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Differentiation of *Trypanosoma cruzi, T. cruzi marinkellei, T. dionisii* and *T. vespertilionis* by monoclonal antibodies

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Summary

Anti-*T. dionisii* and anti-*T. vespertilionis* monoclonal antibodies secreted by 17 hybridoma clones were tested against various strains of *T. dionisii, T. vespertilionis, T. cruzi* and *T. cruzi marinkellei.* Strain and species specific antigens were detected for the homologous immunizing strains. The common antigenic determinants of the tested trypanosome species include a component of the flagellum and different cell structures. Seventeen *T. cruzi* strains could be classified into two groups when tested with anti-*T. dionisii* monoclonal antibodies. The cross reactions between *T. dionisii* and *T. cruzi* demonstrate a strong correlation between *T. dionisii* and *T. cruzi* group 2. On the other hand *T. cruzi* group 1 and *T. cruzi marinkellei* show very similar antigenic character.

Key words: *Trypanosoma cruzi; T. cruzi marinkellei; T. dionisii; T. vespertilionis;* monoclonal antibodies.

Introduction

Bat trypanosomes of the subgenus *Schizotrypanum* have a cosmopolitan distribution. Biometric studies and morphological investigations (Hoare, 1972; Mühlpfordt, 1981) have shown that they are comparable to the etiologic agent of Chagas' disease, *Trypanosoma cruzi,* which infects 20 million people in endemic areas in Latin-America (WHO, 1983). Bat trypanosomes of the Old World are host restricted to bats only, but some American stocks of bat trypanosomes are

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infective to laboratory animals and presumably transmissible by *Triatominae* bugs (Hoare, 1972; Marinkelle, 1982). Using host specificity as a differentiating character is problematic because *T. cruzi* strains of low virulence have been found (Hüther, 1981). Therefore, an accurate identification of *Schizotrypanum* species of bats is important for epidemiological studies of *T. cruzi*.

Several techniques have been used to differentiate bat trypanosomes: buoyant density of DNA, starch gel electrophoresis (Baker et al., 1978), isoelectrofocusing (Ebert, 1983) and lectins (Schottelius et al., 1983).

Monoclonal antibodies have allowed distinction of *T. cruzi* from *T. rangeli* (Anthony et al., 1981) or differentiation of *T. cruzi* strains and clones (Kirchhoff et al., 1984a), and are useful for the development of precise diagnostic assays of *T. cruzi* strains and *T. cruzi* zymodemes (Flint et al., 1984). Furthermore, monoclonal antibodies have been used to characterize stage specific antigens of *T. cruzi* (Snary et al., 1981; Sher and Snary, 1982; Araujo et al., 1982) and to study their physiology (Alves et al., 1983).

This work reports the differentiation of the bat trypanosomes *T. dionisii* and *T. vespertilionis,* and the agent of Chagas' disease, *T. cruzi,* by monoclonal antibodies raised against *T. dionisii* and *T. vespertilionis.*

Materials and Methods

Trypanosomes

The trypanosome strains of *T. dionisii, T. vespertilionis, T. cruzi* and *T. cruzi marinkellei* which were investigated are listed in Table 1. Epimastigote forms of these parasites were cultivated at 26°C in Brain-Heart-Infusion-Agar (Difco) containing rabbit blood.

Hybridoma techniques

Hyperimmunization was done with inbred Balb/c adult female mice inoculated intraperitoneally with live culture forms (5×10^7 epimastigotes washed 3× with phosphate buffered saline, pH 7.4 [PBS]) of *T. dionisii* strain P3 and *T. vespertilionis* strain P14. The inoculation procedure was repeated 5× at an interval of 3 weeks. Five weeks after the last injection the mice were inoculated with 10^8 epimastigotes. Four days later, spleen cells from the boosted mice were fused with the X63 myeloma line following the strategy described by Fazekas de Saint-Groth and Scheidegger (1980).

Screening of monoclonal antibodies

Antibody production by hybrid cells was assessed between 10 and 14 days after fusion. Epimastigote trypanosomes were washed twice with PBS and fixed in 1% formaldehyde in PBS for two hours. Thereafter, they were washed 3× with PBS and used to prepare slides for indirect immunofluorescence (IIF). Slides were air dried and kept at –20°C until used.

Hybridoma supernatants were screened by IIF. The conjugate rabbit anti-mouse immunoglobulin labeled with fluoresceine isothiocyanate (FITC) (Institut Pasteur Production) was diluted in PBS (1:100) containing Evans Blue counterstain (1:10,000).

All the anti-*T. dionisii* (dion) and anti-*T. vespertilionis* (vesp) monoclonal antibodies were tested by IIF several times with 3 *T. dionisii,* 2 *T. vespertilionis,* 17 *T. cruzi* and 2 *T. cruzi marinkellei* strains.

Table 1. Parasite strains used for the production* and characterization of monoclonal antibodies

Species/strain	Locality	Host	Source
T. dionisii			
P2	England, East Anglia	*Pipistrellus pipistrellus*	(1)
P3*	England, East Anglia	*P. pipistrellus*	(1)
P7	England, East Anglia	*P. pipistrellus*	(1)
T. vespertilionis			
P9	England, East Anglia	*P. pipistrellus*	(1)
P14*	England Rollesby, Norfolk	*P. pipistrellus*	(1)
T. cruzi			
Y	Brazil, São Paulo State	patient	(2)
Morcego 1354	Brazil, São Paulo State	*Tadaria laticaudata*	(3)
Esmeraldo	Brazil, Fazenda Velha	patient	(4)
12-SF-2S	Brazil, Bahia State	patient	(5)
WA 301/130	Brazil, Bahia State	*Didelphis azarae*	(4)
MR	Brazil, Rio Grande do Sul	*Triatoma infestans*	(2)
Tehuantepec	Mexico, Tehuantepec area	*Triatoma sp.*	(6)
V	Venezuela, San Juan	patient	(7)
OPS 4	Venezuela, El Yagual, Santa Rosa, Carabobo State	*Didelphis marsupialis*	(7)
OPS 6	Venezuela, La Pavona, San Francisco de Asis, Aragua State	*Didelphis marsupialis*	(7)
OPS 9	Venezuela, Mollejon, Portuguesa State	*Rhodnius prolixus*	(7)
OPS 12	Venezuela, Mollejon, Portuguesa State	*Canis familiaris*	(7)
OPS 13	Venezuela, Palambra del Doctor, Cojedes State	*Rattus rattus*	(7)
OPS 21	Venezuela, Macuayas, Cojedes State	patient	(7)
OPS 22	Venezuela, La Coromoto, Cojedes State	*Panstrongylus geniculatus*	(7)
OPS 53	Venezuela, El Hoyon, Marinas State	*Rhodnius robustus*	(7)
OPS 89	Venezuela, Oritz, Guarico State	*Triatoma maculata*	(7)
T. cruzi marinkellei			
B7	Brazil, Bahia State	*Phyllostomum discolor*	(8)
B9	Brazil, Bahia State	*P. discolor*	(8)

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Results

In order to differentiate the epimastigotes of *T. dionisii, T. vespertilionis* and *T. cruzi,* monoclonal antibodies were raised against *T. dionisii* strain P3 and *T. vespertilionis* strain P14. The IIF method was chosen to detect the epimastigote antigens.

Seventeen positive hybridomas were obtained: six directed against *T. dionisii* and eleven against *T. vespertilionis.* Cross reactions using different *T. dionisii, T. vespertilionis, T. cruzi* and *T. cruzi marinkellei* strains were carried out. Table 2 summarizes the results obtained with all tested trypanosome strains and indicates the existence of species specific and strain specific antigens.

Nine (eight vesp and one dion) monoclonal antibodies were specific for the homologous immunizing strains. A species specific epimastigote antigen for all *T. dionisii* strains was revealed by dion 2.1a. This result demonstrates the homogeneity of the *T. dionisii* strains, while the two *T. vespertilionis* strains showed antigenic polymorphism, when tested with vesp 3.1 and vesp 4.3 monoclonal antibodies.

Three out of 17 monoclonal antibodies (vesp 9.1, vesp 9.3 and dion 10.1b) reacted with all trypanosome strains. A common antigen expressed only by *T. vespertilionis* and *T. dionisii* strains could be demonstrated by vesp 11.4.

The 17 *T. cruzi* strains considered in this study could be classified into two groups when tested against two anti-*T. dionisii* monoclonal antibodies (dion 1.1d and dion 4.6). 12 *T. cruzi* strains (group 1) did not react with either monoclonal antibodies, while the other 5 strains (group 2) reacted with both monoclonal antibodies.

T. cruzi marinkellei strains B7 and B9 behaved very similarly to *T. cruzi* group 1, but they differed partly in cross reaction with monoclonal antibody dion 5.1b, which reacted with all *T. cruzi* strains. In fact, *T. cruzi marinkellei* strain B9 seems to be a variation of the *T. cruzi* group 1.

The raised monoclonal antibodies reacted either with the cytoplasm or with the cell membrane or uniformly stained the whole parasite. Table 3 shows the results of these observations. As exemplified by strains Y (*T. cruzi* group 2), Tehuantepec (*T. cruzi* group 1) and B7 (*T. cruzi marinkellei*) the fluorescence pattern of those monoclonal antibodies which caused cross reactivity was structurally similar to that obtained using the immunizing strains *T. dionisii* P3 and *T. vespertilionis* P14, although the intensity of fluorescence among the various tested parasite strains was different.

Almost all monoclonal antibodies reacted with antigenic structures of the cell membrane. Two monoclonal antibodies (dion 10.1b and vesp 9.3) were specific for a common antigenic determinant present on the surface and in the cytoplasm of all *T. dionisii, T. vespertilionis, T. cruzi* and *T. cruzi marinkellei* strains. The monoclonal antibody vesp 9.1 recognized exclusively a common flagellar antigen expressed by all trypanosome strains. Four monoclonal antibodies stained the cytoplasm: vesp 3.1, vesp 7.1 and vesp 8.2 produced a

Table 2. Indirect immunofluorescence tests of the specificity of monoclonal antibodies raised against *T. dionisii* and *T. vespertilionis*

Strains	Monoclonal antibodies																
	dion						vesp										
	1.1d	2.1a	4.6	5.1b	6.3	10.1b	2.4	3.1	4.3	5.6	6.2	7.1	8.2	9.1	9.3	11.4	14.2
T. dionisii																	
P2	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	+	–
P3 (immunizing strain)	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	+	–
P7	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	+	–
T. vespertilionis																	
P9	–	–	–	–	–	+	+	–	–	+	+	+	+	+	+	+	+
P14 (immunizing strain)	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
T. cruzi (group 1)																	
WA 301/130	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
Tehuantepec	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
V	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 4	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 6	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 9	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 12	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 13	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 21	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 22	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 53	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
OPS 89	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
T. cruzi (group 2)																	
Y	+	–	+	+	+	+	–	–	–	–	–	–	–	+	+	–	–
Esmeraldo	+	–	+	+	+	+	–	–	–	–	–	–	–	+	+	–	–
Morcego 1354	+	–	+	+	+	+	–	–	–	–	–	–	–	+	+	–	–
12 SF-S2	+	–	+	+	+	+	–	–	–	–	–	–	–	+	+	–	–
MR	+	–	+	+	+	+	–	–	–	–	–	–	–	+	+	–	–
T. cruzi marinkellei																	
B7	–	–	–	+	+	+	–	–	–	–	–	–	–	+	+	–	–
B9	–	–	–	–	+	+	–	–	–	–	–	–	–	+	+	–	–

Table 3. Types of IIF staining reaction observed with anti-*T. dionisii* and anti-*T. vespertilionis* monoclonal antibodies

Monoclonal antibodies	Species				
	T. dionisii	*T. vesper-tilionis*	*T. cruzi* (group 1)	*T. cruzi* (group 2)	*T. cruzi marinkellei*
	Strain				
	P3	P14	Tehuantepec	Y	B7
dion					
1.1d	m	–	–	m	–
2.1a	pe	–	–	–	–
4.6	m	–	–	m	–
5.1b	m	–	m	m	m
6.3	m	–	m	m	m
10.1b	m, c	m, c	m, c	m, c	m, c
vesp					
2.4	–	m	–	–	–
3.1	–	c	–	–	–
4.3	–	m	–	–	–
5.6	–	m	–	–	–
6.2	–	m	–	–	–
7.1	–	c	–	–	–
8.2	–	c	–	–	–
9.1	fl	fl	fl	fl	fl
9.3	m, c	m, c	m, c	m, c	m, c
11.4	m	m	–	–	–
14.2	–	m	–	–	–

m = membrane; c = cellbody; fl = flagellum; pe = cytoplasmic structure of the posterior end

homogenous fluorescence, while the *T. dionisii* specific monoclonal antibody dion 2.1a reacted exclusively with cytoplasmic microgranules at the posterior end of all *T. dionisii* strains.

Discussion

The difficulty of distinguishing trypanosome species and subspecies of the subgenus *Schizotrypanum* is a major restraint to progress in epidemiological studies on Chagas' disease. The absence of clear biometric and morphological characteristics of *Schizotrypanum* species (Hoare, 1972; Marinkelle, 1976) does not allow easy differentiation between *T. dionisii, T. vespertilionis* and *T. cruzi.*

Although the ultrastructure of kDNA can be used as a marker to differentiate *T. cruzi* from *T. rangeli, T. conorhini* and *T. lewisi* (Mühlpfordt, 1975), this

method does not differentiate between different bat trypanosomes of the subgenus *Schizotrypanum* and *T. cruzi* (Mühlpfordt, 1981).

T. dionisii could be distinguished from other bat *Schizotrypanum* species by the presence of long thin trypomastigotes in culture (Baker et al., 1978). However, this form may also be found in *T. vespertilionis* cultures after agglutination with an epimastigote stage specific monoclonal antibody (data not shown).

Isoenzyme patterns permit the differentiation between *T. cruzi* and *T. vespertilionis*. *T. dionisii* shows isoenzyme profiles identical with those of *T. cruzi* group 2. *T. cruzi* group 1 is partly identical with *T. cruzi marinkellei* (Ebert, 1983).

Strains of *T. cruzi* and *T. vespertilionis* can be distinguished on the basis of analysis of surface carbohydrate determinants (Schottelius et al., 1983).

Monoclonal antibodies against *T. cruzi* and other *Schizotrypanum* species are ideal tools to study the epidemiology of Chagas' disease. *T. cruzi* zymodeme specific antigens (Flint et al., 1984) and *T. cruzi* and *T. rangeli* specific antigens (Anthony et al., 1981), could be defined with the aid of monoclonal antibodies.

The large degree of polymorphism among trypomastigote surface antigens of *T. cruzi* (Plata et al., 1984) is a further indication of the highly heterogenous nature of *T. cruzi* strains which has been reported earlier in epimastigote culture forms using different methods: isoenzyme profiles (Miles et al., 1977; Ebert, 1982a, b), lectins (Schottelius and Uhlenbruck, 1983) and monoclonal antibodies (Kirchhoff et al., 1984a; Flint et al., 1984). Differences between *T. cruzi* strains and clones using a monoclonal antibody recognizing a carbohydrate epitope on a 72,000 m. w. glycoprotein expressed on the surface are probably based on structural changes or differences in the membrane environment of the molecule (Kirchhoff et al., 1984b).

On the basis of cross reactive anti-*T. dionisii* monoclonal antibodies 17 *T. cruzi* strains could be divided into two groups. These results are in agreement with previous isoenzyme analysis (Miles et al., 1981; Ebert, 1982a, b) and lectin typing (Schottelius, 1982; Schottelius and Uhlenbruck, 1983; Schottelius et al., 1983). *T. cruzi* group 1 correlated with the zymodeme Z1 or isoenzyme group 1 and the PNA-type as investigated for Venezuelan *T. cruzi* strains (Mühlpfordt et al., 1984).

Five out of six *T. dionisii* monoclonal antibodies cross reacted with *T. cruzi* group 2. These results confirmed the close relationship between *T. cruzi* group 2 and *T. dionisii* as was demonstrated by isoelectrofocusing (Ebert, 1983).

T. cruzi marinkellei was correlated to *T. cruzi* group 1 (Ebert, 1983). We could demonstrate by studying epimastigote antigens that strain B9 of this *T. cruzi* subspecies isolated from a bat was different from *T. cruzi*, as has been detected by DNA bouyant density, non-infectivity to mice and low infectivity to *Triatominae* bugs (Baker et al., 1978). However, *T. cruzi marinkellei* strain B7 appeared to be similar to *T. cruzi* group 1.

The present comparative study demonstrated that species specific mono-

clonal antibodies are useful to distinguish *T. cruzi* from other trypanosomes of the subgenus *Schizotrypanum*. Because of their close relationship it will be necessary to test anti-*T. cruzi* monoclonal antibodies in epidemiological studies against *T. dionisii*.

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