Cystitis Induced by Infection with the Lyme Disease Spirochete, \textit{Borrelia burgdorferi}, in Mice

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Previous studies have demonstrated that the urinary bladder is a consistent source for isolating the Lyme disease spirochete, Borrelia burgdorferi, from both experimentally infected and naturally exposed rodents. We examined histopathologic changes in the urinary bladder of different types of rodents experimentally infected with Lyme spirochetes, including BALB/c mice (Mus musculus), nude mice (M. musculus), white-footed mice (Peromyscus leucopus), and grasshopper mice (Onychomys leucogaster). Animals were inoculated intraperitoneally, subcutaneously, or intranasally with low-passaged spirochetes, high-passaged spirochetes, or phosphate-buffered saline. At various times after inoculation, animals were killed and approximately one-half of each urinary bladder and kidney were cultured separately in BSK-II medium while the other half of each organ was prepared for histologic examination. Spirochetes were cultured from the urinary bladder of all 35 mice inoculated with low-passaged spirochetes while we were unable to isolate spirochetes from any kidneys of the same mice. The pathologic changes observed most frequently in the urinary bladder of the infected mice were the presence of lymphoid aggregates, vascular changes, including an increase in the number of vessels and thickening of the vessel walls, and perivascular infiltrates. Our results demonstrate that nearly all individuals (93%) of the four types of mice examined had a cystitis associated with spirochetal infection. (Am J Pathol 1992, 141:1173–1179)

\textit{Borrelia burgdorferi}, the causative agent of Lyme disease, is a tick-borne spirochete infecting humans and a variety of both domestic and wild animals throughout many regions of the world.1–3 In the hyperendemic regions of the northern midwest and northeastern United States, the white-footed mouse, \textit{Peromyscus leucopus}, is the primary reservoir for the spirochete and an important host for immature \textit{Ixodes dammini} ticks that transmit the spirochete.4,5 Lyme disease in humans is a multisystem disorder that varies tremendously in its clinical presentation.2,6–8

Numerous reports have demonstrated the usefulness of various rodents as experimental animals for investigating the infectiousness of \textit{B. burgdorferi},9–13 to examine specific aspects of the pathogenesis of Lyme disease,14–16 to determine \textit{in vivo} antibiotic susceptibility,17 and to examine potential vaccines.18,19 Studies with animals and reports of urine of humans suggest that either the urinary bladder or the urinary tract may be infected with the Lyme spirochete. Previous investigations have shown that \textit{B. burgdorferi} may be present in the urine of naturally infected white-footed mice,20 and that the urinary bladder of experimentally infected and naturally infected rodents often produces spirochetes when this organ is triturated and inoculated into BSK-II medium.10,21–24 Antigens of \textit{B. burgdorferi} have been detected in the urine of both laboratory mice experimentally infected with \textit{B. burgdorferi} and humans with Lyme disease,25,26 and \textit{B. burgdorferi} has been demonstrated in the bladder wall of white-footed mice by direct immunofluorescence staining.19 As well, the amplification of \textit{B. burgdorferi} DNA using the polymerase chain reaction has been used recently to detect spirochetes in the urine of humans with Lyme disease,27 and bladder function disturbance leading to urine retention has also been reported.28 The frequent infection of spirochetes in the uri-
nary bladder of experimental and wild rodents has raised the question as to whether some cases of interstitial cystitis in humans might be caused by *B. burgdorferi*. Because of the previous studies cited above, demonstrating that the urinary bladder of rodents often produces isolations of *B. burgdorferi*, including mice that have been infected for 16 months, the objective of the current study was to determine if infection by Lyme spirochetes resulted in any histopathologic changes in this organ.

**Materials and Methods**

**Animals and Housing Conditions**

The following strains and species of mice were infected with *B. burgdorferi*: laboratory mice (Mus musculus, RML BALB/c/Ann), grasshopper mice (Onychomys leucogaster), white-footed mice, and nude Harlan Sprague-Dawley mice (HSD nu/nu, *M. musculus*). All animals were obtained from the Rocky Mountain Laboratories animal production facility. Both sexes were used, and all animals were 1 to 3 months old when inoculated. RML and HSD nu/nu mice were kept in groups of four to five animals per cage. The grasshopper mice and white-footed mice were kept separately with only one animal per cage.

**Strains of B. burgdorferi and Animal Inoculation**

*Borrelia burgdorferi* strains Sh-2-82, CA-2-87, and ECM-NY-86 were described elsewhere. Briefly, Sh-2-82 originated from *Ixodes dammini* ticks from Shelter Island, New York; CA-2-87 originated from *I. pacificus* ticks from Tulare County, California; ECM-NY-86 originated from a skin biopsy from a EM lesion of a human patient in Southampton, New York. Fresh, low-passaged cultures (passage 5) of Sh-2-82 and ECM-NY-86 were prepared in BSK-II medium, removed from the medium by centrifugation, and resuspended in PBS-5 mmol/l MgCl₂ (pH 7.4), and counted in a Petroff-Hauser bacteria counting chamber. A higher-passaged (passage 15) noninfectious culture of CA-2-87 was prepared in the same manner. Animals infected with the low-passaged strain were inoculated by one of three routes including intraperitoneal, subcutaneous, or intranasal inoculation with approximately $7 \times 10^7$ *B. burgdorferi* in PBS-5 mmol/l MgCl₂ (pH 7.4). Animals inoculated with the high-passaged strain were injected intraperitoneally with the same number of spirochetes and caged separately from mice inoculated with low-passaged spirochetes. Six mice (two of each species) were also inoculated with PBS intraperitoneally for negative controls and kept in separate cages in the same room. The animals were examined twice per week for their health status. Between 14 days and 8 months postinfection, the mice were anesthetized with ether, killed, and the kidney and urinary bladder were removed under sterile conditions. Ten uninoculated nude mice were also examined.

**Cultivation of Borrelia burgdorferi**

One half of each kidney and urinary bladder was used for the histologic examination (see below), and the other half was used to isolate spirochetes as described previously. Briefly, each tissue was triturated in 1 ml BSK-II medium in a glass tissue grinder. Half of this suspension was inoculated into a tube containing 9 ml plain BSK-II medium, and the other half into a tube containing 9 ml BSK-II medium plus phosphomycin (100 μg/ml) and rifampin (50 μg/ml). The cultures were incubated at 34°C and checked by darkfield microscopy every few days for 2 to 3 weeks. Organs of control animals were treated in the same way. Bladders from the 10 uninoculated nude mice were only examined histologically.

**Tissue Processing**

Imprint smears of one half of the urinary bladder and the left kidney from infected and normal animals were fixed with methanol and stained by a direct immunofluorescence assay using rabbit anti-*B. burgdorferi* antibodies labeled with fluorescein isothiocyanate (FITC). Afterwards, the organs were prepared for sectioning using standard methods, cut in 4-μm serial sections and mounted on slides, and examined by light microscopy.

**Staining Procedures**

Hematoxylin and eosin (H&E) stain was used on all slides. Methyl-green-pyronin stain (Sigma) was used to detect plasma cells and mast cells. Immunohistochemical stains (APAAP, ABC peroxidase), direct immunofluorescence with rabbit anti-*B. burgdorferi* antibody labeled with FITC, and indirect immunofluorescence with monoclonal anti-*B. burgdorferi* outer surface protein A antibody H5332, monoclonal anti-flagellin antibody H9724, and polyclonal rabbit anti-*B. burgdorferi* antibody were used to detect *B. burgdorferi* in the tissues.

**Analysis of Histologic Changes in the Urinary Bladder**

Sections from the urinary bladder of 48 mice were examined for histologic abnormalities, including 29 from mice
inoculated intraperitoneally with infectious spirochetes, three from mice inoculated intraperitoneally with noninfectious spirochetes, six from mice inoculated with only PBS, and 10 uninoculated nude mice. Part of the same bladder from all the mice (excluding the 10 nude control mice) were also cultured in BSK-II. The identity of the mice and the results of cultivation were not known when the slides were examined and the histologic changes quantified.

Pathologic changes in the bladder included the number and size of lymphoid aggregates, changes in vasculature, and alterations in bladder tissues. Tissues were scored to quantify the various changes and included the following: the number of lymphoid aggregates per section varied from 0, 1, 2, 3, 4, or more and received scores of 0 to 5, respectively. Sizes of the aggregates measured with an ocular micrometer for relative size to one high-power field (HPF, 400× magnification), varied from less than 25%, 25% to 50%, 50% to 75%, 75% to 100%, and greater than 100% of HPF and received scores of 1 to 5, respectively. Changes in vasculature of any vessel and the value given the change included branching of vessels with lymphoid aggregates = 4, vascular thickening = 3, increase in the number of vessels = 2, and dilation = 1. Changes in the bladder tissue and the value given included vacuolization of the urothelium = 5, perivascular infiltrates = 4, increase of collagen, especially in the lamina propria = 3, submucosal edema = 2, serosal changes, especially edema = 1.

**Results**

None of the mice inoculated with *B. burgdorferi* developed any obvious clinical manifestations during their infection. At autopsy, no gross pathologic changes in the urinary bladder and kidney were evident.

*Borrelia burgdorferi* was isolated from the urinary bladder of all 35 mice inoculated with low-passaged spirochetes (Table 1). The time of isolation varied from only 2 weeks to 8 months postinoculation and resulted from all routes of experimental inoculation including intraperitoneal, subcutaneous, and intranasal. Cultures of bladders from the HSD nu/nu mice were usually positive for spirochetes after only 5 days, whereas cultures of the bladder from the other mice took 1 to 2 weeks before spirochetes were detectable by darkfield microscopy. Spirochetes were not isolated from the bladder of the three grasshopper mice inoculated with the higher-passaged *B. burgdorferi* (CA-2-87). Surprisingly, we were unable to isolate spirochetes from any of the kidneys from the same 35 mice that had an infected bladder.

All attempts to demonstrate spirochetes in paraffin-embedded sections of the urinary bladder using immunohistochemical and immunofluorescence stains were unsuccessful, even though the latter technique has worked for us previously. Spirochetes were detected, however, by immunofluorescence stains used on imprint smears of the bladder fixed with methanol (Figure 1).

The pathologic changes observed most frequently in the bladder of infected mice included the presence of lymphoid aggregates, vascular changes, and perivascular infiltrates (Figures 2–4). Of the 29 mice with infected bladders that were examined histologically after intraperitoneal inoculation, 20 (69%) had lymphoid aggregates, 20 (69%) had perivascular infiltrates, and 20 (69%) had vascular changes; 27 (93%) of the 29 mice had one or more of these alterations, and only 2 (7%) mice had bladders free of such changes. These pathologic changes were not observed in any of 16 control mice that were not infected, including two RML BALB/c mice, two white-footed mice, two grasshopper mice inoculated with PBS; three grasshopper mice inoculated with noninfectious live spirochetes; 10 nude mice 53 to 75 days old that were not inoculated with anything. There was no obvious correlation with the pathologic changes and duration of infection; however, the HSD nu/nu mice killed after only 14 to 21 days were free of these abnormalities. Of the mice with lymphoid aggregates visible in one section of the bladder, nine (45%) of them had only one aggregate,

**Table 1. Isolation of Borrelia burgdorferi from the Urinary Bladder and Left Kidney of Various Mice Species after Different Routes of Inoculation**

<table>
<thead>
<tr>
<th>Mouse species</th>
<th>Strain of inoculum</th>
<th>No. of mice</th>
<th>Route of inoculation</th>
<th>Duration of infection</th>
<th>Urinary bladder</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>RML</td>
<td>Sh-2-82</td>
<td>6</td>
<td>i.p.</td>
<td>8 months</td>
<td>6/6</td>
<td>0/6</td>
</tr>
<tr>
<td>White-footed mice</td>
<td>Sh-2-82</td>
<td>6</td>
<td>i.p.</td>
<td>21 days</td>
<td>6/6</td>
<td>0/6</td>
</tr>
<tr>
<td>White-footed mice</td>
<td>ECM-NY-86</td>
<td>3</td>
<td>s.c.</td>
<td>4 months</td>
<td>3/3</td>
<td>0/3</td>
</tr>
<tr>
<td>White-footed mice</td>
<td>ECM-NY-86</td>
<td>3</td>
<td>i.n.</td>
<td>4 months</td>
<td>3/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Grasshopper mice</td>
<td>Sh-2-82</td>
<td>6</td>
<td>i.p.</td>
<td>4 months</td>
<td>6/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Grasshopper mice</td>
<td>CA-2-87</td>
<td>3</td>
<td>i.p.</td>
<td>4 months</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>HSD nu/nu</td>
<td>Sh-2-82</td>
<td>11</td>
<td>i.p.</td>
<td>14–123 days</td>
<td>11/11</td>
<td>0/11</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>38</td>
<td></td>
<td></td>
<td>35/38</td>
<td>0/38</td>
</tr>
</tbody>
</table>
whereas 11 (55%) mice had two to five aggregates per section. Most of the aggregates were relatively small, constituting less than 25% of 1 HPF. The most common vascular changes included an increase in the number of vessels (18 mice) and thickening of the vessel wall (10 mice). Most of the pathologic changes were seen in the lamina propria. Other types of pathologic changes were observed in some of the mice, including vacuolization of the urothelium, increase in collagen, and submucosal and serosal edema. These abnormalities also were observed in some of the uninfected mice, and it is therefore difficult to ascribe these changes to spirochetal infection. There were no obvious differences observed in the pathologic changes of the bladder among the different species or strains of mice examined. Most individuals in each group of infected mice had sections of the urinary bladder that contained one or more of the abnormalities.
described above. All 6 (100%) of the grasshopper mice infected with low-passaged spirochetes, however, had lymphoid aggregates, whereas only six (55%) of the 11 HSD nu/nu infected mice had such aggregates detectable in sections of the bladder.

A methyl-green-pyronin stain was used to identify mast and plasma cells associated with the B. burgdorferi-induced cystitis in the mice. Although mast cells were seen in the vicinity of perivascular infiltrates, lymphoid aggregates, and few perineural infiltrations, a positive correlation between the number of mast cells and cystitis was not observed. Variation in the number of mast cells appeared to be associated with the species or strain of mouse rather than being associated with infection with B. burgdorferi. Moreover, there was no obvious increase in the number of mast cells in grasshopper mice inoculated with the infectious low-passage strain compared with those animals inoculated with the noninfectious high-passage strain of the spirochete.

**Discussion**

Infection with *Borrelia burgdorferi* in the urinary bladder of 29 mice of different strains and species resulted in a detectable cystitis in most (93%) of the animals. Each of the three most frequently observed pathologic lesions, lymphoid aggregates, vascular branching and thickening, and mononuclear perivascular infiltrates, were equally prevalent (69%) and found most often in the lamina propria. Such changes were not observed in any of 16 control mice. The lymphoid aggregates and perivascular infiltrates consisted primarily of lymphocytes, some plasma cells, and rarely mast cells, characteristic of an inflammation process, and similar to the pathologic changes described for infection by *Treponema pallidum*. This type of cellular and vessel proliferation in the infected urinary bladders is also suggestive of ongoing immunologic stimulation indicative of a chronic type of infection. The low number of plasma cells and the absence of proliferated (= multinucleated giant or epithelioid cells) and activated macrophages suggest that *B. burgdorferi* may not be a potent mediator of inflammation. Additionally, the lymphoid aggregates were not granulomatous. Granulomas are due to the activation and accumulation of T cells, a process that stimulates the fusion of monocytes or macrophages through interleukin-4. Although nude mice are T cell deficient, nearly one-half of the HSD nu/nu mice infected with *B. burgdorferi* produced lymphoid aggregates about the same size (= size 1) as did the immunocompetent mice. Our findings would support that the lack of T cells does not significantly influence either the clinical course or the pathogenesis of Lyme disease, at least as demonstrated by the types of animals used in this study.

In human interstitial cystitis, the histopathology is relatively nonspecific. Round cell infiltrates are observed both diffusely and in aggregates in the bladder lamina submucosa or lamina muscularis accompanied by vasodilation and edema in the lamina submucosa. Fibrosis of the submucosal and muscular layer of the bladder wall is a late sequela of the disease. The histopathologic features of cystitis in mice infected with *B. burgdorferi* were very similar. The most common lesions are one or more lymphoid aggregates, perivascular infiltrates of mononuclear cells, and an increase in the number of vessels. In humans, interstitial cystitis is also clinically defined by sterile urine cultures. Negative urine cultures also have been demonstrated in *B. burgdorferi*-infected *P. leucopus*, *I. agrarius* hamsters, and Lewis rats. The presence of Lyme spirochetes in the urine of wild white-footed mice may have resulted from coinfections with *Babesia microti*, creating an acute catarhalic infection.

A variety of mechanisms may be involved in the development of spirochetal tissue tropism. Certain receptors could be required to mediate *B. burgdorferi* host cell attachment like the outer surface layer of mucopolysaccharide material (hyaluronic acid and chondroitin sulfate) in *T. pallidum* infection. Certain tissues have higher concentrations of mucopolysaccharide ground substance like dermis, testis, or the immediate vicinity of blood vessels, all of which are involved in *B. burgdorferi* infection. Recently, one study demonstrated that the tissue invasiveness of *B. burgdorferi* may be unrelated to hyaluronidase activity. Another important factor could be the fibronectin concentration of the various tissues. Fibronectin staining is dense in the vicinity of basement membranes and other areas in the lamina propria of the urinary bladder. Binding to this major connective tissue protein seems to play an important role in the pathogenesis of syphilis. *Treponema pallidum* may use fibronectin...
to attach to host surfaces, or it may become coated with it and avoid the host’s immune response.\textsuperscript{43}

The pathogenesis of \textit{B. burgdorferi}-induced cystitis reflects invasive properties of the spirochete, but the actual method of invasion into the urinary bladder is unknown. \textit{Borrelia burgdorferi} could gain access to the urinary bladder by the hematogenous route or by urinogen- nous dissemination through the kidney. Because the bladder wall is in direct contact with urine, the causative agent may come from the urine and penetrate into the deeper layer of the bladder, giving rise to inflammation. Yet the relative integrity of the urothelium as seen by light microscopy, and the often negative kidney cultures, suggest that the infection is not through the urine, but more likely through the blood. \textit{Treponema pallidum} can penetrate endothelial monolayer barriers by passing through intercellular junctions,\textsuperscript{44} and there is evidence that \textit{B. burgdorferi} may do the same.\textsuperscript{45}

Again, the urinary bladder was a consistent source for isolating \textit{B. burgdorferi} from additional species and strains of mice as was reported previously for white-footed mice.\textsuperscript{10,23} Our negative cultures of kidney differed, however, from other results both in mice,\textsuperscript{22,23} and rats,\textsuperscript{16,40} in which infection of the kidney varied from 18% to 100% at various times after experimental inoculation. Our results document the histopathologic changes associated with cystitis in mice. The possibility of a similar clinical picture in humans with Lyme disease is worthy of future consideration.

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