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Apparent Exhaustion of the Variable Antigen Repertoires of *Trypanosoma vivax* in Infected Cattle†

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Three groups of cattle, each group comprising six animals, were inoculated intravenously with populations of bloodstream forms of *Trypanosoma vivax*. The first group received *T. vivax* clone ILDat 1.3 derived from an isolate from Nigeria, while the other two received *T. vivax* stocks IL 1875 or IL 2133 isolated from Coast Province, Kenya. One animal from the group that was infected with IL 1875 died 8 weeks postinfection. The remaining 17 animals became aparasitemic in 8 to 12 weeks without intervention by drug therapy. The recovered animals developed serodeme-specific immunity against *Glossina morsitans* subsp. *centralis*-transmitted challenge. There was complete cross-protection between the two East African *T. vivax* stocks, although they were isolated from areas 80 to 90 km apart, indicating that they belong to the same serodeme. Antibodies to the homologous metacyclic variable antigen types (VATs) were not detected in sera from recovered animals, suggesting that the immunity displayed by the recovered animals was directed at the bloodstream and not the metacyclic VATs. It is thus suggested that recovery in these animals is due to exhaustion of the repertoire of bloodstream VATs expressed in the animals by the infecting *T. vivax* clone or stocks.

Infections with African trypanosomes are characterized by a protracted, fluctuating course of parasitemia during which a large repertoire of variable antigen types (VATs) of the parasites are randomly expressed (3, 4). The infected host responds by elaborating protective antibodies against the various trypanosome VATs expressed by the parasites (1, 4, 7, 8, 12). It has been demonstrated also that if cattle survive long enough with *Trypanosoma congolense* or *T. brucei* infections, effective immunity is built up against the full repertoire of VATs expressed by the infecting trypanosomes, leading to spontaneous self-cure (11). The animals undergoing spontaneous self-cure subsequently acquired serodeme (VAT repertoire)-specific immunity (11). Here we report the results of investigations on this phenomenon in cattle infected with *T. vivax* isolates from East and West Africa.

MATERIALS AND METHODS

Animals. Boran cattle (*Bos indicus*, aged 6 to 8 months) were obtained from areas known to be free from trypanosomiasis. They were screened before use for antibodies to *T. congolense*, *T. vivax*, and *T. brucei* by indirect immunofluorescence (14) and were found to be negative. The BALB/c mice used were reared at the International Laboratory for Research on Animal Diseases.

Parasites. A clone from one *T. vivax* stock and three other uncloned stocks were studied. The clone ILDat 1.3 (2) was derived in mice from the first stock Y 486, an isolate from a Zebu cow in northern Nigeria (6). The second *T. vivax* stock, IL 1875, was a population derived from a steer 75 days after tsetse-transmitted infection with KETRI 2375, an isolate from a naturally infected cow in Likoni, about 12 km south of Mombasa, Coast Province, Kenya (13). The third *T. vivax* stock, IL 2133, was isolated from a cow on a Kilifi farm situated 70 to 80 km north of Mombasa. The original isolate was passaged once by syringe in a cow, twice in tsetse, and finally in a goat before cryopreservation. The fourth stock, IL 2337, was a derivative of KETRI 2430 which was isolated from a naturally infected cow on a ranch in Galana, which is about 60 km west of Malindi and 100 km north of Mombasa.

Infection of animals. Six steers were infected with ILDat 1.3, six were infected with IL 1875, and another six were infected with IL 2133 (Table 1). Each animal received 10^7 trypanosomes administered intravenously. All the animals were subsequently bled twice a week, until recovery, to determine the packed erythrocyte volume (PCV) and the level of parasitemia. Parasitemia was detected in the blood buffy coat by phase-contrast microscopy (9). Blood samples for sera were also obtained once a week from each animal and stored at −20°C before use.

Infection of tsetse. *Glossina morsitans* subsp. *centralis* obtained from the International Laboratory for Research on Animal Diseases colony were infected with the three *T. vivax* stocks (IL 1875, IL 2133, IL 2337) by feeding on infected calves or were infected with clone ILDat 1.3 by feeding on infected goats for 25 to 30 days. A random batch of 20 flies from each group were then dissected to determine the infection rates.

Detection of antibodies to metacyclics in serum samples of recovered animals. Serum samples obtained 3 and 6 months postinfection as well as the immediate prechallenge serum sample from each animal were tested for antibodies to homologous metacyclic VATs by the neutralization assay. *G. morsitans* subsp. *centralis* isolates infected with *T. vivax* ILDat 1.3, IL 1875, or IL 2133 were allowed to probe into 600 μl of each serum sample at 37°C in a well of a lymphocyte migration plate. Thirty tsetse were used for each serum sample. The 600 μl containing the parasites was drawn into a syringe, and additional serum was introduced to make a total volume of 1 ml. After a 30-min incubation at room temperature the contents were injected intravenously into two goats, 0.5 ml per goat. The goats were bled from the ear daily, and wet blood smears as well as buffy coats were examined for the presence of parasites for 30 days.

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TABLE 1. Experimental design

Expt	No. of cattle	Initial infection strain	Challenge strain		
			First	Second	Third
A	6	ILDat 1.3	ILDat 1.3	IL 2133	
B	6[a]	IL 1875	IL 1875	IL 2133	IL 2337
C	6	IL 2133	IL 2133	IL 1875	IL 2337

[a] One animal died of severe trypanosomiasis 8 weeks postinfection.

Tsetse-transmitted challenge of recovered animals. After recovery all animals in every group were each challenged first with 20 tsetse infected with the homologous *T. vivax* stock or clone (Table 1). Two challenge control animals were included in each group at each challenge. Parasitemia in the challenged animals was examined daily for 30 days. The animals infected with the two East African *T. vivax* stocks, IL 1875 and IL 2133, were later cross-challenged to determine their relationship with regard to their serodemes. Finally, they were challenged with *T. vivax* IL 2337, using the infected tsetse.

Heterologous challenge of the animals that had recovered from ILDat 1.3 was done with *T. vivax* IL 2133 (Table 1).

RESULTS

Course of parasitemia. The parasitemic profiles of the three groups of animals are shown in Fig. 1, 2, and 3. The infected animals were clear of the parasites 8 to 12 weeks after patency. Only one animal died, 8 weeks postinfection, in the group infected with *T. vivax* IL 1875. This animal died of trypanosomiasis.

In animals infected with ILDat 1.3, there was an initial drop in the PCV, with a maximum decrease of 21% recorded 3 weeks postinfection (Fig. 1). Thereafter, the PCV returned to the preinfection level by week 14. The drop in PCV in animals infected with IL 1875 (Fig. 2) and IL 2133 (Fig. 3) was more pronounced, with the maximum decrease of 41 and 42%, respectively, observed 5 weeks postinfection. Following the clearance of the parasites, the PCV returned to preinfection levels by weeks 23 and 37, respectively (Fig. 2 and 3).

Immunity to tsetse-transmitted challenge. Two animals in the group that recovered from ILDat 1.3 developed a transient infection following homologous tsetse-transmitted challenge, with trypanosomes detectable in the peripheral blood for only 1 day (on day 12 in one and day 21 in the other). All the animals succumbed to heterologous challenge with the Kilifi *T. vivax* IL 2133 (Fig. 1).

All the cattle that recovered from infection with *T. vivax* stocks IL 1875 or IL 2133 displayed complete resistance to challenge by tsetse infected with the homologous stocks (Fig. 2 and 3), whereas all controls developed parasitemia within 7 days postchallenge. The two groups of animals were then cross-challenged to test the relationship with regard to their serodemes. There was complete reciprocal cross-protection (Fig. 2 and 3). The two groups of animals, however, did not show protection against tsetse infected with the *T. vivax* IL 2337 (Fig. 2 and 3).

To determine whether the two stocks (IL 1875 and IL 2133) consisted of more than one serodeme, a steer that had undergone a self-limiting infection following inoculation with an in vitro-derived clone from one of the parental stocks, IL 2133, was challenged with 20 tsetse infected with the parental stock (data not shown). There was no breakthrough parasitemia, indicating that this stock and the Likoni stock (IL 1875), belonged to the same serodeme.

Neutralizing antibodies against metacyclics. To determine whether cattle infected with bloodstream VATs produced antibodies to metacyclics, serum samples obtained 3 and 6 months postinfection, as well as the immediate prechallenge serum sample from recovered animals, were examined for the presence of neutralizing antibodies against homologous and heterologous *T. vivax* metacyclics. Neutralizing antibodies against homologous or heterologous metacyclics were not detected in any of the serum samples tested.

DISCUSSION

Several animals in this study recovered from syringe-passaged infections with East or West African *T. vivax* without the aid of drug therapy. The recovered animals subsequently displayed evidence of serodeme-specific acquired immunity to challenge by infected tsetse, although serum samples obtained from these animals after recovery did not have antibodies to the corresponding metacyclics.

Spontaneous self-cure may be related to the phenomenon of antigenic variation. It has been shown in previous studies (1, 4, 7, 8, 12) that trypanosome-infected animals mount protective antibody responses against the various trypanosome VATs which arise during the course of infection as a result of antigenic variation. It is thus entirely possible that if an infected animal survived long enough to allow the parasites to express their full repertoire of bloodstream VATs, such an animal might build up effective immunity against the whole repertoire of bloodstream VATs expressed, leading to a spontaneous self-cure. This situation, which has also been reported for *T. congolense* and *T. brucei* (11), could have occurred in some of the *T. vivax*-infected animals reported in this study.

The observation that most of the animals that recovered from syringe-passaged infections displayed a solid serodeme-specific acquired immunity to tsetse-transmitted chal-

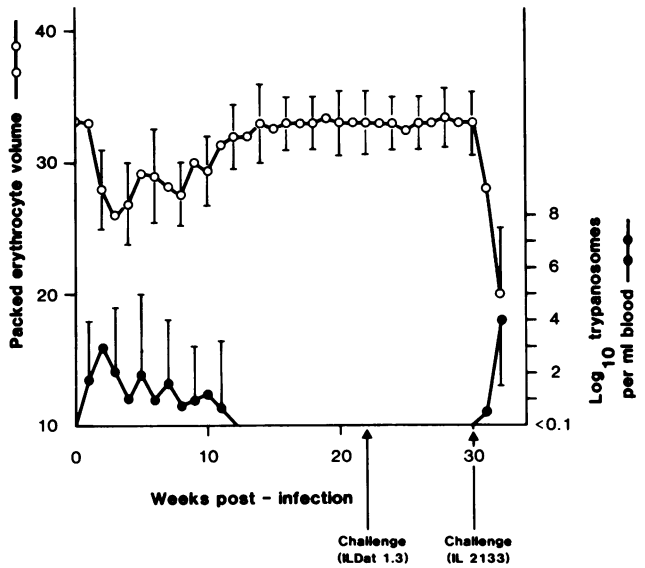

FIG. 1. Immunity of cattle to tsetse-transmitted challenge following self-cure from a syringe-passaged infection with *T. vivax* ILDat 1.3. There was no reduction in the PCV in any of the six animals and only a transient parasitemia in two animals following the homologous challenge, whereas on heterologous challenge with *T. vivax* IL 2133, the PCV dropped precipitously and parasitemia developed in all the animals. Isobars indicate one standard deviation.

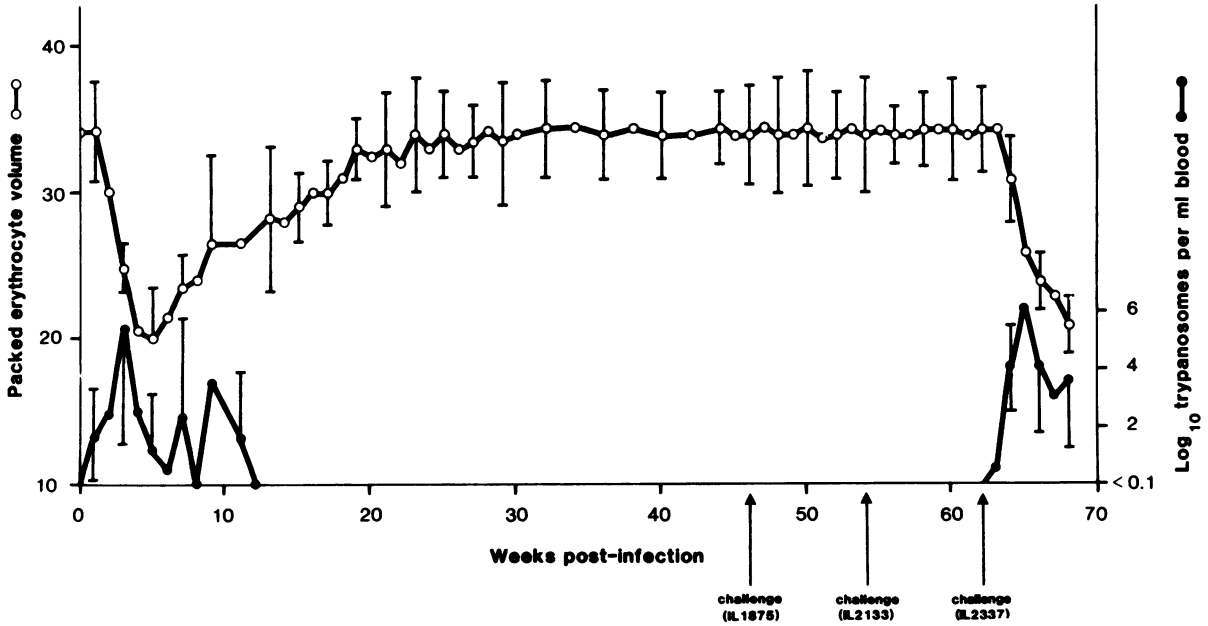

FIG. 2. Immunity of cattle to tsetse-transmitted challenge after self-cure from a syringe-passaged infection with *T. vivax* IL 1875. The PCV did not drop and parasitemia did not develop after homologous challenge. Cross-challenge with *T. vivax* IL 2133 gave similar results, but all five animals succumbed to heterologous challenge with another *T. vivax* stock, IL 2337. Isobars indicate one standard deviation.

lenge, in the absence of neutralizing antibodies to corresponding metacyclics, is consistent with this hypothesis. The bloodstream trypanosome VATs which arise in the animals following transformation of *T. vivax* metacyclics to the bloodstream forms are unpredictably heterogeneous in that their VAT composition does not appear to be limited with regard to number (5). Indeed, in the absence of self-cure, immunity against tsetse challenge does not develop, even in those animals treated with drugs after exposure to several bites by tsetse infected with *T. vivax* (A. L. W. De Gee, Ph.D. thesis, University of Utrecht, The Netherlands, 1980). Thus, the successful solid immunity to tsetse-

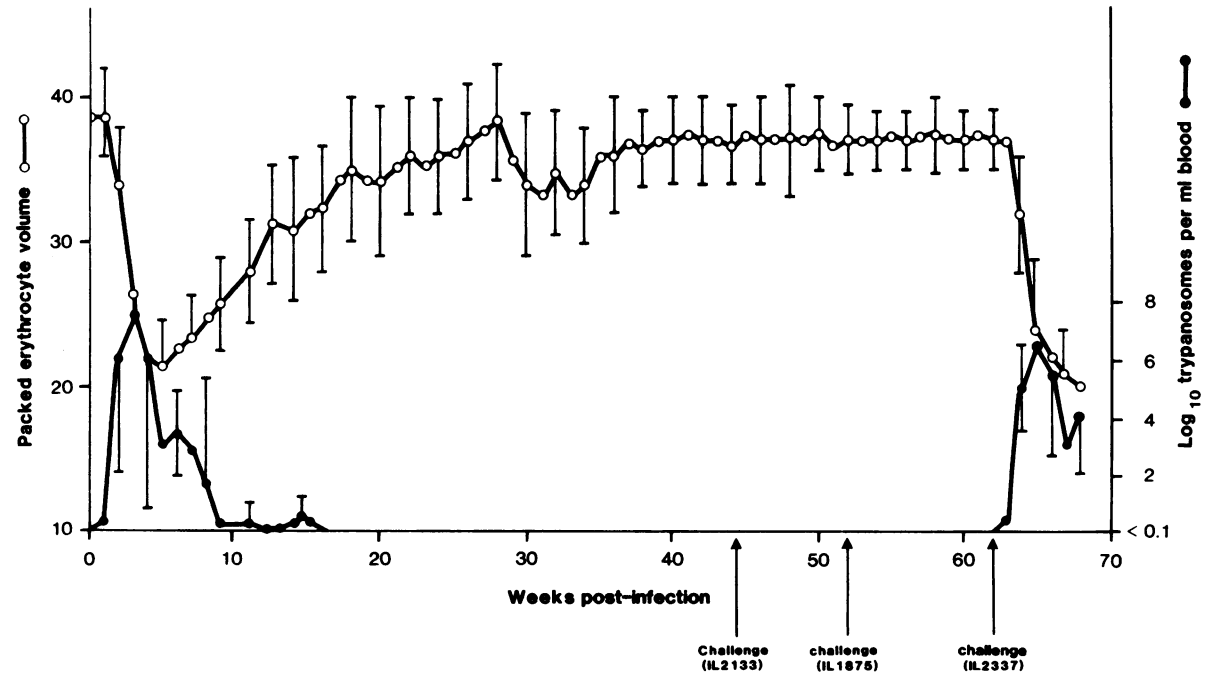

FIG. 3. Immunity of cattle to tsetse-transmitted challenge following self-cure from a syringe-passaged infection with *T. vivax* IL 2133. The PCV did not drop and parasitemia did not develop in any of the six animals on homologous challenge or after heterologous challenge with IL 1875. However, there was no cross-protection against *T. vivax* IL 2337. Isobars indicate one standard deviation.

transmitted challenge which was induced by spontaneous self-cure provides evidence that the immune system of the recovered animals had already come into contact with the full repertoire of bloodstream VATs, leading to a rapid elimination of the VATs expressed by the transformed metacyclics.

It should be pointed out, however, that in some animals spontaneous self-cure may not be associated with an exhaustive expression of the VAT repertoire since it is quite likely that some VATs may not be expressed during the initial infection. Such animals would nevertheless develop immunity against the VATs that were expressed and subsequently undergo spontaneous self-cure. On subsequent tsetse challenge, however, the VATs that were not expressed during the initial infection could then be expressed, giving rise to a transient infection. This indeed could have been the case with the two animals in group A which developed a transient infection following homologous tsetse-transmitted challenge, with trypanosomes detected in peripheral blood for only 1 day.

In this study *T. vivax*-infected cattle took a much shorter period (8 to 12 weeks) to undergo self-cure compared with *T. congolense*- or *T. brucei*-infected cattle, which took 32 and 16 weeks, respectively, to undergo self-cure (11). This observation suggests that for the *T. vivax* stocks and clone used, expression of the VAT repertoires occurred more rapidly or that the VAT repertoires for the *T. vivax* stocks and clone investigated were smaller than those of *T. brucei* or *T. congolense*, leading to an early exhaustion of their VAT repertoires and hence the early self-cure.

In a previous study on *T. brucei* and *T. congolense* (11), it was suggested that the immunity against tsetse-transmitted homologous challenge exhibited by recovered animals was directed against both the bloodstream and metacyclic VATs, since high levels of neutralizing antibodies against homologous metacyclic VATs also appeared in serum samples of the syringe-infected animals. In this study, however, antibodies to metacyclic VATs were not detected in serum samples from syringe-infected animals. The absence of antibodies against the metacyclic VATs in serum samples of recovered animals further suggests that unlike *T. brucei* or *T. congolense* (11), none of the VATs that arose during a syringe-passaged *T. vivax* infection expressed the corresponding metacyclic VAT epitopes. Because the VATs expressing metacyclic epitopes may serve to amplify the immune response of the infected animals to the protective metacyclic surface epitopes (10), their absence in *T. vivax* may offer an explanation, at least in part, for the repeated failure to induce immunity to tsetse-transmitted challenge in animals exposed to infected tsetse bites (De Gee, Ph.D. thesis).

It is also interesting that there was cross-protection between *T. vivax* IL 1875 from Likoni and IL 2133 from Kilifi. Because the protection associated with spontaneous self-cure is serodeme specific, this observation suggests that the two stocks belong to the same serodeme, despite the fact that they were isolated from areas 80 to 90 km apart. This is the first time, to our knowledge, that stocks isolated from areas separated by such a distance have been shown to belong to the same serodeme. This observation may have major implications regarding the extent of the diversity of *T. vivax* serodemes in the coastal areas of Kenya.

In conclusion we have shown in this study that the immunity displayed by animals that recovered spontaneously from *T. vivax* infection is serodeme specific. The serodeme-specific nature of this immunity seems to offer the only approach at present for the identification of *T. vivax* serodemes.

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