The Antigenic Relationship of Strains of *Trypanosoma brucei* Isolated in Nigeria

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SUMMARY

The relationships of eleven strains of *Trypanosoma brucei* were determined by agglutination tests with antisera to the basic and predominant antigens of the strains to assess (a) the relative values of these antigens for classification purposes, and (b) the antigenic diversity of *T. brucei* in Nigeria. The indications of the antigenic relationships of the strains obtained by comparisons based on both types of antigens were very similar, but comparisons of predominant strain antigens were easier to make and were applicable to more strains than were comparisons of basic strain antigens. Clones prepared from four strains isolated in one place in Nigeria were antigenically related, but clones of two other strains from the same area were antigenically distinct. Six strains from widely separated localities were antigenically different and produced few antigens in common.

INTRODUCTION

The numbers of antigenically distinct strains of the common species of pathogenic trypanosomes which can be isolated in the African continent, or in any well-defined geographical locality, have not yet been determined. The collection of such epidemiological information is still generally limited by the variability of trypanosomal antigens and, particularly in the cases of *Trypanosoma vivax* and *T. congolense*, by inadequate methods for isolating and growing strains in the laboratory. Knowledge of the properties of the antigens of *T. brucei*, *T. rhodesiense* and *T. gambiense*, which comprise the 'brucei' subgroup of trypanosomes (Hoare, 1957), has been greatly increased by several recent studies (e.g. Soltys, 1957; Weitz, 1960; Brown & Williamson, 1962; Cunningham & Vickerman, 1962; Seed, 1963), and work on antigenic variation has indicated two ways in which it may be possible to type the more pathogenic strains of these species (Broom & Brown, 1940; Gray, 1965*a*).

First, Broom & Brown (1940) found that although extensive antigenic variation occurred in a strain of *Trypanosoma brucei* in different infected animals, the trypanosomes transmitted by tsetse flies which had fed on such animals were serologically closely related. It has subsequently been shown that such cyclically-transmitted substrains of a strain have at least one antigen in common which has been called the *basic antigen* of the strain (Gray, 1965*a*). Preliminary results have shown that the basic antigens of strains from different localities are antigenically distinct (Broom & Brown, 1940; Gray, 1963), and it has been proposed that they may provide the basis for a serological comparison of strains (Brown, 1963; Gray, 1965*a*).

Secondly, each strain of *Trypanosoma brucei* also produces a group of variant antigens which have been described as *predominant antigens*. These trypanosomal antigens develop at an early stage of infection in animals infected with a strain either by tsetse flies or with strain variants transmitted by syringe (Gray, 1965 *a, b*). The serological specificity of the predominant antigens of different strains has not yet been determined, but if they are strain specific they may provide a means of classifying strains which would avoid the difficulties associated with transmitting trypanosomes through tsetse flies (see Fairbairn & Culwick, 1950). It might also be possible to determine, at least in part, the antigenic relationships of many strains of low virulence, from which antigens for serological tests can only be prepared with considerable difficulty, by testing antisera to such strains for antibodies to predominant antigens of more virulent strains.

This paper describes experiments in which the serological relationships of eleven strains of 'brucei' subgroup trypanosomes were determined by comparing their basic and predominant antigens to explore these potential means of classification and to assess the degree of antigenic diversity of *Trypanosoma brucei* in Nigeria.

METHODS

Strains of trypanosomes

The strains consisted of polymorphic 'brucei' subgroup trypanosomes isolated from domestic livestock by injecting infected blood into rats. With the exceptions of strains 17/8 and 17/58 (see Table 1), the trypanosomes were very pathogenic for mice and rats, causing acute infections, massive parasitaemias and death after 2–3 days. The strains had all been subpassaged by syringe in mice and rats several times between original isolation and their selection for use in the present work. Stocks of the strains were preserved at -80° during the experiments and further passage in mice and rats was limited to the procedures involved in preparing antigens for agglutination tests, agglutinating antisera, and in some instances, clones.

Two groups of strains were used in experiments.

(*a*) *Strains from one area*. Six strains were isolated from different animals during a survey of the incidence of trypanosomiasis in a herd of cattle kept for several years on a Government Farm at Raav, in Benue Province, Northern Nigeria (Fig. 1). Clones were prepared from each of these strains by the method of Inoki (1960) and used to assess the antigenic diversity of *Trypanosoma brucei* in a small area.

(*b*) *Strains from different areas*. Six strains, one of which (17/m) was also used in the preceding group, were obtained from different hosts infected in widely separated places in Nigeria (Fig. 1). Clones prepared from two of these strains, and ordinary populations of trypanosomes chosen at random from rodent-passaged lines of the other four strains, were used in experiments to assess the antigenic diversity of *T. brucei* in different areas of Nigeria.

Species of the trypanosomes. Although there have been several recent reports of attempts to differentiate between the three morphologically identical species *Trypanosoma brucei*, *T. gambiense* and *T. rhodesiense* by cultural, drug-sensitivity and physico-chemical tests (Lehmann, 1964 *a, b*), it is still generally accepted that *T. brucei* can only be distinguished from the other two species with certainty by its

inability to infect man (Ashcroft, 1959). This requirement was satisfied with only one of the eleven strains used; strain 8/18 was non-infective for human volunteers (Godfrey, 1962), and was therefore an authentic strain of *T. brucei*. It may be assumed on epidemiological grounds, however, that the other ten strains were *T. brucei* because they were all isolated from domestic animals in Nigeria, where *T. rhodesiense* does not occur and *T. gambiense* has not been isolated from any naturally-infected vertebrate host other than man.

Fig. 1. Map of Nigeria showing places where strains of *Trypanosoma brucei* were isolated.

Determination of the antigenic relationships of the strains

The antigenic relationships of the strains were determined by agglutination tests using the method of Cunningham & Vickerman (1962) with minor modifications (Gray, 1962).

Antigens for agglutination tests. Attempts were made to infect *Glossina morsitans* and *G. palpalis* with nine of the strains to obtain trypanosomes with basic antigens for use as antigens in agglutination tests. No attempt was made to infect tsetse flies with the clones prepared from strains 17/8 and 17/58 because it was impossible to collect sufficient trypanosomes for agglutination tests on account of the low virulence of the strains for mice.

Glossina morsitans were infected with six of the nine strains and, in these instances,

antigens for agglutination tests were prepared by the method of Cunningham & Vickerman (1962), from cyclically-transmitted trypanosomes isolated during the first 5 days of infections from animals infected by the tsetse flies; these trypanosomes by definition thus possessed basic antigens of the strains. In the other three instances, cyclical transmission was not achieved and antigens for agglutination tests were prepared from syringe-passaged lines of trypanosomes in the hope that they possessed predominant antigens of the strains. The nature of the population of each strain from which an antigen was prepared is shown in Table 1.

Agglutinating antisera. Agglutinating antisera were prepared in rabbits as follows and stored at -20° until required.

(a) *Antisera to strains from one area.* Antisera to the strains isolated from the cattle at Raav were prepared in two ways.

Method 1. Tsetse flies infected with the four more pathogenic clones (17/14, 17/30, 17/70, 17/96) were each fed once on separate rabbits from which sera were collected before infection and afterwards at intervals of 2–3 days for a month. Agglutination tests showed that sera obtained from these animals 10 days after they were bitten by infective flies had high titres of agglutinins to the basic antigens of the infecting trypanosomes.

Method 2. Suspensions of trypanosomes of the six clones were prepared from the blood of infected mice and used to infect rabbits. Each rabbit was given approximately 10 million trypanosomes of one of the clones as a single intravenous injection in 2.0 ml. of Alsever solution. Sera were collected from the rabbits before infection and afterwards at weekly intervals for a month. Thus, although it was not possible to prepare antigens for agglutination tests from clones 17/8 and 17/58 because of their low virulence for mice, enough trypanosomes were obtained to infect rabbits and thus to prepare antisera. Agglutinating antibodies to the variant antigens of trypanosomes persist at a high value in infected animals for several weeks and antisera obtained 3–4 weeks after infection contained antibodies to many of the antigens of the infecting strain including its predominant antigens.

(b) *Strains from different areas.* Antisera to the predominant antigens of this group of strains were prepared as described in *Method 2* above.

RESULTS

The antigenic relationships of strains from one area

The clones prepared from four of the six strains isolated in one area were antigenically related to each other (Table 2). The cyclically-transmitted trypanosomes of clones 17/14 and 17/30 behaved in a similar manner when agglutinated with antisera obtained from rabbits 10 days after infection by tsetse flies infected with the clones; these antisera contained antibodies to the basic antigens of the trypanosomes. The cyclically-transmitted trypanosomes of clones 17/70 and 17/96 differed from each other, and from those of clones 17/14 and 17/30, since 10-day antisera from rabbits infected by tsetse flies with clones 17/70 and 17/96 reacted mainly with the homologous trypanosomes. Although there were at least three antigenic types of trypanosomes in the cyclically-transmitted populations of clones 17/14, 17/30, 17/70 and 17/96, the clones were all able to produce similar antigens, because antisera obtained from rabbits 4 weeks after infection agglutinated the infecting clone and the three

Table 1. *The origins and characteristics of strains of Trypanosoma brucei*

Group of strains	Identity of strains	Origin of strains (see Fig. 1)	Original host	Date isolated	Passages in rats and mice between isolation and use in experiments	Population of strain used in experiments
Strains from one area	17/14	Raav	Ox	28. xi. 63	15	Cyclically-transmitted clone
	17/30	Raav	Ox	28. xi. 63	14	Cyclically-transmitted clone
	17/70	Raav	Ox	28. xi. 63	13	Cyclically-transmitted clone
	17/96	Raav	Ox	28. xi. 63	14	Cyclically-transmitted clone
	17/8	Raav	Ox	28. xi. 63	15	Syringe-passaged clone†
	17/58	Raav	Ox	28. xi. 63	14	Syringe-passaged clone†
Strains from different areas	969	Mokwa	Sheep	22. v. 61	19	Cyclically-transmitted line
	3/749	Funtua	Ox	22. iii. 61	c. 170	Syringe-passaged line
	8/18*	Nsukka	Pig	12. ix. 62	13	Cyclically-transmitted line
	17/14	Raav	Ox	28. xi. 63	15	Cyclically-transmitted clone
	18/182	Damaturu	Ox	8. ii. 64	8	Syringe-passaged clone
	19/170	Ilorin	Ox	24. iii. 64	8	Syringe-passaged line

* Authentic *Trypanosoma brucei*; † antigen not prepared.

heterologous clones. The antigenic similarities of clones 17/14, 17/30, 17/70 and 17/96 were confirmed by the reactions of antisera taken from rabbits 2 and 4 weeks after they had been infected with the clones by syringe (Table 3).

The clones prepared from strains 17/8 and 17/58 were not closely related to clones 17/14, 17/30, 17/70 and 17/96 because antisera from rabbits infected by syringe with clones 17/8 and 17/58 did not react strongly with the antigens of any of the other four clones (Table 3).

Table 2. *Agglutination of clones of Trypanosoma brucei prepared from strains isolated in one area in Nigeria by antisera from rabbits infected with the clones by tsetse flies*

Antigens prepared from clones	Titres of agglutinins in antisera to clones (as reciprocals of serum dilutions)			
	17/14	17/30	17/70	17/96
antisera obtained 10 days after infection				
17/14	160	2560	80	—
17/30	80	1280	160	10
17/70	—	40	5120	—
17/96	—	—	—	320
antisera obtained 4 weeks after infection				
17/14	160	1280	160	320
17/30	320	1280	80	160
17/70	320	640	640	640
17/96	160	80	40	2560

— = no agglutination.

Table 3. *Agglutination of clones of Trypanosoma brucei prepared from strains isolated in one area in Nigeria by antisera from rabbits infected with the clones by syringe*

Antigens prepared from clones	Titres of agglutinins in antisera to clones (as reciprocals of serum dilutions)					
	17/14	17/30	17/70	17/96	17/8	17/58
antisera obtained 2 weeks after infection						
17/14	5120	5120	640	5120	—	—
17/30	5120	2560	640	2560	—	—
17/70	20	20	1280	80	80	40
17/96	10	20	10	5120	20	—
antisera obtained 4 weeks after infection						
17/14	1280	5120	80	640	—	—
17/30	640	2560	80	640	—	—
17/70	320	320	160	160	40	10
17/96	320	—	160	2560	20	—

— = no agglutination.

The antigenic relationships of strains from different areas

The strains of *Trypanosoma brucei* from widely separated areas in Nigeria had few antigens in common. Antisera obtained from rabbits after 2 weeks of infection, which had high titres of agglutinins to the infecting strain, did not react with, or had much lower titres to, other strains (Table 4). Sera obtained from the rabbits 1–2 weeks later, when there had been ample time for antigenic variation in the strains

and for antibody production to predominant variant antigens, also, in general, did not react strongly with the antigens of heterologous strains. In each case the titre of agglutinins to the homologous strain had decreased and a noteworthy increase of antibody titre to a heterologous strain occurred in only one instance. The rabbit infected with strain 969 produced a high titre of agglutinins to strain 19/170, indicating that an antigen related to one of those of strain 19/170 had developed during the infection.

Table 4. *Agglutination of strains of Trypanosoma brucei isolated in different places in Nigeria by antisera from rabbits infected with the strains by syringe*

Antigens prepared from strains	Titres of agglutinins in antisera to strains (as reciprocals of serum dilutions)					
	969	3/749	8/18	17/14	18/182	19/170
antisera obtained 2 weeks after infection						
969	5120	—	—	—	—	160
3/749	160	5120	—	—	—	—
8/18	—	—	5120	—	—	—
17/14	80	—	—	5120	—	160
18/182	—	—	—	—	1280	—
19/170	—	—	80	—	10	1280
antisera obtained 3–4 weeks after infection						
969	320	—	—	20	—	10
3/749	40	1280	—	80	—	—
8/18	—	—	640	—	—	—
17/14	160	—	40	1280	—	320
18/182	—	—	—	—	160	—
19/170	640	—	40	10	—	160

— = no agglutination.

DISCUSSION

Although there are many difficulties associated with transmitting 'brucei' subgroup trypanosomes by tsetse flies in the laboratory (see Fairbairn & Culwick, 1950), comparisons of the basic antigens of strains, which are associated with cyclically-transmitted trypanosomes, may yet prove to be a useful method of determining strain relationships. It was found by this means that four clones prepared from trypanosomes isolated from different animals in one herd were antigenically related but not identical, and that three strains of trypanosomes isolated in different places in Nigeria were antigenically distinct. The former result supports the idea expressed earlier (Gray, 1965*a*), that it may be possible to trace strains in epidemiological studies by means of their basic antigens.

Comparisons based on the predominant antigens of trypanosomes, however, appear to offer a more practical and easier means of general classification than comparisons which depend on basic strain antigens. In every instance, indications of antigenic relationships of strains of *Trypanosoma brucei* from comparisons of their basic antigens were fully confirmed by agglutination tests with antisera to their predominant antigens, showing that predominant antigens, as well as basic antigens, are characteristic for each strain. It was also possible to determine, on the basis of predominant antigens, the antigenic relationships of three strains which could not be transmitted by tsetse flies and those of two strains which caused only

mild infections in mice. It became apparent when studying the relationships of four antigenically similar clones, that more work must be done to develop methods of finding whether strains which are closely related in terms of agglutination tests are antigenically identical or whether they only produce a number of variant antigens in common.

In view of the established capacity of trypanosomes for antigenic variation, and the numerous different antigens a strain can produce (e.g. Ritz, 1916; Osaki, 1959), it was of considerable interest that clones prepared from four strains of *Trypanosoma brucei* isolated in one area were antigenically related. This result is similar to those of Cunningham & Vickerman (1962) and Weitz (1962) who found that several strains of *T. brucei* isolated in South East Uganda had antigens in common. There may thus be some hope of success when attempts are made to immunize animals against a single trypanosomal species in a restricted area. In contrast to these findings, strains of *T. brucei* from widely separated places in Nigeria showed little antigenic relationship. Other authors have also found considerable antigenic differences between strains of *T. brucei* from different geographical localities (Broom & Brown, 1940), and the antigenic dissimilarity of the strains used in the present experiments is supported by experience in the field where it is generally recognized that strains encountered in different areas are liable to be immunologically distinct (see Hornby, 1941).

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