, H5 and H10 may be associated to known genetic markers. This is in agreement with several studies of strains from other geographical areas (8,9,20,25), suggesting that additional genetic markers need to be identified. Interestingly, a recent study of Mexican strains showed the first direct association between genetic markers from RFLP of rRNA gene and the ability of the strains to kill infected mice (31), and another group reported a correlation between genotypes and the vectorial transmissibility of *T. cruzi* strains, measured by its multiplication and development in insect vectors (32).

In conclusion, we described here the *in vivo* biological behaviour in mice of three strains from

the Yucatan peninsula, Mexico. Experimental pathogenicity of the strains H4, H5, and H10 showed a similar variability as the one described in other geographical areas for other strains, even though a direct comparison is difficult due to important differences in methodologies between laboratories. (9,13,20-22,31)

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