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Kinetics of *Borrelia burgdorferi* Dissemination and Evolution of Disease After Intradermal Inoculation of Mice

Stephen W. Barthold,* David H. Persing,† Audrey L. Armstrong,* and Ruth A. Peeples*

From the Section of Comparative Medicine, Yale University School of Medicine, New Haven, Connecticut; and the Department of Laboratory Medicine and Pathology,† Mayo Clinic, Rochester, Minnesota*

Borrelia burgdorferi ***dissemination to selected target organs was examined on days 1, 2, 3, 4, 7, 10, 15, 21, and 30 after intradermal inoculation of 4-week-old C3H mice. Infection was determined by culture (blood, spleen, kidney, ear punch); polymerase chain reaction (PCR) for outer surface protein A (OSP A) DNA (ear punch); histology and spirochete histochemistry (spleen, kidney, skin, heart, joints); and OSP A DNA* in situ *hybridization (joints, heart). Blood or spleen of most mice were culture positive by day 3 and ear punch by day 10. Polymerase chain reaction performed on ear punches was also positive by day 10. Inflammation of joints and tendons began on days 4 through 7 and heart on days 7 through 10, which coincided with colonization of tissues with spirochetes. Spirochetes were multifocal in distribution, with a predilection for collagenous connective tissue of joints, heart, arteries, nerves, muscle, skin, and other tissues. Relative numbers of spirochetes peaked at 15 days, then decreased by 21 days. Gamma M immunoglobulin (IgM) antibody was detectable on immunoblots as early as day 4, with subsequent declining reactivity, and IgG antibody was detectable by day 7, with expanding reactivity to multiple antigens through day 30. (Am J Pathol 1991, 139:263–273)***

Lyme disease (LD) in humans and animals is a multisystemic disorder caused by *Borrelia burgdorferi*.[1] Individuals are infected cutaneously through the bite of an Ixodid tick, or possibly other biting arthropods.[1,2] Mechanisms of LD pathogenesis are not well understood. Evidence that antibiotic therapy stops or reverses the course of LD, followed by diminishing antibody titers over the ensuing months,[3] suggests a direct role of *B. burgdorferi* in the generation and perpetuation of LD. This is reinforced by the visualization of small numbers of spirochetes in autopsy or biopsy specimens,[4,5] as well as isolation of *B. burgdorferi* from human skin lesions months to years after initial infection.[6,7] It is uncertain, however, if the varied manifestations of LD are due to direct effects of the spirochete, host immune response to the spirochete, or both.

With the recent discovery of a laboratory mouse model of LD, an understanding of the mechanisms of LD pathogenesis can be achieved. Immunologically intact, inbred C3H/He mice of all ages are susceptible to infection with *B. burgdorferi*, and develop multisystemic infection with a reproducibly high incidence of polyarthritis and carditis within 4 weeks after inoculation.[8] Arthritis undergoes regression after this interval. Mice are preferentially susceptible to infection by the intradermal route, compared with other routes of exposure,[9] underscoring the importance of skin as a preferred site of spirochete entry. Other strains of mice are susceptible to infection but develop only mild disease.[8] The purpose of the present study was to examine the sequential dissemination of *B. burgdorferi* to various tissues and the evolution of joint and heart lesions within the first 4 weeks of infection.

Materials and Methods

Mice

Random sex, virus-antibody–free C3H/HeNCrlBR (C3H) mice were obtained from Charles River Breeding Laboratories, Portage, Michigan. Mice were shipped in filtered crates and housed in isolator cages (Lab Products, Maywood, NJ). Food (Agway, Syracuse, NY) and water were

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Address reprint requests to Dr. Stephen W. Barthold, Section of Comparative Medicine, Yale University School of Medicine, 333 Cedar St., PO Box 3333, New Haven, CT 06510.

provided *ad libitum*. Animals were killed with carbon dioxide gas, followed by cardiac exsanguination.

Borrelia burgdorferi

The N40 strain of *B. burgdorferi* was grown in modified Barbour-Stoenner-Kelly (BSK II) medium.[10] This strain has proven infectivity and pathogenicity in a variety of laboratory animal species, including mice.[11] Inoculum consisted of spirochetes in their third *in vitro* passage. Tissues to be cultured were collected aseptically, homogenized in BSK II medium, and cultured without antibiotics, as described.[8] Duplicate ear punch samples also were cultured in antibiotic medium containing 50 μg/ml Rifampicin (Sigma Chemical Co., St. Louis, MO).[12] Cultures were incubated at 35°C for 14 days, then examined for spirochetes by dark-field microscopy.

Polymerase Chain Reaction

Borrelia-specific outer surface protein A (OSP A) DNA sequences in ear skin punches were amplified by polymerase chain reaction (PCR). Oligonucleotide primers were prepared and PCR amplifications of stock strains of *B. burgdorferi* were performed as described.[13] For amplification of *Borrelia*-specific sequences from ear punches, punches were first digested in 50 μl of 'K buffer' (10 mmol/l [millimolar] TRIS HCl, pH 8.3, mmol/l KCl, 1.75 mmol/l $MgCl_2$, 0.01% gelatin, 0.5% NP40, 0.5% Tween 20, 50 μg/ml proteinase K). The samples were incubated at 55°C for 1 hour, boiled for 5 minutes, then quenched immediately on ice; 5-μl aliquots were removed for amplification by PCR. One nanogram of control DNA or 5 μl of the ear punch digest was added to a 50 μl PCR reaction containing 10 mmol/l TRIS HCl, pH 8.3, 50 mmol/l KCl, 0.01% gelatin, 200 μmol/l (micromolar) each diethylnitrophenyl thiophosphate and 50 picomoles each of primer OSP A2 and OSP A4,[14] and 1.75 mmol/l $MgCl_2$. All reactions were performed in a Perkin-Elmer-Cetus (Norwalk, CT) thermal cycler; components were denatured at 94°C for 30 seconds, annealed at 55° C for 45 seconds, and extended at 72°C for 1 minute, for a total of 45 cycles. The amplification products were then slot blotted and probed as described.[14]

Histology/Histochemistry/ In Situ *Hybridization*

Tissues were immersion fixed in 10% neutral buffered formalin (pH 7.2). Osseous tissue was demineralized in decalcifying solution (Baxter Health Care Corp., McGaw Park, IL). Tissues were then embedded in paraffin, sectioned, and stained by standard technique. Tissues were stained for spirochetes with a variation of a previously described modified Dieterle stain method,[15] during which uranyl nitrate, gum mastic, silver nitrate, and developer solutions were all preheated to 60°C. Slides were incubated in silver nitrate in total darkness, and silver nitrate solution was prepared and stored in darkness. Tissue sections for *in situ* nucleic acid hybridization were bonded to glass slides with 3-aminopropyltriethoxysilane,[16] deparaffinized in xylene, and rehydrated through graded ethanols. Sections were treated for 30 minutes with proteinase K, then acetylated with acetic anhydride, dehydrated through 100% ethanol, and air dried. Sections were treated with 95% formamide in 0.15 m sodium chloride 0.015 m sodium citrate (SSC) for 15 minutes at 65° C, ice-cold SSC (0.1%), dehydrated through 100% ethanol, then air dried. For synthesis of the probe used for *in situ* analysis, we used primers OSP-A1[13] and OSP-A2[14] to produce a 646-bp amplification product of the OSP-A gene of strain N40. The fragment was gel purified, nick translated, and labeled to a high specific activity (0.5 to 1.0 × 10^9 cpm/μg) with ^{32}P-DCTP by the random priming method. Hybridization mix containing 5 × 10^5 cpm was applied to each slide, and hybridized overnight at room temperature. Slides were then washed, air dried, dipped in Ilford KD-5 emulsion (Poly Sciences, Inc., Warrington, PA) for autoradiography, exposed for 7 days, then developed, fixed, stained, and mounted with coverslips.

Serology

Serum gamma M immunoglobulin (IgM) and IgG antibody titers to *B. burgdorferi* were determined with an enzyme-linked immunosorbent assay (ELISA) using N40 spirochetes as antigen.[17] Immunoblots were prepared by transfer of N40 proteins from sodium dodecyl sulfate polyacrylamide gels to nitrocellulose paper with a Hoefer Transphor cell fitted with a Hoefer power lid (Hoefer Scientific Instruments, San Francisco, CA) for 45 minutes at 0.6 amperes. Nitrocellulose paper strips were processed as described.[17] They were incubated with test serum, diluted 1:50 in TRIS-buffered saline for 2 hours at room temperature on a shaker, washed twice in blocking buffer, then incubated for 2 hours with phosphatase-labeled, goat anti-mouse IgM or IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Strips then were washed twice with TRIS-buffered saline, then stained for color development with the BCIP/NBT phosphatase substrate system (Kirkegaard and Perry Laboratories).

Experimental Plan

The sequential course of *B. burgdorferi* dissemination from an initial cutaneous site of inoculation to selected

Table 1. *Rate of Isolation of* B. burgdorferi *from Selected Tissues and Inflammation in Joints and Heart at Intervals After Intradermal Inoculation*

	Culture					Pathology	
Day	Blood	Spleen	Kidney	Ear	Rx Ear*	Joint	Heart
1	0/5†	0/5	0/5	0/5	0/4	–	–
2	0/5	1/5	0/4	0/5	0/4	–	–
3	2/5	4/5	0/5	0/5	0/4	0/5	0/5
4	3/5	4/5	1/5	0/5	0/4	3/5	0/5
7	3/5	3/4	2/3	0/5	0/4	5/5	1/5
10	5/5	4/4	3/5	3/4	0/4	5/5	5/5
15	5/5	4/4	3/4	1/1	1/5	5/5	5/5
21	3/4	4/4	4/4	4/4	0/3	4/4	4/4
30	4/5	5/5	4/4	–	–	5/5	5/5

* Rx Ear, results of ear cultured in antibiotic medium.
† Number positive/number tested. Contaminated samples were deleted, explaining the variable sample size of some tissues listed in this table.

target organs and the evolution of lesions was followed for up to 30 days in C3H mice inoculated at 4 weeks of age. Dissemination of spirochetes from the site of dermal inoculation was measured by culture of blood, spleen, kidney, and ear punch, because these tissues have been found to be consistently infected in mice.[8,9,18] Heart and joint tissue, but not skin, spleen and kidney, have been shown to develop a high incidence of inflammatory lesions after experimental inoculation of C3H mice.[8] These tissues were selected for microscopic examination to determine the distribution of spirochetes and evolution of lesions. Mice were anesthetized with methoxyflurane, the fur on their back clipped, then each was inoculated intradermally with 10^4 *B. burgdorferi* in 0.1 ml BSK II medium. Five mice were randomly selected for necropsy on days 1, 2, 3, 4, 7, 10, 15, 21, and 30 after inoculation. Heart, blood, skin (ear punch), spleen, and kidney were cultured from each mouse. Knee, tibiotarsal, and tarsal joints from both rear legs, skin, spleen, kidney and heart were processed for histology from day 3 onward. Ear punches were collected for skin culture as well as for PCR analysis. Serum was collected and frozen for subsequent antibody titration.

Results

Based on culture, dissemination of spirochetes was first detectable at day 2 after inoculation, when the spleen of a single mouse was positive (Table 1). By 3 days, blood or spleen of most mice were culture positive, and all blood and spleen samples tested positive on day 10. Spirochetemia persisted through 30 days. Culture of spirochetes from kidney lagged behind blood and spleen. A single mouse kidney was culture positive on day 4, and subsequent intervals showed a majority of kidney samples to be culture positive. Ear punches from three of four mice cultured on day 10 were positive, with all ear punches becoming positive at subsequent intervals. Contamination of some ear punch samples precluded evaluation of all five mice at each interval. Duplicate ear punch samples, cultured in antibiotic-containing medium, yielded only one positive result of 12 samples tested from mice on days 10, 15, and 21. In contrast, eight of nine ear punch samples, cultured in medium without antibiotic, yielded positive results from mice tested on days 10, 15, and 21.

Detection of spirochete dissemination to distant skin sites by PCR analysis of ear punches correlated well with

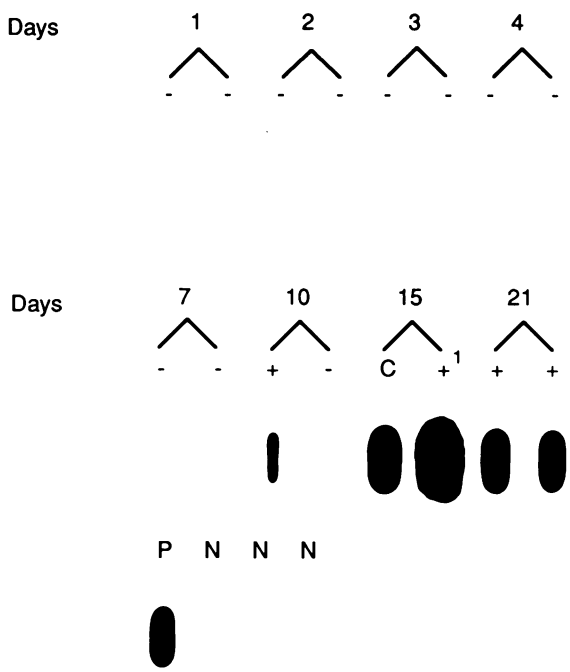


Figure 1. *Slot blots of PCR products from ear punches taken from mice at intervals after inoculation. Culture results (+ or −) of duplicate samples are given below the days after inoculation. Duplicate culture samples labeled as C were contaminated. Culture of one sample designated +1 at day 15 was contaminated, but alternate sample cultured in antibiotic medium was positive. P and N are positive and negative controls, respectively.*

Table 2. *IgM and IgG ELISA Antibody Titers to* Borrelia burgdorferi *at Intervals After Infection*

Days after inoculation	IgM	IgG
1	<80*	<80
2	<80	<80
3	<80	<80
4	<80	<80
7	211 (160–320)	453 (320–640)
10	368 (320–640)	2,941 (1280–10,240)
15	422 (320–640)	20,480 (10,240–40,960)
21	320 (320)	34,443 (20,480–40,960)

* Reciprocal dilution of geometric mean titer (range).

culture results (Figure 1). Duplicate ear punches taken from mice that had culture-negative ear punches were negative by PCR on days 1, 2, 3, 4, and 7. On day 10, a duplicate ear punch from a mouse that had a culture-positive ear punch was PCR positive, whereas the sample from a culture-negative mouse was PCR negative. Ear punch cultures taken on day 15 had a high rate of contamination. A duplicate ear punch taken from a mouse with a contaminated culture was PCR positive. The second day-15 mouse tested positive by PCR. This mouse had a positive culture using antibiotic medium, but its other culture tube was contaminated. On day 21, ear punches from both culture-positive mice were PCR positive. The positive control tested positive and three negative controls tested negative by PCR. These results were confirmed by PCR detection of a second genetic target in the *B. burgdorferi* genome, the 16S ribosomal gene.[19]

All mice developed detectable levels of ELISA IgM antibody to *B. burgdorferi* beginning on day 7, which increased slightly in titer through day 15. Most mice tested on day 7 also had detectable levels of IgG antibody by ELISA, which was present in all mice at subsequent intervals and increased to high titer (Table 2). Immunoblots with sera from all five mice on day 4 showed IgM reactivity against 31-kd Osp A and several other high–molecular-weight bands. Gamma M immunoglobulin reactivity was reduced on day 7, became weaker on day 15, and nearly undetectable by day 21. No IgM reactivity was seen in any of the day 4 sera, but on day 7, all mice had weak reactivity against 31-kD OspA and other antigens. On days 15 through 30, IgG reacted strongly

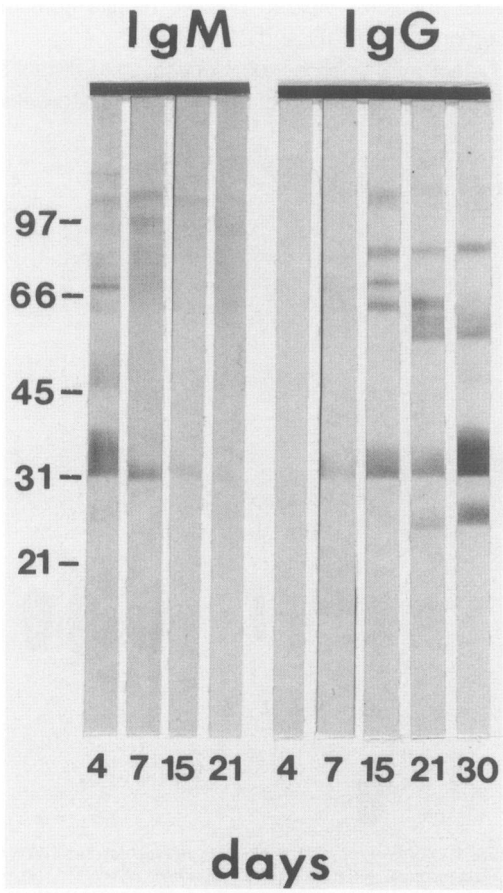


Figure 2. *Representative IgM and IgG immunoblots of serum samples from mice at intervals after inoculation. Days after inoculation are indicated at the bottom of each blot and molecular weights of standards (kilodaltons) are indicated at the right.*

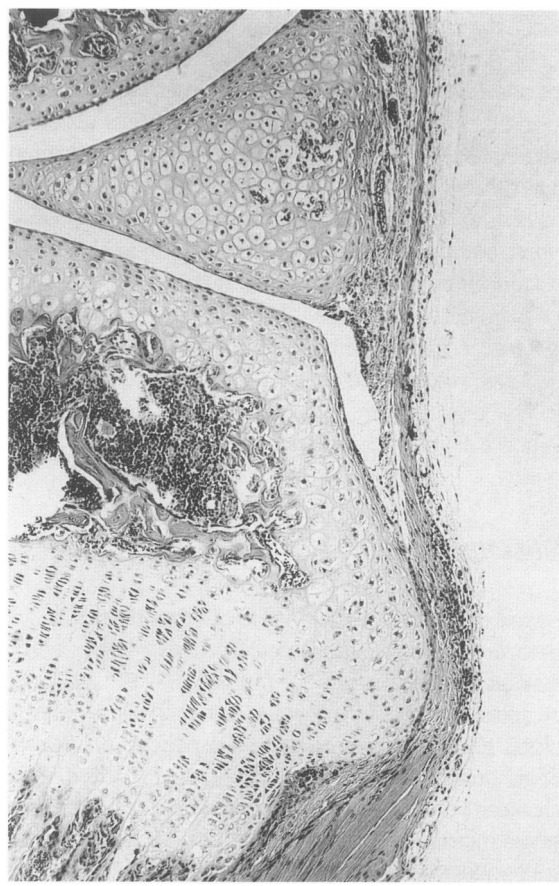
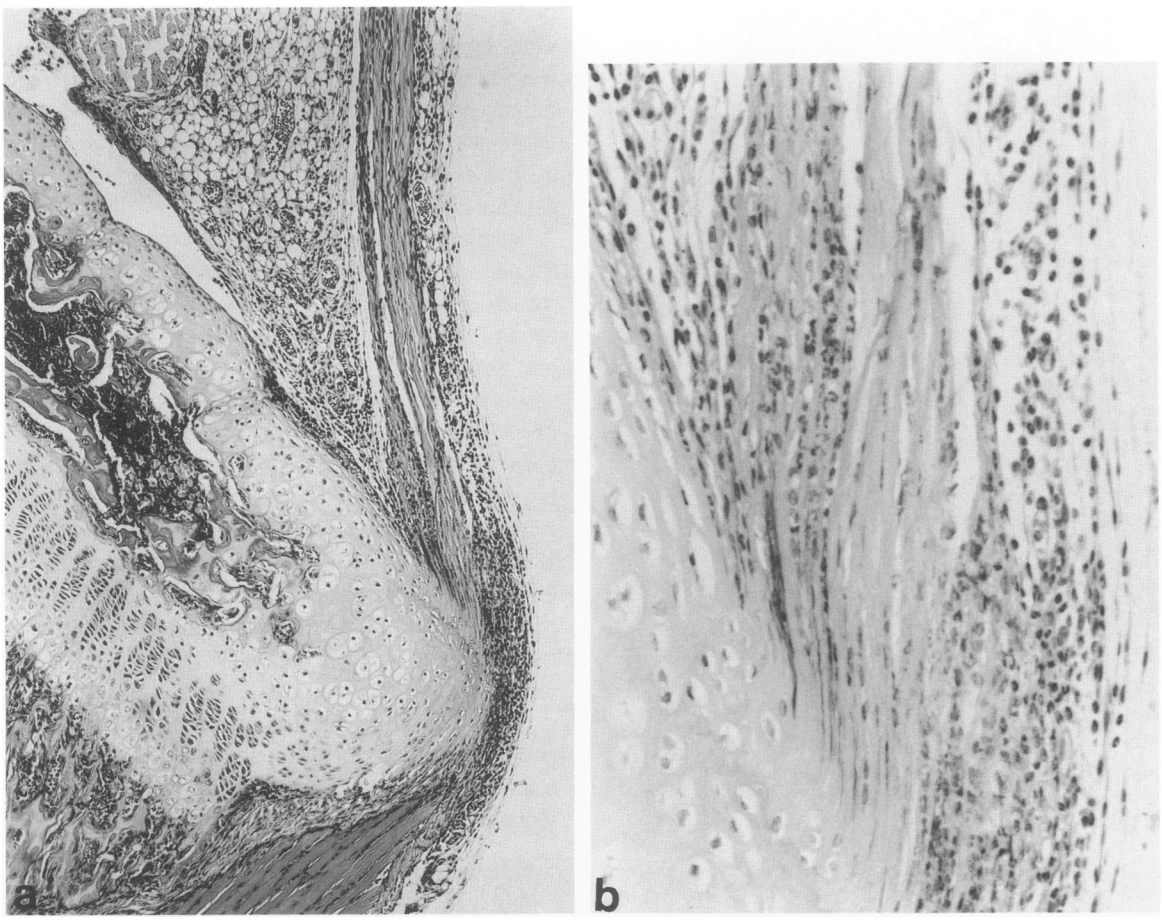


Figure 3. *Early inflammation of the anterior knee joint capsule, at the tibial crest, 4 days after inoculation. H&E, ×60.*



Figures 4. a, b: *Inflammation of anterior knee joint capsule and perisynovial tissue, with early exudation of leukocytes into the synovial lumen (top), 7 days after inoculation. H&E, ×60, 210.*

against several bands, particularly Osp A (Figure 2). Normal mouse serum did not react in immunoblots of N40 antigen, and immune serum did not react in immunoblots prepared with BSK II medium.

Microscopy showed early inflammatory lesions around joints in three of five mice as early as day 4, and all mice had arthritis after day 10 (Table 1). Early (day 4) lesions evolved at the junction of the joint capsule and patellar ligament to tibia, with infiltration of neutrophilic leukocytes into these areas (Figure 3). On days 7 and 10, inflammation became more intense, with expansion into surrounding connective tissue and synovium. Small amounts of exudate could be found in the synovial spaces of these joints (Figures 4, 5). At these intervals, inflammation began around joints, ligaments, and tendons of the tibiotarsal area. Ligament and tendon attachment sites to tibiotarsal bones were often involved, as seen in the knee. On days 10 and 15, ligament and tendon attachment sites had intense fibroblastic proliferation (Figure 6). Arthritis was generally more pronounced, with joint spaces containing conspicuous amounts of fibrin and neutrophils. Synovial lining had become thickened,

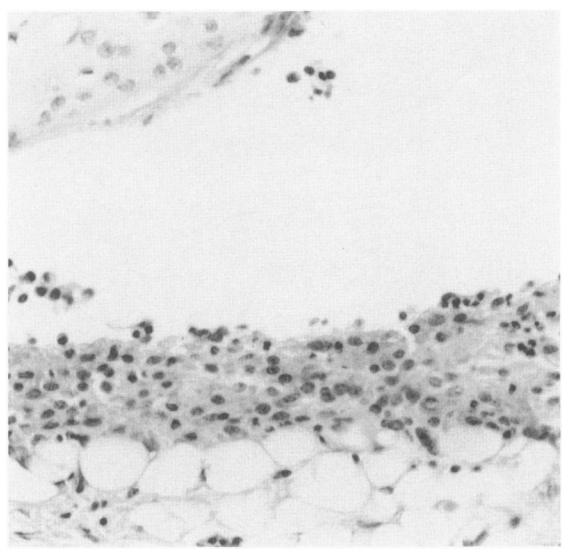


Figure 5. *Synovium of knee joint with mild synovial hyperplasia and neutrophilic leukocyte infiltration, 7 days after inoculation. H&E, ×210.*

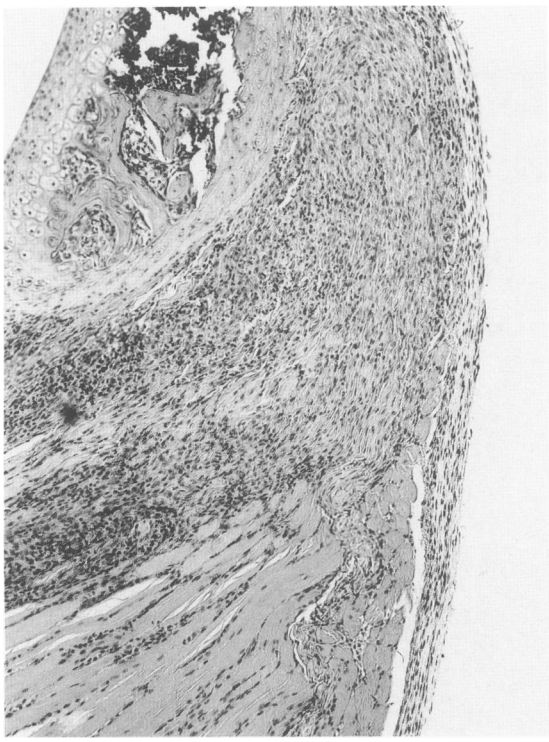
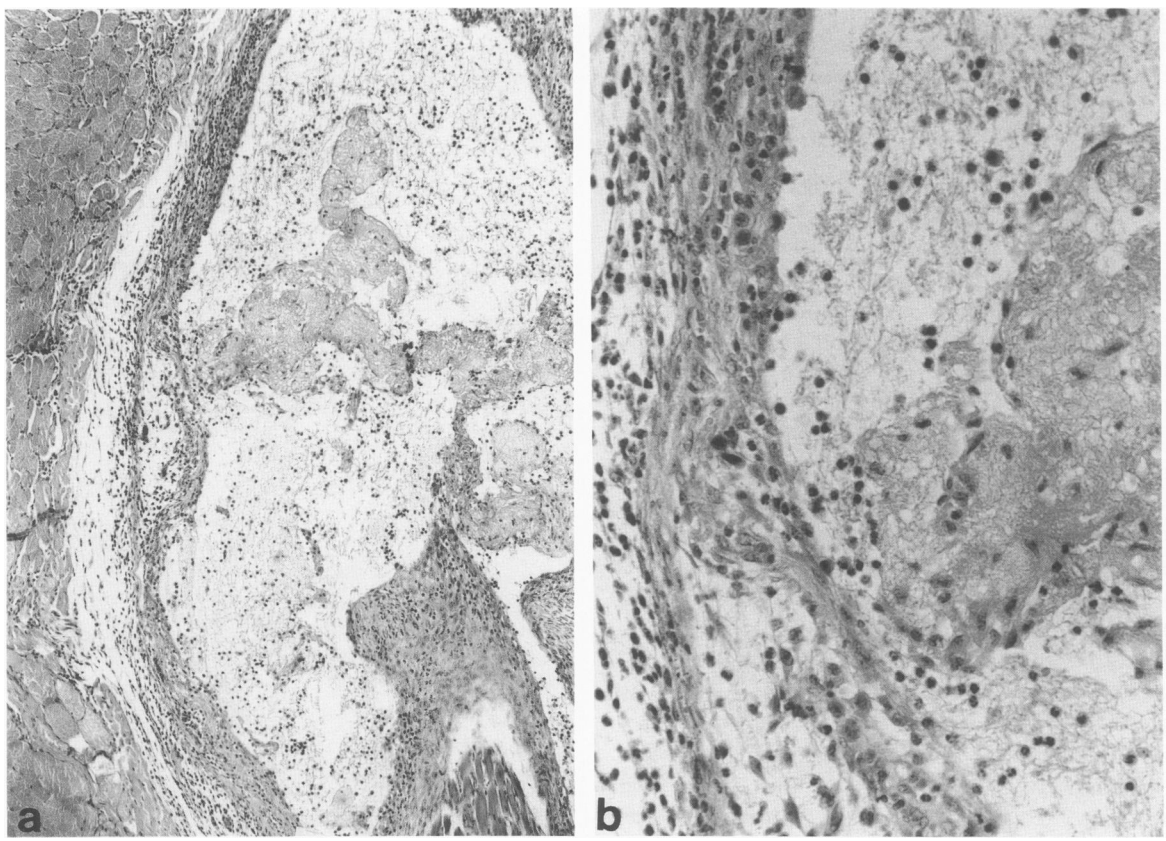


Figure 6. *Fibroplasia and inflammation of patellar tendon and adjacent quadriceps femoris muscle, 15 days after inoculation. H&E, ×60.*

because of proliferation of synovial lining cells. Multiple joints, tendon sheaths, bursae, and periarticular tissues were involved to varying degrees. Arthritis became progressively more severe through days 21 to 30 (Figure 7). Gross swelling of the tibiotarsal area due to periarticular edema and inflammation was most evident at day 15, then subsided by day 21.

Inflammation of cardiac tissues was present in all mice examined at day 10 and beyond, as well as a single mouse on day 7. Day 7 and 10 hearts had focal margination of neutrophils along the endothelium of the aorta and valves at the base of the heart, with infiltration of vascular walls with mixed leukocytes, primarily neutrophils (Figure 8). Leukocytic infiltration extended out into the adventitia and myocardium, especially on day 15 and beyond. At these later intervals, mononuclear leukocyte infiltrates were also apparent in the epicardium of the atria, and to a lesser extent, upper ventricles (Figure 9). On day 30, leukocyte infiltrates were predominantly mononuclear, including plasma cells, and occurred primarily in the periaortic adventitia (Figure 10). No lesions were found in skin, spleen, or kidney.

All tissues (knee, tibiotarsus, skin, heart, spleen, kidney) on three to four mice per interval were Dieterle stained and examined for spirochetes. Numbers of spirochetes varied extensively between tissues, areas within



Figures 7. a, b: *Severe arthritis of the tarsal joint, 30 days after inoculation. The synovium is thickened and the lumen contains exudate with abundant fibrin and leukocytes. H&E, ×60, 330.*

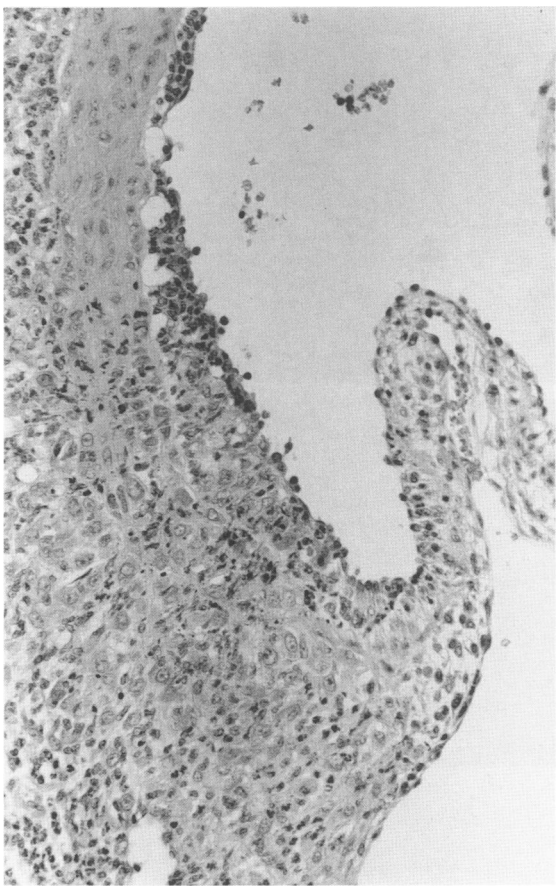


Figure 8. *Aortitis at the heart base, adjacent to aortic valve, 15 days after inoculation. H&E, ×150.*

tissues, and intervals. Numbers and location within tissues therefore could be assessed only by their presence and numbers relative to other intervals and sites. Leg tissue (knee and tibiotarsus) demonstrated small numbers of spirochetes in areas of inflammation on days 4 and 7, with more organisms present on day 10 and the

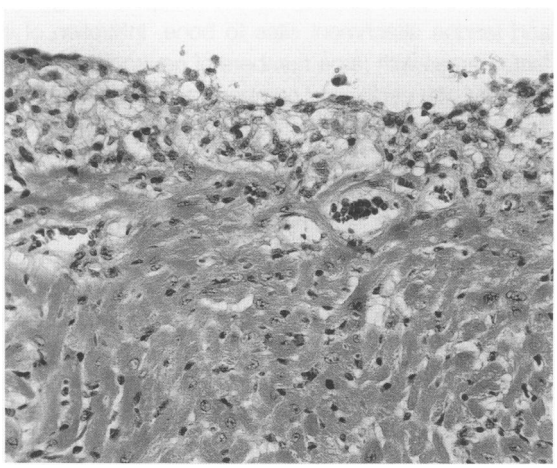


Figure 9. *Infiltration of upper ventricular epicardium with mononuclear leukocytes, 21 days after inoculation. H&E, ×150.*

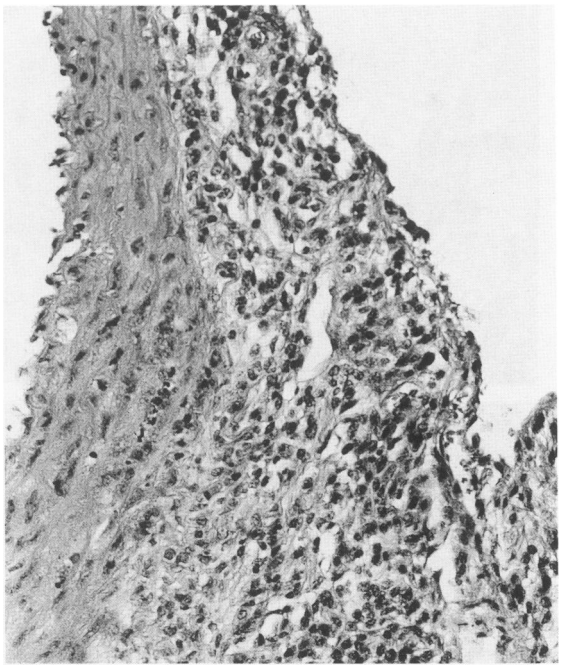


Figure 10. *Infiltration of aortic adventitia at the heart base with mononuclear leukocytes, 30 days after inoculation. H&E, ×150.*

greatest number of spirochetes on day 15. The number of spirochetes diminished significantly thereafter. Spirochetes occurred focally. Multiple organisms were usually observed in an area, whereas other areas were totally devoid of discernible organisms. Their presence around joints was usually associated with areas of leukocyte response. They were found in and around vessel walls (Figure 11a), especially arteries, adjacent connective tissue, synovium (Figure 11b), ligaments and tendons, especially at osseous attachment sites (Figure 11c), and periosteum. In ligaments and tendons, organisms were oriented between collagen fibers in relatively large numbers and did not seem to elicit a response, other than at the site of osseous attachment. Organisms were only rarely found in the synovial space. They also tended to infiltrate perineural connective tissue and muscle connective tissue (Figure 11d) and, rarely, muscle fibers. Popliteal lymph nodes, when represented in tissue sections, often contained spirochetes in medullary sinuses (Figure 11e). At day 21, spirochetes were only rarely present in synovium and surrounding tissues. Small numbers of spirochetes, however, could consistently be found within ligaments and tendons, their osseous insertion sites and adventitia of arteries.

Within the heart, spirochetes were first found on day 7 within the walls of great vessels at the base of the heart, particularly the aorta. At subsequent intervals, they were found in the adventitia of great vessels (Figure 12a) and coronary arteries, connective tissue of atrial and upper ventricular epicardium (Figure 12b), endocardium, chordae tendonae, valves, and the connective tissue in the

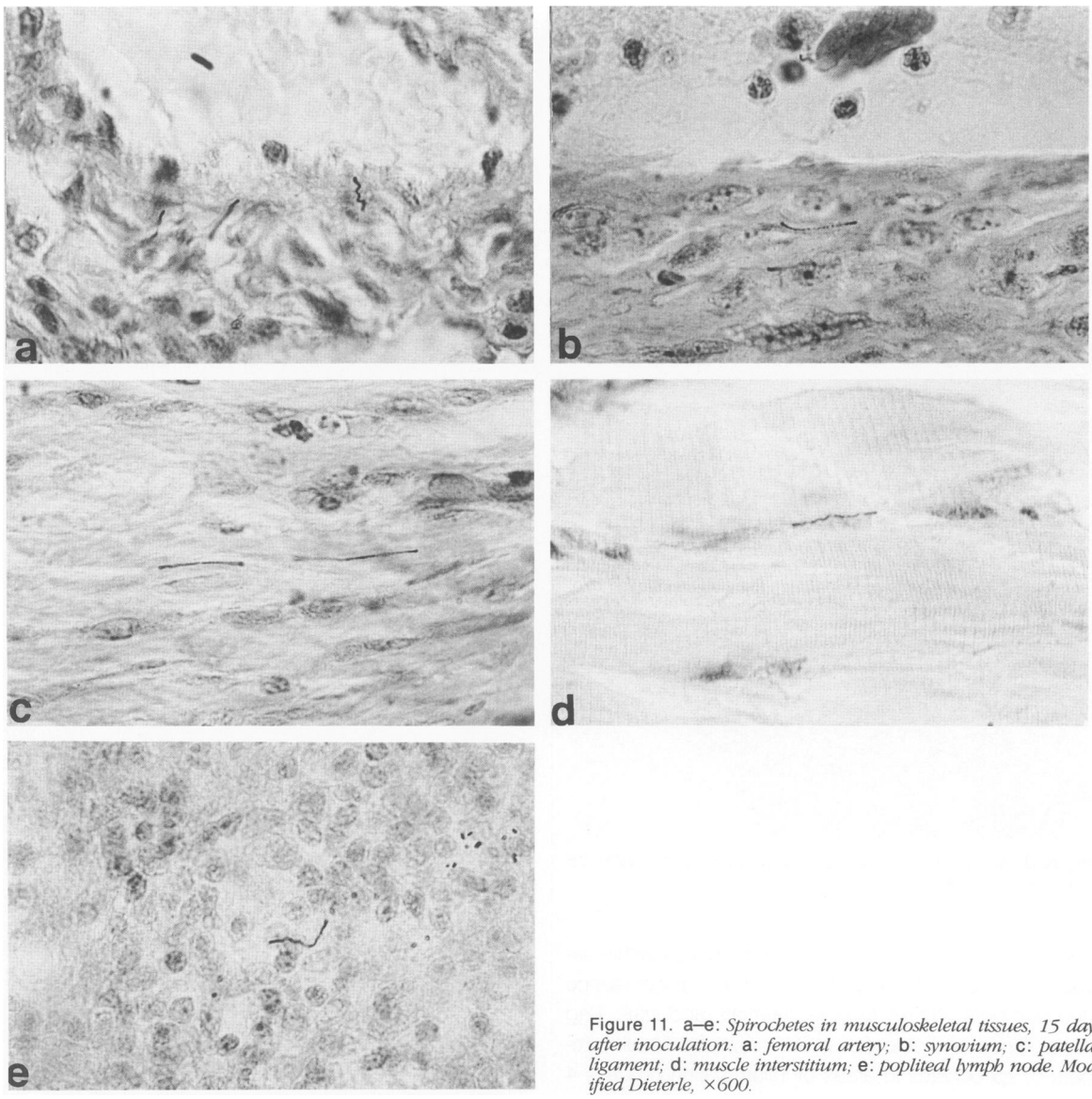


Figure 11. a–e: *Spirochetes in musculoskeletal tissues, 15 days after inoculation:* a: *femoral artery;* b: *synovium;* c: *patellar ligament;* d: *muscle interstitium;* e: *popliteal lymph node. Modified Dieterle, ×600.*

heart base. Fewer organisms were found within the myocardium, and when present, were generally within the interstitium. Mediastinal lymph nodes also contained spirochetes in a high percentage of samples. Spirochetes were most plentiful in heart and aorta on day 15, and were much fewer by day 21.

In other tissues, spirochetes also commonly were found in the dermis in focal areas (Figure 13). They were present around vessels and within the dermal collagen. Their presence in kidney was most often associated with the adventitia of renal arteries, and they tended to occur in the serosal connective tissue of spleen.

Inflammation appeared to be elicited by the presence of spirochetes associated with cardiac, synovial, perisynovial, and periosteal tissues, but little reaction occurred in response to spirochetes within ligaments, tendons, muscle, skin, or other tissues, with the exception of ligament and tendon attachment sites to bone. Infiltration of infected areas with large numbers of neutrophils was associated with diminished numbers of visible spirochetes, and fragments of silver-positive structures, suggestive of spirochetes, were visible within the cytoplasm of phagocytes.

In situ nucleic acid hybridization confirmed culture and histology results. The tarsus of a mouse at day 4 had positive hybridization at the insertion of a ligament to a tarsal bone (Figure 14a) and around blood vessels within the adjacent connective tissue without evidence of inflammation. Hybridization was also demonstrated in and around blood vessels (Figure 14b), synovium (Figure 14c), synovial exudate, periarticular connective tissue; cardiac vessels (Figure 15a), endocardium, epicardium

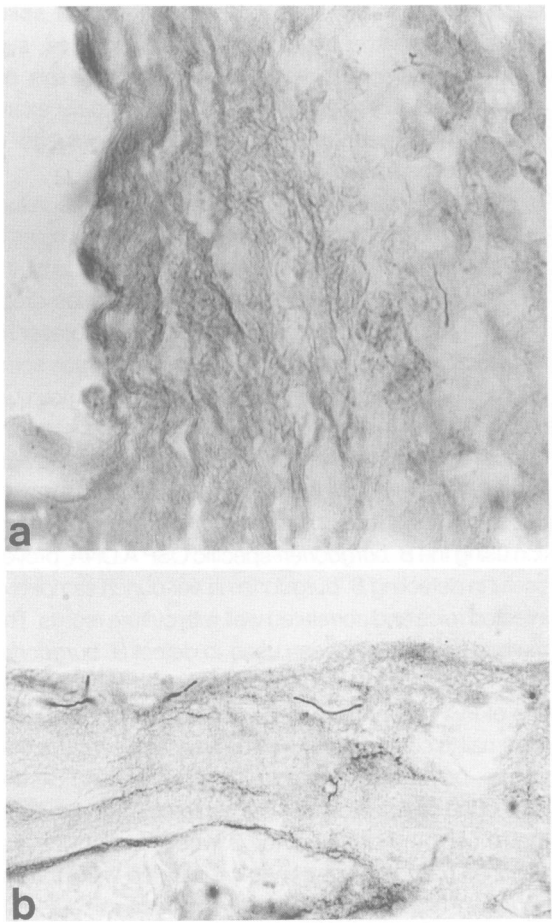


Figure 12. a, b: *Spirochetes in cardiac tissue, 15 days after inoculation:* a: *aortic adventitia;* b: *epicardium. Modified Dieterle, ×600.*

(Figure 15b), myocardium, and skin of other mice examined on day 15.

Discussion

These studies show that *B. burgdorferi* spirochetes disseminate to cause multisystemic infection within a few days after initial infection of the skin. The onset of inflammation in distant target tissues such as joints and heart coincides with the appearance of spirochetes in these sites. The early onset of inflammation and its direct correlation with spirochetes provides strong evidence that the arthritis and carditis of acute Lyme disease are due to direct effects of the spirochete, rather than an immunopathic mechanism. Further support for this contention is provided in studies involving mice with severe combined immunodeficiency, which also develop arthritis and carditis when infected with *B. burgdorferi*.[20,21] Morphologic evidence suggests that the neutrophils and macrophages infiltrating the areas of inflammation are actively involved in bacterial killing and phagocytosis. Neutrophils

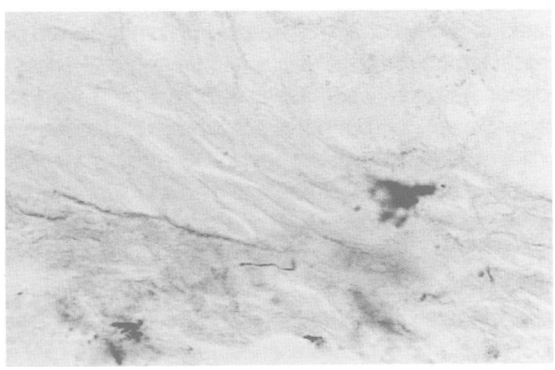


Figure 13. *Spirochete in dermis, 15 days after inoculation. Modified Dieterle, ×535.*

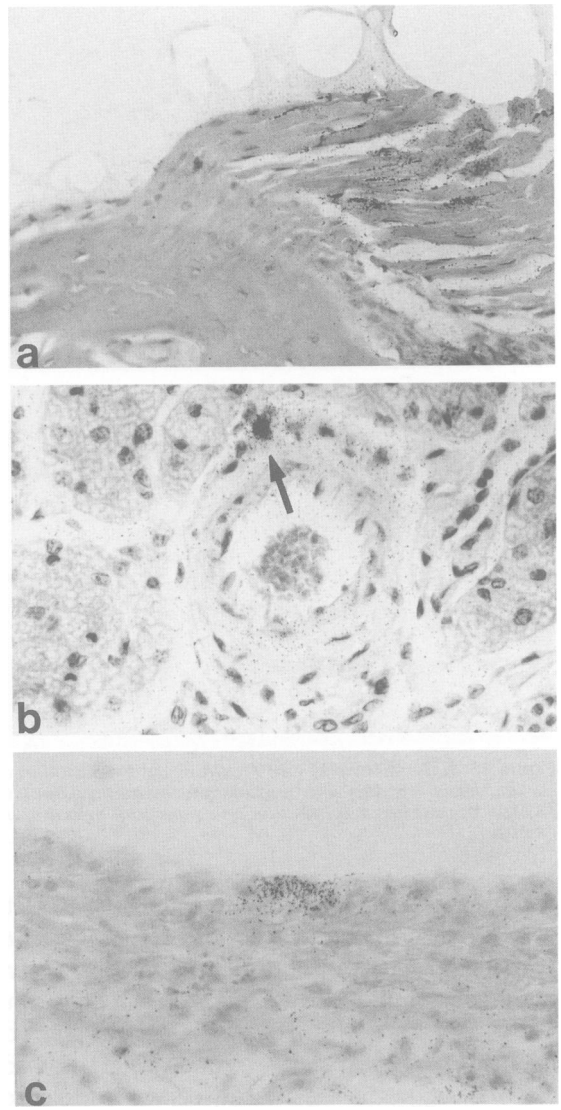


Figure 14. a–c: *^{32}P-labeled OSP A DNA* in situ *hybridization of musculoskeletal tissues, 4 days* (a) *and 15 days* (b, c) *after inoculation:* a: *osseous attachment site of tarsal tendon (×60);* b: *adventitia of femoral artery (×240);* c: *synovium (×240).*

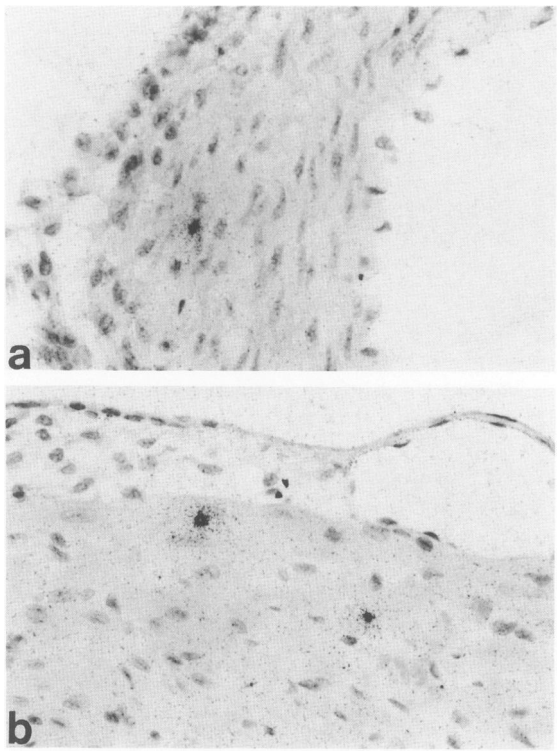


Figure 15. a, b: *^{32}P-labeled OSP A DNA* in situ *hybridization of cardiac tissue, 15 days after inoculation:* **a:** *aortic adventitia (×210);* **b:** *ventricular epicardium and superficial myocardium (×210).*

and macrophages have been found to be chemotactically attracted to and phagocytose *B. burgdorferi in vitro*.[22,23] It is curious that intense inflammation occurs only in target tissues such as heart and joints, despite the presence of spirochetes in other sites, such as skin, kidney, and spleen, with virtually no evidence of host reaction. This suggests a role of locally derived mediators of inflammation, such as interleukin-1 (IL-1), which can be generated by a number of cell types in articular tissue, including synoviocytes, macrophages, and endothelial cells.[22–25]

The distribution of spirochetes in infected mice parallels that seen in human Lyme disease tissue specimens.[4,5] Spirochete distribution was not uniform, but rather occurred in a multifocal pattern. Generally, foci of infection showed multiple spirochetes, whereas adjacent areas were totally devoid of visible organisms. This random pattern may relate to the multifocal nature of secondary cutaneous erythema migrans, the oligoarthritis or polyarthritis, and markedly varied clinical manifestations of Lyme disease in humans.[1] Spirochetes were usually extracellular, although small numbers were found in intracellular locations in these mice, as well as in rats[17] and humans[4,5] with Lyme disease. They had a distinct predilection for connective tissue, rather than parenchymal components of target organs, as described in tissue specimens from patients with Lyme disease.[4,5] We also noted a remarkably predictable association of spirochetes with ligament and tendon insertion sites, synovium, cardiac connective tissue, and blood vessels, especially arteries. Nerves were not systematically examined, but perineurium was also a frequent site of spirochete localization.

A major obstacle in Lyme disease pathogenesis studies or diagnostics is the demonstration of spirochetes in tissue sections with certainty. As noted in this study, the number of visible spirochetes in infected tissues drops considerably as infection progresses. They are present in very small numbers in human Lyme disease tissue specimens,[4,5] and visualization of spirochetes is arduous under the best of circumstances, because organisms are seldom optimally oriented in tissue sections. Nucleic acid hybridization methods therefore are an attractive means of overcoming these obstacles. Polymerase chain reaction using the *B. burgdorferi*-specific OSP A DNA, proved useful in detecting *B. burgdorferi* in ear punch samples of infected mice and correlated well with culture results. This method has recently been used to detect *B. burgdorferi* DNA in naturally infected ticks.[13] This is the first reported use of PCR on Lyme disease tissues. Sample size was too small to conclude if PCR was any more sensitive than culture, but results were equivalent, faster, and circumvented the problem of bacterial contamination of cultures that must be incubated for 2 weeks or longer. Ear punches have been shown to be infected with *B. burgdorferi* in high incidence in *Peromyscus* mice.[18] With PCR, ear punch biopsies can be a noninvasive method to monitor infection in laboratory animals. *In situ* hybridization of tissue sections for OSP A DNA was effective, but offered no advantages over properly executed histochemical methods for spirochete visualization.

The C3H mouse appears to be a useful model system of Lyme borreliosis. We originally demonstrated that C3H/HeJ mice developed more severe joint and heart disease than several other strains of mice and, unlike other laboratory animal models, immunologically intact mice at 3 and 12 weeks of age were susceptible to disease induction without immunosuppression. Mice inoculated at 3 days of age developed uniformly severe disease, regardless of genotype, but genetic differences in disease severity were clearly apparent by 3 weeks of age. Severity of, but not susceptibility to, disease was influenced by advancing age in C3H mice.[8] We have shown also that the intradermal median infectious dose for disease-susceptible C3H and disease-resistant BALB mice is identical, and that arthritis and carditis occur at the infectious dose level.[9] Because C3H/HeJ mice are lipopolysaccharide (LPS) unresponsive, we chose to use C3H/HeN mice in the present study, which are LPS responsive.[26] Thus the current study has demonstrated that the disease susceptibility of C3H mice is not related to LPS responsiveness of this mouse strain. Joint and

heart lesions in the mouse resemble the acute lesions of Lyme disease in a number of species, including humans.[4,5] Vasculitis seems to be a common feature of Lyme borreliosis.

Vascular lesions with onionskinlike intimal thickening and myocardial vasculitis have been observed in joint and heart tissues from human cases of Lyme borreliosis.[4,5] Intimal proliferation and transmural inflammation also have been found in the femoral arteries of C3H mice with experimental Lyme borreliosis,[8] but were not observed in the present study. Vascular tropism of *B. burgdorferi* was clearly evident in hearts and peripheral arteries of virtually all mice. No effort was made to study brain in the present study, because neurologic lesions were not found in mice up to 30 days after inoculation.[8] A remarkable difference in mice compared with humans is the early and strong antibody reactivity to Osp A, which does not occur until late in the course of infection in humans. In humans, the early IgM response is directed primarily against the 41-kd flagellin, whereas this is not a feature of the mouse response. The expanding reactivity to increasing numbers of spirochetal antigens with time occurs in both species.[3]

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