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Comparisons of antigenic types of *Trypanosoma (T.) brucei* strains transmitted by *Glossina m. morsitans*

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Summary

A modified infection and maintenance procedure for the cyclical transmission of *T. (T.) brucei* in *Glossina m. morsitans* is described which produced high mature infection rates in the flies. Freshly extruded metacyclic forms and bloodstream forms were serologically typed, using the indirect fluorescent antibody test (IFAT) and the neutralization infectivity test (NIT). Metacyclic forms of a certain strain and its cloned derivative were antigenically homogenous and of the same antigenic type, whereas metacyclics of different strains were antigenically different. Antigenic variation had occurred in bloodstream forms of mice harvested 36 h after the infectious bite.

Key words

Trypanosoma (T.) brucei – *Glossina m. morsitans* – metacyclic forms – antigenic types.

Introduction

The early work of Broom and Brown (1940) has shown that there were close antigenic similarities between *T. brucei* populations of the same strain transmitted by different tsetse flies. The serological test used in this investigation was the red cell adhesion test. Gray (1965) confirmed these results by using the agglutination test. He found that during cyclical development in the tsetse fly, antigenic variants of a *T. brucei* strain tended to revert to a so-called basic strain antigen. In a recent study with *T. gambiense*, Gray (1975) showed that tsetse flies may transmit trypanosomes of more than one antigenic type. His results showed that the first antibodies produced by cyclically infected animals

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agglutinated more than one serotype antigen. The findings mentioned above derive from serological investigations using bloodstream forms as antigens.

On the other hand, metacyclic forms were serologically typed by Cunningham (1966) using the neutralization test. Cryopreserved metacyclic forms were the source of the antigens. Similar antigenic types have been demonstrated in metacyclic forms of one *T. rhodesiense* strain. Most recently, Honigberg et al. (1976) carried out an antigenic analysis on formalin fixed smears of salivary gland forms of *T. brucei*. The surface antigens of metacyclic forms were different from those of bloodstream forms obtained by injection of a salivary gland suspension into a mouse.

It was the aim of the present study to investigate different antigenic types of cyclically transmitted *T. (T.) brucei* strains and cloned derivatives. In order to obtain sufficient amounts of metacyclic forms for serological tests, attempts were made to raise the mature infection rates in *Glossina m. morsitans*.

Material and methods

1. *Trypanosoma (T.) brucei* strains

STIB 246 was originally isolated from a hartebeest in the Serengeti in 1971 and cryopreserved after one rat passage (Geigy and Kauffmann, 1973).

STIB 348 T is a clone derived from a single metacyclic form of STIB 348 which is a clone of STIB 246 (Geigy et al., 1975).

STIB 101 is a derivative of EATRO 1093 which was isolated from a sable antelope (Geigy et al., 1967).

STIB 366 A is a clone of Molteno S42/030 which was obtained from Dr. G. A. M. Cross, Cambridge (pertinent reference Cross, Klein and Linstead, 1975).

STIB 366 D is a clone derived from a single metacyclic form of STIB 366 A.

STIB 367 H is a clone from a single metacyclic form of LUMP 227 which was obtained from Dr. J. J. Doyle, WHO.

2. Laboratory animals

ICR white mice (20–25 g), SIV white rats (120–160 g) and white New Zealand rabbits (2–2.5 kg) were used throughout the experiments.

3. Tsetse flies

Pupae of *Glossina m. morsitans* were obtained from Dr. A. M. Jordan, Langford, Bristol.

4. Cyclical transmission through tsetse flies

Pupae were kept in sterile sand at 30° C and 80% r. h. in the dark. Teneral flies took the infective bloodmeal within 4 h after hatching (25° C, 80% r. h.). The teneral flies were fed on infected mice at first parasitaemic peak (about 50% stumpy forms). The skin surface of the mice was sterilized by cleaning with 70% alcohol. The engorged flies were immediately transferred to 20° C/80% r. h. and kept for 8 h. After that time the flies were brought to the fly room and maintained at 25° C and 80% r. h. Batches of 20 flies (both sexes) were kept in Geigy cages. The flies were subsequently fed on surface sterilized clean mice every day for 1 week. After that period, the flies were offered a bloodmeal every second day. The flies were transferred to cleansed cages (soaked in 70% alcohol) twice a week.

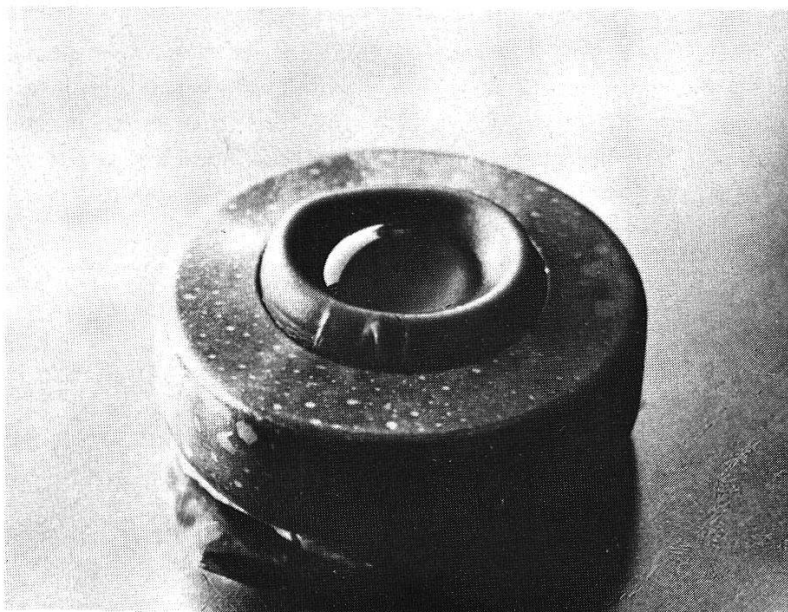


Fig. 1. Nescofilm covered capsule with drop of FCS/PSG solution for the harvest of saliva and metacyclic forms. Magn. $2,5\times$.

Infective flies were found by means of infected feeding mice. Single infective flies were detected by microscopical examination of unstained saliva probes using phase contrast. These positive flies were isolated and transferred to Geigy cages.

Freshly extruded metacyclic forms from single flies were harvested from saliva probes deposited in drops of fetal calf serum (FCS) diluted 1:1 with phosphate-buffered saline-glucose (PSG) 6:4, pH 8. Small drops of this solution were warmed up to 37°C on Nescofilm (Nippon Shoji Kaisha Ltd.), which was slightly stretched over rubber caps normally used for sealing of multidose glass vials. The Nescofilm was stretched over the concave side of the caps and fixed with a metal band in revealing a depression in which the FCS/PSG solution was warmed up (Fig. 1). Immediately after the flies had probed, the metacyclics were cooled to $0-2^{\circ}\text{C}$ and their number estimated.

Metacyclic forms from single flies could be repeatedly harvested during 4–10 weeks.

5. Clones from single metacyclic forms

Clones from single metacyclic forms were obtained as described for bloodstream forms of *T. congolense* by Schläppi and Jenni (1977). All clones used in this experiments were obtained from X-ray irradiated (600 rad) mice (pertinent reference see Schläppi and Jenni, 1977).

6. Antigens

Freshly extruded metacyclic forms as well as bloodstream forms obtained from mice 36 h after the infectious bite were used. The bloodstream forms were separated from blood using DEAE-cellulose (Lanham and Godfrey, 1970). This separation was done at $0-2^{\circ}\text{C}$ as well as the subsequent washing (4 times) by centrifugation in PSG 6:4, pH 8.

Culture forms of all the trypanosome strains were grown in SDM-77 (Brun and Jenni, 1977). The culture forms were air-dried and acetone fixed on glass slides.

7. Antisera

Antisera against metacyclic forms were obtained from mice, rats and rabbits as described by Jenni (1977). All antisera were inactivated at 56°C for 30 min.

8. Serological tests

Indirect fluorescent antibody tests (IFAT) were carried out in suspension using viable metacyclics or bloodstream forms (Jenni, 1977). The number of parasites incubated per test was 500–1000.

Parallel to the IFAT, the neutralization infectivity test (NIT) was used in order to detect neutralizing antibodies against metacyclics and bloodstream forms. The NIT was carried out as described by Schläppi and Jenni (1977). Approximately 1000 parasites were incubated per test. Sera dilutions of 1:2 and 1:10 were applied to the tests.

Results

Several attempts were made to raise the mature infection rates of *T. (T.) brucei* in *G. m. morsitans*. In modifying different parameters, the infection and maintenance procedure for tsetse flies described above was developed and revealed high mature infection rates as shown in Table 1. With this method, STIB 367H which seemed not to be transmissible through flies in earlier experiments could be cyclically transmitted and developed mature infections in 6.9% of flies infected as teneral flies. The duration of the cycle in the flies was very much reduced compared to data (average of 23 days) obtained previously in our laboratory using other infection methods. Flies infected with STIB 348T developed mature salivary gland infections 9 days after the infectious blood-meal which was the lowest value we have ever observed in our studies. On the other hand, the survival rate of the flies was increased and in most of the cages all flies survived for 40 days after which the first flies started to die.

The number of metacyclic forms extruded into the drop of the FCS/PSG solution ranged from 2000–3000 per fly. Epimastigote forms, which could easily be distinguished from metacyclic forms by their morphological appearance and movement, were sometimes also observed in saliva suspensions. Antibodies against surface antigens of metacyclic and bloodstream forms were detected with the IFAT in mice, rats and rabbits 6 days after the infectious bite(s). The antibodies were strain specific and no cross reactions with antigens from different strains occurred.

Animals which were bitten once by one infective fly, developed reciprocal antibody titers of 80–160 within 6 days. Control sera from uninfected animals sometimes showed positive reactions up to a reciprocal titer of 80. The base-line was therefore set at a titer of 160.

Compared to the relatively low antibody response induced by a single infective bite, mentioned above, the corresponding antibody titers were raised when animals were bitten by several flies (up to 15) on one or several (up to 3) successive occasions. In rats and mice which have been bitten twice (day 0 and day 2) by 10–15 infective flies each time, the antibody titers reached values of 1280 on day 9. When the same batches of infective flies were fed three times on rabbits, the corresponding antibody titers were 2560 on day 10.

The IFAT results obtained with rabbit antisera from rabbits challenged with metacyclics using this infection method are shown in Table 2. The results

Table 1. Mature infection rates obtained with *G. m. morsitans* infected with different *T. (T.) brucei* strains and cloned derivatives

Stabilate STIB	Total flies infected	Total flies infective	% infective	First infected fly detected after day
366A	110	23	20,9	13
366D	102	18	17,6	20
246	101	25	24,8	13
348T	91	40	44	9
101	96	15	15,6	11
367H	87	6	6,9	22

Table 2. IFAT in suspension: rabbit antisera (day 10) tested with viable metacyclic forms

Antigen Metacyclics STIB	Rabbit antisera 1:1280 against STIB				
	366A	366D	246	348T	101
366A	+	+	—	—	—
366D	+	+	—	—	—
246	—	—	+	+	—
348T	—	—	+	+	—
101	—	—	—	—	+

— = negative at antisera titer of 1:160

show that surface antigens of metacyclic forms of a certain strain and its cloned derivative were distinct from other strains and clones. No variant antigenic types in metacyclics of one strain could be detected with this IFAT technique. On the other hand, when the corresponding bloodstream forms (36 h) were tested with the same sera, only 10–20% of the trypanosomes showed immuno-fluorescence activity, whereas the remainder were negative.

All antisera were first tested with the IFAT against culture forms in order to detect common antibodies. Only those sera which reacted negatively with culture forms at a reciprocal titer of 320 were applied to the IFAT.

In parallel to the IFAT, NITs were carried out with the same rabbit antisera used for the IFAT. Neutralizing antibodies against metacyclic forms were found 10 days after the first infective bites. The results obtained with the NIT were similar to those of the IFAT. Fresh metacyclic forms of one strain and its cloned derivative were both neutralized by their corresponding homologous antisera (Table 3). These metacyclics were not neutralized with antisera raised against metacyclics of different strains and cloned derivatives. In one case (STIB 367H) fresh metacyclic forms as well as thawed metacyclics from cryo-

Table 3. *NIT*: rabbit antisera (day 10) tested with viable metacyclic forms

Antigen Metacyclics STIB	Rabbit antisera final dilution 1:10 against STIB					
	366A	366D	246	348T	101	367H
366A	+	+	—	—	—	—
366D	+	+	—	—	—	—
246	—	—	+	+	—	—
348T	—	—	+	+	—	—
101	—	—	—	—	+	—
367H	n.d.	n.d.	n.d.	n.d.	—	+ / —

n.d. = not done

+ = neutralized

— = not neutralized

+ / — = fresh metacyclics neutralized but thawed metacyclics from stabilates not neutralized

preserved populations were tested. Whereas the freshly obtained populations were all neutralized by the homologous antisera the thawed populations were not. The corresponding *NIT* with bloodstream forms revealed no neutralization even then when bloodstream forms were incubated with their homologous antiserum which did neutralize the respective metacyclic forms.

Discussion

It is difficult to identify the reasons why the fly infection procedure described above revealed the extremely high salivary gland infection rates. Many parameters are still not well understood. The first interesting point to mention is the fact that the parasite populations which infected our flies best were those which were chosen very shortly after the first parasitaemic peak (8–12 h) in the mouse, with about 50% stumpy forms or even less. We observed that the aged short stumpy forms which developed later, 20–48 h after the parasitaemic peak, died very shortly after the ingestion by the fly and no transformation to the midgut form took place. The second point was that the crop emptying of newly engorged flies was decelerated in response to the drop in temperature (25° C to 20° C) which is in correspondance to the findings of Moloo and Kutzua (1970). According to Harmsen (1973) the trypanosomes should stay at least for 1 h in the neutral environment of the crop, in order to carry out specific transformation steps. The third point concerns the surface sterilization of the feeding mice. We observed that those flies which developed mature infections were almost entirely free of midgut contaminations with bacteria and fungus, whereas negative flies mostly showed heavily contaminated proboscis, fore- and midgut.

The serological tests have shown that metacyclic forms of the different strains were antigenically different. The *NITs* indicated that the metacyclic populations of one strain and its cloned derivative were antigenically homoge-

nous. Minor antigenic variants could not be detected but one might assume they did not exist, for single and not neutralized metacyclic forms can develop a parasitaemia as has been shown by cloning experiments.

The comparisons of metacyclic forms and the corresponding bloodstream forms 36 h after the infectious bite showed that antigenic variation occurred within a short period of time in the mammalian host. The 10–20% of bloodstream forms which exhibited a positive immunofluorescence reaction, were most probably of the same antigenic type as the parent metacyclic forms.

According to these serological results obtained with fresh metacyclic forms, immunization experiments using metacyclic antigens followed by the challenge of an infective tsetse bite will be carried out.

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