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Specific immunity in rats inoculated with *Trypanosoma brucei brucei* derived from tsetse salivary glands and cultured in vitro

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Summary

Twenty rats were each immunized intraperitoneally with 1.25×10^7 formalin-fixed *Trypanosoma brucei brucei* (Stock EATRO 1969) derived from tsetse salivary glands and propagated in culture at 29° C in their infective form for more than 1000 days. Another group, consisting of 10 rats, was similarly immunized with parasites of stock EATRO 999 derived from salivary glands of a tsetse fly and propagated in culture for more than 360 days. Immunized animals were resistant to homologous challenge with 10^4 cultured infective parasites. There was no cross protection between stocks and animals were susceptible to homologous infective tsetse bite challenge.

Key words: *Trypanosoma brucei brucei;* immunization in rats; immunity; susceptibility to homologous tsetse challenge.

Introduction

African trypanosomiasis, due to the capacity of the trypanosomes to undergo antigenic variation has become an immunological enigma and attempts to protect people or livestock against the disease have not been successful. However, a recent report (Jenni and Brun, 1981) indicated that parasites derived from tsetse salivary glands, cultured for 50 h at 37° C, irradiated and then injected into mice, induce immunity against infective tsetse bites. Similar studies (Duxbury and Sadun, 1969; Charoenvit and Campbell, 1981) have shown that immunity can be induced in rats by irradiated bloodstream form parasites. Wellde et al. (1973, 1975) immunized cattle and rats with gamma-

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irradiated *T. rhodesiense*. In this laboratory two stocks of *Trypanosoma brucei brucei* have been grown at 29° C in their infective forms from tsetse salivary glands (Nyindo et al., 1979). In this paper I report on the use of these parasites for experimental immunization studies in rats.

Materials and Methods

Parasites

Trypanosoma brucei brucei, stock EATRO 1969 and stock EATRO 999, were grown in their vertebrate infective form from tsetse salivary glands as previously described (Nyindo et al., 1979). Stock EATRO 1969 was originally isolated from blood of a female hyena (*Crocuta crocuta*) in Tanzania. Stock EATRO 999 was derived from bovine blood in 1964 in Kenya. Briefly, salivary glands of tsetse flies, *Glossina morsitans morsitans*, containing metacyclic trypanosomes, were explanted into sterile T-flasks containing a monolayer of bovine embryonic spleen cells at 29° C in RPMI 1640 medium supplemented with 20% foetal bovine serum. Cultured parasites of stock EATRO 1969 on days 1147, 1150, 1158, 1161 and 1169 were centrifuged and the pellet on each occasion was resuspended in 5 ml phosphate saline glucose (PSG) pH 8.0 (Lanham and Godfrey, 1970). An equal volume of 2% formaldehyde in PSG was added, and the parasites were fixed at 4° C for 30 min, centrifuged, washed twice in PSG and finally resuspended in 5 ml PSG. Parasites derived from stock EATRO 999 on days 366, 368, 382 and 385 of cultivation were handled in a similar way.

Animals

Twenty white rats (*Rattus rattus*), 3 months old, were each inoculated intraperitoneally with 0.25 ml of the fixed parasites from stock EATRO 1969 on each day of fixation. Twenty other rats were each inoculated with 0.25 ml PSG and served as controls. Ten rats were each similarly inoculated with parasites of stock EATRO 999 origin. Ten control animals were each injected with 0.5 ml PSG. Total number of parasites for stock EATRO 1969 inoculated into each rat was 12.50×10^6, while each animal injected with parasites of stock 999 received 10.0×10^6 parasites.

Tsetse flies

One rat was inoculated with 10^6 cultured parasites from stock EATRO 1969 on day 1145 of cultivation. At the development of the first peak parasitemia, blood was taken and parasites were isolated by the method of Lanham and Godfrey (1970). Five rats were each inoculated intraperitoneally with a clone of the bloodstream form parasites (Lumsden et al., 1973). One hundred and fifty teneral *Glossina morsitans morsitans* (flies) were fed on one of the five rats inoculated with a clone at first peak parasitemia. Metacyclic trypanosomes were detected in the salivary glands of 5 flies by day 21 by examination of their saliva and transmission studies. Thirty-nine days later the infected flies were each used to challenge 5 animals immunized with cultured parasites from stock EATRO 1969 and 5 control animals.

Challenge exposures

a) *Animals immunized with stock EATRO 1969.* Two days after the last immunization each rat was challenged intraperitoneally with 10^4 EATRO 1969 cultured parasites on day 1171 of cultivation. The 20 control animals were also inoculated with 10^4 parasites. Appearance of parasites in peripheral blood was followed for 30 days by examination of fresh preparation of tail blood as wet mounts. After this time, 5 of the immunized animals which did not develop parasitemias were challenged with metacyclic forms of parasites by infected flies, and another 5 were inoculated with 10^4 cultured EATRO 999 parasites.

Table 1. Results of immunization and challenge exposure with cultured parasites and infected tsetse flies

Stock used for immunization	No. of animals immunized	Animals becoming parasitaemic after challenge exposure with		
		Stock 1969	Stock 999	Fly
1969	20	2/20 (7–9)	5/5 (16)	5/5 (4–5)
999	10	5/5 (7–9)	2/5 (12)	not done

All 20 control animals inoculated with cultured parasites of stock EATRO 1969 became parasitaemic in 7–9 days. The control animals inoculated with cultured parasites stock EATRO 999 became parasitaemic in 16 days. The 5 control animals challenged with metacyclic trypanosomes from tsetse flies were parasitaemic by day 4 to day 5. Numbers in parentheses indicate the prepatent period in days.

b) *Animals immunized with stock EATRO 999.* Five of the 10 rats immunized with EATRO 999 were given an homologous challenge with 10^4 cultured parasites on day 388 of cultivation and the remaining 5 were challenged with 10^4 EATRO 1969 cultured parasites grown for 1171 days. The 10 control animals were also inoculated with 10^4 cultured EATRO 999 parasites. No fly challenge was done in this group of animals immunized with EATRO 999.

Results

All 20 control animals inoculated with 10^4 cultured parasites from stock EATRO 1969 showed parasites in their peripheral blood in 7–9 days. Out of the 20 immunized animals only 2 showed parasites in their peripheral blood after challenge (Table 1). When 5 of the immune animals were later challenged with infected tsetse flies all of them developed parasitemias by day 4 to day 5 similar to the controls. When 5 of the 13 remaining immune animals were challenged with EATRO 999 cultured parasites they all become positive by day 16 as did the controls.

Two out of 5 rats immunized with EATRO 999 and later exposed to homologous challenge became positive in 12 days. All 5 animals immunized with EATRO 999 and challenged with EATRO 1969 became positive in 7–9 days. All 10 control animals became positive by day 12 after inoculation with EATRO 999 cultured parasites.

Discussion

Immunity was induced in rats by inoculation with formalis-fixed infective parasites derived from tsetse salivary glands and cultured for 1169 or 385 days in vitro. No protection against a heterologous challenge or challenge with metacyclic trypanosomes from infective tsetse bites occurred. Jenni and Brun (1981) demonstrated similar stock specific immunity in mice.

It has been shown by Le Ray et al. (1978) that metacyclic trypanosomes in tsetse salivary glands are heterogenous with respect to the variable surface antigen types (VATs). Hence as shown in this study, rats were susceptible to homologous tsetse bites. Since animals immunized with the cultured parasites were susceptible to challenge exposure with infective tsetse bites from the same stock, it is likely that our culture system does not support the growth of all possible trypanosome VATs found in the tsetse salivary glands.

Nyindo and Rurangirwa (1981) showed that infective salivary gland parasites grown at 29° C remained antigenically stable but started to modulate their surface coat antigens when transferred to 37° C. Antigenic stability of bloodstream form parasites grown at 37° C was not demonstrated by Doyle et al. (1980). It therefore appears that parasites grown at 29° C would be better for experimental immunization than parasites grown at 37° C, yet even with the former parasites protection is only possible against a homologous challenge only.

Charoenvit Y., Campbell G. H.: Immunization of mice with *Trypanosoma rhodesiense* exposed to ultraviolet irradiation. Amer. J. trop. Med. Hyg. *30*, 1198 (1981).

Doyle J. J., Hirumi H., Hirumi K., Lupton E. N., Cross G. A. M.: Antigenic variation in clones of animal infective *Trypanosoma brucei* derived and maintained in vitro. Parasitology *80*, 359 (1980).

Duxbury R. E., Sadun E. H.: Resistance produced in mice and rats by inoculation with irradiated *Trypanosoma rhodesiense*. J. Parasit. *55*, 858 (1969).

Jenni L., Brun R.: In vitro cultivation of pleomorphic *Trypanosoma brucei* stocks: a possible source of variable antigens for immunization studies. Trans. roy. Soc. trop. Med. Hyg. *75*, 150 (1981).

Lanham S. M., Godfrey D. G.: Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. Exp. Parasit. *28*, 521 (1970).

Le Ray D., Barry J. D., Vickerman K.: Antigenic heterogeneity of metacyclic forms of *Trypanosoma brucei*. Nature (Lond.) *273*, 300 (1978).

Lumsden W. H. R., Herbert W., McNellage F. J. C.: Techniques with trypanosomes, p. 45. Churchill Livingstone, Edinburgh 1973.

Nyindo M., Rurangirwa F. F.: *Trypanosoma brucei* continuous cultivation of antigenically stable parasites at 29° C and induction of antigenic variants at 37° C. Insect sci. Appl. *2*, 263 (1981).

Nyindo M., Patel N., Darji N., Golder T. K.: *Trypanosoma brucei*. In vitro propagation of metacyclic forms derived from the salivary glands of *Glossina morsitans*. J. Parasit. *65*, 751 (1979).

Wellde B. T., Duxybury R. E., Sadun E. H., Langbehn H. R., Lotzsch R., Deindl G., Warui G.: Experimental infections with African trypanosomes. IV. Immunization of cattle with gamma-irradiated *Trypanosoma rhodesiense*. Exp. Parasit. *34*, 62 (1973).

Wellde B. T., Schoenbechler M. J., Diggs C. L., Langbehn H. R., Sadun H.: *Trypanosoma rhodesiense*. Variant specificity of immunity induced by irradiated parasites. Exp. Parasit. *37*, 125 (1975).