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Short Communication

The NIH-3 Immunodeficient Mouse Is a Model for Lyme Borreliosis Myositis and Carditis

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Experimental infection of immunodeficient NIH-3 (N:NIH-bg-nu-xid) mice with* Borrelia burgdorferi *was found to result in multisystem histopathologic lesions. In addition to T-cell deficiency due to the nude mutation, these mice have an x-linked defect affecting the B-cell maturation and the beige mutation resulting in the absence of NK cells. NIH-3 mice were susceptible to progressive infection with* B. burgdorferi *resulting in pancarditis, synovitis, and skeletal interstitial myositis whereas controls remained normal. Cardiomyopathy was characterized by inflammatory mononuclear infiltration and fibrillar necrosis. Synovial hyperplasia and inflammation were seen in the tibiotarsal and ulna-carpal joints. Advanced myositis was observed in peripheral skeletal muscle. Gastrointestinal submucosa, heart, and skeletal muscle were heavily colonized with* B. burgdorferi. *This mouse is proposed as a model for Lyme borreliosis carditis, synovitis, and myositis. (Am J Pathol 1992, 141:3–10)

Lyme borreliosis (LB) is a multisystem illness caused by the spirochete *Borrelia burgdorferi*[1,2] and transmitted to humans primarily by ticks of the *Ixodes ricinus* complex.[3] Acute LB is often manifested by the dermatologic lesion erythema migrans.[4] In the absence of resolution by antibiotic therapy, disseminated LB may result from hematogenous spread of the organism. This phase may include musculoskeletal, cardiovascular, and nervous system disorders.[5] In early LB, musculoskeletal manifestations entail myalgia and muscle fatigue, whereas debilitating swelling and resting pain occur in advanced disease. Lyme myositis has been recognized as a distinct clinicopathologic entity that may or may not be accompanied by peripheral neuropathy. Microscopically, it usually consists of necrotic, focal nodular, or interstitial lesions with spirochetes present.[6–8] Cardiac involvement is seen clinically as tachycardia and atrioventricular block.[9] Transvenous cardiac biopsies reveal lymphocytic endocardial infiltration and myositis.[10] Spirochetes have been demonstrated in endomyocardial biopsies histologically and by culture.[11,12] Fatal pancarditis has been reported.[13]

In an effort to advance the understanding of LB pathogenesis, several animal models have been investigated. Although rats, hamsters, and gerbils are susceptible to spirochetemia and systemic infection, only minor lesions are seen in these models.[14–16] Components of multisystem disease most consistent with human LB pathology have been easiest to demonstrate in hosts lacking a completely developed immune system. Polyarthritis, carditis and myositis have been described in *B. burgdorferi* infected scid mice.[17,18] After infection with *B. burgdorferi,* arthritis in the form of mild-to-moderate synovial thickening and ingress of mononuclear inflammatory cells has also been shown to occur in weanling infected Lew/N rats,[19,20] inbred mice,[21] and irradiated LSH hamsters.[22,23] Although spirochetes are demonstrable in some tissues of these hosts they are often rare or present in small numbers. These observations and the paucity of spirochetes in human tissue biopsies suggest that a functional immune system limits the growth of tissue-bound spirochetes in Lyme borreliosis.

To test the hypothesis that decreased or deficient host cell immunity might favor an increase in numbers of spi-

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rochetes in the tissue, we investigated the susceptibility of immunodeficient NIH-3 mice to *B. burgdorferi* infection. In addition to the nude condition (*nu*) rendering these mice athymic, this strain has an x-linked defect (*xid*) affecting B-cell maturation and the beige (*bg*) mutation resulting in the absence of natural killer (NK) cells.[24–26] We report that NIH-3 mice develop pronounced multisystem histopathologic lesions during experimental infection with *B. burgdorferi*. Our findings confirm this mouse to be a model for synovitis, pancarditis, and myositis. Furthermore, chronic infection results in colonization of the heart, peripheral muscle, and gastrointestinal system with large numbers of spirochetes. This model expands the opportunities for the study of Lyme carditis and myositis as well as mammalian tissue adaptation and colonization by *B. burgdorferi*.

Materials and Methods

Animals

NIH-3 (N:NIH-*bg-nu-xid*) homozygous adult female and male mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. Mice were reared in specific pathogen-free conditions.

Bacteria

Strain 297 of *B. burgdorferi* was isolated from human spinal fluid.[2] Strain CT-1 was isolated from an adult *Ixodes dammini* tick collected in Wisconsin. NCH-1 is a human skin isolate obtained from a patient in Wisconsin. Spirochetes were grown in Barbour-Stoenner-Kelly (BSK) medium[27] at 30°C. Virulence of isolates was maintained by passage in Syrian hamsters. Virulent isolates were not subcultured more than three times before use. Avirulent strain 297 was prepared by repeated passage *in vitro* and is no longer infectious for the Syrian hamster. *Borrelia* were heat-killed by incubation at 56°C for 30 minutes and inactivation of spirochetes confirmed by absence of growth in BSK medium. Mice were inoculated with 1×10^7 cells of log phase *B. burgdorferi* in approximately 0.1 mL of medium. Mice were injected intramuscularly at the base of the tail. Control mice were injected with 0.1 mL of BSK medium alone. At various time points in experiments, mice were randomly selected for culture or histopathology and killed by inhalation of carbon dioxide. For culture, blood and a 1:10 homogenate of tissues were used to inoculate duplicate tubes of BSK medium. Cultures were incubated at 30°C and checked regularly for the presence of spirochetes using darkfield microscopy.

Serology

Blood was drawn from mice by cardiac puncture. The presence of specific antibodies was detected using an indirect immunofluorescent assay (IFA) for IgG as previously described.[28]

Tissue Preparation

For all studies, mice were coded and this designation maintained on sections and slides throughout the study. Histopathology, including the silver stains, was interpreted by one of us (PHD) and formally reported to the other investigators upon completion without knowledge of experimental conditions for each animal.

All mice were necropsied with dissecting blade changes before sectioning each separate organ or tissue system. The skin, paraspinous muscle, joints, intestine, urinary bladder, popliteal lymph nodes, and all internal organs as well as the eye and brain were sampled. Tissue samples were processed and stained for histologic examination in the conventional manner. Modified Dieterle silver impregnation stains were used for visualization of spirochetes.[15] With the exception of the liver, all organs and viscera were totally embedded for histologic examination.

Results

Culture and Serology

Mice infected for 30 days with strain 297 ($n = 1$) and for 60 days with strain CT-1 ($n = 1$) were bled, and tissues were cultured. Strain 297 was recovered from the blood, spleen, and urinary bladder but not the left kidney, whereas for CT-1, the spleen, urinary bladder, and left kidney were culture positive and the blood was negative. No antibody to *B. burgdorferi* was detected by IFA in these mice.

Gross Findings

All mice survived for the duration of the experiment. Mice injected with media only or with heat-killed or high-passage avirulent spirochetes appeared normal at all times. All infected mice began to show clinical signs after 14–21 days. These mice appeared morbid and less active than controls. Marked bilateral swelling and induration of the tibiotarsal and ulna-carpal joints was accompanied by moderate difficulties in ambulation. The appearance of the joints did not change significantly after

the first 30 days, although lameness progressed considerably. Dramatic kyphosis was seen in the thoracic vertebrae of two mice infected for 250 days. Moderate scoliotic deformation was also apparent in both thoracic and coccygeal vertebrae of these mice and was confirmed at necropsy.

Histopathologic Findings

All results were compiled without knowledge of the infectious status of any mouse studied. Mice injected with media alone for up to 250 days showed no histologic abnormalities. Mice infected with heat-killed or avirulent spirochetes appeared normal or had mild cystitis. The first significant histologic changes observed were in the spleens of mice infected for 30 days. Spleens from these mice had depleted white pulp relative to uninfected NIH-3 mice. When infection persisted for 90 days, white pulp depletion was accompanied by nuclear karyorrhexis. In contrast, spleens of chronically infected mice at day 250 had no abnormal histopathologic findings and lymphoid follicles were intact suggesting restoration of these tissues in advanced infection (Table 1). No pathologic changes were seen in the pancreas or lymph nodes of any mouse. Few abnormalities were noted in the lungs or brains except in two mice infected with strain 297 for 90 days. These organs showed increased tissue mast cells and plasma cells with rare spirochetes. Increased mast cell infiltration was also seen in the random sections of skin and muscle of mice inoculated with live virulent spirochetes.

Mild cystitis seen within the first month of borreliosis progressed in severity in chronically infected mice and at 90 days vasculopathy (luminal sclerosis) and plasmacytosis were observed in small vessels of the urinary bladder. The upper gastrointestinal tract had submucosal mononuclear inflammatory cell infiltrates in the gastroduodenal submucosa accompanied by interstitial edema. Inflammatory infiltrates were not seen in any section of gastrointestinal mucosa above the muscularis zone. Moderate gastric submucosal edema with increased mononuclear cells was also seen. The submucosal infiltrates did not include plasma cells. Presence of spirochetes correlated with zones of interstitial expansion by edema and mononuclear cell infiltrates. Both the submucosal interstitial mesenchyme of the upper gastrointestinal tract and the urinary bladder were demonstrated to be colonized with large numbers of *B. burgdorferi* but the bladder appeared to be involved to a lesser extent.

Histologic alterations of the tibiotarsal joints of mice infected for 250 days were limited to moderate synovial hyperplasia and inflammation with a significant number of organisms seen. Examination of the paraspinous muscle of kyphotic, chronically infected mice revealed advanced interstitial myositis. This condition was characterized by an intense mononuclear infiltrate and clusters of perivascular plasmacytoid cells. Kyphosis appeared to be a result of extensive myositis of the paraspinous muscle. Spirochetes were abundant throughout muscle interstitium in an extracellular location (Figure 1).

A severe cardiomyopathy was seen to progress in *B. burgdorferi*-infected NIH-3 mice. Myocardial interstitial inflammation and aortitis were detected in mice infected for 30–90 days (Figure 2). Mononuclear inflammation was pronounced in the atrial chamber where spirochetes were prominent in the interstitium. Significant infiltrates and necrotic changes were observed in the epicardium (Figure 2). In progressive infection, focal myonecrosis and endocarditis were sometimes accompanied by polymorphonuclear infiltrates. As the course of infection progressed, isolated myonecrosis correlated with neutrophils in some areas.

Short- and long-term myocarditis had distinctive histologic findings. Carditis in mice infected for 12 weeks or less had mononuclear interstitial infiltrates primarily at the base of the heart and inferior portion of the aortic root, largely sparing the ventricles, whereas mice with long-term infection had more diffuse interstitial pancarditis with extensive involvement of the ventricular myocardium. Moreover, the chronically infected mice had much greater numbers of extracellular spirochetes than found in the acutely infected mice. Plasmacytoid lymphocytes were not visible in long-term carditis despite the marked interstitial mononuclear cell response.

Spirochetes were seen in muscle, joints, gastrointestinal tissue, urinary bladder, heart, lung, and brain. *Borrelia* were sparse to rare in the lung and brain of all mice and joints of mice infected for 90 days or less. We were unable to find organisms in the spleen, liver, lymph nodes, or pancreas. Conversely, muscle, heart, and the gastrointestinal tract and urinary bladder contained increased numbers of spirochetes later in infection. In mice chronically infected with *B. burgdorferi,* these tissues contained a profusion of spirochetes with tangled clusters noted in the gastrointestinal tract, muscle, and heart (Figure 3). Sites of colonization were always interstitial, often associated with collagen. No evidence for an intracellular location of spirochetes was seen in any tissues examined.

Discussion

This investigation shows the NIH-3 mouse to be susceptible to progressive infection with *B. burgdorferi* resulting in pancarditis, synovitis, and skeletal interstitial myositis. In the heart, spirochetal infection induced inflammatory

Table 1. *Histopathologic Findings of* B. burgdorferi*-infected NIH-3 Mice*

B. burg. strain or inoculum	Days PI*	n	Nerve and skin	Muscle	Tibio-tarsal joints	GI tract	Urinary bladder	Pancreas	Lymph nodes	Spleen	Liver	Heart	Lung	Brain
297 avir	30	2	0†	0	0	0	13	0	0	0	0	0	0	0
297 vir	30	2	0	0	0	10	0	0	0	7	1	4	0	0
NCH-1	30	2	0	0	0	0	13	0	0	7	0	0	0	0
Media	90	1	0	0	0	0	0	0	0	0	0	0	0	0
297 HK	90	2	0	0	0	0	13	0	0	0	0	0	0	0
297 avir	90	2	0	0	0	0	0	0	0	0	0	0	0	0
297 vir	90	2	0	8	0	0	13,14	0	0	6,7	1	4,12	11,12	12,+
NCH-1	90	2	0	8	5	0	13,14	0	0	6,7	1	2	0	0
Media	250	1	0	0	0	0	0	0	0	0	0	0	0	0
297vir	250	1	9	8	5,+	10,++	13,+++	0	0	0	0	2,3,+++	0	0
CT-1	250	1	9,11	8,11,+++	5,++	10,+++	12,13,+++	0	0	0	0	3,4,+++	0	0

* Postinoculation.
† No histologic abnormalities.

Histopathologic findings are as follows: 1, hepatitis; 2, carditis; 3, carditis with myonecrosis; 4, focal carditis with aortitis; 5, synovitis; 6, white pulp karyorrhexis; 7, white pulp depletion; 8, peripheral interstitial myositis; 9, skin changes; 10, inflammation; 11, increase in mast cells; 12, plasmacytoid infiltrates; 13, cystitis; 14, vasculopathy. The presence of spirochetes is noted as follows: +, rare spirochetes seen; ++, 1–5 spirochetes seen per high power field (HPF); +++, more than 5 spirochetes per HPF.

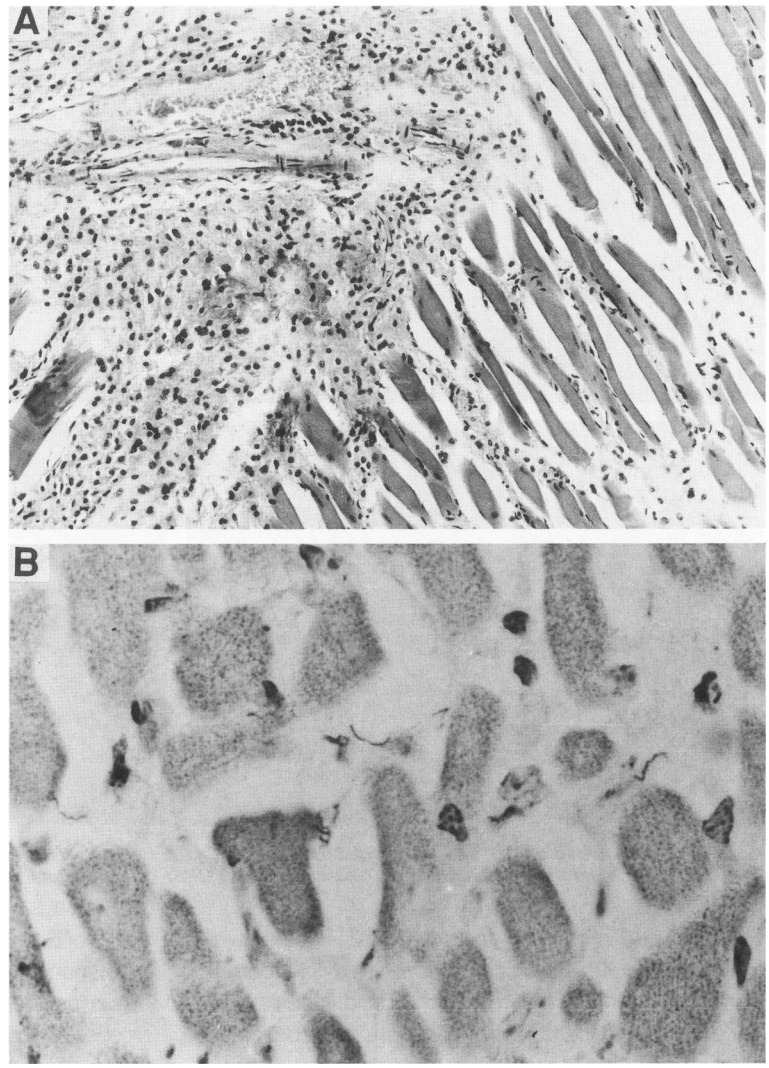

Figure 1. a: *Paraspinous muscle of NIH-3 mouse infected for 250 days with strain CT-1 revealing myonecrosis and dense mononuclear infiltrate (×200 H&E).* b: *Spirochete colonization of paraspinous interstitium (×800 modified Dieterle).*

lesions of the endocardium, pericardium, myocardium and aorta. Human Lyme carditis is characterized histopathologically by interstitial lymphocytic infiltrates including T and B lymphocytes and plasma cells.[10,29] In humans, few spirochetes can sometimes be detected by silver stain of endocardial biopsy.[11] Myocardial cell degeneration, expanded myocardial interstitial spaces, and striking numbers of spirochetes in long-term infection with *B. burgdorferi* in this model contrast with findings in immune-competent rodent models. Infection of the scid mouse with *B. burgdorferi* has also been reported to result in pancarditis.[17,30] The pathology of the heart in these two models has similarities.[17,30]

Myositis observed in the peripheral skeletal muscle was pronounced and in some areas paralleled the extent seen in the myocardium. Additionally, focal myonecrosis associated with inflammatory interstitial lesions occurred. Spirochetes were seen throughout the muscular tissue and were commonly found interspersed within extracellular matrices (Figure 1). With the exception of the density of spirochetes, microscopic observations of the paraspinous muscle are consistent with histopathology of human Lyme myositis. In human cases of myositis, biopsies have confirmed infiltrates of mononuclear and mast cells, fibrillar necrosis, and the observation of spirochetes.[6–8] Mild-to-moderate histopathologic lesions of the skeletal muscle have been reported in *B. burgdorferi*-infected gerbils,[16] and myositis has been reported in scid mice.[17,18] The presence of necrotic focal lesions and abundant spirochetes in skeletal muscle of the NIH-3 mouse suggests that this model is useful for human Lyme borreliosis myositis.

Clinical signs of polyarticular arthritis were noted in NIH-3 mice and included the swelling of multiple joints, predominantly the tibiotarsal, ulna-carpal, and metatarsal. Mice infected for 90 or more days exhibited synovial hyperplasia with mononuclear infiltrates. Arthritis has been induced in weanling rats,[19,20] irradiated LSH ham-

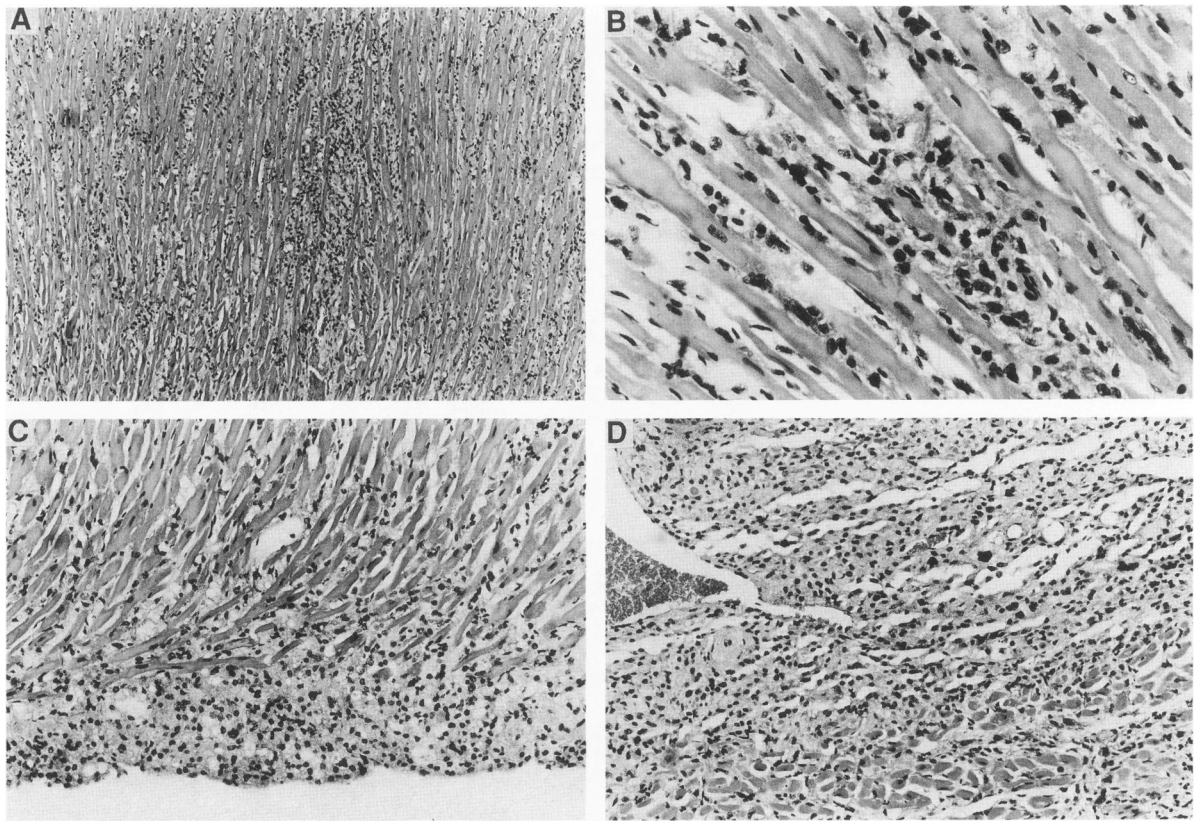

Figure 2. **a**: *Myocarditis of NIH-3 mouse infected for 250 days (×200 H&E).* **b**: *Focal myofibrillar necrosis with mononuclear infiltration (×800 H&E).* **c**: *Infiltration and necrosis of epicardium (×400 H&E).* **d**: *Endocardial mononuclear inflammation (×400 H&E).*

sters,[22,23] and in inbred[21] and scid mice[17] infected with *B. burgdorferi*. The arthritic component of NIH-3 borreliosis was milder than that reported for scid mice that develop bone and cartilage degradation.[17] In contrast to other models, spirochetes were abundant in joints of mice when infected for 250 days.

Our observations revealed several unique characteristics of *B. burgdorferi* infection in NIH-3 mice. In contrast to immune-sufficient rodents infected with *B. burgdorferi*, such as the natural reservoir host, the white-footed mouse,[31] gerbil,[16] and Syrian hamster,[15] few spirochetes were observed in the spleen, kidney, and liver of the NIH-3 mice. Extensive colonization by *B. burgdorferi* was observed in the heart, paraspinous muscle, and upper

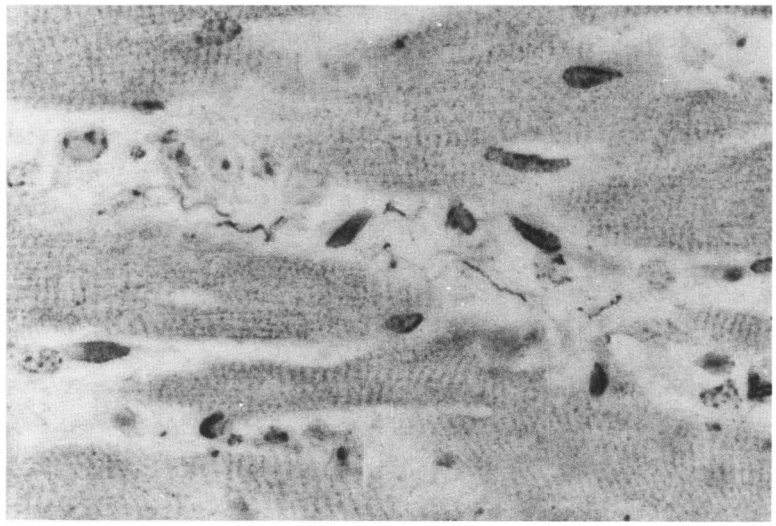

Figure 3. *Numerous spirochetes in the interstitium of myocardium from a mouse infected with strain 297 of* B. burgdorferi *for 250 days (×800 modified Dieterle).*

gastrointestinal submucosal mesenchyme and, to a lesser extent, the urinary bladder. To our knowledge, observations of colonization of gastrointestinal mesenchyme by *B. burgdorferi* is unique. Of infected scid tissues examined, only the heart had large enough numbers of organisms to be seen histologically.[17] The differences in infection in these two immunodeficient mice may be attributable to the fact that NIH-3 mice lack NK cells. These cells have been shown to have potent extracellular bactericidal activity.[32,33] Zimmer et al reported the presence of NK-like cells in the myocardium of infected scid mice.[30] These cells may control spirochete colonization in these hosts.

The inflammatory lesions and necrosis observed in this host evidently emerged as a result of colonization by spirochetes and nonlymphocytic pathophysiologic responses. In human Lyme disease, T lymphocytes appearto be the major responder in the central nervous system and elsewhere, whereas B lymphocytes and plasma cells are responsive in the fascia, myocardium, dermis, and synovium.[29] Immune complexes and humoral responses can at times be demonstrated as a prominent feature of the histogenesis of borreliosis.[29,34] The histopathologic features of this study are consistent with responses mediated by the macrophage, dendritic and mast cells, and complement. Inflammatory processes may be sustained through induction of a range of cytokines such as IL-1 and TNF by colonization with viable spirochetes. These factors could conceivably then influence secondary mediators such as collagenase, proteases, and prostaglandins.

In conclusion, we propose the immunodeficient NIH-3 mouse as a model for Lyme myositis and various aspects of the pathogenesis of Lyme disease. We also show that this animal is highly susceptible to colonization by *B. burgdorferi*. As the immune deficiencies of the NIH-3 mouse are a result of specific mutations, these mice should provide further elucidation of the role of the lymphocytic component of this infection by comparison of infection in various genotypes.

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