

Use of CB hamsters in the study of *Treponema pertenu*

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SUMMARY The CB/Ss LAK strain of inbred hamster was used as a model for studies of infection with *Treponema pertenu* and of acquired resistance to it. When infected, this strain developed cutaneous lesions which lasted for six to seven months, even in the presence of peak titres of antitreponemal antibody. The rate of appearance and resolution of these lesions varied with the size of the inoculum. The infected hamsters' inguinal lymph nodes increased significantly in weight and teemed with treponemes for several weeks. Animals infected for eight or 10 weeks obtained quick resolution of their lesions by treatment with penicillin and were thereafter resistant to reinfection.

Introduction

For investigations of the immune mechanism of frambesia (yaws), inbred strains of hamsters are becoming recognised as the animal model of choice. Two such studies with inbred golden hamsters have been reported (Guerraz *et al.*, 1977; Schell *et al.*, 1978).

We have found that CB/Ss LAK (CB) strain of hamster responds especially rapidly to infection with *Treponema pertenu*, the causative agent of frambesia, and that pathological changes induced in this strain can be readily quantitated. For these reasons we are now using the CB strain in experimental studies of passive transfer of resistance to *T. pertenu*.

The advantages of the hamster model—and of the CB strain in particular—are presented in this report.

Materials and methods

ANIMALS

Five inbred hamster strains were studied initially: CB, MHA/Ss LAK, LSH/Ss LAK, PD4/LAK, and LHC/LAK. These were obtained from Charles River Breeding Laboratories Inc., Wilmington,

Massachusetts. Hamsters weighing 80-100 g were housed five or six per cage at an ambient temperature of 18°C, a condition which facilitates the development of cutaneous lesions (Hollander and Turner, 1954).

ORGANISM

T. pertenu strain Haiti B (obtained from Paul H. Hardy, Jr., Johns Hopkins University) was maintained by passage in hamsters. The inguinal lymph nodes were removed aseptically three to four weeks after intradermal infection, teased apart in sterile saline, and filtered through 60-mesh steel wire. After centrifugation at 270 × g for three minutes to remove cellular debris, the number of treponemes in the supernatant was determined by darkfield microscopy.

COUNTING OF TREPONEMES

The approximate number of treponemes per lymph node was determined according to the procedure described by Miller (1971). Briefly, duplicate slides of each homogenised lymph node were prepared and 120 fields per slide were examined for treponemes by darkfield microscopy.

SEROLOGICAL TEST

The Sera-Tek treponemal antibody test (manufactured by Fujizoki Pharmaceutical Co. Ltd., Shinjuku-ku, Tokyo, Japan) was used. The test was

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performed as specified by the manufacturer, except that sera obtained from frambesial hamsters were serially diluted with absorbing diluent to yield quantitative titres.

STATISTICAL ANALYSIS

Fischer's least significant difference test (Steel and Torrie, 1960) was used to examine pairs of means when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 before the experiments started.

Results

APPEARANCE OF CUTANEOUS LESIONS

Ten hamsters from each of the five strains were infected intradermally in the inguinal region with 1×10^6 *T. pertenu* and examined weekly for development of cutaneous lesions.

Three weeks after infection lesions were found in all CB hamsters, in one MHA/Ss LAK hamster, and in none of the remaining strains (Table 1). Although more lesions developed in each of the other strains up to 12 weeks after infection, in only the CB strains were all test animals affected. Similar results were obtained when this study was repeated, and no new lesions occurred in any strain after 12 weeks. All further experiments were conducted with the CB strain.

Table 1 Cumulative number of hamsters from five inbred strains developing cutaneous lesions

Strain (n = 10)	Weeks after infection			
	3	5	8	12
CB/Ss LAK	10	10	10	10
MHA/Ss LAK	1	6	8	8
LSH/Ss LAK	0	3	3	5
PD4/LAK	0	2	3	7
LHC/LAK	0	5	7	7

EFFECT OF INOCULUM SIZE

Groups of six CB hamsters were inoculated intradermally with 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , or 1×10^6 treponemes. The rate of development of cutaneous lesions was directly dependent on the number of treponemes injected (Table 2). All hamsters given $\geq 1 \times 10^4$ treponemes, however, formed lesions within six weeks. In groups given $\leq 1 \times 10^3$ treponemes, few or no animals showed lesions even after observation for 12 weeks.

Hamsters inoculated with 1×10^5 or 1×10^6 treponemes developed extensive, chronic, skin lesions, which usually resolved six to seven months after infection. In a few (10%) hamsters the lesions remained nine months after infection. In contrast,

Table 2 Cumulative number of CB hamsters developing cutaneous lesions after varying inocula of *T. pertenu*

Weeks after infection	Inoculum (six animals/group)				
	10^6	10^5	10^4	10^3	10^2
1					
2	1				
3	6				
4	6	4	1		
5	6	6	2		
6	6	6	6	2	

lesions regressed rapidly in hamsters inoculated with 1×10^3 or 1×10^4 treponemes. Thus inoculum size influenced not only the development but also the resolution of cutaneous lesions.

LYMPH NODE WEIGHTS AND TREPONEMAL COUNTS

Thirty-six hamsters were infected in the inguinal region with 1×10^6 *T. pertenu*. At weekly intervals for 12 weeks three frambesial hamsters were bled by cardiac puncture and killed. The inguinal nodes were removed aseptically, and the approximate number of treponemes per node was determined by darkfield microscopy. Three age-matched, non-infected hamsters were killed each week to determine the weights of normal inguinal lymph nodes.

The inguinal nodes from frambesial hamsters rapidly increased in weight, reaching a peak five weeks after infection (Figure). The number of treponemes detected in the lymph nodes increased concomitantly (Table 3). Six weeks after infection the

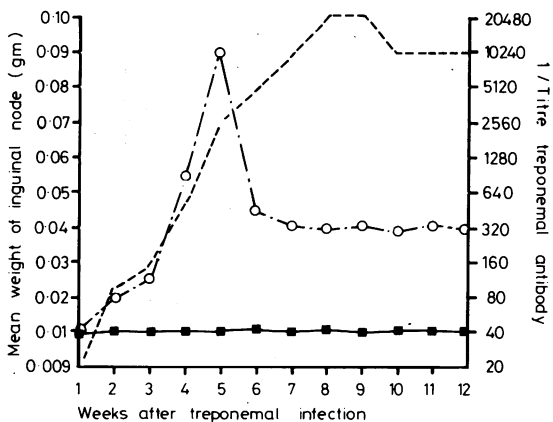


Figure Mean weights of inguinal lymph nodes in normal CB hamsters (■) and in hamsters infected with 1×10^6 *T. pertenu* (○). (Standard error for each mean = 0.009). Broken line shows antitreponemal antibody response in infected animals.

Table 3 *Approximate number of treponemes in inguinal lymph node after infection with T. pertenuae*

Week after infection	Mean No. of treponemes $\times 10^4$ *
1	5.1
2	58.8
3	729.0
4	996.0
5	1610.0
6	633.0
7	209.0
8	131.0
9	128.0
10	111.0
11	98.0
12	120.0

*Three hamsters per group interval

weight decreased by 50% and the number of treponemes declined sharply.

ANTIBODY RESPONSE

Antitreponemal antibody titre increased slowly during the first three weeks after infection (Figure). Thereafter the titre increased rapidly, reaching a peak eight to nine weeks after infection. Infected hamsters retained this peak titre nine months after infection. When the experiment was repeated, similar results were obtained.

RESISTANCE TO REINFECTION

At two-week intervals three groups of six hamsters each were infected intradermally with 1×10^6 *T. pertenuae*. At week 10 (that is, after six, eight, or 10 weeks of infection) 3000 units of penicillin were administered to all three groups plus a fourth group of six non-infected hamsters. Ten days later all four groups were challenged intradermally with 1×10^6 *T. pertenuae*.

Lesions developed after 17 ± 2 days in the controls and after 28 ± 3 days in animals which had previously been infected for six weeks. In contrast, no cutaneous lesions were detected even after prolonged observation (total seven weeks) in hamsters that had been infected previously for eight or 10 weeks.

Twenty-eight days after reinfection three hamsters from each group were killed. No treponemes were detected in the lymph nodes of hamsters previously infected for eight or 10 weeks. A large number of treponemes was detected in the lymph nodes of controls (2.3×10^7) and of hamsters previously infected for six weeks (1.2×10^6). The weight of the lymph nodes averaged 0.06 g in controls, 0.04 g in animals previously infected for six weeks, and 0.01 g in animals previously infected for eight or 10 weeks (standard error for each mean = 0.009). The reduction in weight in the latter groups (eight or 10

weeks) compared with that in the controls was statistically significant ($P < 0.001$).

These results showed that after eight weeks of frambesial infection the hamsters had developed an effective immune response.

Discussion

For experimental studies of the immune mechanism of frambesial infection, hamsters have several distinct advantages:

- (1) Inbred CB hamsters are readily available and are required for immunological studies of the way resistance may be transferred to normal recipients with sera or cells from animals immune to frambesial infection.
- (2) Infection of inbred hamsters with *T. pertenuae* consistently produces extensive, chronic, skin lesions that resemble human frambesial infection (Williams, 1935).
- (3) Frambesial lymph nodes can be easily detected in hamsters, and their increased weight is a useful measure of pathogenicity and infectivity.
- (4) Since the lymph nodes of frambesial hamsters teem with treponemes their numbers can be readily estimated by darkfield microscopy.
- (5) The hamster has no known treponemal disease that might influence an experimental infection (Willcox and Guthe, 1966).
- (6) The inbred hamster is relatively inexpensive and does not require elaborate animal facilities.

The ultimate goal of treponemal research is to develop an effective vaccine. For this purpose it is especially important that treponemal infections of vaccinated and non-vaccinated inbred hamsters can be compared in three parameters: (1) lymph-node weight, (2) number of treponemes per inguinal lymph node, and (3) development of cutaneous lesions. In the frambesial rabbit model only the cutaneous lesions (Eagle, 1948) can be readily measured.

Although Geiman and McKee (1950) and several other investigators (Guimaraes, 1953; Rosenau, 1953; Hill and Gordon, 1954; Turner and Hollander, 1957; Blom *et al.*, 1976; Hovind-Hougen *et al.*, 1976) have shown that outbred hamsters can be infected with *T. pertenuae*, only one inbred strain capable of such infection has been reported. These are the golden hamsters used by Guerraz *et al.* (1977). Our results show that the CB hamster is also an excellent model for treponemal studies. When infected, it regularly developed cutaneous lesions, which normally persisted for six to seven months, even in the presence of peak anti-treponemal antibody titres. The rate of appearance of cutaneous lesions and of

their resolution varied measurably with the size of the inoculum. In addition, the infected CB hamster's inguinal lymph nodes increased significantly in weight and teemed with treponemes for several weeks. Similar results were reported for outbred strains by Geiman and McKee (1950), Turner and Hollander (1957), and Rosenau (1953).

The cutaneous lesions resolved rapidly after treatment with penicillin. When CB hamsters are so treated after eight or 10 weeks of infection and are subsequently reinfected with *T. pertenu*, no frambesial lesions develop. The ability to acquire resistance to reinfection makes this strain an appropriate one for studies of passive transfer of resistance, which are now under way in our laboratory (Schell *et al.*, 1978).

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