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Studies on *Trypanosoma (nannomonas) congolense*

IV. Experimental immunization of mice against tsetse fly challenge

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SUMMARY

Groups of mice were exposed to multiple bites by tsetse flies (*Glossina morsitans morsitans*) infected with a clone of *Trypanosoma congolense* spread over a period of 8 days. The mice were subsequently treated with Berenil 10 days after the first fly bite as were uninfected control mice. The group of mice which received 12–15 infectious fly bites on two occasions, 21 days apart, were subsequently resistant to infection when re-challenged by flies infected with the same clone of *T. congolense*. These mice were also immune to challenge by flies infected with a different bloodstream variable antigen type derived from this same stock. The immunity was stock-specific and directed against the metacyclic forms of the parasite, but was short-lived.

INTRODUCTION

It has been recognized since 1909 (Levaditi & McIntosh) that salivarian trypanosomes can apparently evade the development of a successful host immune response by the process of antigenic variation. Recently, it has also been shown in man (Greenwood, Whittle & Molyneux, 1973) and laboratory animals (Allt, Evans, Evans & Targett, 1971; Goodwin, Green, Guy & Voller, 1972; Urquhart, Murray & Jennings, 1972; Longstaffe, Freeman & Hudson, 1973; Murray, Urquhart, Murray & Jennings, 1973; Murray, Jennings, Murray & Urquhart, 1974*a b*; Jayawardena & Waksman, 1977) that one consequence of such infection is a profound suppression of various aspects of the host's immune response. In spite of these two impediments to the development of a successful protective immune response, it has, however, been previously recorded (Wilson, 1971; Wilson & Cunningham, 1971, 1972) that Ndama and Zebu cattle can successfully control their initial infection and subsequently show no detectable parasitaemia.

Effective protection against bloodstream trypanosome challenge can be induced experimentally by immunizing animals with whole bloodstream trypanosomes or fractions of their homogenates containing the surface variant antigen. However, such protection has been shown to be effective only against challenge with the

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homologous trypanosome populations. Significant cross-protection has not yet been convincingly demonstrated between heterologous variants of the same stock or between heterologous stocks of the same species (Doyle, 1977; Murray & Urquhart, 1977).

In an earlier paper in this series (Nantulya, Doyle & Jenni, 1980) we reported that when mice are exposed to several infectious tsetse bites they form neutralizing antibodies to the metacyclics from corresponding flies, as shown by *in vitro* neutralization tests. The following experiments were subsequently designed to investigate whether such neutralization could also be achieved *in vivo*.

MATERIALS AND METHODS

Parasites

The stocks of *T. congolense*, STIB 228 and STIB 249, were isolated from different lions in the Serengeti area (Tanzania) by Geigy & Kauffmann (1973). Clones 228B and 228C are different variable antigen types of STIB 228A which was itself derived from STIB 228 (Nantulya *et al.* 1980). Metacyclics of these 3 derivatives of STIB 228 had earlier been shown to cross-react completely with respect to their variable antigen types. Derivatives of STIB 249 had also been shown to revert to a characteristic metacyclic variable antigen composition which was different from that of STIB 228.

Tsetse flies

Flies were infected with STIB 228A, 228B, 228C and 249 and infected flies were subsequently identified as described by Nantulya, Doyle & Jenni (1978).

Laboratory animals

White female ICR mice (25–30 g) were used.

Experimental design

Twenty-five mice were divided into 3 groups and exposed to increasing numbers of bites by flies infected with *T. congolense* clone 228B and were subsequently treated with Berenil (Diaminazine aceturate, Hoechst Pharmaceuticals) at a dose of 50 mg/kg body weight administered intraperitoneally (Lumsden, Herbert & Hardy, 1965). Following treatment, they were challenged either by the same flies or by flies infected with a different clone derived from the same stock or by flies infected with another stock of *T. congolense*. Protection was said to have occurred if the animals showed no parasitaemia over a period of 30 days as judged by the haematocrit centrifuge technique (Woo, 1971).

RESULTS

Immunization with a single course of 5–12 infectious fly bites

The first group (7 mice) was exposed to 5 infectious fly bites/mouse, spread over a period of 8 days and then treated 10 days after the first fly bite. The second group (8 mice) received 10–12 infectious fly bites over the same time period of

8 days and were subsequently treated 10 days after the first fly bite. Six control mice exposed to 10 uninfected fly bites were treated similarly.

All mice were challenged once only, 1 week after Berenil treatment, by 1–2 flies infected with the same clone 228B. They all became infected and the pre-patent period of the immunized mice (10–12 days) was similar to that of the control group.

Immunization with 2 courses of 12–14 infectious bites

Fourteen mice were exposed to a total of 12–14 infectious fly bites over a period of 8 days using the same flies as used to immunize the first 2 groups of mice and were then similarly treated. Three weeks later, all 14 mice were each exposed to another course of 12–15 infectious fly bites and again treated with Berenil. Six control mice received 2 courses of 15 uninfected fly bites each followed by Berenil treatment, as did the 14 test mice.

Of the 14 immunized mice, 6 were challenged once only, 1 week after the second Berenil treatment by flies infected with clone 228B; 4 were similarly challenged by flies infected with a different clone 228C derived from the same stock, and the rest were challenged by flies infected with a different stock of *T. congolense* (STIB 249). The control mice received 1–2 infectious bites by flies infected with clone 228B.

All 6 mice that received a homologous challenge did not show parasitaemia, whereas all controls developed detectable parasitaemia within 10–12 days after challenge. The 4 mice challenged by flies infected with a different clone 228C derived from the same stock did not become infected, indicating that immunity against metacyclics of clone 228B also protects against challenge by flies infected with different variable antigen types of this stock of *T. congolense*. When these 10 mice were kept without any further exposure to infectious fly bites for a period of 7 months and then challenged by flies infected with the same clone 228B, however, they all showed infection within 10–12 days after challenge, indicating that the initial immunity had already waned to non-protective levels.

The 4 mice challenged by flies infected with a different stock of *T. congolense* (STIB 249) all became infected, showing that protection against tsetse fly challenge was stock-specific.

DISCUSSION

In an earlier paper in this series (Nantulya *et al.* 1980) we reported that metacyclics of a stock of *T. congolense* have a characteristic variable antigen composition. This observation suggested to us that vaccination against metacyclics would offer 2 important advantages: firstly, it would induce protection against the stock and secondly, it would prevent the development of bloodstream infection with its consequence of antigenic variation and possible immunosuppression.

Our present study has shown that mice immunized using a single course of 5–12 infectious fly bites did not show protection against an homologous cyclical challenge given 1 week after Berenil treatment as did the controls. The mice exposed to 2 courses of 12–15 infectious fly bites each, however, displayed a sterile immunity while the appropriate controls became infected within 10–12 days after challenge. Such mice were also protected against challenge by flies infected with a different

bloodstream variable antigen type derived from the same stock of *T. congolense* but succumbed to challenge by flies infected with a known unrelated stock. These observations are in agreement with the results of the *in vitro* tests (Nantulya *et al.* 1980) which showed that this stock of *T. congolense* reverts to a characteristic metacyclic antigen composition, and that metacyclics of these 2 stocks differ antigenically. This immunity to cyclical challenge was, however, short-lived.

The failure of mice receiving a total of 12 infectious fly bites to develop demonstrable protection is perhaps surprising in view of the fact that such an immunization schedule produces antisera capable of neutralizing 50–100 metacyclics of the homologous population *in vitro* (Nantulya *et al.* 1980). This may indicate that such serum antibody levels are not sufficient to neutralize the metacyclics deposited in the dermal tissues following the infectious fly bite (Luckins & Gray, 1978) before the parasites have undergone antigenic variation.

An additional point of interest is that Berenil, which at the dose used in the present study, has been reported to have prolonged residual prophylactic activity against *T. brucei* in mice (Lumsden *et al.* 1965) does not appear to have a similar action against *T. congolense*.

These protection experiments should be repeated using larger numbers of experimental animals and various doses of challenge, and using other stocks of *T. congolense*, before extending them to determine whether cattle can develop effective sterile or concommitant immunity to tsetse fly challenge under field conditions. Such investigations could be important to the understanding of the mechanisms of acquired resistance to trypanosome infections in cattle. Such investigations, however, are likely to be complicated by the multiplicity of species and strains of trypanosomes in the field, together with the possible mechanical transmission of the parasite by other blood-sucking *Diptera*.

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