A Guinea Pig Model for Lyme Disease

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We report that outbred Hartley guinea pigs are susceptible to Borrelia burgdorferi. We recovered spirochetes from 57 of 60 (95%) guinea pigs inoculated when ≤3 months of age. In contrast, animals inoculated when ≥6 months of age were resistant to infection as defined by recovery of organisms at ≥4 weeks postinoculation. Infection was widely disseminated: B. burgdorferi was recovered from 83% of bladders, 64% of knee joints, 57% of hearts, 48% of spleens, and 38% of spinal cords examined within 4 weeks of inoculation. Histopathologic changes were common in the heart (88%) (preferential involvement of perinervial tissues near the annulus fibrosus) and bladder (76%) and were also noted in a minority of spinal cords (13%) and knee joints (9%). Western immunoblots demonstrated an immunoglobulin G response to B. burgdorferi, particularly to the 24-, 31- (OspA), 39-, and 41-kDa (flagellin) antigens. Infection was cleared from most tissues with the passage of time; spirochetes were recovered from 63% of tissues removed from guinea pigs at ≤4 weeks after inoculation but from only 32% at ≥8 weeks postinoculation (P < 0.001). An exception was the failure to clear spirochetes from infected knees, 90% of which were culture positive even when evaluated at ≥8 weeks postinoculation. The guinea pig provides a new model useful for studying host-spirochete interactions in Lyme disease.

Lyme disease is caused by the spirochete Borrelia burgdorferi and is transmitted to humans by Ixodes ticks. After initial skin involvement, untreated infection usually disseminates and may later cause disease manifestations in the heart, nervous system, and joints. Although the factors involved in spirochetal dissemination and virulence are largely unknown, the host immune response can be protective in animals (17) but is often slow to develop in humans (34) and may be involved in producing chronic disease manifestations (37).

Experimental B. burgdorferi infections in Syrian hamsters (16, 25), rabbits (12, 27), rats (7, 8), immunodeficient mice (14, 31), C3H mice (5, 6, 9), and other inbred mice (5, 32) have been described. The C3H mouse has been the only well-characterized immunocompetent small animal model in which infection is accompanied by histopathologic abnormalities.

The guinea pig immune system is thought to be similar to that of humans (40), and the guinea pig has a relatively long life span, which may be important in allowing the development of late disease manifestations. The guinea pig has proven to be a useful model for other human infectious diseases. For example, the guinea pig has been the only small animal model to spontaneously develop recurrent genital herpes simplex (36). The guinea pig has been employed in immunologic studies of leptospirosis (1). The C4-deficient guinea pig has been shown to develop cutaneous infection with syphilis (40-43), a spirochetal disease with clinical similarities to Lyme disease, including poor immunogenicity and the late development of disease manifestations. The guinea pig has a long gestational period and placenta similar to that of humans and has provided a useful model of congenital cytomegalovirus infection (25).

For these reasons, we investigated whether the guinea pig might provide a suitable animal model for the study of infection with B. burgdorferi. Although skin lesions have been reported in guinea pigs following ixodid tick bites (28), the susceptibility of guinea pigs to B. burgdorferi infection has not, to our knowledge, been previously documented. We report that the guinea pig demonstrates age-dependent susceptibility to infection with B. burgdorferi. Infected animals develop pathologic changes relevant to human Lyme disease and mount a host immune response which is ultimately effective in clearing spirochetes from most involved tissues.

MATERIALS AND METHODS

Guinea pigs. Outbred Duncan-Hartley guinea pigs were obtained from Sasco Breeding Laboratory, Omaha, Nebr., or Harlan Sprague Dawley, Inc., Indianapolis, Ind. C4-deficient guinea pigs were obtained originally from the National Institutes of Health and bred in our facilities. Animals included males and females ranging in age from 1 to 9 months. Control and infected animals were housed separately in plastic tubs in groups of two to four.

B. burgdorferi strains and animal inoculation. The CT-1 strain of B. burgdorferi was originally isolated from an adult female Wisconsin Ixodes dammini tick and employed in most experiments. Where noted, we also used a recent skin isolate from a biopsy of an erythema migrans lesion from a patient (patient 18) in Long Island, New York (10), passed twice in vitro in modified Barbour-Stoenner-Kelly (BSK) medium (4, 26, 39). Virulent low-passage (maximum of three in vitro subcultures before inoculation) spirochetes were grown in BSK medium. Spirochetes were grown at 34°C to a density of 107 to 10⁸ organisms per ml, and 1-ml samples were administered to guinea pigs in the right flank subcutaneously or intradermally with a 25-gauge needle. Control animals were inoculated with 1-ml portions of BSK
medium from the same batch used to prepare the inoculum, and in some cases, uninoculated animals also served as controls.

In one experiment, to ascertain that spirochetes alone could induce pathologic changes, the inoculum was prepared without BSK medium. Spirochetes grown in BSK medium were pelleted by centrifugation, resuspended in 0.01 M phosphate-buffered saline (PBS), repelleted, and washed twice in PBS. Animals were inoculated with PBS alone, PBS with spirochetes (still actively motile by dark-field examination), or spirochetes suspended in BSK medium.

Cultivation of guinea pig tissues for spirochetes. Animals were euthanized with carbon dioxide at 2, 4, 8, and 16 weeks after inoculation. Whole blood was obtained for culture and serologic studies by cardiocentesis. Organs (bladder, spleen, liver, heart, knee joint, spinal cord, cerebrum, and ear lobe; not all organs in every experiment) were transferred to sterile plastic bags (Tekmar Stomacher Co., Cincinnati, Ohio) containing 6-ml portions of BSK broth and homogenized manually and with a Tekmar Stomacher. Ear lobes were processed as described elsewhere (35). A 1:10 dilution of the tissue homogenate in BSK agar was also prepared for culture in duplicate tubes. Two drops of whole blood were inoculated into BSK agar for culture. Cultures were incubated at 30°C and evaluated by dark-field microscopy at 4 weeks postinoculation.

PCR identification of isolated spirochetes as B. burgdorferi. Selected positive cultures were confirmed to be B. burgdorferi by adding 25 µl of the cultured spirochetes in BSK medium to a polymerase chain reaction (PCR) mixture. PCR was performed with B. burgdorferi-specific chromosomal primers as previously described (22).

Pathologic studies. Bladder, heart, liver, left knee, spinal cord, and cerebrum samples were fixed with formalin, decalcified when indicated (knee), embedded in paraffin, and stained with hematoxylin and eosin. Sections from selected blocks were stained with a modified Dieterle technique. The identity of tissues as either control or infected was masked, and the slides were reviewed by one of us (J.C.M.) without knowledge as to which animals had been infected and which served as controls.

SEROLOGIC STUDIES. Serum samples were obtained from animals at the time of sacrifice. In addition, in one experiment, serum samples were obtained from infected and control animals at 0, 7, 15, 23, 30, and 60 days postinoculation by pricking the lateral metatarsal vein of animals briefly anesthetized with Innovar-Vet (Pitman-Moore, Inc.).

Western immunoblotting. Virulent low-passage B. burgdorferi CT-1 was grown in BSK broth to a concentration of 10^7 to 10^9 organisms per ml. The spirochetes were pelleted by centrifugation, resuspended, and washed three times in 0.01 M PBS. A detergent-compatible protein assay (Bio-Rad, Richmond, Calif.) was used to measure 300 µg of protein per trough (10 to 12 µg per strip). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transfer were performed as previously described (19). Briefly, proteins were electrophoresed for 1.5 h at 35 mA on a 7.5 to 15% polyacrylamide linear gradient gel (0.75 mm thick). Proteins were electrotetransferred onto Immobilon-P (Millipore, Bedford, Mass.) for 30 min at 1.01 A with a Transphor Electrophoresis Unit (Hoefer Scientific Instruments, San Francisco, Calif.). Strips were incubated for 1 h with 2-ml samples of test guinea pig serum diluted 1:200. Positive-control sera were also diluted in BLOTTO as follows: rabbit serum, 1:2,000; human serum, 1:400; monoclonal antibody to OspA (H5332), 1:40; and monoclonal antibody to OspB (HSTS), 1:20 (both monoclonal antibodies provided by Rocky Mountain Laboratories, Hamilton, Mont.). Strips were washed twice with 0.1% Tween 20-Tris-buffered saline (TTBS) and incubated for 1 h with the appropriate alkaline phosphatase conjugates (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) diluted in BLOTTO as follows: goat anti-guinea pig immunoglobulin G (IgG) diluted 1:2,000, goat anti-rabbit IgG (Sigma Chemical Company, St. Louis, Mo.) diluted 1:8,000, goat anti-human IgG diluted 1:5,000, and goat anti-mouse IgG diluted 1:1,000. Strips were washed twice with TTBS, twice with TBS, and twice with barbital buffer and then developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate for 20 min.

RESULTS

Initial experiments to document susceptibility and basic pathologic changes were followed by systematic experiments of infected cohorts monitored over time. To simplify the presentation, data from initial and subsequent experiments are combined, except where noted.

Susceptibility of guinea pigs to B. burgdorferi. Following subcutaneous inoculation with the CT-1 strain of B. burgdorferi, spirochetes were isolated from at least one organ in 43 of 46 (93%) Hartley guinea pigs of ≤3 months of age at the time of inoculation. In contrast, cultures of all organs from all 12 animals of ≥6 months of age at the time of inoculation were negative (Table 1, P < 0.001, χ^2 test). In addition, three of four C4-deficient guinea pigs inoculated at 1 to 2 months of age were infected while one 7-month-old animal was not.

To determine whether guinea pigs are susceptible to infection following intradermal inoculation and to infection with another B. burgdorferi strain, 1-month-old guinea pigs were inoculated subcutaneously or intradermally with either the CT-1 or patient 18 strain (seven animals each). B. burgdorferi was recovered from at least one organ in each of these 14 animals, regardless of the strain or the route of inoculation, and there were no discernible differences in either the number or distribution of infected organs or the pathology observed (see below).

Disseminated tissue infection of the guinea pig. A total of 60 Hartley guinea pigs under the age of 3 months were inoculated either subcutaneously or intradermally with either the CT-1 or patient 18 strain of B. burgdorferi and dissected 2 to 16 weeks after inoculation. B. burgdorferi was cultivated from 29 of 60 hearts (48%) 32 of 57 bladders (56%), 16 of 46 spleens (35%), 27 of 34 knee joints (79%), 6 of 9 quadriceps muscles (67%), and 3 of 29 spinal cords (10%) studied (Table 2). Results were similar whether the B. burgdorferi inoculum was suspended in BSK medium or in CBS. Cultures were negative for all 8 cerebrums, 10 ear lobes, and 4 livers examined. Cultures of the same organs from 19 control animals inoculated with uninfected BSK medium were all

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of animals infected/total no. of animals inoculated</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>7</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hartley</td>
<td>37/38</td>
<td>3/4</td>
<td>3/4</td>
<td>0/3</td>
<td>0/4</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>C4^D</td>
<td>2/2</td>
<td>1/2</td>
<td>ND</td>
<td>ND</td>
<td>0/1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>C4^-D</td>
<td>3/4</td>
<td>0/1</td>
<td>ND</td>
<td>ND</td>
<td>0/1</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* Animals inoculated subcutaneously with CT-1 strain.

b C4-deficient guinea pigs from the National Institutes of Health.

ND, not done.

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negative. Whole-blood samples were obtained for culture at 7, 15, 23, 30, and 60 days postinoculation and were negative among 34 animals evaluated. To confirm that spirochetes recovered in culture were <i>B. burgdorferi</i>, selected positive cultures were tested by PCR, which confirmed the presence of <i>B. burgdorferi</i> DNA (not shown).

<i>B. burgdorferi</i> infection was usually disseminated to multiple organs; 65% of infected guinea pigs had two or more culture-positive organs. The proportion of organs that were culture positive for <i>B. burgdorferi</i> diminished with the passage of time after inoculation. While 72 of 115 (63%) organs from animals dissected 2 or 4 weeks after inoculation were culture positive, only 35 of 111 (32%) organs from animals dissected at 8 or 16 weeks remained culture positive (<i>P < 0.001</i>, χ² test). In addition to the aggregate data, we found similarly diminished recovery of spirochetes with time after inoculation in two experiments in which animals from the same infected cohorts were dissected at different intervals after inoculation. In those cohort experiments, 34 of 53 (64%) organs were culture positive in animals dissected at ≤4 weeks after inoculation versus 22 of 58 (38%) in animals dissected at ≥8 weeks after inoculation (<i>P < 0.01</i>, χ² test). Spirochetal clearance of spirochetes occurred in all tissues cultured sequentially except knee joints for which 18 of 20 (90%) were still culture positive at ≥8 weeks postinoculation (Table 2). Of the three animals dissected 4 months after inoculation, cultures were negative for all bladders, hearts, spleens, and spinal cords but spirochetes were recovered from the knee joints of two animals. Furthermore, among four animals dissected 6 months after inoculation, tissue cultures were all negative except for the knee from which <i>B. burgdorferi</i> was recovered in three animals.

**Histopathologic findings.** (i) **Heart.** Pathologic changes were noted on hematoxylin-and-eosin-stained slides in 22 of 25 (88%) hearts examined from animals inoculated subcutaneously with the CT-1 strain of <i>B. burgdorferi</i>. The histologic findings were similar in all hearts from infected animals, demonstrating multifocal myocarditis with lymphoplasmacytic infiltrates at multiple levels of sectioning (Fig. 1a). Pathologic abnormalities were localized preferentially to the annulus fibrosus, to the subendocardium, and to areas containing neural elements including ganglion cells and conduction fibers (Fig. 1b). Tissue macrophages were frequently abundant in areas of myocarditis. Focal pericarditis consisting of lymphoplasmacytic infiltrates was frequently noted (not shown). The infiltrates were abundant in perivascular areas and were occasionally associated with myocardial cell necrosis. There was no evidence of vasculitis. Three animals had evidence of recent myocardial infarction in the lower septum or the base of the heart (not shown). <i>B. burgdorferi</i> was isolated from 16 of 22 (73%) hearts with abnormal histologic findings. Minimal focal abnormalities were noted in 2 of 22 negative-control animals (inoculated with BSK medium or PBS). One had minimal pericarditis and a mild lymphocytic infiltrate in the left ventricle and papillary muscle, and the other had a lymphocytic infiltrate at the root of the aortic valve. Deeper sections of cardiac tissue from these two control animals were normal. These hearts, like all controls, were culture negative.

(ii) **Bladder.** Histologic abnormalities were present in 19 of 25 (76%) bladders from guinea pigs evaluated at 1, 2, or 4 months after inoculation. Interstitial and perivascular lymphoplasmacytic infiltrates were seen in the lamina propria (Fig. 1c) and were similar in all affected animals. Numerous spirochetes were noted in the involved areas on modified Dieterle stains (Fig. 1d). Inflammatory infiltrates often persisted following host clearance of spirochetes. For example, among 14 animals dissected 4 weeks after inoculation who had abnormal bladder histology, 11 were culture positive whereas all 5 guinea pigs with abnormal histology at ≥8 weeks had negative cultures. The transitional epithelium, muscular wall, and bladder mucosa were morphologically normal. All 22 control animals were histologically normal and had negative cultures.

(iii) **Spinal cord.** Histopathologic abnormalities were found in the spinal cords of 2 of 16 (13%) animals examined. Both were dissected 4 weeks after inoculation with the CT-1 strain and represented two of four examined at 4 weeks. In these animals, one of which was also spinal cord culture positive, focal meningoitis was present and consisted predominantly of lymphocytes with a few neutrophils (Fig. 1e). No animal examined 8 or 16 weeks after inoculation (<i>n = 12</i>) had pathologic changes. All 14 spinal cords from control animals were histologically normal and culture negative.

(iv) **Knee joint.** Abnormal histology was noted in 2 of 22 (9%) knees evaluated. In one animal, focal lymphoplasmacytic infiltrates were present in the synovium (Fig. 1f). In this animal, an area of articular surface had been eroded and replaced by granulation tissue and covered by new synovial lining (Fig. 1g). The opposite articular surface was normal. In another infected animal, focal abscess formation was present in soft tissues surrounding the synovial membrane; and the synovial lining itself showed areas of sloughing with hyperplastic changes (Fig. 1h). Both animals found to have arthritis had been dissected 1 month after infection and had <i>B. burgdorferi</i> isolated from cultures of the affected joints. In 1 of 22 control animals, there was a focal area of cartilage degeneration and macrophage remodelling without inflammation or fibrosis (not shown). All control animals had negative cultures.

(v) **Liver and cerebrum.** These organs were histologically normal in all infected animals evaluated (four livers and eight cerebrums).

The pathologic changes described above in various organs of <i>B. burgdorferi</i> infected animals occurred whether the inoculum was prepared in BSK medium or PBS.

**Antibody response.** Western immunoblotting demonstrated that all infected guinea pigs developed an IgG antibody response to <i>B. burgdorferi</i> proteins (Fig. 2). Infected guinea pigs began to develop detectable Western blot reac-

### Table 2. Recovery of <i>B. burgdorferi</i> from the organs of outbred Hartley guinea pigs

<table>
<thead>
<tr>
<th>Organ or aggregate data</th>
<th>≤4 weeks postinoculation</th>
<th>≥8 weeks postinoculation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>29/35 (83)</td>
<td>3/22 (14)</td>
<td>32/57 (56)</td>
</tr>
<tr>
<td>Heart</td>
<td>20/35 (57)</td>
<td>9/25 (36)</td>
<td>29/60 (48)</td>
</tr>
<tr>
<td>Spleen</td>
<td>11/23 (48)</td>
<td>5/23 (22)</td>
<td>16/46 (35)</td>
</tr>
<tr>
<td>Knee</td>
<td>9/14 (64)</td>
<td>18/20 (90)</td>
<td>27/34 (79)</td>
</tr>
<tr>
<td>Muscle</td>
<td>ND</td>
<td>6/9 (67)</td>
<td>6/9 (67)</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>3/38 (8)</td>
<td>0/21 (0)</td>
<td>3/29 (10)</td>
</tr>
</tbody>
</table>

Total no. of animals positive 34/35 (97) 23/25 (92) 57/60 (95)

Total no. of organs positive 72/115 (63) 35/111 (32) 107/226 (47)

* The guinea pigs were ≥3 months old at time of inoculation.

* Value for ≤4 weeks significantly different (<i>P < 0.001</i>) from value for ≥8 weeks by the χ² test.
Myocardial lymphoplasmacytic infiltrates (large arrows) and myocardial cell damage (small arrows) located in the base of ventricular septum (magnification, ×25); (b) myocardial lymphoplasmacytic infiltrates particularly prominent around nerves and parasympathetic ganglia (arrows) (×18.7); (c) perivascular lymphoplasmacytic infiltrates (arrows) in the lamina propria of the bladder (×6.25); (d) spirochetes in the bladder lamina propria (arrow) (modified Dieterle stain) (×900); (e) focal lymphocytic meningitis (arrows) (×25); (f) focal lymphoplasmacytic infiltrate of knee synovium (arrows) (×33); (g) erosion of articular knee cartilage surface (lower) with fibrovascular granulation tissue formation and new synovial lining (arrows) (×25); (h) focially sloughed synovial lining (lower left) and new synovium with hyperplastic changes (upper right, arrows) (×25).

FIG. 1. Representative histopathologic findings in infected guinea pigs. (a) Focal myocarditis characterized by lymphoplasmacytic infiltrates (large arrows) and myocardial cell damage (small arrows) located in the base of ventricular septum (magnification, ×25); (b) myocardial lymphoplasmacytic infiltrates particularly prominent around nerves and parasympathetic ganglia (arrows) (×18.7); (c) perivascular lymphoplasmacytic infiltrates (arrows) in the lamina propria of the bladder (×6.25); (d) spirochetes in the bladder lamina propria (arrow) (modified Dieterle stain) (×900); (e) focal lymphocytic meningitis (arrows) (×25); (f) focal lymphoplasmacytic infiltrate of knee synovium (arrows) (×33); (g) erosion of articular knee cartilage surface (lower) with fibrovascular granulation tissue formation and new synovial lining (arrows) (×25); (h) focially sloughed synovial lining (lower left) and new synovium with hyperplastic changes (upper right, arrows) (×25).

tivity by day 15 which became progressively more intense at days 23 and 30 (Fig. 2a). There was no major change noted in the intensity or specificity of the immune response from days 30 to 60 (Fig. 2b). The most abundant IgG antibodies noted reacted with proteins migrating at 24, 31 (OspA), 39, and 41 (flagellin) kDa. A single nonspecific band was noted at approximately 46 kDa in all guinea pigs, including controls. All eight CT-1-infected guinea pigs evaluated developed detectable antibody to the 31-kDa (OspA) protein by day 15 (Fig. 2a). Only 2 of 11 guinea pigs developed detectable antibody to the 34-kDa (OspB) protein even by day 30, and a third animal developed a very faint band at day 60 (Fig. 2b).

DISCUSSION

We have demonstrated that the guinea pig is susceptible to infection with B. burgdorferi and may provide a useful new small animal model for the study of Lyme disease. Of 60 Hartley guinea pigs inoculated at ≤3 months of age, 57 (95%) were found to be infected. B. burgdorferi infection disseminated to a variety of tissues, including the heart, bladder, knee, spinal cord, spleen, and muscle. Spirochetemia was not documented, although blood cultures were not obtained during the first week after inoculation, when transient spirochetemia most likely occurs. Immunoblots documented a specific antibody response to multiple B. burgdorferi proteins. We did not observe clinical evidence of arthritis or erythema migrans among infected guinea pigs, although it is possible that clinical disease manifestations occurred but were transient and were missed or that they occur ≥4 months after infection.

The susceptibility of guinea pigs was age dependent; animals of ≥6 months of age at the time of inoculation were resistant to infection. Thus, it appears likely that unknown factors in the guinea pig immune system mature between 3 and 6 months and confer host resistance to B. burgdorferi infection. Guinea pigs have been reported to develop resistance to infection with leptospires because the humoral immune system matures at 7 to 10 days after birth (1), much earlier than the development of resistance noted in our studies. Age has previously been described as a factor in determining disease severity in experimental B. burgdorferi infection of Lewis rats (7, 8, 29) and C3H mice (5).

We found that the guinea pig commonly develops histopathologic changes in the heart and bladder. In the heart,
lymphoplasmacytic infiltrates preferentially involved the annulus fibrosus, subendocardium, and areas containing abundant conductive fibers and neural elements such as ganglion cells. This localization of pathologic changes is consistent with cardiac conduction disturbances seen clinically in humans with acute Lyme disease. Tropism of spirochetes for cardiac tissues in hamsters (21) and mice (3) has also been documented.

Guinea pigs demonstrated a surprisingly high percentage of positive knee joint cultures (27/34 = 79%). No other animal model has been reported to yield such a high frequency of positive joint cultures. The most obvious explanation for this finding is a tropism for articular tissue. Although this would be consistent with the clinical findings of human Lyme disease and unpublished studies employing PCR (11), the isolation of B. burgdorferi from human joints has been very rare. It is possible that some knee joints in these guinea pigs may have been culture positive because of spirochetes localized within adherent muscle. We did find that quadriceps muscle was frequently infected with B. burgdorferi (6/9 = 67%). However, in the group of animals for which muscle was cultured, all three animals with negative muscle cultures had positive knee joint cultures. Despite the high frequency of positive knee joint cultures and the apparent role of the knee as a sanctuary for persistent infection, significant pathologic changes were observed in only 2 of 22 (9%) knees. In humans, Lyme arthritis usually occurs months to years after initial infection. Whether a higher proportion of guinea pigs would develop pathologic changes with the passage of more time or after tick infection remains to be determined.

Lymphocytic meningitis and/or other central nervous system manifestations occur in approximately 10% of untreated cases of human Lyme disease. While an acute and transient lymphocytic meningitis has been described in Lewis rats inoculated intravenously with B. burgdorferi (18), no animal model of neuroborreliosis has yet been described. We found that 3 of 29 (10%) spinal cords from infected guinea pigs had B. burgdorferi isolated by culture and 2 of 16 animals examined pathologically had meningitis. These animals with meningitis represented two of four animals whose spinal cords were examined pathologically 4 weeks after inoculation. Further studies are in progress to ascertain the frequency of meningitis at early time points.

While cystitis is not a clinical feature of human Lyme disease, tropism of B. burgdorferi for the bladder has been previously documented in Peromyscus leucopus (33), hamsters (21), and C3H mice (6). We found the guinea pig bladder to be frequently infected (32/57 = 56%) and noted histopathologic changes in most animals (19/25 = 76%). Bladder infection is relevant to diagnostic studies, because urine is readily available and has potential diagnostic utility for detection of spirochetal components by PCR (22) and antigen detection methods (15, 24). As reported for C3H mice, pathologic changes were localized to the lamina propria and did not involve the mucosa itself or muscular...
The cultures. Postinoculation. Positive human urine. Histologic changes, particularly in immunocompetent hosts. Like the C3H mouse, the guinea pig develops pathologic changes in tissues relevant to human disease, but, in addition, then mounts an immune response which is associated with the clearance of organisms from most infected tissues. The guinea pig is apparently unique among small animal models with respect to the spirochetal persistence noted in infected knee joints despite clearance elsewhere.

Both increasing postinfection immunity and apparent age-related maturation of the immune system may be important in the spirochetal clearance which we observed. Finally, the guinea pig antibody response also appears relevant to that observed in humans, in whom responsiveness to Osp proteins may not appear for months to years (13). In our studies, the response to proteins other than OspA and OspB are dominant in the first 3 weeks after infection. While a detectable OspA response was noted within 3 weeks, the response to OspB was not striking and occurred in only 2 of 11 animals by 30 days. Although our initial studies to document guinea pig susceptibility to B. burgdorferi infection have utilized needle inoculation, it is now well recognized that tick infection may affect both the antibody response (20, 30) and the disease manifestations produced (2). For these reasons, it will also be of interest to study the pathogenesis of B. burgdorferi infection in guinea pigs following infection by ticks.

In conclusion, the guinea pig provides a promising new experimental model of B. burgdorferi infection which demonstrates relevant histopathologic changes, an active immune response, and spirochetal persistence in knee joints. This model may be useful in complementing existing small animal models by allowing the determination of specific components of the host response critical in the limitation of infection and the pathogenesis of disease manifestations.

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