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The appearance of *Trypanosoma (Duttonella) vivax* in lymph following challenge of goats with infected *Glossina morsitans morsitans*

Short communication

D. L. EMERY, J. D. BARRY, S. K. MOLOO

From the absence of extensive tissue lesions, it has been suggested that *Trypanosoma (Duttonella) vivax* remains within the blood vascular system of infected animals (reviewed by Losos and Ikede, 1972). However, a number of studies document the presence of *T. vivax* in lymph nodes after challenge with infected tsetse in the field (Adams, 1936; Losos and Ikede, 1972), and in lymph nodes (Bungener and Mehltz, 1977; Masake and Morrison, submitted) and tissue including the heart (Morrison et al., 1979; Murray et al., 1979), following the intramuscular inoculation of blood stream forms of the parasite. In addition, *T. vivax* is located extravascularly in the local skin reaction ("chancre") which results from challenge with infected tsetse and in skin biopsies sampled at the time of the peaks of parasitaemia (Emery and Moloo, in press). This report describes the movement of *T. vivax* from the site of inoculation by the infected tsetse through the local draining lymph nodes. The lymphocytic response which was provoked during the development of the skin reaction was monitored in the efferent lymph.

The efferent lymphatic duct from the prefemoral lymph node of 4 East African × Galla goats was cannulated by modification of the technique described for sheep (Hall, 1967). Each goat was bitten 48 h later on the ipsilateral flank to the operation by one of 4 *G. m. morsitans* infected with a clone of a serodeme of *T. (D.) vivax* stock Y486 (Barry and Gathuo, in preparation). Infection was established in 3 of the goats, and the subsequent enumeration of trypanosomes in lymph and blood employed the darkground technique of Murray et al. (1979). Lymphatic lymphocytes were counted in a haemocytometer and cells with a diameter greater than 12 µm were classed as lymphoblasts. Two addi-

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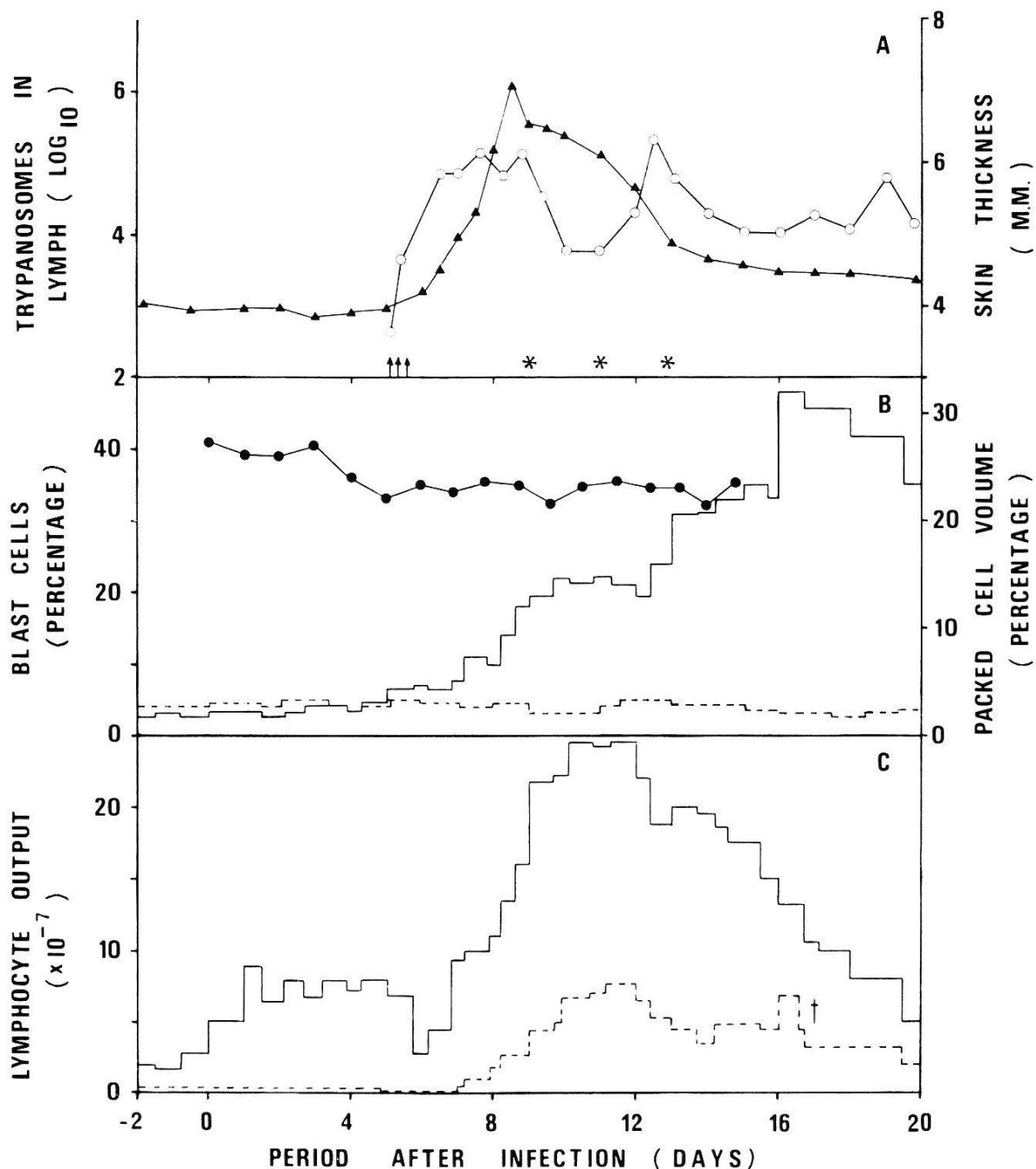


Fig. 1. The movement of *T. (D.) vivax* and host lymphocytes in efferent lymph from the regional lymph node in relation to development of the local skin reaction and parasitaemia following challenge of 3 goats with infected tsetse.

A. ▲ = Changes in skin thickness (mean); ○ = mean average numbers of trypanosomes in lymph; ↑ = initial detection of trypanosomes in lymph; * = initial detection of parasitaemia.

B. ● = Changes in PVC (mean); — = percentage blast cells in lymph from infected goats; ---- = percentage blast cells in lymph from 2 goats bitten by uninfected tsetse.

C. — = Mean average lymphocyte output per hour; --- = mean average blast cell output per hour; † = death of host.

tional goats were also cannulated 12 days after an intravenous inoculation of 10^5 bloodstream forms of the same clone of *T. vivax*.

The development of the local skin reaction which presented after 7 days as a discrete nodule at the site of challenge was similar to the lesion elicited with *T. vivax* in previous work (Emery and Moloo, in press). After reaching a maximum on day 9, the skin reaction subsided gradually over the next 6 days (Fig. 1 A). The output of lymphocytes from the draining lymph node increased slightly during the first 6 days after challenge (Fig. 1 C). At the time of the initial development of the chancre, a depression of 24-h duration in the lymphocyte traffic was observed; this feature was reminiscent of the "shutdown" reported in lymphocyte recirculation following antigenic challenge of lymph nodes in sheep (Hay and Morris, 1976). A dramatic increase in the output of total and blast cells occurred over the next 4 days, the peak of which reached 5–6 times the output of cells from the unstimulated node. During the same period, the proportion of blast lymphocytes in the efferent lymph increased from 3–5% to almost 40% of the cells collected around day 16 after challenge (Fig. 1 B). A large proportion (20–37%) of these blast cells stained positive by immunofluorescence for intracellular immunoglobulin.

Trypanosomes (approx. 600 per ml) appeared in lymph from the 3 goats simultaneously at the collection period 120–128 h (5–6 days) after challenge. Their appearance preceded by 24 h the first clinical evidence of the chancre and $1\text{--}5 \times 10^6$ trypanosomes were collected in lymph during this interval. The numbers of *T. vivax* reached a peak of approximately 10^5 per ml of lymph and a total of $1.3\text{--}7.5 \times 10^7$ were collected prior to the maximum development of the chancre (Fig. 1 A). After their initial detection, the average number of trypanosomes collected during the course of the infection followed the same pattern in each of the 3 goats (Fig. 2). Although with this clone of *T. vivax* in goats, the parasitaemia is usually first detected 8–9 days after tsetse challenge (Barry, unpublished), the removal of lymph-borne cells and trypanosomes ($3\text{--}12 \times 10^7$ before parasitaemia) appeared to delay the development of a detectable parasitaemia until 9, 11 and 13 days after challenge. The first peak of parasitaemia reached $5\text{--}8 \times 10^6$ trypanosomes per ml 15–16 days after challenge. The packed cell volume (PCV) did not alter appreciably during the first 16 days after infection (Fig. 1 B), and the goats died at 19, 25 and 26 days after challenge. One goat, in which the cannula blocked after day 12, died earliest at day 19 at the second peak of parasitaemia (Fig. 2 top). In the goats which were infected intravenously with bloodstream forms of *T. vivax*, the first peak of parasitaemia occurred on day 10, and trypanosomes were detected in central lymph from the day of cannulation (day 12) until the respective goats died 15 and 17 days after challenge. The concentration of trypanosomes in the lymph was only 5–10% fewer than that in blood, and followed quantitatively the pattern of the parasitaemia.

It is evident from this study that after inoculation by the infected tsetse a major route for the dissemination of *T. vivax* is through the regional lymph

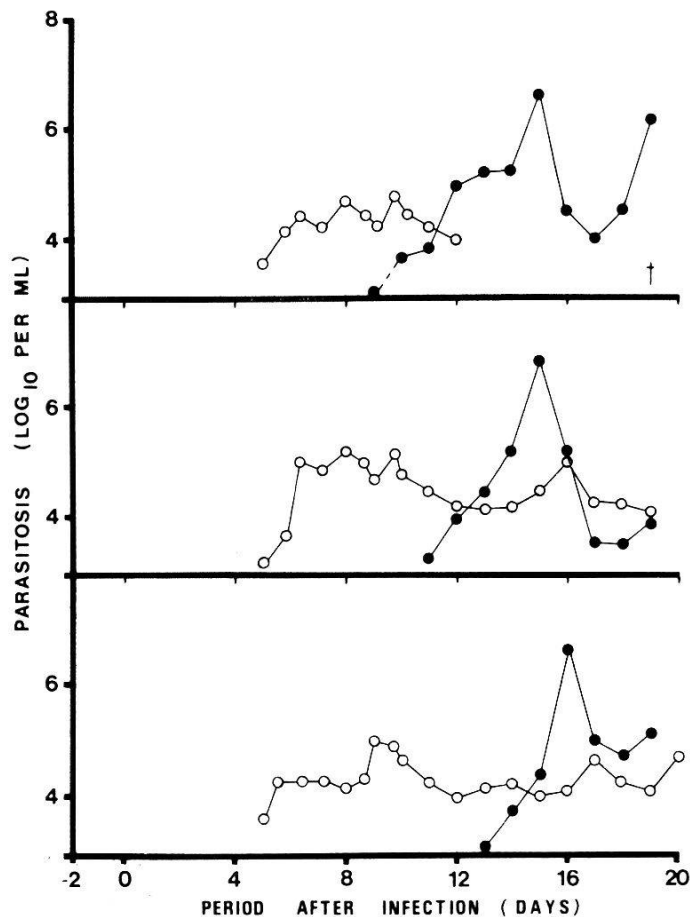


Fig. 2. The number of trypanosomes in lymph and blood following challenge of 3 goats with tsetse infected with *T. (D.) vivax*. ○ = Average number of trypanosomes in lymph from individual goats; ● = average parasitaemia in individual goats.

node and into the central lymph stream. This migration together with the local proliferation of the parasite at the site of inoculation provokes an initial host reaction in the formation of the chancre and a substantial enlargement of the regional lymph node, which is reflected as changes in the quality and quantity of the efferent lymph-borne cells. It has been suggested (Emery and Moloo, 1980) that trypanosomes might be contained in the chancre as a prelude to the induction of the host's immune response; it is clear from this study that *T. vivax* leaves the site of challenge in large numbers before the local skin reaction is formed. The early increase in the numbers of trypanosomes in lymph, and their detection in several tissues suggests that *T. vivax* is not an obligate parasite of the bloodstream. Following the first peak of parasitaemia, *T. vivax* recirculates through the lymph nodes and into the lymph stream; during this period when the chancre subsides, the local skin reaction probably contributes a decreasing proportion of the trypanosomes collected from the draining lymph node.

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