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Analysis of the variable antigen composition of *Trypanosoma brucei brucei* metacyclic trypanosomes using monoclonal antibodies

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Summary

The metacyclic trypanosomes of a *Trypanosoma brucei brucei* clone (ILTat 2.1) were analysed with regard to their variable antigen (VAT) composition using monoclonal antibodies. The metacyclic population was antigenically heterogeneous. Despite the heterogeneity, however, the overall VAT composition of the metacyclic population appeared to be limited in number. A similar pattern of reactivity was observed when the monoclonal antibodies were tested on metacyclics of another clone (ILTat 2.2) derived from a rabbit 30 days after infection with ILTat 2.1 as well as those of the parent stock (STIB 247). The VAT characteristics of the metacyclics of this serodeme were consistent regardless of whether they were transmitted by *Glossina morsitans morsitans* or *G.m. centralis*. The monoclonal antibodies also reacted with some of the bloodstream VATs isolated within 72 h from cyclically infected mice. None of the monoclonal antibodies, however, reacted with metacyclics of a different stock (LUMP 227).

Key words: *Trypanosoma brucei brucei* metacyclics; variable antigen types; serotyping with monoclonal antibodies; limited repertoire.

Introduction

African trypanosomiasis is one of the important vector-borne diseases of man and livestock in tropical Africa. The disease is characterized by a relapsing course of parasitaemia which is associated with a successive emergence of numerous variable antigen types (VATs) from the infecting trypanosomes (Doyle, 1977). This continuous emergence of different VATs frustrates the im-

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mune system of the host since antibodies to one VAT do not confer protection against other VATs. Recent studies have shown, however, that in the case of *Trypanosoma (Trypanozoon) brucei* and *T. (Nannomonas) congolense*, if such VATs are derived initially from one trypanosome then passaged through tsetse, they revert to an antigenically identical population of metacyclics (Jenni, 1977a, b; Nantulya et al., 1980a). Thus immunization of mice (Nantulya et al., 1980b; Jenni and Brun, 1981) or goats and cattle (Emery et al., 1980) with metacyclic antigens obtained from tsetse infected with one VAT induces protection against subsequent challenge by tsetse infected with any other member of the VAT repertoire belonging to the same serodeme.

Subsequent studies (Le Ray et al., 1978; Gardiner et al., 1980; Crowe et al., 1981; Esser et al., 1981) have shown that metacyclic populations of the *T.(T.) brucei* complex are themselves heterogeneous with respect to their VAT composition. The number of different VATs in such metacyclic populations, however, is not known. The use of monoclonal antibodies, with their epitope specificity, could allow analysis of the number of VATs present in a metacyclic population. We present here evidence using monoclonal antibodies, which shows that although the metacyclics of *T. brucei brucei* are antigenically heterogeneous, the total number of different metacyclic VATs associated with a given serodeme is limited and characteristic for the serodeme, regardless of whether the serodeme is transmitted by *Glossina morsitans morsitans* or *G.m. centralis*.

Materials and Methods

1. *Trypanosomes.* – *Trypanosoma b. brucei* clone ILTat 2.1 was derived in lethally irradiated Balb/C mice from STIB 247, an isolate (Geigy and Kauffmann, 1973) from the Serengeti area of Tanzania. ILTat 2.2 is a clone derived similary from a rabbit 30 days after infection with ILTat 2.1. STIB 367H is a population derived in mice from a single metacyclic form of LUMP 227 (Jenni, 1977a).

2. *Laboratory animals.* – Balb/C mice (weighing 20–25 g) were used throughout the experiments.

3. *Tsetse flies.* – *Glossina morsitans morsitans* and *G.m. centralis* were obtained from ILRAD tsetse colonies.

4. *Cyclical transmission through tsetse.* – Tsetse were infected with clones and parent stocks of *T.b. brucei*, and the infected tsetse identified and maintained on rabbits following described methods (Jenni, 1977a):

5. *Production of monoclonal antibodies against metacyclics.* – Ten *G.m. morsitans* flies, infected with ILTat 2.1, were allowed to feed on individual normal Balb/C mice which were subsequently treated with Diminazene aceturate (Berenil – Hoechst, Frankfurt, Germany) after 24 h. The dose used was 40 mg/kg body weight, administered intraperitoneally. Two weeks later, each mouse was rechallenged with the same 10 flies and sacrificed 3 days later to obtain immune spleen cells which were subsequently fused to NSI myeloma cells using polyethylene glycol 1550 (Galfre et al., 1977). Positive hybrids were detected by indirect immunofluorescence. The hybrids were then grown and cloned twice in macrophage-containing soft agarose before determining the Ig class of each monoclonal antibody (Pearson et al., 1980). The hybrid cells were then inoculated into Balb/C mice to obtain ascitic fluid (Pearson et al., 1980) which was fractionated by the Protein-A-Sepharose technique (Ey et al., 1978). Positive Ig fractions were conjugated to fluorescein isothiocyanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC) by the dialysis method (Clark and Shepard, 1963).

Table 1. The variable antigen types of *T.b. brucei* metacyclics revealed by indirect immunofluorescence

		% metacyclics stained (mean ± 1 standard deviation)			
Monoclonal antibody	Ig class	ILTat 2.1	ILTat 2.2	STIB 247	STIP 367H
Tb3/38.10.2	IgG_1	25 ± 10	34 ± 8	29 ± 11	0
Tb3/33.3.23	IgG_1	18 ± 9	16 ± 12	16 ± 11	0
Tb8/16.37.15	IgG_1	19 ± 5	15 ± 6	16 ± 7	0
Tb8/16.32.5	IgG_{2b}	15 ± 7	14 ± 12	14 ± 10	0
Tb9/17.3.23	IgM	13 ± 4	15 ± 5	14 ± 6	0
Tb2/17.56.8	IgG_{2a}	< 1	< 1	< 1	0

Each value represents the results of assays performed at least 10 times. The percentage was calculated on the basis of the number of metacyclics showing a positive reaction per 200 metacyclics examined.

Table 2. The reactivity of anti-metacyclic monoclonal antibodies with bloodstream variable antigen types (VATs) isolated from mice cyclically infected with ILTat 2.1 as demonstrated by indirect immunofluorescence

	% VATs (mean ± 1 standard deviation) showing positive staining in populations isolated after	
Monoclonal antibody	48–72 h	5 days
Tb3/38.10.2	12 ± 3	0
Tb3/33.3.23	8 ± 2	0
Tb8/16.37.15	25 ± 5	0
Tb8/16.32.5	11 ± 4	0
Tb9/17.3.23	< 5	0
Tb2/17.56.8	< 5	0

Each value represents the results of assays performed 3 times. The percentage was calculated on the basis of the number of trypanosomes showing a positive reaction per 200 trypanosomes examined.

6. *Serological tests.* – The indirect immunofluorescent antibody tests (IFAT) on metacyclic trypanosomes were carried out on fresh unfixed tsetse salivary probe materials following the method described by Nantulya et al. (1980a). All ascitic fluids from mice previously inoculated with antibody-producing hybridomas were first heat-inactivated (56° C for 30 min) before use in the IFAT. The dilution used was 1:100 for each ascitic fluid. To determine whether any two monoclonal antibodies stained different metacyclic populations, the FITC-conjugated Ig fraction of one monoclonal antibody was mixed with a TRITC-conjugated Ig fraction of the other and then applied to the salivary probe materials directly (direct immunofluorescence).

Results

The staining patterns of six monoclonal antibodies that have so far been characterized are summarized in Tables 1 and 2. Each monoclonal antibody

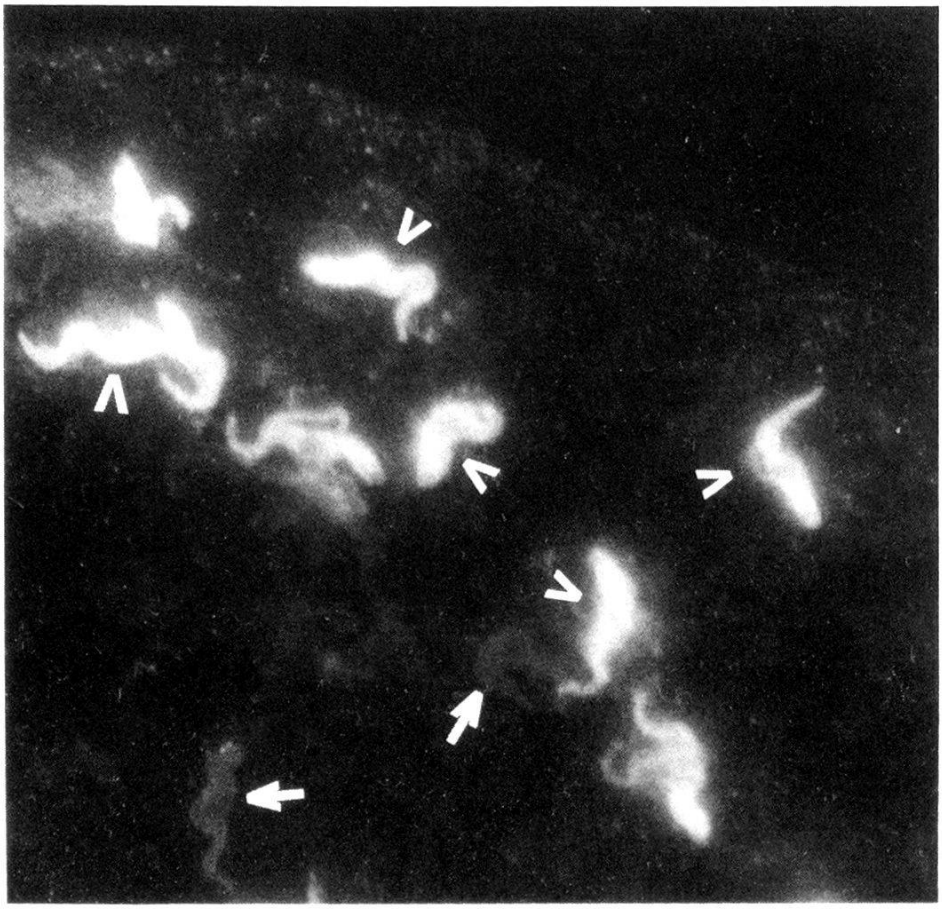

Fig. 1. Antigenic heterogeneity amongst *T.b. brucei* metacyclic trypanosomes (ILTat 2.1) as demonstrated by direct immunofluorescence using TRITC-labelled monoclonal antibody Tb3/38.10.2. Positive (>) and negative (→) trypanosomes are shown. Magnification: 500×.

stained a different percentage of the population of metacyclics from tsetse infected with ILTat 2.1. Double labelling studies confirmed that *T.b. brucei* metacyclics are antigenically heterogeneous with respect to their VATs. The percentage of metacyclics stained by each monoclonal antibody was markedly variable. When mixed together, however, the six monoclonal antibodies stained 80–90% of the metacyclic population extruded by any tsetse on any occasion. None of the monoclonal antibodies stained the bloodstream VAT (ILTat 2.1) which was initially ingested by tsetse. When metacyclics of ILTat 2.1 were compared with those of the original stock and another clone ILTat 2.2, they were all found to be antigenically similar. These results were consistent regardless of whether the two clones and the parent stock were transmitted by *G.m. morsitans* or *G.m. centralis*. However, metacyclics obtained from tsetse infected with a clone (STIB 367H) derived from a different stock (LUMP 227) were not stained by any of the six monoclonal antibodies (Table 1).

Tsetse infected with ILTat 2.1 were then allowed to feed on non-irradiated Balb/C mice and the trypanosome populations isolated from these mice were analysed for the possible presence of VATs that could be stained by the monoclonal antibodies. It was found that those populations isolated 48–72 h after

infection contained variable proportions of VATs that were stained by the monoclonal antibodies to metacyclics, whereas the population obtained 5 days after infection did not contain any such VATs (Table 2). Two monoclonal antibodies, Tb8/16.37.15 and Tb2/17.56.8, stained a higher percentage of trypanosomes from the early bloodstream forms than the metacyclics (Tables 1 and 2).

Discussion

The present work has shown that the metacyclic population of a serodeme of *T.b. brucei* is heterogeneous with regard to the VAT composition, in that each monoclonal antibody stained a different percentage of metacyclics. Despite the heterogeneity, however, the overall VAT composition of the metacyclics comprising this stock would appear to be constant and limited in number in that the six monoclonal antibodies stained 80–90% of the metacyclics extruded by any tsetse on any occasion. Thus even though the remaining 10–20% of the metacyclics of this serodeme could also be heterogeneous, the number of VATs in this fraction of the population may also be limited. A possible limitation of the number of VATs in *T.b. brucei* metacyclic populations is a tenable hypothesis in view of the evidence from immunization trials which has shown that protective immunity against *T.b. brucei* metacyclics can readily be induced in mice (Jenni and Brun, 1981) or in goats and cattle (Emery et al., 1980).

Bloodstream populations isolated 48–72 h from cyclically infected mice contained VATs that were recognized by the monoclonal antibodies against homologous metacyclics while the populations obtained 5 days post-infection did not contain any such VATs. These results show that some of the early bloodstream forms of *T.b. brucei* bear variable surface antigens that are identical to those of the corresponding metacyclics with regard to their surface epitopes. Similar observations have been reported by Jenni (1977a) who found that 10–20% of the bloodstream VATs isolated after 36 h from cyclically infected mice were recognized by infection serum against homologous metacyclics. Furthermore, antibodies to metacyclics have been demonstrated in infection sera from rabbits (Le Ray et al., 1978; Barry eta al., 1979) and cattle (Nantulya et al., in preparation) chronically infected with *T.(T.) brucei* bloodstream VATs, showing that some of the bloodstream VATs do indeed possess surface epitopes in their variable surface glycoproteins which are identical to the corresponding metacyclic trypanosomes. It is suggested that the emergence of bloodstream forms of trypanosomes which bear variable surface antigens that are similar to metacyclics is advantageous to the mammalian host in that it appears to allow the development of effective immunity against cyclical challenge even in animals exposed to syringe-passaged infections (Nantulya et al., in preparation).

The finding that none of the monoclonal antibodies against ILTat 2.1 metacyclics reacted with any of the metacyclics of Clone 367H, derived from a different stock, emphasizes the earlier conclusions that for a given *T.(T.) brucei*

serodeme the metacyclics are characteristic and unique with regard to the VAT repertoire (Jenni, 1977a, b). These results thus indicate that serological typing of metacyclics may offer an important tool for serodeme analysis.

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