Variant Responses of Mice to *Borrelia burgdorferi* Depending on the Site of Intradermal Inoculation

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Received 15 March 1993/Returned for modification 15 April 1993/Accepted 2 July 1993

C3H/He mice inoculated intradermally at one of two sites with *Borrelia burgdorferi* responded differently to infection. Shoulder-inoculated mice developed spirochetemia, *B. burgdorferi*-specific antibody, and arthritis earlier than foot-inoculated mice. Lymphocyte populations derived from spleen tissue were elevated in the shoulder- but not the foot-inoculated mice, and those from lymph nodes were increased in both groups. Lymphocytes derived from blood and spleen tissue showed impaired proliferative responses to all mitogens for shoulder-inoculated mice only, whereas proliferation of lymph node cells was not affected, regardless of route. These results demonstrate that the site of initial *B. burgdorferi* inoculation is an important determinant in the pathogenesis of *B. burgdorferi* infection.

Lyme disease manifestations, caused by tick-borne *Borrelia burgdorferi*, are extremely variable among humans, partly because of age and genetic factors (20, 21). In the experimental laboratory mouse model of Lyme borreliosis, disease severity is influenced by differences in the isolates of *B. burgdorferi*, as well as by host factors (1, 2, 4–9). Mice can be infected with 1 to 10 spirochetes when inoculated intradermally, whereas a higher dose is required for infection by intraperitoneal inoculation (4). Furthermore, skin is among the most consistent sites for spirochete isolation during the course of chronic infection (6, 7). We recently observed that C3H mice inoculated intradermally with *B. burgdorferi* developed impaired splenocyte responses to both T- and B-cell mitogens and that this impairment appeared to be localized to the spleen and was not present in peripheral lymph nodes (13).

The present study was undertaken to evaluate the site of intradermal inoculation as a parameter in the response of C3H mice to infection with *B. burgdorferi*, since skin appears to be a critical initiating and perpetuating target organ in Lyme borreliosis and since tick bites are variable in their location. The kinetics of *B. burgdorferi* infection were evaluated following intradermal inoculation of mice in either the dorsal midline scapular region, to represent exposure on the trunk, or the dorsal metatarsal region of the left foot, to represent exposure of a peripheral limb.

Pathogen-free C3H/HeNCrIbr (C3H) mice were purchased from Charles River Laboratories and maintained as described elsewhere (5, 12, 13). Low-passage *B. burgdorferi* (isolate N40) with previously proven infectivity and pathogenicity in mice (2, 5–9) was cultured in modified Barbour-Stoenner-Kelly (BSKII) medium as described previously (3, 5–9, 13). Four-week-old mice were inoculated intradermally in the shoulder or foot with 10^4 viable *B. burgdorferi* cells in a 0.1-ml volume with a 26-gauge needle. Four mice from each group were killed with carbon dioxide gas at 2, 3, 4, 7, 10, and 14 days after inoculation. Blood, spleen, and bladder tissues (days 2 to 10) were aseptically collected and cultured as described elsewhere (7). Immunoglobulin M (IgM) and IgG titers against *B. burgdorferi* were measured for the sera (all intervals) by an enzyme-linked immunosorbent assay (ELISA) as previously described (5, 13). Antibody titer was determined as the highest dilution of serum that gave a net value (antigen-positive well minus antigen-negative well) of 0.04, based on results for 40 antibody-negative mice.

The joints (both knees and tibiotarsi) and heart were examined for pathology on days 7, 10, and 14. Tissues were fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin as described previously (2, 8). Tibiotarsal joints were blindly scored for arthritis severity on a scale of 1 (mild) to 3 (severe). For each mouse the most severe of the scores for the tibiotarsal joints was used for statistical analysis (Student’s unpaired *t* test).

Peripheral blood cells (PBC), lymph node cells (LNC), or spleen cells (SC) were collected and pooled from four to five mice for each treatment group (naive age-matched controls, shoulder-inoculated, and foot-inoculated) on days 7 and 14 after inoculation. Heparinized blood was diluted 1:4 in phosphate-buffered saline (PBS), lymph nodes (prescapular, axillary, popliteal, and inguinal) were ground with glass slides, and spleens were triturated in Ten Broeck tissue grinders. Cell suspensions were applied to lymphocyte separation medium, washed in PBS, and resuspended at 2 × 10^6 viable cells per ml in RPMI 1640 medium containing 5% fetal bovine serum, 0.05 mM 2-mercaptoethanol, 2 mM L-glutamine, 100 U of penicillin, and 100 μg of streptomycin per ml. Cells (2 × 10^5) were cultured in 96-well plates at 37°C in 5% CO₂ with final concentrations of 2 μg of concanavalin A (con A), 25 μg of lipopolysaccharide (LPS), or 0.5 μg (1 × 10^5 cells) of *B. burgdorferi* per ml. Spirochetes used for proliferation assays were washed and enumerated as described elsewhere (12, 13). Final culture volumes were 200 μl. Cultures were pulsed with 1 μCi of tritiated thymidine 20 h before counting by liquid scintillation spectroscopy. Cells were harvested with a semiautomated cell harvester (Cambridge Technology, Watertown, Mass.). Each sample was assayed in triplicate. Data are presented as the difference (mean ± standard deviation) in counts per minute between stimulated and unstimulated (diluent-treated) cultures at 72 h. Differences between mean values were analyzed with Student’s two-tailed *t* test.

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**TABLE 1. Rates of *B. burgdorferi* isolation from tissues of mice at intervals after intradermal inoculation of shoulder or foot with 10^6 *B. burgdorferi* cells**

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>Rate of isolation from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>Shoulder</td>
</tr>
<tr>
<td>2</td>
<td>0/4</td>
</tr>
<tr>
<td>3</td>
<td>0/4</td>
</tr>
<tr>
<td>4</td>
<td>4/4</td>
</tr>
<tr>
<td>7</td>
<td>2/4</td>
</tr>
<tr>
<td>10</td>
<td>0/4</td>
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</tbody>
</table>

* Number of culture-positive mice/number of mice inoculated. Denominators vary because of contamination of some samples.

*B. burgdorferi* was not isolated from the blood, spleen, or bladder until day 4, at which time shoulder- but not foot-inoculated mice were culture positive, indicating a delay in dissemination in the latter group (Table 1). Shoulder- but not foot-inoculated mice developed IgM reactivity to *B. burgdorferi* by day 7 (Table 2). IgG seroconversion occurred among shoulder-inoculated mice on day 10 and among foot-inoculated mice on day 14. IgM titers were higher in shoulder- than in foot-inoculated mice, while IgG titers appeared equivalent. All four shoulder-inoculated mice had arthritis in one or more joints, compared with only one of four foot-inoculated mice on day 7 (chi square P ≤ 0.05) (Table 3). All of the mice, regardless of inoculation site, had arthritis on day 10, with no difference in severity. On day 14, all mice had arthritis in one or more joints but shoulder-inoculated mice had consistently milder arthritis (mean score, 0.7) than foot-inoculated mice (mean score, 2.7; P ≤ 0.05). Furthermore, foot-inoculated mice had more affected joints than shoulder-inoculated mice after this interval (Table 3). Mice that were inoculated in the left foot did not develop earlier or more severe arthritis in the left hind leg than in the right hind leg. Carditis was first detected on day 10 in some mice from both inoculation groups and in all mice on day 14, with no apparent difference in severity between groups.

Lymph nodes yielded greater numbers of LNC from shoulder-inoculated (P ≤ 0.05) and foot-inoculated mice than from controls (Table 4). Both groups showed significant increases in LNC relative to uninfected controls on day 14, with the greatest yields from the shoulder-inoculated group. Shoulder- but not foot-inoculated mice infected for 14 days had increased numbers of SC relative to controls (P ≤ 0.05).

Proliferative responses were found to be dependent upon the lymphoid organ sampled as well as the site of inoculation. Lymphocytes derived from the spleens of mice infected for 7 days showed impaired proliferative responses to all mitogens tested in the shoulder- but not the foot-inoculated groups (Fig. 1). Lymphocytes derived from lymph nodes did not demonstrate impaired responses to any of the mitogens tested, regardless of inoculation site. Blood lymphocyte responses to con A were impaired. Responses to *B. burgdorferi* as a mitogen were nonspecific, occurring in uninfected controls, and were most marked with SC. Splenocytes continued to show impaired responses to con A in the shoulder- but not the foot-inoculated group on day 14 (Fig. 2). LNC showed no impaired responses to con A, regardless of inoculation site, after the 14-day interval. Day 14 SC but not LNC proliferative responses to LPS were marginally impaired for both inoculation site groups. Lymphocytes derived from blood of infected mice showed a level of impairment similar to that of SC (as performed in a separate assay [data not shown]). Responses to LPS and *B. burgdorferi* of LNC derived from the foot-inoculated group showed hyperresponsiveness relative to controls (Fig. 2).

Proliferative responses of SC and LNC pooled from groups of three mice either uninoculated or inoculated intradermally in the shoulder or foot with sterile BSKII medium were also measured 7 or 14 days after inoculation. Stimuli included con A, LPS, and *B. burgdorferi* at the same concentrations used in the assays with cells from infected mice. At 7 days after foot inoculation with BSKII medium, LNC proliferative responses to all stimuli were approximately twice those of cells from shoulder-inoculated or uninoculated mice. After the same interval, SC from shoulder-inoculated mice exhibited somewhat elevated responses to con A compared with responses of SC from foot-inoculated or uninoculated mice, but responses to LPS and *B. burgdorferi* were similar among the three groups. Responses of SC and LNC collected at 14 days could not be distinguished on the basis of inoculation status or route; proliferative responses to each of the three stimuli were similar.

**TABLE 2. ELISA titers of IgM and IgG antibodies to *B. burgdorferi* in serum of mice at intervals after intradermal inoculation of shoulder or foot with 10^6 *B. burgdorferi* cells**

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>IgM titer*</th>
<th>IgG titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoulder</td>
<td>Foot</td>
</tr>
<tr>
<td>2</td>
<td>&lt;80</td>
<td>&lt;80</td>
</tr>
<tr>
<td>3</td>
<td>&lt;80</td>
<td>&lt;80</td>
</tr>
<tr>
<td>4</td>
<td>&lt;80</td>
<td>&lt;80</td>
</tr>
<tr>
<td>7</td>
<td>226 (160-320)</td>
<td>&lt;80</td>
</tr>
<tr>
<td>10</td>
<td>538 (320-640)</td>
<td>160</td>
</tr>
<tr>
<td>14</td>
<td>2,560 (1,280-5,120)</td>
<td>381 (320-640)</td>
</tr>
</tbody>
</table>

* Titers are expressed as reciprocal geometric means of four serum samples (range of titers).
among un inoculated, shoulder-inoculated, and foot-inoculated mice.

Results of this study reveal that in mice the site of intradermal inoculation significantly influences the kinetics of dissemination, disease expression, and host immune response to *B. burgdorferi*. Clearly, intradermal inoculation in the scapular region results in earlier spirochetemia, dissemination, development of arthritis, and effects upon lymphoid organs (including seroconversion to *B. burgdorferi*, changes in lymphoid organ cell populations, and nonspecific responses to B- and T-cell mitogens, including *B. burgdorferi* itself) than does inoculation in the foot. The culture, disease, and seroconversion data obtained for mice inoculated in the scapular region are complementary to previously reported observations from our laboratory on these parameters in the C3H mouse model (2, 6-9). Some of the differences noted between the two inoculation sites may be temporal in nature, with infection of skin in the peripheral limb taking longer to disseminate and producing secondary effects. The delay in immune response in mice inoculated in the peripheral limb may explain why these mice developed more severe arthritis, as arthritis progression is abrogated by immunity (9). These differences are important considerations in obtaining reproducible results with this model and may explain the variable expression of symptoms in Lyme disease in humans.

The finding of impaired SC proliferative responses from the shoulder-inoculated group at 7 and 14 days is similar to previous findings from our laboratory (13), for which the same inoculation site was also used. We now extend the study of the impaired mitogenic response to PBC. We and colleagues have also previously documented normal or near-normal mitogen-induced proliferation of LNC (13). This phenomenon does not seem to be restricted to *B. burgdorferi* infection of C3H mice, as similar results have been reported in mice infected with *Trypanosoma cruzi*. C3H mice infected subcutaneously with *T. cruzi* had different responses to mitogens, depending on the lymphoid organ examined, with SC but not LNC from infected mice responding poorly to phytohemagglutinin (17). The mouse model of *T. cruzi* infection is similar with respect to host strain patterns of disease resistance and susceptibility (17), and as we and colleagues have shown with *B. burgdorferi* (7), *T. cruzi* infection of inbred laboratory mice is persistent, despite antibody production (14). Our laboratory has previously documented that at least part of this impaired response to nonspecific mitogens is attributable to prostaglandins (13). It may be that higher concentrations of prostaglandins are secreted in the spleen than in the lymph nodes, hence the more impaired proliferation to mitogens by cells from the former. Whether prostaglandin secretion is responsible for impaired proliferation of PBC of infected mice is presently unknown. Impaired responses to mitogens by SC and PBC from mice infected in the scapular region did not seem to affect antibody production. Also, despite normal responses to mitogens by lymphocytes from the mice infected in the foot, the levels of *B. burgdorferi*-specific antibody were lower.

The lack of impaired responses to mitogens seen in the foot-inoculated group cannot be attributed to a kinetic effect,
since extending the time of infection to 14 days did not result in defective lymphocyte proliferation in this group. A frequent but inconsistent finding is that LNC from infected mice tend to show enhanced responses to B-cell mitogens. This was seen in the foot-inoculated group and marginally in the shoulder-inoculated group infected for 14 days. *B. burgdorferi* is a B-cell mitogen (12, 18), and it may be that the spirochete results in enhanced numbers of B lymphocytes in the peripheral lymph nodes.

The current findings indicate that lymphocyte proliferative responses to *B. burgdorferi* are complicated by the site of lymphocyte collection and the site of *B. burgdorferi* infection of the host, as well as by the direct B-cell mitogenic effect of the organism (12, 13, 18). There have been conflicting reports on the response of human PBC to nonspecific mitogens (10, 11, 15, 16, 18, 22, 23), which may be explained by these variables.

This work was supported by the National Institute of Allergy and Infectious Diseases (NIH grants AI 26815 and AI 30548).

### REFERENCES


