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THE EFFECT OF NORMAL HUMAN SERUM ON TRYPANOSOMES OF DISTINCT ANTIGENIC TYPE (ETAT 1 TO 12) ISOLATED FROM A STRAIN OF *TRYPANOSOMA BRUCEI RHODESIENSE*

by

N. VAN MEIRVENNE, E. MAGNUS & P. G. JANSSENS

Summary — Clone populations of the Edinburgh Trypanozoon antigenic types ETat 1 to 12, derived from a strain of *T. b. rhodesiense*, were tested on their sensitivity to normal human serum. The trypanosomes of clone ETat 10 were serum-resistant. The other clones contained only a small minority of resistant individuals. These were shown to be ETat 10 contaminants. By selective elimination of the privileged variant, serum-resistant populatons of other antigenic types were obtained, suggesting that each variant of the strain may exist in two forms.

KEYWORDS : Trypanosoma brucei brucei, Trypanosoma brucei rhodesiense, Antigenic variation, Infectivity, Natural immunity.

Introduction

The taxonomic relationships of *T. brucei brucei*, which is by definition not infective to man, and the human-infective *T. brucei rhodesiense* have been a constant source of discussion. Until recently, the only means of determining the subspecific identity of *T. (T.) brucei* strains isolated from animal hosts, has been a test of infectivity in human volunteers. A promising indirect method, the blood incubation infectivity test (BIIT) has been introduced by Rickman and Robson (1970a, 1970b). This test depends on the principle that the infectivity of *T. b. brucei* and *T. b. rhodesiense* to susceptible laboratory animals is differentially affected by the trypanocidal action of human serum : trypanosomes of the former subspecies are neutralized, those of the latter are not. However, upon maintenance in laboratory animals, strains of proven *T. b. rhodesiense* may become serum-sensitive and give equivocal BIIT results (Geigy *et al.*, 1973, Targett and Wilson, 1973). Hawking (1973) showed that the serum-resistance of populations of *T. b. rhodesiense* often depends on a small minority of individuals.

The present paper reports investigations on the relationships between antigenic variation and sensitivity to human serum in a strain of *T. b. rhodesiense.*

Materials and Methods

Trypanosomes

The 12 ETat clone populations examined were obtained in mice by a few passages at 2 or 3-day intervals of the LUMP stabilates listed in figure 1. Detailed pedigrees of these stabilates have been published by Lumsden and Herbert (1975). Essentially, they are descendants of a strain of *T. b. rhodesiense*, isolated from *Glossina pallidipes* in Uganda (McNeillage *et al.*, 1969). The infectivity to man has been demonstrated by an accidental laboratory infection (Robertson and Pickens, 1975).

Tests with normal human serum

A pool of freshly collected serum from healthy adult individuals was prepared. It was used immediately or stored in small aliquots at — 80 °C and used within 2 weeks (at this temperature the trypanocidal activity remains unchanged for some months). The standard technique of testing for trypanocidal action was a serum-incubation-infectivity-test. Using heparinized blood of a heavily infected mouse, approximately 2.10^6 trypanosomes were suspended in 1 ml of serum and incubated at 36 °C for 5 hours. The survival rate of the incubated trypanosomes was checked under the miscroscope (phase contrast, 10 × 25) by screening 1,000 organisms. About 0.5 ml aliquots of the suspension were injected intraperitoneally into each of two mice. The blood of the animals was examined for trypanosomes daily.

Variant specific immunofluorescence

The antigenic variant composition of trypanosome populations was studied by means of immunofluorescence tests (Van Meirvenne *et al.*, 1975a).

Variant specific neutralization and infectivity tests

Selective elimination of the antigenic type ETat 10 from variant populations was accomplished by lytic neutralization using a specific 6-day antiserum from a clone-infected rabbit (Van Meirvenne *et al.*, 1975a). An infected heparinized mouse blood sample, containing about 2.10^6 trypanosomes, was suspended in 1 ml of a tenfold dilution of antiserum in complement-rich guinea-pig serum. The suspension was incubated at room temperature for 2 hours, examined under the microscope (phase contrast, 10 × 25), and injected intraperitoneally into two mice, each of which receiving about 10^6 incubated organisms. The blood of the mice was examined for trypanosomes daily.

Variant specific trypanolysis tests with human antisera

In 1974 a laboratory worker of the University of Glasgow who had been in contact with the clones ETat 2, 7, 10 and 12, was found to be infected with trypanosomes. Four serum samples from this patient, taken respectively

on the day of diagnosis and 3, 23, and 75 days later, were tested on the presence of lytic antibodies against trypanosomes of the 12 ETat clones. Tests were carried out in small « U »-plates (Microtiter, Cooke) using guinea-pig serum (complement) as diluent (Van Meirvenne *et al.*, 1975a). Briefly, 50 μl samples of serial twofold dilutions of antiserum (1/10 up to 1/2048) containing about 50,000 trypanosomes (10^6 per ml) were incubated at room temperature for two hours. The percentage of lysis was examined under the phase contrast microscope (10 × 25) by screening 1,000 organisms.

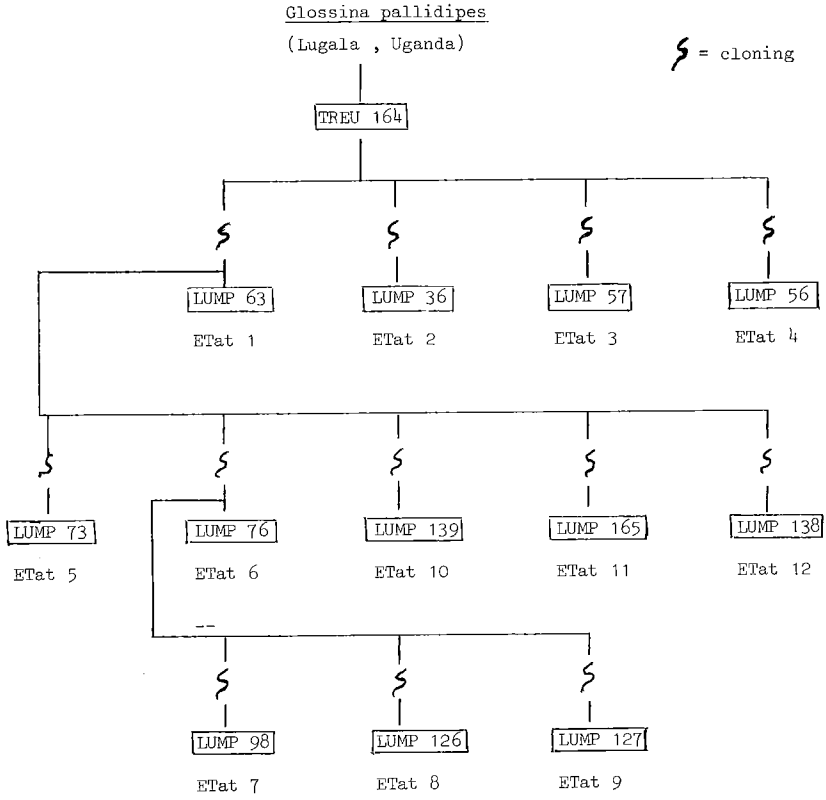

Figure 1

Simplified pedigrees of the ETat clone populations
(for details, see McNeillage, Herbert and Lumsden, 1969; Lumsden and Herbert, 1975).

Results

Sensitivity of the ETat clones to normal human serum

The results of the serum-incubation-infectivity-tests are shown in table 1. Nearly all trypanosomes of clone ETat 10 remained unaffected by human serum. In the incubated suspensions of clones ETat 9, 11, and 12, a small

minority of motile organisms was found, but most of these showed a vacuole in the cell region between the nucleus and the kinetoplast. In the preparations of clones ETat 1 to 8, no surviving trypanosomes were seen. However, all suspensions caused infection in mice. The first trypanosome population appearing in the blood of one of the mice of each test group was used for further experiments. These 12 populations were designated A 1 to A 12; details on their origin are given in table 1.

TABLE 1

The lytic and neutralizing effect of normal human serum upon the ETat clone populations and origin of the A-populations

Clone population examined (stabilate reference and number of supplementary mouse passages)	Results of incubation-infectivity tests			
	Survival *in vitro* (per 1,000)	Prepatent period (days, 2 mice)	A-populations Obtained on day	Code
ETat 1 (Lump 63/P_4)	0	5 - 5	6	A_1
ETat 2 (Lump 36/P_5)	0	4 - 6	6	A_2
ETat 3 (Lump 57/P_4)	0	3 - 4	5	A_3
ETat 4 (Lump 56/P_3)	0	4 - 4	5	A_4
ETat 5 (Lump 73/P_2)	0	4 - 4	5	A_5
ETat 6 (Lump 76/P_4)	0	3 - 3	5	A_6
ETat 7 (Lump 98/P_4)	0	3 - 4	5	A_7
ETat 8 (Lump 126/P_2)	0	3 - 4	5	A_8
ETat 9 (Lump 127/P_4)	3*	3 - 4	5	A_9
ETat 10 (Lump 139/P_2)	> 95 %	1 - 1	2	A_{10}
ETat 11 (Lump 165/P_2)	16**	3 - 4	5	A_{11}
ETat 12 (Lump 138/P_4)	1*	4 - 4	6	A_{12}

* all showing vacuole.
** 11 showing vacuole, 5 completely normal.

Properties of the populations A 1 to A 12

a) *Sensitivity to human serum.* More than 95 per cent of the trypanosomes of all the populations remained unaffected by human serum *in vitro*; infectivity tests were not done.

b) *Antigenic type of the organisms.* The results of the immunofluorescence tests are shown in table 2; all the populations contained more than 99 per cent of ETat 10; findings of other ETa-types were very exceptional.

c) *Infectivity tests following specific neutralization of ETat 10.* The object of these experiments was to set up variant populations from minor antigenic types, present in the A-populations. The results are shown in table 3. More than 99 per cent of the trypanosomes of all the A-populations were lysed by ETat 10-antiserum. Most of the suspensions however remained infective to mice. The resulting populations were designated B 1 to B 12.

TABLE 2

Antigenic variant composition of the A-populations from table 1 (immunofluorescence)

Population examined	Number of different ETat types per 1,000 trypanosomes*											
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
A 1	0	0	0	0	0	0	0	0	0	MT	0	0
A 2	0	0	0	0	0	0	0	0	0	MT	0	0
A 3	0	0	0	0	0	0	0	0	0	MT	0	0
A 4	0	0	0	0	0	0	0	0	0	MT	0	0
A 5	0	0	0	0	0	0	0	0	0	MT	0	0
A 6	0	0	0	0	0	0	0	0	0	MT	0	0
A 7	0	0	0	0	0	0	0	0	0	MT	0	0
A 8	0	0	0	0	0	0	0	0	0	MT	0	0
A 9	0	0	0	0	0	0	0	0	0	MT	0	0
A 10	0	0	0	0	0.2	0	0	0	0	MT	0	0
A 11	0	0	0	0	0	0	0	0	0	MT	0	0
A 12	0.6	0	0	0	0	0	0	0	0	MT	0	0

MT = major antigenic type, present for 99 per cent or more.
* = ratio determined by checking 2,000 or 5,000 organisms.

TABLE 3

The lytic and neutralizing effect of ETat 10-antiserum upon the A-populations and origin of the B-populations

Starting population	Neutralization-infectivity tests		B-populations	
	Survival *in vitro* (per 1,000)	Prepatent period (days, 2 mice)	Obtained on day	Code
A 1	0	5 - 5	6	B 1
A 2	0	4 - 5	6	B 2
A 3	0	5 - 5	6	B 3
A 4	0	4 - n.i.	7	B 4
A 5	0	n.i. - 5	7	B 5
A 6	0	n.i. - n.i.	—	—
A 7	0	4 - n.i.	7	B 7
A 8	0	4 - n.i.	7	B 8
A 9	0	5 - n.i.	7	B 9
A 10	0	5 - 5	7	B 10
A 11	0	n.i. - n.i.	—	—
A 12	0	5 - 5	6	B 12

n.i. = not infected.

a) *Sensitivity to human serum.* Before being tested on serum-sensitivity, the populations B 1, B 2, B 7, and B 12 had been sub-passaged in new mice for two days. All the other B-populations were tested immediately. Only *in vitro* tests were done. The results are shown in table 4. The populations B 2 to B 11 contained a large majority of serum-resistant trypanosomes. In the populations B 1 and B 12 however, about 80 per cent of the trypanosomes were serum-sensitive.

TABLE 4

The effect of human serum upon the B-populations *in vitro* (per cent of intact trypanosomes after 5 hours)

B 1*	B 2*	B 3	B 4	B 5	B 7*	B 8	B 9	B 10	B 12*
20	85	> 95	80	> 95	> 95	> 95	> 95	> 95	20

* Tested after one supplementary mouse passage of 2 days.

b) *Antigenic type of the organisms.* Trypanolysis tests with ETat 10-antiserum gave negative results. The antigenic variant composition, as judged by immunofluorescence tests, is shown in table 5. It may be seen that many populations were mixture of several ETa-types. In the populations B 4, B 9 and B 10, a large amount of unidentified variant types was present. The unexpected finding of ETat 10 in the population B 1 (0.5 per cent) may be explained by supplementary antigenic variation in the test mouse.

TABLE 5

Antigenic variant composition of the B-populations from table 3 (immunofluorescence)

Population examined	Number of different ETat types per 1,000 trypanosomes*											
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
B 1	5	900	1	35	0	0	0	0	0	5	0	0
B 2	0	0	92	330	0	0	556	0	0	0	0	0
B 3	0	0.4	0.6	0	MT	0	0	0	0	0	0	0
B 4	0	450	0.4	0.2	1	0	30	0	0	0	0	70
B 5	0	0.4	0	0	370	470	0	0	0	0	0	0.2
B 7	0	0	0	0	0	950	0	0	0	0	0	0.4
B 8	0	0.2	0	0.4	MT	0	0	0.2	0	0	0	0
B 9	0	1	0.2	0	0	0	0	0	0	0	0	0
B 10	0	1	360	2	90	0	0	0	0	0	0	100
B 12	140	580	90	150	0	0	0	0	0	0	0	0

MT = major antigenic type, present for about 99 per cent.
* = ratio determined by checking 200 to 5,000 organisms.

The protective effect of normal human serum in infected mice

Two mice, infected since 3 days with trypanosomes of clone ETat 9 (4th passage of stabilate LUMP 127), and showing a parasitaemia of about 40.10^6 trypanosomes per ml of blood, were injected intraperitoneally with respectively 0.5 and 1 ml of normal human serum. On the next day, only a few trypanosomes per microscopic field were seen in the wet blood film preparations from both animals. The parasitaemia then quickly rose again. On the 6th day after infection, the 2 relapse populations were examined by immunofluorescence tests. Once again serum-resistant ETat 10 was found to be present for more than 99 per cent.

Trypanolysis tests with human antisera

The results of these experiments (table 6) strongly suggest that the antigenic type that caused the human infection was ETat 10 and that some other variants (ETat 2, 4, and 7) appeared later on. Two normal human sera, tested in the same conditions, gave entirely negative results.

TABLE 6

Lytic antibody titers in sera from the patient (B. L.), infected with ETtat trypanosomes

Serum samples examined	Titer of lytic antibodies against twelve ETat types											
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
B. L. 1	0	0	0	0	0	0	0	0	0	320*	0	0
B. L. 2	0	0	0	0	0	0	0	0	0	640*	0	0
B. L. 3	0	40*	0	10*	0	0	160*	0	0	320*	0	0
B. L. 4	0	10*	0	0	0	0	10*	0	0	40*	0	0
Normal 1	0	0	0	0	0	0	0	0	0	0	0	0
Normal 2	0	0	0	0	0	0	0	0	0	0	0	0

0 = negative result (less than 5 per cent of lysis at all dilutions).
* = lytic end-titer of positively reacting serum (endpoint : 95 per cent).

Discussion

In terms of antigenic variation, the 12 ETat variants represent predominant antigenic types of their particular serodeme, i.e. they appear at an early stage of infection. The clone populations of each of them contain a small minority of the heterologous members of the series (Van Meirvenne *et al.*, 1975b). The present results indicate that nearly all the trypanosomes of clone ETat 10 are resistant to normal human serum whereas the partly resistance of the 11 other clones depends on the presence of ETat 10 contaminants.

In view of the characteristics of the B-populations, listed in table 5, and of the human antisera (table 6), it must be assumed that several ETa-types may exist in a human-serum-resistant « rhodesiense » (r) and a human-serum-sensitive « brucei » (b) form. As compared with its partners, ETat 10 shows a marked propensity to produce r-forms in mice. One possibility is that the r and b individuals of a given antigenic type differ in their infectivity to mice, so that one form may be overgrown by the other. Alternatively, both forms may arise with very different frequencies depending on the antigenic type of the organism, a switch from one antigenic type to another being perhaps prerequisite for a turn-over of the b or r character of a trypanosome. In order to check the validity of each of these theories clone populations of serum-resistant ETat 2 and ETat 6 have recently been prepared.

In the light of the present observations, the pertinent question arises whether *T. brucei rhodesiense* in its natural animal hosts also produces trypanosomes, differing in sensitivity to human serum. It is even thinkable that « rhodesiense » forms are more frequently produced in some natural or experimental host species than in others.

It seems almost impossible to prove that a strain of *T. (T.) brucei*, which has been isolated from animal hosts, is entirely sensitive to human serum, i.e. that it belongs to *T. brucei brucei*. The least one can do is to check a large number of different antigenic variant populations. In this connection, it must be emphasized that the « rhodesiense » identity of the ETat serodeme would not have been detected quickly if ETat 10 had been an exceptional antigenic type. Moreover, large samples of each variant population should be tested. For this purpose, mouse protection tests, carried out at peak parasitaemia, seem more valuable than the incubation-infectivity tests currently used.

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Samenvatting — **De invloed van normaal mensenserum op trypanosomen van verschillend antigeentype (ETat 1 tot 12), afkomstig van een stam van** *Trypanosoma brucei rhodesiense.*

Kloonpopulaties van de Edinburgh Trypanozoon antigeentypen ETat 1 tot 12, afkomstig van een stam van *T. brucei rhodesiense,* werden getest op hun gevoeligheid aan normaal mensenserum. De kloon ETat 10 was nagenoeg volledig resistent. Alle andere kloons bevatten een kleine minderheid serum-resistente trypanosomen van het antigeentype ETat 10. Na selektieve verwijdering van de bevoorrechte variant werden ook serumresistente populaties van andere antigeentypen bekomen, wat laat vermoeden dat elke variant van de bestudeerde stam in twee verschillende vormen kan voorkomen.

Résumé — **Effet du sérum humain normal sur des trypanosomes de type antigénique différent (ETat 1 à 12), provenant d'une souche de** *Trypanosoma brucei rhodesiense.*

Des populations clônées des types antigéniques « Edinburgh Trypanozoon », ETat 1 à 12, provenant d'une souche de *T. brucei rhodesiense,* ont été examinées pour leur sensibilité au sérum humain normal.

Le clône ETat 10 a montré une résistance presque complète.

Tous les autres clônes contenaient une petite minorité d'organismes sérum-résistants du type ETat 10.

Par élimination sélective du variant privilégié, des populations sérum-résistantes d'autres types antigéniques ont été obtenues. Les résultats suggèrent que chaque variant de la souche peut se présenter sous deux formes différentes.

P. G. Janssens (Director), N. Van Meirvenne and E. Magnus : Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium.
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