NOTES

Active Immunization of Hamsters against Experimental Infection with *Borrelia burgdorferi*

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The immunogenicity of a single dose of a whole-cell vaccine of inactivated *Borrelia burgdorferi* HSF (human spinal fluid isolate) was evaluated. Syrian hamsters were vaccinated subcutaneously and challenged by the intraperitoneal injection of 1,000 50% infectious doses of *B. burgdorferi* HSF 30 or 90 days postvaccination. Animals were sacrificed 14 days after challenge, and the kidneys and spleens were examined for spirochetes by cultural procedures. At 30 days postvaccination, 86 to 100% protection against infection was achieved in hamsters receiving 50 and 100 μg (dry weight) of vaccine. Protection was decreased to 60% with 25 μg of vaccine and was absent with 10 μg of vaccine. Resistance to infection decreased to 25, 40, and 5% for the 100-, 50-, and 25-μg vaccine doses, respectively, at 90 days postvaccination.

Lyme borreliosis (Lyme disease and related disorders) is a zoonosis characterized by a number of variable syndromes (2, 11, 20). The etiological agent of this disease is *Borrelia burgdorferi* (4, 14, 19), which is primarily transmitted by Ixodes ticks (1, 5–8, 18). Lyme disease is presently the most commonly reported tick-borne disease in the United States (9).

Control of Lyme borreliosis, a tick-borne zoonosis, is difficult. Elimination of the rodent reservoir, such as the white-footed mouse, is impractical, and deer herd size reduction would only be applicable on a local basis. Large-scale use of acaricides, especially in suburban areas, is generally unacceptable and is of questionable effectiveness. Vaccination of high-risk individuals and of susceptible domestic animals could be a practical measure for the prevention of Lyme disease. We performed vaccine trials with the Syrian hamster as the experimental animal. The Syrian hamster is useful for studying experimental infection by *B. burgdorferi* (10, 13). Our studies on the passive protection of hamsters against experimental infection with *B. burgdorferi* indicated that circulating antibodies play a major role in immunity to this illness (12).

*B. burgdorferi* HSF (originally isolated from human spinal fluid [19]) was isolated from the spleen of an experimentally infected Syrian hamster and was grown in Barbour-Stoenner-Kelly medium (3) at 30°C to a cell density of 10⁸ spirochetes per ml. Cells were harvested by centrifugation (10,000 × g for 30 min); the unwashed cells were suspended in a small volume of distilled water and lyophilized. The vaccine was prepared by suspending the appropriate dry weight of cells in sterile saline containing a 1:10,000 dilution of thimerosal. Male and female hamsters, 5 to 10 weeks of age, were given a single dose of vaccine subcutaneously in the nape of the neck. At 30 or 90 days after vaccination, hamsters were challenged by the intraperitoneal injection of 10⁸ cells of a hamster spleen isolate of *B. burgdorferi* HSF which had not been subcultured more than three times. This challenge dose represents approximately 1000 50% infectious doses of this spirochete (12). The hamsters were killed, and the kidneys and spleens were cultured at 14 days postchallenge. Individual hamster organs were placed in 6 ml of Barbour-Stoenner-Kelly medium and homogenized with a Stomacher Lab-Blender (Tekmar Co., Cincinnati, Ohio). The larger tissue debris was allowed to settle, and duplicate 1:10 dilutions of the supernatant were made in the isolation medium, which consisted of Barbour-Stoenner-Kelly medium plus 0.15% agarose (SeaKem LE; FMC Corp., Rockland, Maine). Cultures were examined for spirochetes after 3 weeks of incubation at 30°C. An animal was considered culture positive if spirochetes could be isolated from one or more organs.

Hamsters vaccinated with a single dose of various amounts of the vaccine preparation were challenged at 30 days postvaccination in experiments 1 and 2 (Table 1). All of the control hamsters receiving saline injections were culture positive. The smallest dose of vaccine tested, 10 μg, did not provide protection from challenge. Increasing the amount of vaccine to 25 μg elicited a protective response in 68% of the vaccines. From 86 to 100% of the hamsters receiving 50 μg of the cell preparation were protected from challenge. A further increase in the amount of vaccine to 100 μg provided a degree of protection similar to that achieved with 50 μg. The protective response induced in hamsters receiving a single dose of vaccine decreased significantly by 90 days postvaccination. Protection decreased to 5, 40, and 25% for the 25-, 50-, and 100-μg doses of vaccine, respectively (Table 1). The spleen was most frequently culture positive. Hamster blood was not examined for anti-*B. burgdorferi* antibodies during the course of the vaccination studies because of the possibility of compromising the results of the experiments. Blood was obtained by cardiac puncture from a group of five hamsters vaccinated with 100 μg of the cell preparation. The indirect immunofluorescent-antibody titers (12) of the pooled sera were 1:8 at day 0, 1:128 at day 14, 1:256 at day 30, and 1:64 at day 90 postvaccination.

The effect of a second vaccination and the use of adjuvants on the duration of immunity should be investigated. However, these studies should be conducted in potential candi-
### Table 1: Active immunization of hamsters against experimental infection with *B. burgdorferi*

<table>
<thead>
<tr>
<th>Vaccine prepn (μg [dry wt])</th>
<th>No. of culture-negative animals/total no. of animals (% protection)</th>
<th>1a</th>
<th>2a</th>
<th>3a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0/20 0/15 1/15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0/10 (0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>13/19 (68)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>20/20 (100)</td>
<td></td>
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</tr>
<tr>
<td>100</td>
<td>18/20 (90)</td>
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</tr>
<tr>
<td>200</td>
<td>5/20 (25)</td>
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</tr>
</tbody>
</table>

* Notes: a Animals challenged 30 days postvaccination. b Animals challenged 90 days postvaccination.

dates for the vaccine, such as the dog (15-17). Although the duration of immunity elicited by a single vaccination was short-lived in the hamster, this study does indicate the feasibility of vaccination as a method for the prevention of Lyme disease. Our study of the passive immunization of hamsters against infection with *B. burgdorferi* (12) indicated that isolates from the north central and northeastern United States produced mutually protective antibodies in the rabbit. These results suggest that a monovalent vaccine should be effective in these two geographical areas.

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### Literature Cited