Antigenic relatedness of stocks and clones of *Trypanosoma vivax* from East and West Africa

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(*Accepted 11 May 1989*)

SUMMARY

The antigenic relationships of 7 stocks and 7 clones of *Trypanosoma vivax* from East and West Africa were compared by immune lysis. Sera from goats infected with different stocks and clones of *T. vivax*, collected on days 40 and 80 after infection, were used in the immune lysis test with homologous and heterologous stocks and clones of trypanosomes. Sera from infected cattle were included to compare stocks and clones from Kenya. The parasites that were used as antigen in the immune lysis tests were collected from infected mice when variable antigen type (VAT) homogeneous populations were used, from goats for infection with stocks and clones from Nigeria, The Gambia and Uganda, and from cattle for Kenyan stocks. Reciprocal cross-reactivity between sera and parasites was found between all the stocks and clones from Nigeria and The Gambia with the exception of one clone from Nigeria that was not recognized by antisera to a clone from The Gambia. There was also cross-reactivity between a stock and clone from Uganda and stocks and clones from Nigeria and The Gambia. Sera from goats infected with stocks and clones from Nigeria, The Gambia and Uganda recognized parasite populations that were homogeneous for one VAT (ILDat 1.2) of the rodent infective stock from Nigeria. Some antisera to West African stocks recognized another stable variant from a Ugandan stock adapted to rodents (ILDat 2.1), indicating that these VATs were expressed in the repertoires of the heterologous stocks. There was no cross-reaction between stocks from Nigeria, The Gambia or Uganda with Kenyan stocks. A stock from Galana (Kenya) and Bamburi (Kenya) showed reciprocal cross-reactivity. Two other Kenyan stocks, from Kilifi and Likoni, also showed cross-reactivity by immune lysis but showed no antigenic relationship with the other Kenyan stocks.

Key words: *Trypanosoma vivax*, stocks, clones, antigenic relationships, cross-reactivity.

INTRODUCTION

The African trypanosomes demonstrate an extensive capacity for antigenic variation of their major cell surface protein. They can elaborate large numbers of antigenic variants which are generally restricted to the antigenic repertoire (or serodeme) in which they are found (Gray & Luckins, 1976). In *T. congolense*-infected cattle it was shown that after primary infection followed by treatment, the cattle could resist challenge with a clone that belonged to the same serodeme as the infecting clone but not with a clone from an unrelated serodeme (Akol & Murray, 1983).

In *T. vivax* infections this approach to determining relationships between serodemes has been more difficult to realise. Investigations in goats that were infected with *T. vivax*, demonstrated that these hosts could not resist challenge with the same clone, or a clone of the homologous serodeme, after termination of the primary infection by chemotherapy (De Gee, Shah & Doyle, 1980; Emery, Moloo & Murray, 1987; Vos, Moloo & Gardiner, 1988). Cattle that had been infected with *T. vivax* stocks, however, and which had self-cured their infection, subsequently resisted challenge with the homologous stocks (Barry, 1986; Nantulya, Musoke & Moloo, 1986) and, in one case, resisted challenge with a stock from a different geographical locality (Nantulya *et al.* 1986) demonstrating the serodeme identity of those two stocks.

Dar, Paris & Wilson (1973) have demonstrated serological cross-reactivity between populations of *T. vivax* from Kenya and Uganda derived by inoculation of cryopreserved tsetse proboscides into cattle. Sera of naturally infected cattle from Zambia have also been demonstrated to possess low lytic antibody titres to 4 Nigerian *T. vivax* stocks and an East African stock (Murray & Clarkson, 1982), and the sera of naturally infected cattle from Kenya lysed parasites from a Nigerian stock (Barry, 1986). This degree of cross-reactivity between parasite populations from different parts of Africa has led to the supposition that the number of antigenic types of *T. vivax* existing within Africa may be relatively limited and that immunoprophylaxis against this species of trypansome might be possible (Murray & Clarkson, 1982; Barry, 1986).

T. vivax is mainly restricted to ruminant hosts, in which only low levels of parasitaemia are usually attained. However, we have been able to establish some clones of the parasite in goats, in mice or *in vitro*. In the present study we sought to evaluate further the extent of the antigenic relatedness of *T. vivax* stocks, by comparing the reactivity of stocks and clones of *T. vivax* from East and West Africa with sera from goats or cattle infected with the same populations.

Table 1. Antigens and sera employed in the immune lysis test

Trypanosoma vivax	Trypanosome population isolated for antigen		Antisera taken	
	From	On day	From	On day
IL 2160	Mice	3	Goats	12
ILDat 1.2	Mice	5	Goats	40, 80
ILDat 2.1	Mice	3	Goats	41, 81
IL 2946	Goat	8	Goats	21, 40
IL 3185	Goat	9	Goats	40
IL 3184	Goat	8	Goats	40, 80
IL 3200	Goat	8	Goats	40
IL V-25	Goat	7	Goats	41, 80
IL 2133	Steer	13	Goats	42, 80
IL 2969	Steer	12	Goats/cattle	42, 80/56
IL 1875	Steer	14	Goats/cattle	42, 83/21, 42
IL 3060	Steer	24	Goats/cattle	48/51
IL 2337	Steer	14	Goats/cattle	42, 82/41, 80

MATERIALS AND METHODS

Experimental animals

Adult castrated male East African cross-bred goats and male Boran and Ayrshire cattle, 6 months of age, were obtained from areas in Kenya known to be free of trypanosomiasis and were kept in fly-proof isolation facilities.

Male and female C3H/He mice, aged between 4 and 8 weeks, were obtained from the ILRAD breeding colony. Before infection, mice were exposed to 650 rad. of gamma-irradiation from a ^{137}Caesium source.

Trypanosomes

The following *T. vivax* stocks were used. IL 2160 is a derivative of Y486 isolated from an infected Zebu cow in Zaria, Nigeria (Leeflang, Buys & Blotkamp, 1976). ILDat 1.2 is a clone of bloodstream forms derived after several passages from Y486 (Barry & Gathuo, 1984). IL 2946 is a derivative of EATRO 1721, isolated from a naturally infected Zebu in Nigeria (Gardiner, Thatthi & King, 1988). IL 3185 was cloned in a goat from IL 2946 (Vos, unpublished results). IL 3184 was cloned in a goat from an isolate obtained from slaughterhouse material (kindly donated by Professor B. Ikede) in Ibadan, Nigeria. IL 3200 was cloned from IL 3171 (Vos, unpublished results), originally isolated from a naturally infected N'Dama in Buruku, The Gambia (kindly donated by Dr R. Dwinger, International Trypanotolerance Centre). IL V-25 is a derivative of EATRO 1262, isolated from a naturally infected Zebu in Lugala, Uganda (Gathuo, Nantulya & Gardiner, 1987) and ILD 2.1 (Gardiner & Clarke, 1987) is a clone from this stock. IL 3060 was isolated from a naturally infected Friesian cow in Bamburi, Kenya (Schonefeld, Rottcher & Moloo, 1987). Il 2133 was isolated from a naturally infected cow in Kilifi, Kenya (Nantulya *et al.* 1986) and IL 2969 is a clone derived in culture from this stock (Vos *et al.* 1988). IL 1875 is a derivative of KETRI 2375, isolated from a naturally infected cow in Likoni, Kenya (Nantulya *et al.* 1986). IL 2337 is a derivative of KETRI 2430, isolated from a naturally infected cow in Galana, Kenya (Nantulya *et al.* 1986).

Immune lysis test (ILT)

The immune lysis test was performed as described by Barry & Gathuo (1984). The trypanosomes that were used in this test were isolated from infected mice, goats or steers during the first peak of parasitaemia as indicated in Table 1. The immune lysis test was carried out at a standard serum dilution of 1:5 and the results read after 30 min at room temperature. The number of lysed parasites/100 total parasites was counted. Lysis by pre-infection sera did not exceed 3%.

Experimental design

The parasites and sera employed in this study are detailed in Table 1. Groups of 4 goats were infected intravenously with stocks and clones of *T. vivax* from West Africa and by tsetse with East African stocks. For the tsetse infection, teneral male *Glossina morsitans centralis* from the ILRAD colony were infected by feeding on an infected steer. Twenty-five days after the initial feed the tsetse with mature infections were identified by examination of salivary probes, and 20 tsetse were used to infect each experimental animal.

Sera of infected goats were collected as indicated in Table 1 and used in the immune lysis test. The sera were tested against trypanosome populations of

Table 2. Results of immune lysis tests between sera of goats infected with stocks or clones of *Trypanosoma vivax* and homologous and heterologous parasites

Goat sera from infections initiated by	*T. vivax* stock used as antigen						
	IL 2160 Nigeria Zaria	IL 2946 Nigeria	IL V-25 Uganda Lugala	IL 2133 Kenya Kilifi	IL 1875 Kenya Likoni	IL 3060 Kenya Bamburi	IL 2337 Kenya Galana
IL 2160 (Nigeria)	+++*	++	0	0	0	0	0
IL 2946 (Nigeria)	++	+++	0	0	0	0	0
IL V-25 (Uganda)	++	++	+++	0	0	0	0
IL 2133 (Kenya)	0	0	0	++	+	0	0
IL 1875 (Kenya)	0	0	0	0	0	0	0
IL 3060 (Kenya)	0	0	0	0	0	0	0
IL 2337 (Kenya)	0	0	0	0	0	0	0
ILDat 1.2 (Nigeria)	+++	++	+	0	0	0	0
IL 3184 (Nigeria)	++	++	0	0	0	0	0
IL 3185 (Nigeria)	+	+++	0	0	0	0	0
IL 3200 (Gambia)	++	++	++	0	0	0	0
ILDat 2.1 (Uganda)	++	++	+++	0	0	0	0
IL 2969 (Kenya)	0	0	0	++	+	0	0

*0, No lysis; +, 3–19% lysis; ++, 20–50% lysis; +++ > 50% lysis; homologous combinations are underlined.

the different stocks and clones. These populations were usually obtained from goats that were infected intravenously with the same stabilate as that used to infect animals for serum collection. ILDat 1.2 and ILDat 2.1 trypanosomes were obtained from infected mice, to obtain VAT homogeneous populations. In both cases the population was 98–100% homogeneous in VAT composition when tested by immune lysis using reference mouse antisera (Barry & Gathuo, 1984). In the case of goats that were infected with these two clones for serum collection, the initial inocula were of a similar antigenic homogeneity. Trypanosome populations of stocks or clones from Kenya (IL 2133; IL 2969; IL 1875; Il 3060 and IL 2337) were obtained from cattle that had been infected by tsetse. This was done because the parasitaemia in goats infected with these stocks was too low for use in the immune lysis test.

Because the sera of goats infected with stocks from Kenya did not always lyse their homologous trypanosome population, sera of cattle infected with these stocks were included in the experiment.

The stocks and clones from West Africa and Uganda were tested for the presence of ILDat 1.2 and ILDat 2.1 using reference antisera.

RESULTS

Trypanosome populations for use as antigen were obtained from the first peak of parasitaemia when the numbers of parasites in the blood were sufficient for use in the immune lysis test (approximately 10^6 trypanosomes/ml of blood). This was later in animals infected by tsetse with *T. vivax* stocks from Kenya (12–24 days) than in animals infected with the other stocks (7–8 days) (Table 1.).

Antisera to 14 stocks and clones of *T. vivax* were collected from infected goats, on day 40 and 80 after infection (Table 1). The antisera were tested by immune lysis for their reactivity with the 13 stocks and clones. In Tables 2 and 3, it is shown that the stocks and clones from Nigeria showed a relationship based on serological cross-reactivity. Antiserum to a clone from The Gambia (IL 3200) recognized parasites in the Nigerian stocks and clones except clone IL 3184. The parasite populations that were obtained from goats after infection with stocks and clones from Nigeria, The Gambia and Uganda were tested for the presence of the VATs ILDat 1.2 and ILDat 2.1 using reference mouse sera (Table 4). Clones IL 3184 and IL 3185 from Nigeria, clone IL 3200 from The Gambia and the Ugandan stock IL V-25 did not contain either the VATs ILDat 1.2 or ILDat 2.1 but IL 2160 consisted of 50% ILDat 1.2 (Table 4). The stock IL V-25 from Uganda, and a clone derived from this stock (ILDat 2.1) were recognized by the sera of goats that were originally infected with ILDat 1.2. Antisera to IL 3200 from The Gambia, lysed parasites of the Ugandan stock (IL V-25) but not the clone (ILDat 2.1).

There was apparently no antigenic similarity between stocks and clones from West Africa with those from Kenya, or reactivity between parasites and sera from Uganda and Kenya. Antisera to Kenyan stocks from Likoni (IL 1875), Bamburi (IL 3060) and Galana (IL 2337) that were obtained from infected goats, did not lyse parasites of their homologous stocks (Tables 2 and 3). However, similar antisera taken from infected cattle always lysed their homologous trypanosome populations (Table 5) but otherwise reacted with the same specificities towards the stocks from West Africa and

Table 3. Results of immune lysis tests between sera of goats infected with stocks or clones of *Trypanosoma vivax* and homologous and heterologous clones

Goat sera from infections initiated by	*T. vivax* clone used as antigen					
	ILDat 1.2 Nigeria Zaria	IL 3184 Nigeria Ibadan	IL 3185 Nigeria	IL 3200 Gambia Buruku	ILDat 2.1 Uganda Lugala	IL 2969 Kenya Kilifi
ILDat 1.2 (Nigeria)	+++*	++	++	++	+	0
IL 3184 (Nigeria)	++	++	++	+	+	0
IL 3185 (Nigeria)	++	+	+++	+	0	0
IL 3200 (Gambia)	++	0	++	+++	0	0
ILDat 2.1 (Uganda)	++	++	++	+	+++	0
IL 2969 (Kenya)	0	0	0	0	0	++
IL 2160 (Nigeria)	+++	++	++	0	0	0
IL 2946 (Nigeria)	++	+	+++	+	+	0
IL V-25 (Uganda)	++	++	++	+++	++	0
IL 2133 (Kenya)	0	0	0	0	0	++
IL 1875 (Kenya)	0	0	0	0	0	0
IL 3060 (Kenya)	0	0	0	0	0	0
IL 2337 (Kenya)	0	0	0	0	0	0

* 0, No lysis; +, 3–19% lysis; ++, 20–50% lysis; +++ > 50% lysis; homologous combinations are underlined.

Table 4. Percentage lysis of stocks and clones of *Trypanosoma vivax* from West Africa and Uganda by mouse reference sera against ILDat 1.2 and ILDat 2.1

Reference serum against	IL 2160 Nigeria Zaria (stock)	ILDat 1.2 Nigeria Zaria (clone)	IL 2946 Nigeria (stock)	IL 3185 Nigeria (clone)	IL 3184 Nigeria Ibadan (clone)	IL 3200 Gambia Buruku (clone)	IL V-25 Uganda Lugala (stock)	ILDat 2.1 Uganda Lugala (clone)
ILDat 1.2	50	98	0	0	0	0	0	0
ILDat 2.1	0	0	0	0	0	0	0	100

Table 5. Results of immune lysis tests between sera of cattle infected with *Trypanosoma vivax* stocks from Kenya and homologous and heterologous parasites

Cattle sera from infections initiated by	*T. vivax* used as antigen			
	IL 2969 Kilifi	IL 1875 Likoni	IL 3060 Bamburi	IL 2337 Galana
IL 2969	+++*	—	—	—
IL 1875	+	+++	—	—
IL 3060	—	—	+++	++
IL 2337	—	—	++	+++

* —, No lysis; +, 3–19% lysis; ++, 20–50% lysis; +++ > 50% lysis; homologous combinations are underlined.
N.B. These cattle sera reacted negatively (like the goat sera) with *T. vivax* stocks from outside Kenya (see Tables 2 and 3).

Uganda as did the goat sera. There were cross-reactivities between the stocks from Likoni (IL 1875) and Kilifi (IL 2133 and IL 2969) and also between the stocks from Galana (IL 2337) and Bamburi (IL 3060) (Tables 2, 3 and 5).

DISCUSSION

T. vivax populations that were isolated from goats infected with stocks and clones from Nigeria, The Gambia, and Uganda showed antigenic relatedness as judged by the immune lysis test. These results confirm the suggestion that overlap exists in the VAT composition of antigenic repertoires (sero-

demes) from distinct geographical areas. The parasite populations of the stocks and clones from West Africa and Uganda were screened for the presence of the VAT ILDat 1.2 from the ILDAR 1 (ILRAD *Duttonella* antigen repertoire) serodeme (Barry & Gathou, 1984) and the VAT ILDat 2.1, employing mouse reference sera in immune lysis tests. None of the stocks or clones from West Africa or Uganda that were used as antigen in the immune lysis tests was directly shown to contain the VAT ILDat 1.2, with the exception of the stock from which this clone was derived. However, all sera from goats infected with the stocks and clones from Nigeria, The Gambia and Uganda lysed ILDat 1.2 trypanosomes. ILDat 2.1, was absent from all stocks and clones from West Africa when tested with reference mouse antiserum, and even from the related stock from Uganda. A population of ILDat 2.1 trypanosomes was, however, recognized by sera from goats originally infected with ILDat 1.2, IL 2946 and its clone IL 3185 from Nigeria, and the stock from Uganda. This indicates that similar VATs to ILDat 1.2 and ILDat 2.1, must have been expressed during infection by the mentioned stocks and clones from Nigeria, The Gambia and Uganda.

Murray & Clarkson (1982) showed cross-reactivity between 4 stocks from Nigeria and found lytic antibody titres in the sera of naturally infected Zambian cattle to these Nigerian stocks and to one East African stock. Antigenic similarities between trypanosome populations from Kenya and Uganda have previously been reported by Dar *et al.* (1973). Barry & Gathuo (1984) demonstrated lytic antibodies in the sera of naturally infected Kenyan cattle to a number of VATs of the ILDAR 1 serodeme, indicating similarities between *T. vivax* stocks from East and West Africa.

In our studies, however, there was no cross-reactivity between the stocks and clones from West Africa and Uganda and stocks and clones from Kenya using either cattle or goat sera. Sera from goats infected with Kenyan stocks did not always lyse their homologous populations. It is possible that, because the parasites were isolated from a steer and not from goats, different VATs had been expressed in the two hosts. However, the Kenyan stocks showed much less virulent infections in goats than in cattle, with extremely low parasitaemias that were rapidly suppressed. Consequently, the numbers of VATs that could have been expressed in the infected goats were probably very low and their sera therefore would not contain antibodies against a large number of VATs. There was cross-reaction between a stock from Likoni and a stock from Kilifi, both from Kenya but from areas that are about 80 km apart. Trypanosomes from the Likoni stock (IL 1875) were lysed by sera from goats infected with a stock (IL 2133) or a clone (IL 2969) from Kilifi and sera of cattle infected with IL 1875 from Likoni lysed parasites from Kilifi. Nantulya *et al.* (1986) showed that there was complete cross-protection between these two stocks (IL 1875 and IL 2133) in cattle that had self-cured their infections and were subsequently challenged by tsetse infected with either stock.

There was no relationship between the stocks from Likoni or Kilifi with those from Galana or Bamburi which are also located in the Coast Province of Kenya. Similarly, no cross-protection between the stocks from Likoni or Kilifi and the stock from Galana was noted by Nantulya *et al.* (1986). However, there was reciprocal lysis between the stock from Bamburi (IL 3060) and Galana (IL 2337). Both these latter two stocks cause haemorrhages in cattle (Gardiner *et al.* 1989; Assoku & Gardiner, 1989; Schonefeld *et al.* 1987).

The antigenic relatedness between stocks and clones from a wide geographical area in Africa that has been demonstrated in this study, and by other authors (Dar *et al.* 1973; Murray & Clarkson, 1982; Barry, 1986), suggests that the number of antigenic variants common to different stocks of *T. vivax* in Africa may be quite high. The concept of serodeme, in relation to the number of antigenic repertoires of *T. vivax* which exist in Africa, therefore becomes difficult to define for this parasite. In *T. b. brucei*, *T. b. rhodesiense*, *T. b. gambiense* and *T. evansi* isotypic VATs (isotypes) have been described. Isotypes are cloned populations derived from widely different original field isolates which cross-react in immune lysis and immunofluorescence tests and therefore appear to possess cross-reactive epitopes carried on their variable surface antigens (VSGs) (Van Meirvenne, Magnus & Vervoort, 1977). The VSGs of the isotypes studied had the same molecular weight but different isoelectric points and contained not only the cross-reacting determinants found in all other *T. brucei* VSGs (Barbet & McGuire, 1978) but also additional cross-reacting determinants (Vervoort *et al.* 1981). The extensive cross-reactivity between *T. vivax* stocks from different areas that was found in the present study implies that identical, or isotypic VATs, occur frequently in the antigenic repertoires of these stocks.

The senior author was supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO). The authors thank Dr Moloo and his staff for carrying out the tsetse infections.
This is ILRAD publication number 708.

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