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Maintenance of *Glossina morsitans morsitans* on antiserum to procyclic trypanosomes reduces infection rates with homologous and heterologous *Trypanosoma congolense* stocks

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Summary

Three experimental groups of male *Glossina morsitans morsitans* were infected at their first feed with procyclic forms of different stocks of *Trypanosoma congolense* and subsequently maintained on a diet containing rabbit antiserum to one of these stocks. Control flies were similarly infected but were then maintained on normal rabbit serum. Dissection of the flies 19–21 days post infection showed a reduction in both immature and mature infection rates in all groups fed on antiserum by comparison with control flies. These results suggest that vaccination with a single procyclic *T. congolense* strain could reduce transmission of both homologous and heterologous *T. congolense* strains which might, in certain epidemiological circumstances, provide an alternative or additional control method for African trypanosomiases.

Key words: *Glossina morsitans; Trypanosoma congolense;* infection rates; African trypanosomiasis; vaccination; control.

Introduction

The salivarian trypanosomes have evolved a mechanism for eluding the immune system of their mammalian hosts which involves the production of a succession of variable antigenic types (VATs (Vickerman, 1978). The antigenic properties of the bloodstream and metacyclic forms of the trypanosome reside in its surface coat, which is made up of glycoprotein molecules, the composition of which differs for each VAT (Cross, 1975). The expression and control of the

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genes involved in the production of these antigenic variants has recently been the subject of intense research which has shown such variation results from genetic rearrangements within the trypanosome genome (Borst and Cross, 1982; Englund et al., 1982; Turner, 1982). It appears that this antigenic heterogeneity is acquired as soon as the metacyclic trypanosomes mature in the tsetse fly (LeRay et al., 1978). These findings have profound implications for the development of a vaccine designed to protect mammals against trypanosome infection, suggesting as they do that a very complex "cocktail" of antigens would be necessary to provide effective protection. The present work examines an alternative approach to trypanosomiasis control which would be to use a vaccine which elicits an antiserum effective against the procyclic trypanosomes found in the tsetse fly midgut. These trypanosomes have lost the antigenically variable protective surface coat (Barry and Vickerman, 1979) and become vulnerable to such an antiserum when ingested by the fly as part of a blood meal (Murray et al., 1980). Such an "altruistic" approach to vaccination, through transmission blocking immunity, has also been suggested for the control of malaria as anti-gamete antibodies raised in the mammalian host prevent gamete fertilization in the midgut of mosquitoes (Gwadz, 1976; Rener et al., 1980).

Materials and Methods

A single stock of *Trypanosoma congolense* (LUMP 82, derived from TREU 621 isolated in Uganda in the 1960's) in procyclic culture (Evans, 1979) was used to raise antiserum in a rabbit. The rabbit was immunized by intradermal injection with 0.5 ml of a 1% suspension of frozen (−80 °C) and thawed *T. congolense* procyclic forms in RPMI 1640 emulsified with an equal volume of Freunds complete adjuvant. Booster injections consisting of 0.5 ml of a 6% suspension of frozen and thawed *T. congolense* procyclic forms in RPMI 1640 emulsified with an equal volume of Freunds incomplete adjuvant were given 5 and 15 weeks after primary immunization. A test serum sample, taken at 17 weeks, was immunofluorescence test (Miller and Turner, 1980) positive on suspensions of *T. congolense* procyclic forms but not on similar suspensions of *T. brucei*. The rabbit was exsanguinated 24 weeks after primary immunization and the serum heat inactivated and freeze dried. After reconstitution in distilled water, the serum was sterilized by filtration and then diluted 1:1 with normal rabbit serum which had been similarly treated; control serum from untreated rabbits was heat inactivated and treated identically to the experimental serum. To determine whether this antiserum to *T. congolense* LUMP 82 procyclic forms could eliminate trypanosome infections in the vector, it was tested in flies against the homologous stock (LUMP 82) and two heterologous stocks of the same species: TRPZ 133 isolated from a dog in Zambia in 1981 and 1/148 FLY isolated from a cow in Nigeria in 1960.

Male *Glossina morsitans morsitans* in groups of 200 were infected, 18–30 h following emergence from the puparium, with procyclic culture forms of *T. congolense* in whole pig blood. In the first experiment (LUMP 82) the infecting dose was 6.4×10^6 trypanosomes/ml blood; in experiment 2 the dose of TRPZ 133 was 1.6×10^6/ml and in experiment 3 the dose of 1/148 FLY was 6.4×10^6/ml. Flies which refused the infective feed were removed from the experiment. For subsequent feeds flies were fed six days a week on a diet of one volume of pig red cells (washed 3× in phosphate buffered saline) mixed with two volumes of either rabbit antiserum to LUMP 82 (test flies) or normal rabbit serum (control flies). Flies were infected and maintained on an artificial membrane feeding system (Mews et al., 1977). 19–21 days post infection flies were dissected and the

gut, labrum and hypopharynx of each were examined for trypanosomes by phase contrast microscopy (×400).

Results

The results of the dissections are given in Table 1 which shows that antiserum raised against a single strain of *T. congolense* can significantly reduce infection rates in flies infected with heterologous *T. congolense* stocks as well as in those infected with the homologous stock. In experiment 1, using the homologous stock, gut and labral infection rates were significantly reduced in flies fed on antiserum when compared with the control flies. In addition, the mature (hypopharyngeal) infection rate was halved, although statistical significance was not demonstrable with the small numbers involved. [In a preliminary experiment with LUMP 82, in which control flies were fed foetal calf serum instead of rabbit serum, results were closely similar to those shown here; when pooled these data demonstrated a significantly reduced mature infection rate, from 14% (162 flies) in the controls to 5% (171 flies) in the experimental flies (χ^2 = 5.4, $0.05>P>0.01$).] The second experiment produced only a small reduction in gut TRPZ 133 infection rates but a significant reduction in mature infections, from 21% in the control flies to 8% in the experimental flies. The results of the third experiment were similar to those of the first, with 1/148 FLY gut and labral infection rates significantly reduced compared with control flies; the reduction in maturation rate was not statistically significant, again probably due to the small numbers involved.

Table 1. Percentage infection rates in midgut (G), labrum (L) and hypopharynx (H) of three experimental groups of male *G. m. morsitans* infected with three different stocks of *T. congolense* and fed on diet containing rabbit antiserum to a single stock (LUMP 82) or, control diet containing normal rabbit serum. Gut (G) infection rates refer to the proportions of flies with trypanosomes in the midgut whether or not the mouthparts were also infected. Numbers of flies dissected in each group given in parentheses. Statistical comparisons by χ^2 tests

Expt. No.	Trypanosome stock		Diets		Probabilities
			Experimental	Control	
1	LUMP 82	G	34 (89)	64 (92)	<0.001
		G, L	19	46	<0.001
		G, L, H	8	15	not significant
2	TRPZ 133	G	43 (95)	52 (95)	not significant
		G, L	35	42	not significant
		G, L, H	8	21	$0.01>P>0.001$
3	1/148 FLY	G	22 (94)	52 (92)	<0.001
		G, L	2	13	$0.01>P>0.001$
		G, L, H	0	3	–

Discussion

The present work has shown that *T. congolense* infection rates can be significantly reduced by feeding flies on antiserum raised against a single stock of cultured procyclic trypanosomes. This is consistent with theoretical considerations which suggest that the procyclic culture form is equivalent to the gut form of the trypanosome, having an antigenically invariable surface, unlike bloodstream and metacyclic trypomastigotes (Vickerman, 1970). Murray et al. (1982) have recently shown that antibody raised against a single *T. congolense* procyclic stock (ILRAD C24) will reduce infection rates in flies infected with the same and different stocks of *T. congolense*. The present results show that antiserum to a single procyclic stock affects both the establishment of procyclic infections and the subsequent maturation of both homologous and heterologous stocks in tsetse. Reduced procyclic infection rates may result directly from antibody lysis of trypanosomes, or indirectly from the action of tsetse gut enzymes or serum lipids on antibody-agglutinated trypanosomes (Maudlin et al., 1984). It is likely that reduced maturation rates follow from inactivation of trypanosomes moving forward to the proboscis; alternatively, antibody may inhibit trypanosome morphogenesis as described in *Trypanosoma cruzi* (Sher and Snary, 1982).

These results also point to a possible alternative method of trypanosomiasis control, in particular of sleeping sickness if similar results can be obtained with *T. brucei gambiense*. For example, in parts of the Ivory Coast where *G. palpalis palpalis* may take more than 80% of their feeds from domestic pigs (Gouteux et al., 1982), trypanosome infection rates in the fly population might be reduced significantly by vaccinating domestic livestock with a simple procyclic antigen. Similarly, for bovine trypanosomiasis, the trypanosome "challenge" (Whiteside, 1958) in a ranching area might be significantly reduced by vaccinating herds of cattle with a procyclic antigen and we are examining this by computer simulation. The number and frequency of feeds that infected flies must take from animals immunized with procyclic antigen to reduce infection rates significantly, and the frequency of host vaccination required to maintain effective antiserum levels, are currently being investigated; clearly these are likely to be important determinants of the efficacy of a procyclic vaccine.

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