Resistance of Cattle to Tsetse-Transmitted Challenge with *Trypanosoma brucei* or *Trypanosoma congolense* After Spontaneous Recovery from Syringe-Passaged Infections

VINAND M. NANTULYA, ANTONY J. MUSOKE, FREDERICK R. RURANGIRWA, AND SHAMSHUDEEN K. MOLOO

International Laboratory for Research on Animal Diseases, Nairobi, Kenya

Received 20 June 1983/Accepted 4 November 1983

Groups of cattle were inoculated intravenously with cloned populations of bloodstream forms of *Trypanosoma brucei* or *Trypanosoma congolense*. All five steers infected with *T. brucei* ILTat 2.1 and six of the eight steers infected with *T. congolense* IL 13-E14 became aparasitemic within 16 and 32 weeks postinfection, respectively. Examination of sera from animals infected with *T. brucei* by indirect immunofluorescence and neutralization assays revealed the presence of antibodies against all the metacyclic variable antigen types (VATS) of the infecting clone. The neutralizing capacity of the sera increased with the course of infection from 1:10 at 2 months to 1:100 at 3 to 4 months postinfection. The recovered animals were completely immune to challenge by *Glossina morsitans* subsp. *centralis* infected with clone ILTat 2.1, which had initiated the infection, as well as with another clone (ILNat 3.1) belonging to the same serodeme, but they were susceptible to a tsetse-transmitted heterologous challenge with isolate STIB 367-H. Similar results were obtained with sera from *T. congolense* IL 13-E14-infected steers. The six steers infected with a different *T. congolense* ILNat 3.1 clone did not recover spontaneously; however, 2 months postinfection, sera from five of them also contained neutralizing antibodies against ILNat 3.1 metacyclic VATs. These results indicate that some of the bloodstream VATs that arise during the course of a chronic infection possess surface epitopes in their variable surface glycoproteins that are identical to those of the metacyclic VATs. It is suggested that in chronic infection, the infecting trypanosomes could exhaust their VAT repertoire, including those that cross-react with metacyclics, thereby leading to both "self-cure" and subsequent immunologic cyclization to homologous cyclically transmitted challenge.

Chronic African trypanosomiasis is associated with protracted fluctuating parasitemia, with the infecting trypanosomes expressing several different variable antigen types (VATS) (5). The number of different VATs that can arise during a single infection is not known, but it is possibly in excess of 100 (4). Despite the emergence of this large repertoire of VATs, some infected cattle can recover spontaneously (14, 15, 22). It has also been reported that such recovered animals are largely resistant to rechallenge by tsetse carrying the same trypanosome serodeme (22). The mechanisms underlying these phenomena are, however, unknown. We have also observed this "self-cure" phenomenon and resistance to reinfection in many steers experimentally infected with cloned bloodstream forms of *Trypanosoma brucei* or *Trypanosoma congolense*. Data are presented here to explain the possible mechanisms responsible for both the self-cure phenomenon and the immunity arising therefrom and their implications with regard to livestock management in areas of trypanosomiasis challenge.

MATERIALS AND METHODS

**Animals.** Steers (Zebu × Charolais), aged 6 months, were obtained from areas known to be free from trypanosomiasis, screened for antibodies to *T. congolense*, *T. brucei*, and *Trypanosoma vivax* by indirect immunofluorescence (23) before use, and found to be negative. International Laboratory for Research on Animal Diseases (ILRAD)-bred BALB/c mice used were 4 months old and weighed 25 g.

**Parasites.** *T. brucei* clone ILTat 2.1 was derived from STIB 247, an isolate (7) from the Serengeti area of Tanzania. As follows: STIB 247 was passaged three times at 2- to 4-day intervals in nonirradiated BALB/c mice and then cloned into irradiated (900 roentgens [R]) BALB/c mice. Parasitemia was detected in one mouse 4 days after intraperitoneal inoculation with a single trypanosome. This clone was immediately recloned into an irradiated BALB/c mouse, passaged twice at 4- and 6-day intervals into irradiated (900 R) rats, and stabilized as IL 1114 (ILTat 2.1). ILTat 2.2 was a different clone derived from an irradiated BALB/c mouse inoculated with a single trypanosome from a rabbit with ILTat 2.1 30 days postinfection, whereas STIB 367-H is a population from a mouse infected with a single metacyclic trypanosome of an unrelated stock LUMP 227 (9).

*Trypanosoma congolense* clone ILNat 3.1 was derived from STIB 212. STIB 212, an isolate from a lion in the Serengeti National Park (7), was passaged 3 times in nonirradiated BALB/c mice and cloned in an irradiated (500 R) BALB/c mouse. The clone, detected 11 days postinoculation in a mouse, was passaged once into a similarly irradiated BALB/c mouse and then stablilized as IL 933. Clone IL 933 was then inoculated into an irradiated (500 R) BALB/c mouse and recloned 3 days postinfection. The subsequent recloned population was stablilized after 11 days as IL 968 (ILNat 3.1). *T. congolense* clone IL 13-E14 was prepared similarly from IL 5-EL2, a stock from EATRO 209 (13).

**Infection of animals.** Five steers were infected with ILTat 2.1, eight with *T. congolense* IL 13-E14, and six with ILNat 3.1. The dose used was 10⁵ viable trypanosomes administered intravenously. The animals were subsequently bled twice a week to determine the packed erythrocyte volume (PCV) and level of parasitemia. Parasitemia was determined...
by examination of theuffy coat (16) of a 15-µl microhematocrit tube.

Serum samples were also obtained weekly from each animal until death or self-cure, heat inactivated at 56°C for 30 min, stored at −20°C, and later tested for the presence of antibodies to homologous metacyclic trypanosomes by indirect immunofluorescence (18) and neutralization assays.

Infected cattle were considered to have undergone self-cure if they displayed the following characteristics for a period of at least 2 months: absence of parasites on direct examination of peripheral blood; repeated failure of development of parasitemia in groups of mice subinoculated weekly with blood from the infected cattle, each mouse receiving 0.5 ml of bovine blood intraperitoneally; and the return of the PCV to the preinfection levels.

**Infection of tsetse.** Glossina morsitans subsp. centralis obtained from the ILRAD colony were fed on mice infected with the trypanosome clones (9), and the infected tsetse were identified by the warm-slide probe method (3) and phase-contrast microscopy.

**Cyclically transmitted challenge.** After self-cure from *T. brucei* infection, all animals were challenged each with 20 tsetse infected with the clone, ILTat 2.1, that had initiated the infection and another clone, ILTat 2.2, belonging to the same serodeme. Later, the same animals were exposed to challenge by tsetse infected with a clone, STIB 367-H, belonging to a different serodeme. For each challenge, two serologically negative control animals were included. Similarly, the animals that had recovered from syringe-passaged *T. congolense* IL 13-E14 infection were challenged both homologously and heterologously by using tsetse infected with IL 13-E14 and ILNat 3.1, respectively.

**Detection of antibodies to *T. brucei* or *T. congolense* metacyclic VATs in sera from infected cattle.** The presence of antibodies to *T. brucei* or *T. congolense* metacyclic VATs in sera from ILTat 2.1-infected steers was determined by two assays, neutralization and indirect immunofluorescence. The assay for neutralizing antibodies against *T. brucei* metacyclics was performed as follows. Forty tsetse infected with this clone were allowed to probe into 5 ml of phosphate saline glucose (pH 8.0). The number of parasites was estimated by the hemacytometer method, and the concentration was adjusted to give 3 × 10⁵ metacyclic trypanosomes per 200 µl. Each test serum was diluted 1:5, 1:25, and 1:50. A 200-µl amount of each serum dilution was added into each of six 3-ml plastic test tubes. An equal volume (200 µl) of the parasite suspension was then added into each tube and left on ice for 30 min, after which the contents of each tube were inoculated separately into six BALB/c mice by the intraperitoneal route. The mice were then observed for 30 days for the development of parasitemia. If parasitemia did not develop in any of the six mice in a group, the test was considered positive for that particular serum dilution. If parasitemia developed in one or more mice in a group, the test was considered negative.

Assays for neutralizing antibodies against *T. congolense* metacyclics were performed differently since their small number precludes estimation by the hemacytometer method. Five infected tsetse in single holding tubes were allowed to probe singly into 20 µl of pre- or postinfection serum in wells of a lymphocyte migration plate, and the volume was made up to 1.5 ml with the same serum. After incubation on ice for 30 min, the suspension was inoculated, in equal portions, into three BALB/c mice. Each serum was tested twice. The criteria for neutralization were as described in the assay for antibodies to *T. brucei* metacyclic trypanosomes. The indirect immunofluorescent antibody tests on metacyclic trypanosomes were carried out with fresh unfixed tsetse salivary probes by a previously described procedure (18).

**RESULTS**

**Parasitemia.** The parasitemic profile of *T. brucei*-infected steers is shown in Fig. 1. The animals became aparasitemic within 16 weeks postinfection. Six of the eight steers infected with *T. congolense* IL 13-E14 recovered, but they took a longer period, 32 weeks, to clear their parasitemia (Fig. 2). The remaining two animals died 10 weeks postinfection. The six steers infected with ILNat 3.1 died 12 to 16 weeks postinfection.

PCV. In the groups of animals that recovered spontaneously, an initial fall in PCV, which was more severe in *T. congolense*-infected steers, was followed by a gradual return to preinfection levels (Fig. 1 and 2).

**Homologous cyclically transmitted challenge.** The cattle which recovered from *T. brucei* infection showed complete resistance to challenge by 20 tsetse infected with clones ILTat 2.1 or ILTat 2.2, whereas all controls became infected (Fig. 1). Likewise, cattle recovering from *T. congolense* IL 13-E14 did not develop parasitemia when challenged with a similar number of tsetse infected with IL 13-E14 (Fig. 2), whereas the controls did.

**Heterologous cyclically transmitted challenge.** Animals recovering from infection with *T. brucei* clone ILTat 2.1 were not immune to cyclical challenge with *T. brucei* STIB 367-H belonging to a different serodeme (9). Similarly, the steers recovering from the syringe-passaged infection with *T. congolense* IL 13-E14 were susceptible to challenge with tsetse infected with ILNat 3.1 (Fig. 2).

**Antibodies against metacyclic VATs.** To assess whether antibodies against metacyclics appear in cattle infected with bloodstream clones, sera from all infected animals were tested for the presence of such antibodies by indirect immunofluorescence (18). In the case of *T. brucei*, sera collected 1 month postinfection contained antibodies against only a small proportion (<20%) of the homologous metacyclic population, whereas samples obtained 2 months postinfection stained all the homologous (ILTat 2.1, ILTat 2.2, and STIB 247) but not heterologous (STIB 367-H) metacyclics.

![FIG. 1. Immunity of cattle to tsetse-transmitted challenge after self-cure from a syringe-passaged infection with a bloodstream *T. brucei* clone (ILTat 2.1). The PCV did not fall after challenge, and parasitemia did not develop. Isobars indicate one standard deviation.](http://iai.asm.org/Downloaded from September 20, 2017 by guest)
FIG. 2. Immunity of cattle to a tsetse-transmitted challenge after self-cure from a syringe-passaged infection with a bloodstream *T. congolense* clone (IL 13-E14). There was no reduction in the PCV and no parasitemia on homologous challenge with IL 13-E14, whereas on heterologous challenge with ILNat 3.1, the PCV dropped precipitously, and parasitemia developed. Isobars indicate one standard deviation.

The results in Table 1 show that these sera contained neutralizing antibodies against homologous metacyclic trypanosomes. Serum samples obtained 2 months after infection completely neutralized $3 \times 10^9$ metacyclic trypanosomes, and the neutralizing capacity of the sera increased with the course of infection as evidenced by the rising neutralization titers (Table 1). Neutralization did not occur, however, when metacyclic trypanosomes of a different serodeme, STIB 367-H, were incubated in sera from these animals. Essentially similar results were obtained when sera from *T. congolense*-infected steers were tested by indirect immunofluorescence and neutralization assays against metacyclics of IL 13-E14 and ILNat 3.1 (Table 2), except that neutralizing antibody activity against homologous metacyclics was demonstrable in sera obtained as early as 1 month postinfection in some animals.

**DISCUSSION**

Cattle infected with cloned bloodstream *T. brucei* or *T. congolense* displayed complete immunity against tsetse-transmitted homologous challenge after a spontaneous elimination of trypanosomes from peripheral blood circulation. The animals were also immune to challenge by tsetse infected with other clones belonging to the same serodeme but remained susceptible to similarly transmitted heterologous serodemes. Sera obtained from these animals before challenge contained neutralizing antibodies against metacyclic VATs of the homologous but not heterologous serodeme.

Although these animals might have continued to harbor trypanosomes in some privileged tissue sites (11), the apparently complete restoration of their physical well-being to preinfection levels after the elimination of trypanosomes from peripheral blood circulation would suggest that spontaneous self-cure (14, 15, 22) could have occurred, at least in some animals. Such a self-cure phenomenon could be related to the process of antigenic variation. Cattle infected with *T. brucei* or *T. congolense* produce high levels of neutralizing and phagocytosis-promoting antibodies against the infecting as well as subsequent VATs that arise during the infection (12, 17, 21). If immunity built up against the entire repertoire of pathogenic bloodstream VATs expressed in the bovine host by the infecting trypanosome clones, spontaneous self-cure might occur. The animals which did not recover from infection might have had less effective immune responses or parasite clearance mechanisms or both, giving rise to prolonged parasitemia and, eventually, death of the host.

The occurrence of antimetacyclic antibodies in cattle infected with bloodstream clones, also reported in rabbits (2, 10), would suggest that some of the trypanosomes that arise during the course of chronic infection possess surface epitopes in their variable surface glycoproteins which are identical to those of metacyclic trypanosomes. Recent studies with monoclonal antibodies (16, 20) have shown that in the case of *T. brucei* and *T. rhodesiense*, such bloodstream VATs do indeed exist. The rising titer of neutralizing antimetacyclic antibodies in sera obtained 3 to 4 months postinfection is an indication that the bloodstream VATs responsible for the production of antimetacyclic antibodies reappear (19) or that other closely related VATs emerge during the course of infection (1, 19). The immunity exhibited by these animals against homologous challenge could, therefore, be directed at the metacyclic or the bloodstream VATs or both.

Under field conditions, *T. brucei* and *T. congolense* are transmitted principally by tsetse, although the possibility of mechanical transmission has also been suggested (7, 8). The observation, however, that serodeme-specific immunity to tsetse-transmitted challenge readily develops in livestock regardless of the mode of transmission has significant practical implications. It indicates that under natural conditions of challenge, livestock could, over a course of time, acquire resistance against both bloodstream and metacyclic VATs of the local trypanosome serodemes. The observations of several workers, summarized by Murray et al. (15), do indeed suggest that cattle, maintained with the aid of trypanocidal drugs in areas of trypanosomiasis challenge, acquire immu-

<table>
<thead>
<tr>
<th>TABLE 2. Neutralizing antibodies against <em>T. congolense</em> metacyclic VATs in sera from cattle syringe infected with bloodstream clones IL 13-E14 and ILNat 3.1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosome clone</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>IL 13-E14</td>
</tr>
<tr>
<td>ILNat 3.1</td>
</tr>
</tbody>
</table>

* Neutralization was considered to have occurred if parasitemia did not develop in any of the six BALB/c mice inoculated with metacyclics incubated in undiluted postinfection serum. If parasitemia developed in one or more mice in the group, the test was considered to be negative.

b ND. Not done. All cattle had died.

seremun. Sera obtained from these animals before challenge contained neutralizing antibodies against metacyclic VATs of the homologous but not heterologous serodeme.

Although these animals might have continued to harbor trypanosomes in some privileged tissue sites (11), the apparently complete restoration of their physical well-being to preinfection levels after the elimination of trypanosomes from peripheral blood circulation would suggest that spontaneous self-cure (14, 15, 22) could have occurred, at least in some animals. Such a self-cure phenomenon could be related to the process of antigenic variation. Cattle infected with *T. brucei* or *T. congolense* produce high levels of neutralizing and phagocytosis-promoting antibodies against the infecting as well as subsequent VATs that arise during the infection (12, 17, 21). If immunity built up against the entire repertoire of pathogenic bloodstream VATs expressed in the bovine host by the infecting trypanosome clones, spontaneous self-cure might occur. The animals which did not recover from infection might have had less effective immune responses or parasite clearance mechanisms or both, giving rise to prolonged parasitemia and, eventually, death of the host.

The occurrence of antimetacyclic antibodies in cattle infected with bloodstream clones, also reported in rabbits (2, 10), would suggest that some of the trypanosomes that arise during the course of chronic infection possess surface epitopes in their variable surface glycoproteins which are identical to those of metacyclic trypanosomes. Recent studies with monoclonal antibodies (16, 20) have shown that in the case of *T. brucei* and *T. rhodesiense*, such bloodstream VATs do indeed exist. The rising titer of neutralizing antimetacyclic antibodies in sera obtained 3 to 4 months postinfection is an indication that the bloodstream VATs responsible for the production of antimetacyclic antibodies reappear (19) or that other closely related VATs emerge during the course of infection (1, 19). The immunity exhibited by these animals against homologous challenge could, therefore, be directed at the metacyclic or the bloodstream VATs or both.

Under field conditions, *T. brucei* and *T. congolense* are transmitted principally by tsetse, although the possibility of mechanical transmission has also been suggested (7, 8). The observation, however, that serodeme-specific immunity to tsetse-transmitted challenge readily develops in livestock regardless of the mode of transmission has significant practical implications. It indicates that under natural conditions of challenge, livestock could, over a course of time, acquire resistance against both bloodstream and metacyclic VATs of the local trypanosome serodemes. The observations of several workers, summarized by Murray et al. (15), do indeed suggest that cattle, maintained with the aid of trypanocidal drugs in areas of trypanosomiasis challenge, acquire immu-

<table>
<thead>
<tr>
<th>TABLE 1. Neutralizing antibodies against <em>T. brucei</em> metacyclic VATs in sera from cattle syringe infected with a bloodstream clone IL.Tat 2.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time postinfection (mo)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

* Neutralization was considered to have occurred if parasitemia did not develop in any of the six BALB/c mice inoculated with $3 \times 10^9$ metacyclic trypanosomes incubated in the serum at the dilution indicated. If parasitemia developed in one or more mice in the group, the test was considered to be negative for that serum dilution.
nity to trypanosomiasis, as evidenced by the progressive lengthening of the interval between treatment and reinfection. The observations reported here, therefore, underscore the potential for the practical approach to livestock management in trypanosomiasis areas. Immunity so acquired, however, should be viewed in the context of a local situation since it would probably break down on translocation of resistant animals to areas with different trypanosome sero-
demes.

ACKNOWLEDGMENTS
We thank G. Kamunya and D. Lugo for technical assistance and Marion Kanyugo for typing the manuscript. This is ILRAD publication serial no. 247.

LITERATURE CITED