American Journal of Pathology, Vol. 139, No. 6, December 1991


# Rapid Communication

## Myositis in Mice Inoculated with *Borrelia burgdorferi*

**Crisan Museteanu,* Ulrich E. Schaible,* Thomas Stehle,* Michael D. Kramer,† and Markus M. Simon***

*From the Max-Planck-Institut für Immunbiologie,* Freiburg, and Institut für Immunologie und Serologie der Universität Heidelberg,† Heidelberg, Federal Republic of Germany*

***The authors describe the appearance of myositis in immunocompetent and immunodeficient mice after subcutaneous inoculation with* Borrelia burgdorferi *by histology and immunohistology. Experimental infection of mice 1) causes inflammation of striated but not smooth muscles, 2) affects the entire musculoskeletal system, and 3) is characterized by perivascular and interfascicular infiltration of mononuclear leukocytes in the striated muscle leading to necrosis as well as disruption of muscle fibers. The lesions found in striated muscle specimens were most pronounced in immunodeficient (SCID), less severe in T-cell-deficient* nu/nu *(BALB/c, C57BL/6) and marginal to moderate or almost not present in immunocompetent AKR/N and C.B-17 mice, respectively. (Am J Pathol 1991, 139:1267–1271)***

Lyme disease, which is caused by the tick-borne spirochete *Borrelia burgdorferi* (*B. burgdorferi*), is associated with dermatologic, rheumatic, and neurologic abnormalities.[1,2] In humans, early in the infection, muscle pains, debilitating malaise, and fatigue are the most frequent clinical features.[3–5] The musculoskeletal pain of Lyme disease is intermittent and generally migratory in joints, tendons, and muscles. Histologic examination of muscle biopsies taken at early or late stages of *B. burgdorferi* infection revealed both noninflammatory myositis with muscle-fiber necrosis[6] as well as inflammatory myositis with infiltrations of B and T lymphocytes.[7,8] Spirochetes were rarely seen *in situ*[7,8] and their cultivation from muscle was unsuccessful.[8]

We and others have previously shown that mice,[9–11] rats,[12] and hamsters[13] experimentally inoculated with *B. burgdorferi* develop arthritis, carditis,[9–13] and hepatitis.[14] It was found that the clinical symptoms were much more pronounced in immunodeficient[14] or immunocompromised[12,13] as compared with normal animals indicating a crucial role of the immune system in the control of the disease. However, more detailed studies on a panel of inbred strains of mice revealed that mice with certain haplotypes such as H-$2^k$ (AKR/N, C3H/He, B10.BR), H-$2^b$ (C57BL/6), H-$2^j$ (B10.WB), H-$2^r$ (B10.R111), and H-$2^s$ (B10.S) developed arthritis of variable duration and intensity on inoculation with *B. burgdorferi*, whereas those of the haplotype H-$2^d$ (BALB/c, C.B-17, B10.D2, DBA/2, Cal-20) did not show any signs of disease.[10,15] This suggest that immune response genes are not only operative in protection but also in pathogenesis of *B. burgdorferi* infection.

Preliminary data in severe combined immunodeficiency (SCID) mice indicated that the inflammatory pathologic changes induced by spirochetal infection in the periarticular tissues also included skeletal muscle.[14] We present a more detailed report on the appearance of myositis in *B. burgdorferi* infected immunocompetent and immunodeficient mice.

### Materials and Methods

#### Animals and Experimental Inoculation

Adult mice (8–12 weeks of age) of strains AKR/N (H-$2^k$), BALB/cByJ-nu (BALB/c *nu/nu:* H-$2^d$), C57BL/6ByJ-nu (C57BL/6 *nu/nu:* H-$2^b$), C.B-17 (H-$2^d$), and C.B-17IcRJ-scid (SCID; H-$2^d$ homozygous for the SCID mutation[16]) were bred at the Max-Planck-Institut für Immunbiologie (Freiburg, FRG) under specific pathogen-free conditions.

Supported in part by the Bundesministerium für Forschung und Technologie (01 KI 8909).

Accepted for publication October 9, 1991.

Address reprint requests to Mr. Markus Simon, Max-Planck-Institut für Immunbiologie, Stübeweg 51, Freiburg, FRG.

Female animals were inoculated with $1 \times 10^8$ viable *B. burgdorferi* organisms subcutaneously in the tail. Numbers of animals used to perform the histopathologic studies are listed in Table 1. Noninfected age-matched animals of the same strains kept under similar conditions served as controls.

### Bacteria

The low passage *B. burgdorferi* (two *in vitro* passages) strain ZS7 used in this study was originally isolated from a female *Ixodes ricinus* tick in Freiburg, FRG, as described recently.[9] Spirochetes were cultivated in modified Kelly's medium and harvested as described previously.[17] Reisolation of spirochetes from blood of infected mice has been described in detail.[17] To avoid loss of virulence of spirochetes, aliquots from a frozen stock (second passage, −70°C) were used throughout the study.

### Preparation of Tissue for Histology and Immunohistology

At various timepoints after inoculation with *B. burgdorferi* (Table 1), mice were killed and both, front and hind legs as well as tail (striated skeletal muscle specimens) and 5 mm pieces of the small and large intestines (smooth muscles specimens) were removed from each mouse and fixed in 5% formaldehyde in PBS for embedding in paraffin or they were frozen in liquid nitrogen for preparations of cryostat sections. As controls, muscle specimens from noninfected mice were investigated in parallel. Formaldehyde-fixed extremities were decalcified in 5% trichloroacetic acid in PBS for 1 hour. Sections (4–6 μm thick) were stained with hematoxylin and eosin (H&E) embedded in Entellan (E. Merck, Darmstadt, FRG). Samples were properly coded and examined under double-blind conditions. Immunohistology was performed using a rat anti-mouse Mac-1 monoclonal antibody (MAb, 18) and the streptavidin-peroxidase system as described in detail.[14]

## Results and Discussion

Mice of strains SCID, C.B-17, BALB/c *nu/nu,* C57BL/6 *nu/nu,* and AKR/N were inoculated subcutaneously with $10^8$ viable *B. burgdorferi* organisms of the low passage European isolate ZS7 and analyzed for the development of clinical arthritis and myositis. As shown before, all SCID mice developed a progressive multisystem disease after inoculation with *B. burgdorferi* affecting the joints, heart, and liver.[14] The first clinical symptoms of arthritis were characterized by reddening and swelling of the tibiotarsal joints starting at day 7 after inoculation. In contrast, immunocompetent coisogenic C.B-17 mice did not develop clinical signs of arthritis and showed, if at all, only marginal histopathologic alterations in the joints. Mice of strains BALB/c *nu/nu* and C57BL/6 *nu/nu* developed an intermittant and more often unilateral clinical arthritis, which however appeared only later during infection (around day 23). Although all tested *nu/nu* mice showed joint lesions, macroscopic arthritis was not always apparent. In AKR/N mice the first symptoms of clinical arthritis were observed on day 14 postinoculation in 10% of the infected animals and on day 45 postinoculation all mice were positive (see 15). Spirochetes could be readily isolated from blood of SCID mice at any stage of infection (32/33; and 9, 14), but were only occasionally recovered from similar specimens of C57BL/6 *nu/nu* (1/5) mice and not at all from those of either BALB/c *nu/nu* (0/14), AKR/N (0/5) or C.B-17 (0/4) mice during the entire observation period.

Inflammatory pathologic changes of skeletal muscle were noted in 21/32 SCID mice when tested between days 7 and 60 postinoculation (Table 1). Later on (days 71, 87, 115), all mice tested (3/3) were similarly affected. Lesions evolved primarily within the striated muscle ad-

**Table 1.** *Development of Myositis in Immunodeficient SCID,* nu/nu *and Immunocompetent C.B-17 and AKR/N Mice Experimentally Inoculated with* B. burgdorferi *Strain ZS7*

| Mouse strain | No. of animals tested | Development of myositis (days after inoculation) | |
|---|---|---|---|
| | | <60 days | >60 days |
| SCID | 35 | 21/32 | 3/3 |
| C.B-17 | 8 | 0/3 | 0/5* |
| BALB/c *nu/nu* | 15 | 8/11 | 3/4 |
| C57BL/6 *nu/nu* | 5 | 3/5 | nd |
| AKR/N | 14 | 0/7* | 2/7 |

* Only marginal histopathologic alterations were found in periarticular muscle tissues of some of these mice.
nd, not determined.

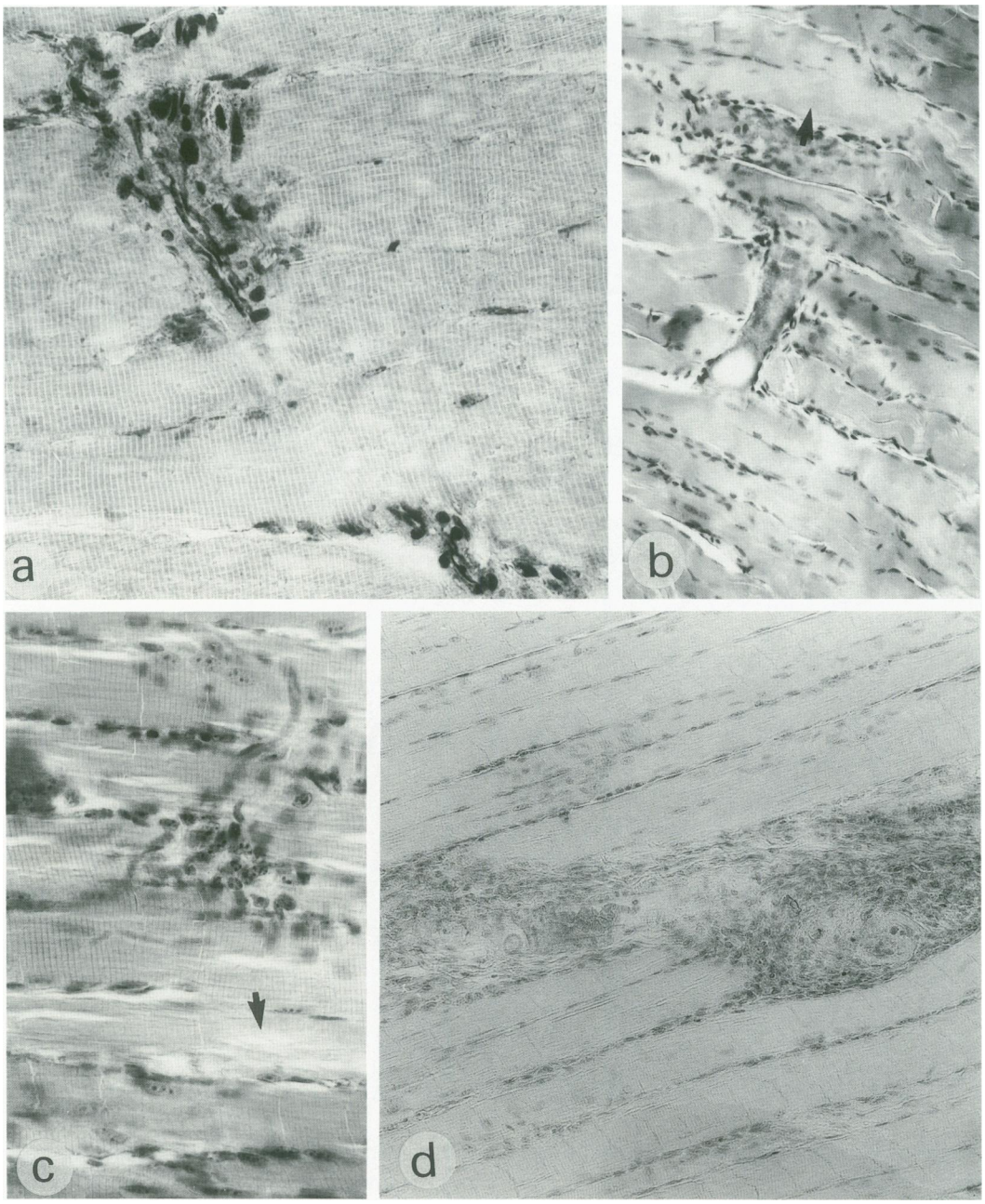


**Figure 1.** **a:** *Skeletal muscle of a SCID mouse (biceps) 21 days p.i. of $10^6$* B. burgdorferi *ZS7. Perivascular and interfascicular infiltration of mononuclear leukocytes in the muscle (magnification ×400).* **b, c:** *Skeletal muscles of a SCID mouse 21 days p.i. of $10^8$* B. burgdorferi *ZS7. Infiltration of mononuclear leucocytes between and inside the muscle fibers leading to partial necrosis (arrow) of the muscle fibers (magnification ×200* **(b)**, *×400* **(c)**. **d:** *Skeletal muscle of a SCID mouse 35 days p.i. of $10^8$* B. burgdorferi *ZS7. Perivasculitis characterized by perivascular cuffing of mononuclear leucocytes located around the muscle arteries and swelling of the endothelia (magnification ×200).* **e:** *Skeletal muscle of a SCID mouse 77 days p.i. of $10^8$* B. burgdorferi *ZS7. Interstitial infiltration of mononuclear leukocytes between the muscle fibers (magnification ×200).* **f:** *Skeletal muscle of a BALB/c* nu/nu *mouse 179 days p.i. of $10^8$* B. burgdorferi *ZS7. Unusual infiltration of mononuclear leukocytes inside and outside the muscle fibers leading to partial necrosis (arrow) as found in some of these mice late after infection (magnification ×400).* **g:** *Skeletal muscle of a SCID mouse 71 days p.i. of $10^8$* B. burgdorferi *ZS7. Cellular infiltrations between the muscle fibers stain positive for Mac-1, a marker for macrophages and monocytes (18; biotin-avidin-complex, peroxidase staining; magnification ×100).*

jacent to the joints but were also seen though in more moderate forms in other striated muscles. The inflammation was characterized by infiltration of the interfibrilar muscle interstitium (Figure 1a, 1e) and perivascular cuffing (Figure 1a, 1d; vasculitis) with mononuclear cells. The cellular infiltration mainly consisted of Mac-1$^+$ cells, most probably macrophages ([18]; Figure 1g) which is similar to previous findings in the heart.[14] Obviously, the infection dispersed along the vessels and progresses longitudinal and transversal to muscle fibers. Subsequently inter-

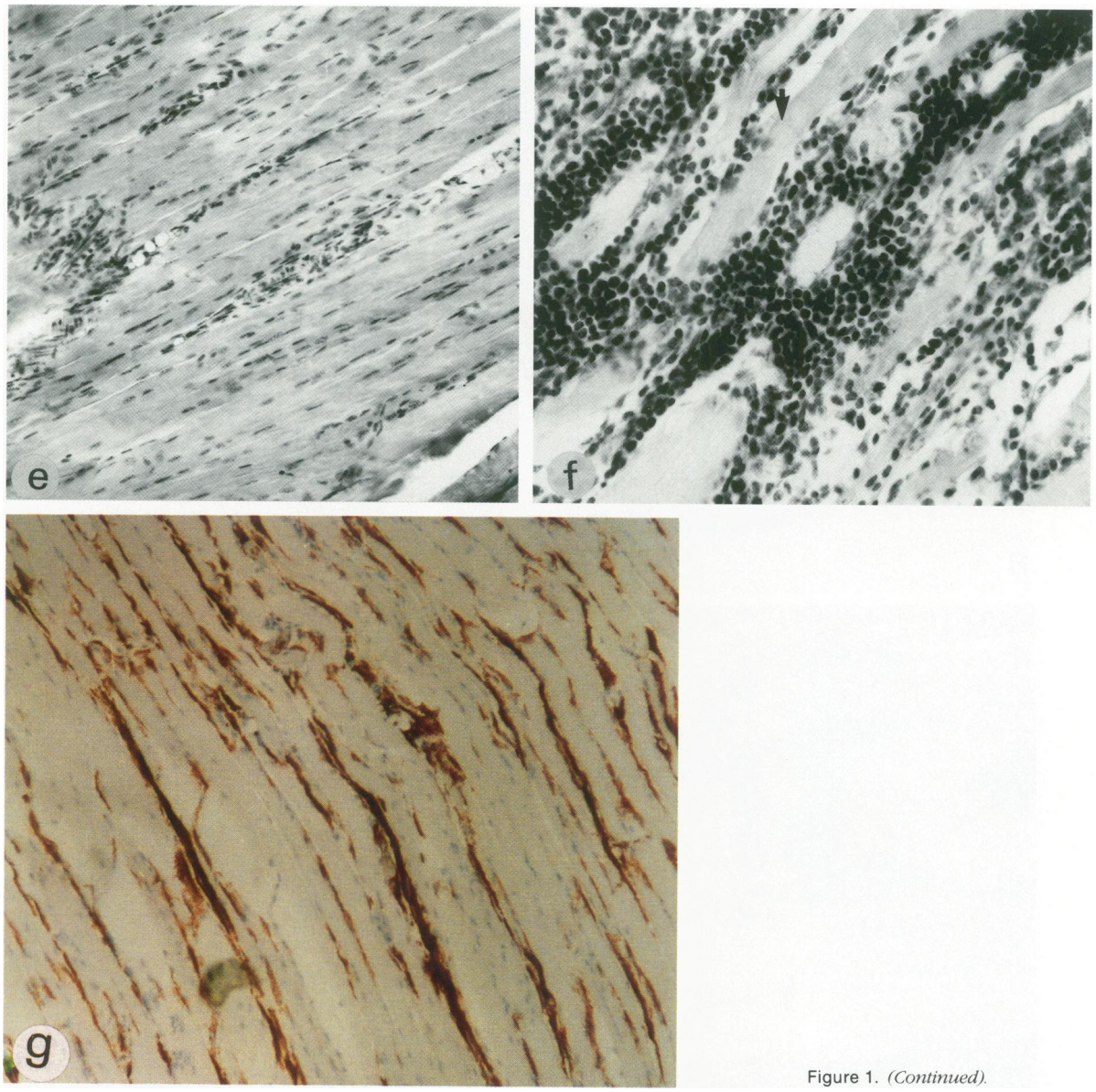


Figure 1. *(Continued).*

fibrilar infiltration and necrosis (Figure 1b, 1c; arrows) of muscle fibers occurred.

Inflammatory foci in striated muscle were also observed in 8/11 BALB/c *nu/nu* mice when tested up to day 60 postinoculation and in 3/4 at later stages of infection (days 78, 87, 170). Early during infection, lesions were moderate compared with those in SCID mice with infiltration of mononuclear cells confined predominantly to interfascicular areas. Later on, inflammation became more intense and was accompanied by necrosis (arrow) of muscle fibers. The infiltration observed in some BALB/c *nu/nu* mice at later stages of infection are more pronounced than in SCID mice (Figure 1f). Similar alterations were observed in muscle tissue from infected C57BL/6 *nu/nu* mice (3/5, Table 1). Only 2/7 AKR/N mice showed significant histopathologic alterations of striated muscles when examined at day 60 or later (up to day 170 postinoculation) after inoculation with *B. burgdorferi*. Only marginal alterations were observed in these mice when tested up to day 60 postinoculation.

In general, the lesions contained rather small mononuclear cell infiltrates including $Mac1^+$ cells. The presence of B- and/or T lymphocytes in the inflammatory foci observed in *nu/nu* or AKR/N mice has not been tested. Significant histopathologic lesions of the striated muscles were not found in any of the C.B-17 mice tested. No inflammation was seen in smooth muscle specimens from any of the five mouse strains analyzed (data not shown).

As shown previously, infection of SCID and immunocompetent mice with the nonvirulent *B. burgdorferi* strain B31 did not lead to the development of clinical symptoms and pathologic alterations including myositis (data not

shown and 9, 17). In addition, histologic analysis of uninfected SCID (n = 12), BALB/c *nu/nu* (n = 4), C57BL6 *nu/nu* (n = 3), AKR/n (n = 3), and C.B-17 (n = 5) mice revealed no or only marginal lesions in skeletal muscles.

Together with previous findings[14] the data show that experimental inoculation of mice with *B. burgdorferi* leads to a multisystem infection involving the entire musculoskeletal system in addition to other organs. The inflammation of skeletal muscle was most severe in immunodeficient (SCID; H-$2^d$), less pronounced or at least delayed in BALB/c *nu/nu* (H-$2^d$) and absent in C.B-17 (H-$2^d$) mice. These results are in line with previous findings showing that *B. burgdorferi* infection in mice is controlled, at least in part, by specific immune responses, most probably antibodies.[19] In addition, the observation that AKR/N (H-$2^k$) but not C.B-17 (H-$2^d$) mice develop musculoskeletal lesions corrborates recent studies showing that H-$2^k$, but not H-$2^d$ mice are susceptible to the development of Lyme arthritis[15] and argues for the contribution of immunocompetent cells in both protection and pathogenesis of *B. burgdorferi* infection in mice.

Vasculitis, a common feature of Lyme Borreliosis in humans[2] is shown in the present study also to be a frequent occurrence in the mouse experimental model. Most probably, infiltration of perivascular areas by mononuclear cells is one of the first events leading to myositis and possibly also the other organ manifestations.[14] Our data in the SCID mouse indicate that macrophages play an important role in the development of myositis but do not necessarily exclude the participation of other cell types including T and B cells in this process.

A high proportion (74%) of patients with Lyme disease complain of chronic fatigue[4] and muscular pain (15–43%; [4,20]), which are common clinical signs of myositis. Our experimental data together with these and other observations[7,8] would suggest that myositis is a frequent component of *Borrelia burgdorferi* infection.

## Acknowledgments

The authors thank Dr. J. Langhorne and N. Honarvar for critically reading the manuscript. This study was in part supported by grants of the Bundeministerium für Forschung und Technologie (BMFT, 01 -KI 9001; M.M.S) and the Deutsche Forschungsgesellschaft (DFG, Kr 931/2-1).

## References

1. Steere AC: Lyme disease. N Engl J Med 1989, 321:586–596
2. Duray PH, Steere AC: Clinical pathologic correlations of Lyme disease by stage. Ann NY Acad Sci 1988, 539:65–79
3. Steere AC, Bartenhagen NH, Craft JE, Hutchinson GJ, Newman JH, Rahn DW, Sigal LH, Spieler PN, Stenn KS, Malawista SE: The early manifestations of Lyme disease. Ann Intern Med 1983, 99:76–82
4. Logigian EL, Kaplan RF, Steere AC: Chronic neurologic manifestations of Lyme disease. N Engl J Med 1990; 21:1438–1444
5. Naglo AS, Wide K: Borrelia infection in children. Acta Paediatr Scand 1989, 78:918–922
6. Schoenen J, Sianard-Gainko J, Carpenter M, Reznik M: Myositis during Borrelia burgdorferi infection (Lyme disease). J Neurol Neurosurg Psych 1989, 52:1002–1005
7. Atlas E, Novak SN, Duray PH, Steere AC: Lyme Myositis: Muscle invasion by Borrelia burgdorferi. Ann Intern Med 1988, 109:245–246
8. Reimers CD, Pongartz DE, Neubert U, Pilz A, Hübner G, Naegele M, Wilske B, Duray PH, de Koning J: Myositis caused by Borrelia burgdorferi: report of four cases. J Neurol Sci 1989, 91:215–226
9. Schaible UE, Kramer MD, Museteanu C, Zimmer G, Mossmann H, Simon MM: The severe combined immunodeficiency (SCID) mouse: A laboratory model for the analysis of Lyme arthritis and carditis. J Exp Med 1989, 170:1427–1432
10. Barthold SW, Beck DS, Hansen GM, Terwilliger GA, Moody DK: Lyme Borreliosis in selected strains and ages of laboratory mice. J Infect Dis 1990, 162:133–138
11. Barthold SW, Persing, DH, Armstrong AL, Peeples RA: Kinetics of Borrelia burgdorferi dissemination and evolution of disease after intradermal inoculation of mice. Am J Pathol 1991, 139:263–273
12. Barthold SW, Moody KD, Terwilliger GA, Jacoby RO, Steere AC: An animal model for Lyme arthritis. Ann Acad Sci 1988, 539:264–273
13. Hejka A, Schmitz JL, England DM, Callister SM, Schell RF: Histopathology of Lyme arthritis in LSH hamsters. Am J Pathol 1989, 134:1113–1123
14. Schaible UE, Gay S, Museteanu C, Kramer MD, Zimmer G, Eichmann K, Museteanu U, Simon MM: Pathogenesis of Lyme Borreliosis in the severe combined immunodeficiency (SCID) mice. Am J Pathol 1990, 137:811–820
15. Schaible UE, Kramer MD, Wallich R, Tran T, Simon MM: Experimental Borrelia burgdorferi infection in inbred mouse strains: Antibody response and association of H-2 genes with resistance and susceptibility to development of arthritis. Eur J Immunol 1991, 21:2337–2405.
16. Bosma GC, Custer RP, Bosma MJ: A severe combined immunodeficiency mutation in the mouse. Nature 1983, 301:527–530
17. Schaible UE, Kramer MD, Justus CWE, Museteanu C, Simon MM: Demonstration of antigen-specific T cells and histopathological alterations in mice experimentally inoculated with Borrelia burgdorferi. Infect Immunol 1989, 57:41–47
18. Springer T, Galfre G, Secher DS, Milstin C: MAC-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur J Immunol 1979, 9:301–306
19. Schaible UE, Kramer MD, Eichmann K, Modolell M, Museteanu C, Simon MM: Monoclonal antibodies specific for the outer surface protein A (OspA) of Borrelia burgdorferi prevent Lyme Borreliosis in severe combined immunodeficiency (scid) mice. Proc Natl Acad Sci USA 1990, 87:3768–3772
20. Wörth WD: Die Erythema-migrans-Krankheit (Lyme-Krankheit, Erythema-migrans-Borreliose). Med Klin 1986, 81:464–469