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Suppression of cyclical development of *Trypanosoma brucei brucei* in *Glossina morsitans centralis* by an anti-procyclics monoclonal antibody

V. M. NANTULYA, S. K. MOLOO

Summary

Five hundred and sixty teneral male *Glossina morsitans centralis* were fed, at the height of parasitaemia, on a goat infected with *Trypanosoma brucei brucei*. Thereafter, the tsetse were divided into 4 equal groups. Group I was fed in vitro once weekly for 4 weeks and Group II twice weekly for 4 weeks on fresh defibrinated ox blood containing 2 mg/ml purified monoclonal antibody against *T. b. brucei* procyclics, while Group III was fed twice a week for 4 weeks on blood containing 2 mg/ml anti-*T. vivax* monoclonal antibody. The last group was fed on a rabbit. The tsetse were dissected on day 31 and the percent salivary gland infection rates observed were 18.2, 18.6, 39.8 and 40.8, respectively. In another experiment, 2 groups of tsetse, 120 per group, were fed on fresh defibrinated ox blood containing 2 mg/ml anti-*T. b. brucei* (test group) or anti-*T. vivax* (control group), on days 3, 6 and 9 following the infected feed. Dissection of the tsetse on day 31 revealed salivary gland infection rates of 0% in the test group and 6.5% in the control group. Thus the monoclonal antibody had a marked, specific suppression of the cyclical development of *T. b. brucei* in the tsetse vector.

Key words: *T. b. brucei*; *G. m. centralis*; monoclonal antibody; cyclical development; suppression.

Introduction

Trypanosoma brucei group bloodstream trypanosomes possess an electron opaque surface coat (Vickerman, 1969) consisting of a 12–15 nm layer of tightly packed variable antigen-specific surface glycoprotein (VSG) molecules (Cross,

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1975). The organisms have the innate ability to vary their VSG by switching on or off the genes for the expression of different VSGs. This phenomenon, known as antigenic variation, occurs in approximately one in every 10^5 dividing organisms (Miller et al., 1984), thus giving rise to a sequential expression of large numbers of different antigenic variants (Capbern et al., 1977; Van der Ploeg et al., 1982). This phenomenon is the principal obstacle to development of an effective vaccine against African trypanosomiasis.

When bloodstream trypomastigote forms are ingested by the tsetse fly, they lose the surface coat in the gut and transform to midgut trypomastigotes (procyclics). Thus, the uncoated procyclic trypomastigotes in the gut lumen could be vulnerable to the action of antibodies against common, non-variable surface antigens if such antibodies were ingested by the tsetse fly, leading to the suppression of cyclical development of the trypanosomes in the vector.

That this could occur has been noted previously in tsetse fed on goats immunized with whole procyclics (Murray et al., 1985) and in tsetse flies fed *in vitro* on rabbit anti-*T. congolense* procyclic sera (Maudlin et al., 1984). These studies, however, did not utilize antigens or antibodies of defined specificities. In pursuing a vaccine based on transmission-blocking, it would be desirable to identify and characterize the antigens involved, to facilitate investigations into the prospects for a large-scale production of these antigens and for studies on the best adjuvants for use in stimulating the production of highly potent antibodies.

Recently, we reported the derivation of monoclonal antibodies against *T. b. brucei* procyclics (Nantulya et al., 1987). The monoclonal antibodies identify a protein antigen which is expressed on the surface of procyclics of several *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* stocks isolated from different African countries. In the present study, we have examined the ability of this monoclonal antibody to block the development of *T. b. brucei* in *Glossina morsitans centralis*.

Materials and Methods

Tsetse

General male *G. m. centralis* were from the ILRAD-bred colony.

Trypanosomes

The *T. b. brucei* stock investigated was STIB 247, a 1971 isolate from a Coke's hartebeest in the Serengeti region of Tanzania (Geigy and Kauffmann, 1973).

Animals

Adult male castrated goats (crossbreeds between East African Maasai and Galla) weighing 20–25 kg, were bought from an area of Kenya known to be free from trypanosomiasis. Prior to use, they were screened for antibodies to *T. vivax*, *T. congolense* and *T. b. brucei* by the indirect immunofluorescent antibody test following the procedure described by Katende et al. (1987) and shown to be negative.

Table 1. Experimental design

Experiment A

Tsetse group	Number of tsetse used	Antibody incorporated in bloodmeal	Antibody-containing bloodmeal administered according to schedule below
I	140	TB7/8.1.48	Once a week for 4 weeks, beginning on day 3 post-infection (p.i.)
II	140	TB7/8.1.48	Twice a week (Monday and Thursday), for 4 weeks, beginning on day 3 p.i.
III	140	TV8/8.5.38	Control antibody given as in II above
IV	140	None	Maintained by in vivo feeding on a rabbit every 3 days

Experiment B

Tsetse group	Number of tsetse used	Antibody incorporated in bloodmeal	Antibody-containing bloodmeal taken on post-infection days shown below		
I	120	TB7/8.1.48	3	6	9
II	120	TV8/8.5.38	3	6	9

TB7/8.1.48 = IgM monoclonal antibody to *T. b. brucei* culture procyclics

TV8/8.5.38 = IgM monoclonal antibody to *T. b. vivax* culture procyclics

Antibodies

The monoclonal antibody used, TB7/8.1.48, was an IgM antibody made against in vitro-derived procyclics of *T. b. brucei* LUMP 427, isolated in 1960 from a sheep in Uganda (Cunningham and Vickerman, 1962). This monoclonal antibody recognizes an antigen on the surface of live procyclics of all *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* stocks (Nantulya et al., 1987). The control monoclonal antibody, TV8/8.5.38, was an IgM antibody specific for *T. vivax* procyclics (Nantulya et al., 1987). The IgM fractions of the monoclonal antibodies were purified from the ascites fluid of pristane-treated Balb/c mice by gel filtration through sepharose 6B (Rurangirwa et al., 1986). These fractions were tested for activity against *T. b. brucei* STIB 247 culture procyclics by indirect immunofluorescence performed on live organisms (Nantulya et al., 1987), and also by the agglutination test (Cunningham and Vickerman, 1962).

Experimental design

Five hundred and sixty teneral male tsetse (Experiment A, Table 1) were fed once on a goat infected with *T. b. brucei* STIB 247. These were then divided into 4 groups, each comprising 140 flies. Group I was fed once weekly for 4 weeks while Group II was fed twice weekly for 4 weeks on antibody-containing fresh defibrinated bovine blood containing 2.0 mg/ml purified monoclonal antibody (TB7/8.1.48) against procyclics of a different *T. b. brucei* stock, LUMP 427 (Table 1). In between the antibody-containing bloodmeals the tsetse were maintained by feeding on uninfected rabbits. The 2 control groups were as follows: Group III was fed in vitro on defibrinated bovine blood containing a non-crossreacting monoclonal antibody to *T. vivax* procyclics, while Group IV tsetse were maintained on rabbits every 3 days. The tsetse in this experiment were dissected 31 days post-infection to determine the infection rates.

Since *T. b. brucei* procyclics multiply for 3–10 days in the gut lumen of the vector before they enter the ectoperitrophic space, the second experiment (Experiment B) was designed to determine whether the administration of an antibody-containing bloodmeal in the first 9 days would have any suppressive effect on cyclical development. Teneral male *G. m. centralis*, were allowed to feed once on a goat infected with *T. b. brucei* STIB 247. Thereafter, they were divided into 2 groups of 120 tsetse each (Experiment B, Table 1). The test group was subsequently fed 3 times, in vitro, on fresh defibrinated bovine blood containing 2.0 mg/ml purified monoclonal antibody (TB7/8.1.48) against procyclics of a different *T. b. brucei* stock, LUMP 427. The antibody-containing bloodmeal (2 mg of antibody per ml defibrinated bovine blood) was given at 3 day intervals beginning 3 days after the infected feed. A control group of 120 tsetse was treated similarly except that a monoclonal antibody against *T. vivax* was used. Both the test and control groups of tsetse were subsequently maintained on normal rabbits, starting 3 days after the last in vitro feed. The tsetse were dissected 31 days post-infection to determine the infection rates.

The results of the 2 experiments were analysed by the chi-square test of association.

Results

The anti-*T. b. brucei* monoclonal antibody had strong fluorescence (Fig. 1a) and agglutination (Fig. 1b) activity on *T. b. brucei* procyclics at the concentration (2 mg/ml) used for incorporation in the tsetse bloodmeals. The control anti-*T. vivax* antibody had no such activity (Fig. 1c).

The gut and salivary gland infection rates in the tsetse in the first experiment (Experiment A) are given in Table 2. There were highly significant differences ($p < 0.001$) in the rates of salivary gland infection for tsetse fed once weekly (Group I) or twice weekly (Group II) for 4 weeks on anti-*T. b. brucei* antibody-containing bloodmeal, compared to the controls. The salivary gland infection rates in the test groups were 18.2% for Group I and 18.6% for Group II, compared to the infection rates in the 2 control groups of 39.8% for Group III and 40.8% for Group IV, although the gut infection rates were high in both the control and the test groups (Table 2).

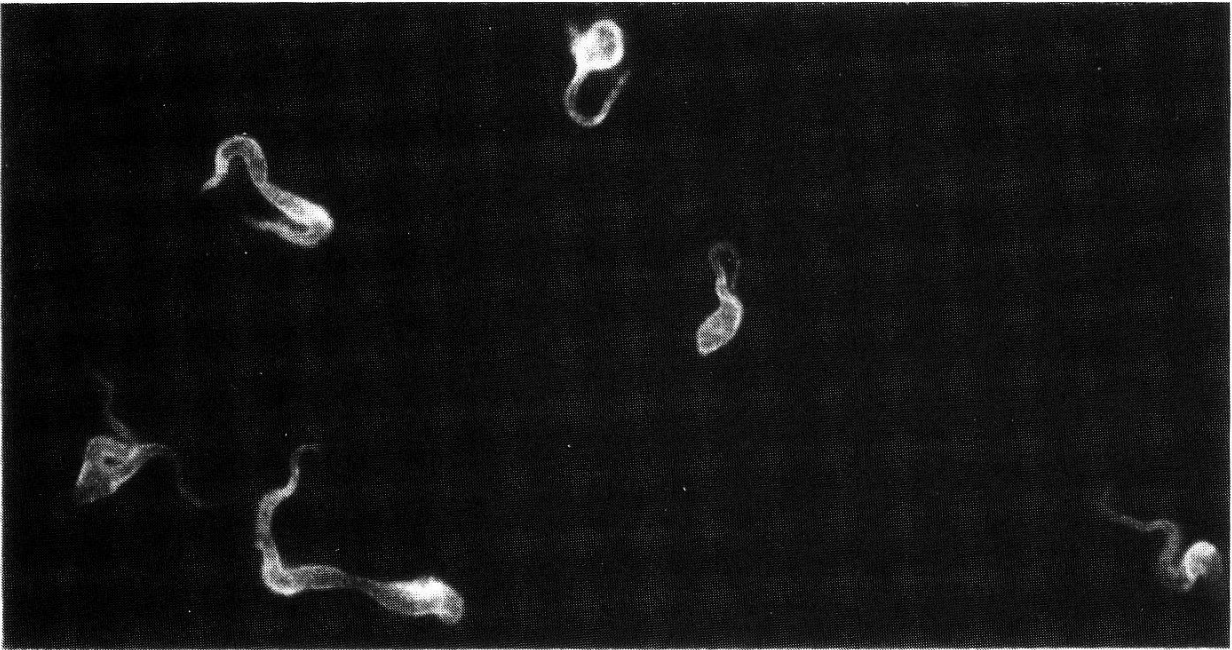
In the second experiment (Experiment B, Table 2), the salivary gland infection rate for the test group was 0% compared to 6.5% observed in the control group.

There were also differences in the survival rates of the test groups of tsetse when compared to the controls, with test groups giving lower survival rates (Tables 1 and 2).

Discussion

This study has shown that the development of a *T. b. brucei* stock STIB 247 in tsetse was suppressed by a monoclonal antibody raised against procyclics of a different *T. b. brucei* stock, LUMP 427. Since the antigen recognized by this

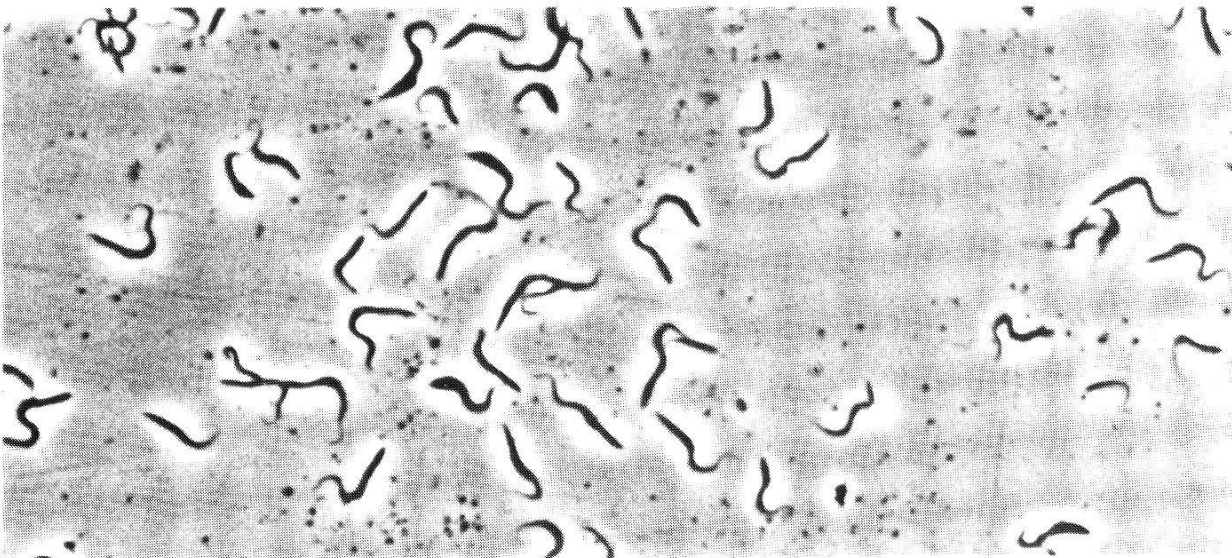
Fig. 1. Indirect immunofluorescent staining (Fig. 1a, 500 \times) and direct agglutinating effect (Fig. 1b, 200 \times) of anti-*T. b. brucei* monoclonal antibody on live cultured procyclics of *T. b. brucei* STIB 247; the control anti-*T. vivax* monoclonal antibody had no agglutinating effect (Fig. 1c, 200 \times).



a



b



c

Table 2. The effect of anti-procyclic monoclonal antibody on cyclical development of *T. b. brucei* in *G. m. centralis*

Experiment A

Tsetse group	Antibody used	No. of antibody bloodmeals	No. tsetse infected	No. surviving tsetse dissected	Infection rates (%)	
					Gut	Salivary glands
I	TB7/8.1.48	4	140	88	51.1	18.2
II	TB7/8.1.48	8	140	86	46.5	18.6
III	TV8/8.5.38	8	140	128	69.5	39.8
IV	None	None	140	120	63.3	40.8

Experiment B

Tsetse group	Antibody used	No. tsetse infected	No. surviving tsetse dissected	Infection rates (%)	
				Gut	Salivary glands
I	TB7/8.1.48	120	69	44.9	0.0
II	TV8/8.5.38	120	108	58.3	6.5

TB7/8.1.48 = IgM monoclonal antibody to *T. b. brucei* culture procyclics

TV8/8.5.38 = IgM monoclonal antibody to *T. b. vivax* culture procyclics

monoclonal antibody has been demonstrated to be expressed also by procyclics of *T. b. rhodesiense* and *T. b. gambiense* (Nantulya et al., 1987), it is possible that this antibody could also block cyclical development of *T. b. rhodesiense* and *T. b. gambiense*.

A similar level of reduction in the infection rates was reported for *T. b. brucei*, *T. vivax* and *T. congolense* in tsetse maintained, after the initial infected feed, on goats immunized with corresponding lyophilized procyclic trypanosomes (Murray et al., 1985), and in tsetse maintained by in vitro feeding on bloodmeals containing rabbit anti-*T. congolense* procyclics hyperimmune serum (Maudlin et al., 1984). However, Maudlin et al. (1984) and Murray et al. (1985) fed the tsetse daily either on the immunized goats or in vitro on antibody-containing bloodmeals.

The strategy adopted in the present study was that of assessing the effect of providing an antibody to a defined procyclic surface antigen during the early phase of the development of *T. b. brucei* in the vector, and at varied time intervals. The results of this study are encouraging in that they show that ingestion of antibody to a defined procyclic surface antigen can significantly reduce cyclical development of *T. b. brucei*. Furthermore, the level of suppression of the cyclical development was similar to that reported by Maudlin et al.

(1984) and Murray et al. (1985), even though the frequency of antibody-containing bloodmeals was reduced to only once a week or only 3 bloodmeals given in the first 9 days after the initial infected feed.

The precise mechanism by which the monoclonal antibody blocked cyclical development is not known. It is possible, however, that the antibody exerted its effect by inducing agglutination of most of the parasites in the lumen of the tsetse gut, thereby, interfering with their further development, since the antibody fraction used had a high agglutinating activity against *T. b. brucei* procyclics. This agglutination may also have affected, in some way, the survival rate of tsetse in the test groups.

These results may have important implications with regard to control strategies for the *gambiense* sleeping sickness. Firstly, the antigen recognized by the monoclonal antibody is expressed on the surface of procyclics of all *T. b. gambiense* stocks (Nantulya et al., 1987). Secondly, it is known that in certain situations in the field, particularly in some areas of Central and West Africa, livestock provide the bulk of bloodmeal source for the peridomestic *G. palpalis* and *G. tachinoides* (Baldry, 1980). It is thus tempting to speculate that in such a situation immunization of livestock which also may serve as the reservoir of infection (Gibson et al., 1978; Moloo et al., 1986), could lead to a reduction in *T. b. gambiense* transmission.

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