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LSH Hamster Model of Syphilitic Infection

RONALD F. SCHELL,[1,2]* JACK L. LEFROCK,[3] JOHN K. CHAN,[1,2] AND OMAR BAGASRA[2,3]

Department of Microbiology and Immunology[1] and Department of Medicine[3] (Division of Infectious Diseases), Albany Medical College, and Clinical Microbiology Laboratory,[2] Albany Medical Center Hospital, Albany, New York 12208

The inbred LSH/Ss LAK strain of hamster can be infected with *Treponema pallidum* Bosnia A, the causative agent of endemic syphilis. When infected, this strain consistently produced extensive chronic skin lesions that persisted for 6 to 9 months, even in the presence of peak antitreponemal antibody titers. The lymph nodes increased in weight and contained measurable numbers of treponemes. This infection also gave the hamsters cross-immunity to *T. pallidum* Nichols and *Treponema pertenue*, the causative agents of venereal syphilis and frambesia, respectively. LSH hamsters are thus an excellent model to study the immune response mechanisms to syphilitic infection.

In studies of syphilis a major obstacle has been the unavailability of a suitable inbred animal model for elucidating the role of humoral and cell-mediated immunity in the host's resistance to infection. Treponemal disease is not regularly induced in guinea pigs (19) or mice (2, 4, 9, 13, 18), although some success has been achieved with inbred strains (5, 19). Inbred rabbits can regularly develop clinical manifestations of syphilis (1), but these signs are limited to the development of lesions. In any case, commercially inbred rabbits are difficult to obtain.

We have previously reported (12) the suitability of inbred hamsters, especially the CB/Ss LAK strain, as models of the related treponemal disease frambesia (yaws). Infection of these hamsters with *Treponema pertenue* elicits three responses, which are amenable to quantitation: (i) development of cutaneous lesions, (ii) changes in lymph node weights, and (iii) growth of treponemes in the lymph nodes. By these parameters it has been possible to compare frambesial infections in normal animals and animals that have been transfused with immune serum and cells (3, 11).

Continued studies have now shown that another strain of inbred hamster, LSH/Ss LAK (LSH), is especially suitable for investigations with *Treponema pallidum*. These hamsters can be infected with *T. pallidum* Bosnia A, the causative agent of endemic syphilis. The infected hamsters consistently produce extensive chronic skin lesions that persist for 6 to 9 months. In addition, the lymph nodes increase in weight and teem with treponemes. Since LSH hamsters infected with *T. pallidum* Bosnia A are resistant to reinfection with homologous or heterologous virulent treponemes, we are now using this model to determine whether resistance to syphilis can be passively transferred with immune serum and cells (10).

MATERIALS AND METHODS

Animals and infection. Five inbred hamster strains (LSH; MHA/Ss LAK [MHA]; CB/Ss LAK [CB]; PD4/LAK [PD4]; and LHC/LAK [LHC]) were obtained from Charles River Laboratories, Inc., Wilmington, Mass. The animals from each strain were randomized for assignment to cages and treatment groups. Housing conditions have been described previously (3, 10). All animals were shaved and kept hair-free by weekly clipping before infection. Treponemes were injected intradermally in the inguinal region.

Organisms. *T. pallidum* Bosnia A, *T. pallidum* Nichols, and *T. pertenue* Haiti B were obtained from Paul H. Hardy, Jr. (Johns Hopkins University) and were maintained by passage in hamsters. The inguinal lymph nodes were removed aseptically 3 to 4 weeks after intradermal infection, teased apart in sterile saline, and filtered through 60-mesh stainless-steel screens. After centrifugation at 270 × *g* for 3 min to remove cellular debris, the number of treponemes in the supernatant was determined by dark-field microscopy (8).

Evaluation of treponemal infections. Counting of treponemes in the lymph nodes, use of the Sera-Tek Treponemal Antibody test (Fujizoki Pharmaceutical, Tokyo, Japan; obtained from Ames, Elkhart, Ind.), and statistical evaluation by Fischer's least-significant-difference test have been described (3, 10).

RESULTS

Variations in lesions by strain. Five hamsters from each strain were infected with 4×10^5 *T. pallidum* Bosnia A and examined daily for inflammation. Each site of inoculation became erythematous 3 weeks after infection in all LSH hamsters (Table 1). The intensity of erythema increased until day 26 after infection, at which

time the skin appeared roughened and scaly. Within 24 to 48 h thereafter the skin ulcerated and became either a larger crusted lesion or an open lesion, while the periphery of the lesion continued to expand. The initial erythematous lesions did not appear in all CB hamsters until week 5, or in all MHA, PD4, or LHC hamsters until week 12. Central healing of the lesions in the LSH strain began 4 months after infection, and the margin of the lesions still exhibited marked erythema (Fig. 1). The lesions were not resolved until 6 to 9 months after infection. Similar results were obtained when this study was replicated.

TABLE 1. *Cumulative number of hamsters from five inbred strains developing cutaneous lesions after infection with T. pallidum Bosnia A*

Strain ($n = 5$)	No. of hamsters at weeks after infection:			
	3	5	8	12
LSH	5	5	5	5
MHA	1	3	3	5
CB	3	5	5	5
PD4	2	2	2	5
LHC	3	3	3	5

All further experiments were conducted with the LSH strain.

Variations in lesions by inoculum size. Groups of six LSH hamsters were inoculated intradermally with varying numbers of virulent treponemes. The rapidity of the appearance of

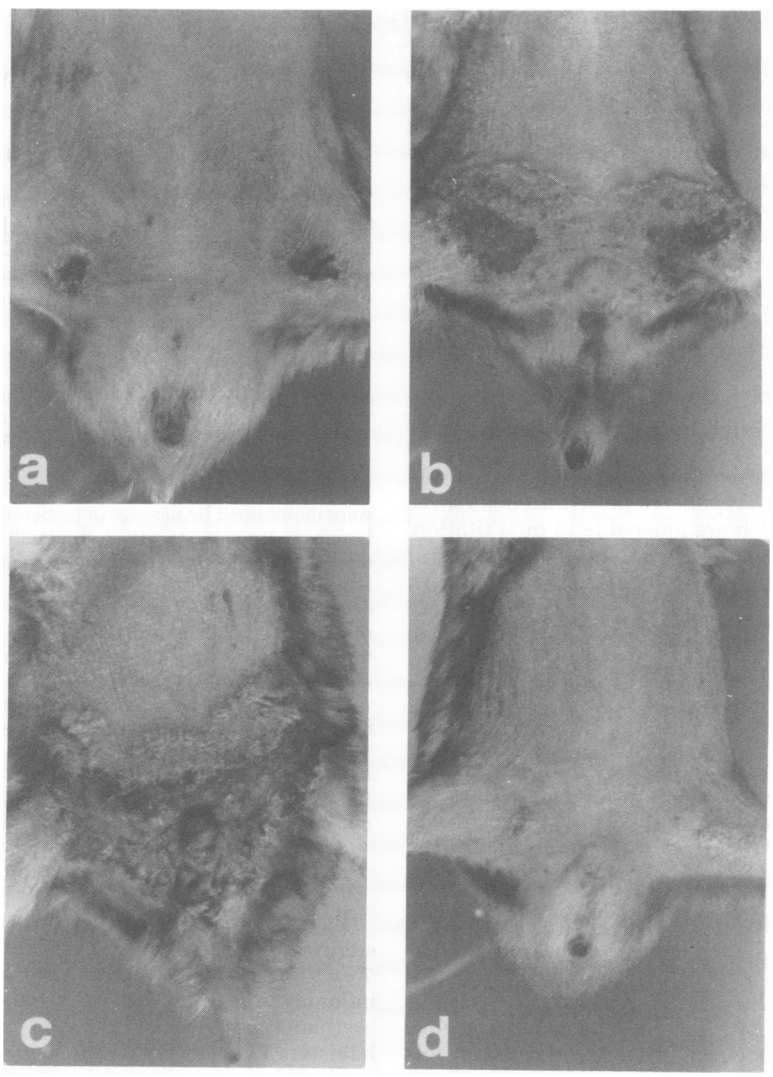

FIG. 1. *Development and regression of cutaneous lesions in representative LSH hamsters at (a) 4, (b) 8, (c) 16, and (d) 36 weeks after infection with T. pallidum Bosnia A.*

cutaneous lesions was directly dependent on the number of treponemes injected (Table 2). A minimum dose of 10^4 treponemes was required for all animals to develop lesions, but these lesions regressed within 3 months. Doses of 10^5 and 10^6 treponemes produced lesions in all animals by 4 and 3 weeks, respectively, and the lesions persisted for 6 to 9 months.

Lymph node weights and treponemal counts. Thirty-six hamsters were infected with 4×10^5 *T. pallidum* Bosnia A. Each week for 12 weeks three of these syphilitic hamsters were sacrificed, along with three age-matched, noninfected hamsters as controls. From week 3 on there was a rapid increase in the weights of the inguinal lymph nodes of the syphilitic hamsters (Fig. 2) and in the number of treponemes in the nodes (Table 3). After 10 weeks, both the node weights and the number of treponemes had begun to decrease.

Antibody response. After a slow rise during the first 4 weeks after infection, the antitreponemal antibody titer increased rapidly (Fig. 2). A peak titer was reached 8 to 9 weeks after infection and was maintained until 9 months after infection. Similar results were observed when the experiment was replicated.

Resistance to reinfection with homologous and heterologous treponemes. Infections with 4×10^5 *T. pallidum* Bosnia A were timed so that 12 groups of experimental hamsters could be studied simultaneously after 6, 8, 10, and 16 weeks of infection. For each time period there were three groups of six hamsters each. Three groups of six normal hamsters each were also included as controls.

Each animal in the 15 groups (12 infected, 3 control) was given 4,000 U of penicillin. Ten days later each was challenged with 10^6 *T. pallidum* Bosnia A, *T. pallidum* Nichols, or *T. pertenue*.

Reinfection with *T. pallidum* Bosnia A produced lesions after 21 ± 4 days in the controls and after 31 ± 3 days in most animals that had been previously infected with this strain for 8 weeks or less (Table 4). In contrast, hamsters that had been infected with this strain for 10 to 16 weeks developed no lesions during a prolonged observation period of 20 weeks after reinfection.

Twenty-eight days after reinfection, three hamsters from each experimental group and three control hamsters were sacrificed. Very few treponemes were detected in the lymph nodes of hamsters previously infected for 6, 8, or 10 weeks, compared to controls (Table 4). No treponemes were detected in hamsters previously infected for 16 weeks.

Thus, after 10 weeks of infection with *T. pallidum* Bosnia A, the hamsters had mounted an effective immune response to this strain.

Hamsters infected with *T. pallidum* Bosnia A also developed a substantial resistance to heterologous reinfection. The number of *T. pallidum* Nichols or *T. pertenue* treponemes de-

TABLE 2. *Cumulative number of LSH hamsters developing cutaneous lesions after varying inocula of T. pallidum Bosnia A*

Weeks after infection	No of hamsters at inoculum[a]:				
	10^6	10^5	10^4	10^3	10^2
1	—	—	—	—	—
2	4	—	—	—	—
3	6	4	1	—	—
4	6	6	3	—	—
5	6	6	6	3	—
6	6	6	6	4	—

[a] Six animals per group. —, No lesions were detected.

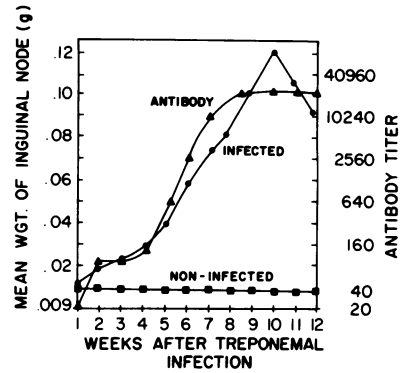

FIG. 2. *Mean weights of inguinal lymph nodes in normal LSH hamsters (■) and in hamsters infected with 4×10^5 T. pallidum Bosnia A (●). Standard error for each mean = 0.005. Antitreponemal antibody response is also shown (▲).*

TABLE 3. *Approximate number of treponemes in inguinal lymph node after infection with T. pallidum Bosnia A*

Week after infection	Treponemes ($\times 10^3$)[a]
1	9
2	2
3	90
4	330
5	2,000
6	3,000
7	2,500
8	2,000
9	1,000
10	700
11	400
12	200

[a] Three hamsters per week.

TABLE 4. *Development of resistance in hamsters infected with T. pallidum Bosnia A to reinfection with the same or heterologous treponemes*

Week after initial infection	*T. pallidum* Bosnia A		*T. pallidum* Nichols[a]		*T. pertenue*	
	Lesions/sites[b]	Treponemes ($\times 10^3$) per node	Lesions/sites	Treponemes ($\times 10^3$) per node	Lesions/sites	Treponemes ($\times 10^3$) per node
6	12/12	5.6	0/12	67	12/12	100
8	8/12	2.2	0/12	49	12/12	78
10	0/12	1	0/12	2	4/12	4
16	0/12	0	0/12	0	0/12	0
Controls	12/12	310	0/12	150	12/12	1,750

[a] Lesions rarely develop in hamsters infected with *T. pallidum* Nichols, but the lymph nodes teem with treponemes.

[b] Six animals per group. Each animal was inoculated at two sites.

tected 28 days after reinfection was lower in all experimental groups than in controls (Table 4). Hamsters previously infected for 16 weeks with *T. pallidum* Bosnia A developed no lesions and had no treponemes after reinfection with *T. pallidum* Nichols or *T. pertenue*. It should be pointed out that infection of "control" hamsters with *T. pallidum* Nichols rarely resulted in development of lesions. These results demonstrated that hamsters exposed to endemic syphilis are protected from challenge with other virulent treponemes.

DISCUSSION

The LSH strain of inbred hamster responds rapidly to infection with *T. pallidum* Bosnia A, and pathological changes induced in this strain can be readily quantitated. In our study the LSH hamster regularly developed cutaneous lesions that persisted for 6 to 9 months, even in the presence of peak antitreponemal antibody titers. The timing of the appearance of cutaneous lesions and their resolution varied with the inoculum size. In addition, the infected hamsters' inguinal lymph nodes increased significantly in weight and contained sizable numbers of treponemes for several weeks.

Our results further demonstrate that among the virulent treponemes cross-resistance to infection can develop in hamsters. Infection of hamsters with *T. pallidum* Bosnia A induced a substantial resistance to challenge with *T. pallidum* Nichols, the strain most commonly used to study venereal syphilis, or with *T. pertenue*, the causative agent of frambesia (yaws). Hamsters infected for 16 weeks developed no lesions, and their lymph nodes contained no treponemes after reinfection with homologous or heterologous treponemes.

The converses of this experiment have been previously reported (6, 7, 14, 16). For example, Turner and Hollander (15) showed that rabbits inoculated intratesticularly with the Nichols strain were resistant to challenge with *T. pallidum* Bosnia A. No lesions developed, although control rabbits developed lesions. Likewise, rabbits infected with yaws or syphilis showed a substantial immunity to reinfection with heterologous treponemes (6, 7, 14, 16, 17).

Attempts to elucidate the mechanism by which animals respond to syphilitic infection have been hindered by the unavailability of suitable inbred animals. This study clearly demonstrates that inbred LSH hamsters acquire resistance after infection with *T. pallidum* Bosnia A and are resistant to reinfection with this strain and other virulent treponemes. Experimental infection of the LSH hamster with *T. pallidum* Bosnia A is thus an excellent model to study immune response mechanisms to syphilitic infection, with the ultimate goal of developing an effective vaccine.

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