MULTINUCLEAR FORMS IN A DYSKINETOPLASTIC STRAIN OF TRYPANOSOMA EVANSI IN MICE

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INTRODUCTION

Trypanosoma evansi which causes the disease Surra is one of the most economically important parasitic protozoa infecting domestic animals such as camels, horses, buffalo and deer in the People’s Republic of China. T. evansi is generally recognized as monomorphic as only « slender forms » are observed in the blood of an infected host (Bruce, 1911; Liu, 1980). There are numerous reports, however, of « short-stumpy » and « intermediate » forms in old strains which have been maintained by serial passage in laboratory animals for several years (Lavier, 1933; Hoare, 1956, 1972; Miles, 1970; Lun, 1988). Recently, De Diego et al. (1985) reported multinuclear forms and stumpy-like forms in division in T. b. brucei from infected mice. Our study reveals that multinuclear forms are also present in the dyskinetoplastic and normal kinetoplastic strains of T. evansi. When we tested the survival time of T. evansi in different lower animals other than mammals we found that numbers of such forms can be increased if healthy mice are inoculated with blood containing trypanosomes transfer back to mice. The multinuclear trypanosomes showed more nuclei than flagella indicating that disruption of the normal cell cycle had taken place and not simply inhibition of cleavage. A Chinese kinetoplastic T. evansi treated similarly rarely produced stumpy or multinuclear forms.

MATERIALS AND METHODS

The dyskinetoplastic strain of T. evansi was obtained from the Department of Veterinary Medicine, South China Agriculture University, 1979 and stored in liquid nitrogen. This strain was isolated from a horse in the outskirts of Guangzhou, in 1962 and maintained by continuous passage monthly alternating between rabbits and mice. No multinuclear forms in this strain were observed in rabbits. Cryostabilates were thawed at 37° C and inoculated into mice. Gecko lizards (Gekko japonicus) (collected on campus) were inoculated intraperitoneally with 0.5 ml mouse blood containing 10⁸ trypanosomes/ml. Blood smears of geckos indicated that there were no blood parasitic protozoa in the peripheral blood of these lizards before they were infected with T. evansi. Geckoes were kept at 35°-37° C in cages made of wood and nylon net for a week before and after inoculation, and fed with Culex or Aedes mosquitoes. Blood from the tails of infected geckoes was inoculated into mice every day for 7 days after infection of the gecko. Two days after inoculation, blood smears of infected mice were made every day until the mice died. A normal kinetoplastic strain of T. evansi isolated from a buffalo in Yang Jiang County, Guangdong Province, in 1982, was used as control. Both kinetoplastic and dyskinetoplastic strains were highly virulent in experimental animals killing mice 3 to 5 days after inoculation with 10⁶ parasites. All blood smears were fixed in methanol and stained with 10 % Giemsa for examination by light microscopy.

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RESULTS AND DISCUSSION

Short stumpy and multinuclear forms (Fig. 1) were found in the blood smears of mice infected with dyskinetoplastic and normal kinetoplastic strain of T. evansi, especially in the dyskinetoplastic one (Table I).

All Chinese strains of T. evansi hitherto isolated from buffalo, horses and horse-deer were claimed by their investigators (Liu, 1980; Gu, 1982) to be monomorphic as defined by Bruce (1911). Inconstant pleomorphism of T. evansi has been reported however in African and Indian strains (Hoare, 1952, 1956; Miles, 1970). Some strains proved to be highly pleomorphic (50 percent stumpy forms) even in clones (Miles, 1970). Miles (1972) also demonstrated that the mitochondrial marker enzyme, NAD diaphorase, was found in the short form of T. evansi SAK strain suggesting that the stumpy forms in this species correspond physiologically to those of T. brucei (Vickerman, 1965).

In our present work, it was very interesting to find peculiar multinuclear forms (with 6 to 10 nuclei, Fig. 1 C, D, E) in the blood smears of mice inoculated with trypanosomes from geckoes. These lizards, when inoculated with the strains of T. evansi will harbour the parasites for about 7 days, though the trypanosomes do not multiply in the poikilothermic host. Most multinuclear forms were observed in the mouse blood smears taken on the 4th to 6th day after inoculation. What was peculiar about the multinuclear trypanosomes was that the number of nuclei was in excess of the number of flagella, showing that the normal sequence of events in the cell cycle (replication of flagellum, replication of kinetoplast-mitochondrion, nuclear division, cleavage) had been disrupted (Table II).

Recent work using in vitro isolation of such bizarre trypanosomes has demonstrated that they are viable i.e. can give rise to normal trypanosomes once more.

<table>
<thead>
<tr>
<th>Number of flagella</th>
<th>Number of nuclei</th>
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<tbody>
<tr>
<td>3</td>
<td>4 2</td>
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<tr>
<td>4</td>
<td>1 1</td>
</tr>
<tr>
<td>5</td>
<td>2 7</td>
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<tr>
<td>6</td>
<td>3 3</td>
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Multinuclear forms were rarely found in the kinetoplastic strain when it was treated in the same way. Results from the stocks of cloned trypanosomes were similar to those for uncloned ones. Previous investigators have noted that pleomorphism is more readily induced in dyskinetoplastic strains of T. evansi (Hoare, 1972).

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REFERENCES