LYME BORRELLIOSES, caused by the spirochete *Borrelia burgdorferi*, is one of the most frequently reported tick-borne infectious diseases (1). Since a reliable control of this multisystemic disease through antibiotic therapy is questionable, present investigations are mainly concerned with the role that the infectious agent itself, as well as the host's immune response, plays in the expression of the various clinical manifestations of this disease.

During our studies on the induction of *B. burgdorferi*-specific immune responses in mice, we have found that experimental inoculation of several inbred mouse strains with a high-passage isolate of *B. burgdorferi* leads to moderate but significant pathomorphological changes in various organs, such as brain, heart, lungs, kidneys, and spleen, which are comparable with those found in patients with Lyme disease (2). We now report that mice with severely impaired T cell and B cell functions, i.e., the severe combined immunodeficiency (scid) mouse (3), develop a multisystemic disease with a preponderance for polyarthritis and carditis after inoculation with a low-passage tick isolate of *B. burgdorferi*. In addition, the data indicate that scid mice are a suitable source to propagate infective *B. burgdorferi* organisms in vivo. This mouse model should prove useful to elucidate the role of cellular and humoral immune responses in the pathogenesis of Lyme Borreliosis.

**Materials and Methods**

*Mouse and Inoculation with B. burgdorferi.* Adult mice of strains C.B-17 scid (homozygous for the scid mutation) and C.B-17 were bred at the Max-Planck-Institut für Immunbiologie, Freiburg, FRG. Female and male animals between 6 and 10 wk of age were used in this study.

Mice were inoculated with either $10^5$, $5 \times 10^5$, $10^6$, or $10^8$ viable or inactivated (UV irradiation) *B. burgdorferi* organisms subcutaneously in the tail.

*Bacteria and Isolation of B. burgdorferi from Mice and Ticks.* The high passage *B. burgdorferi* strain B31 (ATCC 35210) was obtained from Dr. A. Vogt, Institut für Immunologie der Universität, Freiburg, FRG. *B. burgdorferi* strains ZS7 and ZQ1 (low-passage isolates) were isolated from two female *Ixodes ricinus* ticks collected by flagging in the Freiburg area. All *B. burgdorferi* strains were grown in modified Kelly's medium as described (2).

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**Serological Tests.** The detection of *B. burgdorferi*-specific as well as total (IgM, IgG) antibodies was performed in a conventional solid-phase ELISA system as described elsewhere (4).

**Enrichment of *B. burgdorferi* Organisms from Blood.** 50 μl of blood was pipetted into a hemocrit tube (Becton Dickinson & Co., Heidelberg, FRG) and centrifuged at 5,000 g in a hemocrit centrifuge (method from L. Gern, Institut Zoologie, Université de Neuchatel, Switzerland, personal communication). Tubes were cut at the interphase between serum and erythrocytes, and 5 μl of serum was mounted onto adhesion slides (Superior, Bad Mergenthaim, FRG).

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**Immunofluorescence and Giemsa Stain.** Adhesion slides mounted with serum samples were air dried and fixed in 100% ethanol for 1 min at −20°C. After incubation with rabbit anti-*B. burgdorferi* hyperimmune serum (diluted 1:100 from stock) in a wet chamber for 1 h at room temperature, the slides were washed five times in PBS and then stained with FITC-conjugated goat anti-rabbit antiserum (diluted 1:20 from stock; The Jackson Laboratory, Bar Harbor, ME) for 1 h. Slides were washed and embedded in Kaiser’s Glycerol-Gelatine (Merck, Darmstadt, FRG), and immediately examined using a fluorescence microscope (Leitz, Wetzlar, FRG).

Whole blood smears were air dried, fixed in methanol, stained with Giemsa (0.1%; Merck), destained in PBS, and embedded in Entellan (Merck).

**Histologic Preparations and Staining Procedures.** Different internal organs were removed from mice at different time intervals after inoculation and stored either in liquid nitrogen for preparation of cryosections or in 5% formaldehyde (in PBS) for embedding in paraffin or methacrylate (Kulzer, Friedrichsdorf, FRG). Sections (4-7 μm) were stained with hematoxylin-eosin and embedded in Entellan (E. Merck AG, Darmstadt, FRG). Immunohistology was performed using the streptavidin-biotin-peroxidase system as described in detail (4).

**Results**

C.B-17 **scid** or control C.B-17 mice were inoculated subcutaneously with $10^5-10^8$ organisms of either of the two recently obtained low-passage tick isolates of *B. burgdorferi*, ZS7 and ZQ1, or with $10^8$ *B. burgdorferi* B31 organisms. In addition, ZS7 spirochetes, previously inactivated by UV irradiation, were used for inoculation. *B. burgdorferi* organisms of all three tick isolates, ZS7, ZQ1, and B31, were consistently detected in the blood of **scid** mice previously inoculated with viable organisms (Table I) during the entire observation period between days 7 and 87 post-inoculation. However, only spirochetes derived from isolates ZS7 and ZQ1, but not those from strain B31, could be recultured in vitro from either whole blood and/or synovial fluid (Table I). When compared with the primary isolate of *B. burgdorferi* ZS7, no changes in spirochetal proteins or in their plasmid profiles were observed in any reisolate (data not shown).

In contrast to control C.B-17 mice, no or only marginal levels of total or *B. burgdorferi*-specific antibodies of either isotypes, IgM and/or IgG, were found in *B. burgdorferi*-inoculated scid mice during the entire observation time (Table I). Moreover, splenic cells of the infected scid mice did not contain mature T and B cells (data not shown), which is in agreement with previous findings (3).

Clinical signs of arthritis in response to both *B. burgdorferi* strains ZS7 and ZQ1 were observed in **scid** mice between days 7 and 20, depending on the number of spirochetes inoculated ($10^5-10^8$). Mice had difficulty in walking and showed reddening and swelling of both tibiotarsal joints, which increased and did not resolve during the entire observation period (up to day 87; Table I). The progressive arthritis was characterized by synovial hyperplasia, infiltration of mononuclear leukocytes into inflamed synovium, and pannus formation (compare Fig. 1 a and Fig. 1, b, c, and d) with cartilage destruction and joint erosion (data not shown). In addi-
Inoculation of C.B-17 scid and C.B-17 Control Mice with B. burgdorferi: Reisolation of Spirochetes from Tissues, Antibody Tilters, and Development of Arthritis

Detected by Giemsa stain or immunofluorescence. Isolation from blood (b), joint (j).

+ , reddening and swelling of tibiotarsal joints.

§ - , < 7.5 μg/ml serum.

**TABLE I**

Inoculation of C.B-17 scid and C.B-17 Control Mice with B. burgdorferi: Reisolation of Spirochetes from Tissues, Antibody Tilters, and Development of Arthritis

| Mouse strain | B. burgdorferi strain | Number inoculated | Days after inoculation | Detection in blood* | Isolation† | Arthritis | Antibodies‡ | Specific Total Ig | Specific Ig | μg/ml |
|--------------|----------------------|------------------|-----------------------|---------------------|------------|-----------|------------|----------------|------------|-------|
| C.B-17      | ZS7 5 x 10⁵           | 7                | +                     | -                   | -          | -         | -          | -              | -          | 20    |
| scid        |                      |                  |                       |                     |            |           |            |                |            |       |
| (n = 1)     |                      |                  |                       |                     |            |           |            |                |            |       |
| C.B-17      | ZS7 10⁸              | 7                | +                     | -                   |  +         |  +        | -          |  +             | -          | 108   |
| scid        |                      |                  |                       |                     |            |           |            |                |            |       |
| ZS7 10⁸     | 23                   | +                | -                     | +                   | -          |  +        |  +         | -              | -          | 54    |
| ZS7 10⁸     | 29                   | +                | j                     | +                   | -          | -         | -          | -              | -          | -     |
| ZS7 10⁸     | 87                   | b/j              | +                     | -                   | -          | -         | -          | -              | -          | -     |
| ZS7 10⁸     | 16                   | -                | ND                    | ND                  | +          | ND        | ND         | ND             | ND         | -     |
| ZS7 10⁸     | 22                   | -                | ND                    | ND                  | +          | ND        | ND         | ND             | ND         | -     |
| ZS7 10⁸     | 29                   | +                | -                     | +                   | -          | -         | -          | -              | -          | -     |
| ZS7 10⁸     | 87                   | +                | b/j                   | +                   | -          | -         | -          | -              | -          | -     |
| ZS7 10⁸     | 57                   | +                | j                     | +                   | -          | -         | -          | -              | -          | -     |
| B31 10⁹     | 22                   | +                | -                     | -                   | -          | 26        | 37         | -              | -          | -     |
| ZS7uvir     | 29                   | +                | -                     | -                   | -          | -         | -          | -              | -          | -     |
| ZQl 10⁹     | 29                   | +                | -                     | -                   | -          | -         | -          | -              | -          | -     |
| ZS7 10⁸     | 16                   | -                | ND                    | ND                  | +          | ND        | ND         | ND             | ND         | -     |
| C.B-17      | ZS7 10⁸              | 24               | -                     | -                   | -          | -         | -          | -              | -          | 2,145 |
| (n = 7)     |                      |                  |                       |                     |            |           |            |                |            |       |
| C.B-17      | ZS7 10⁸              | 24               | -                     | -                   | -          | -         | -          | -              | -          | 304   |
| scid        |                      |                  |                       |                     |            |           |            |                |            |       |
| C.B-17      | ZS7 10⁸              | 24               | -                     | -                   | -          | -         | -          | -              | -          | 216   |

- Detection by Giemsa stain or immunofluorescence.
- Isolation from blood (b), joint (j).
- + , reddening and swelling of tibiotarsal joints.
- § - , < 7.5 μg/ml serum.

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FIGURE 1. Paraffine sections of tibiotarsal joints from noninfected and B. burgdorferi-infected scid mice. (a) Tibiotarsal joint from a noninfected scid mouse. HE stain, x400. (b) Tibiotarsal joint from a scid mouse, 7 d after subcutaneous inoculation with B. burgdorferi strain ZS7. There is an initial phase of pannus formation with infiltrating mononuclear leukocytes in the inflamed synovium. HE stain, x400. (c) Tibiotarsal joint from a scid mouse, 49 d after subcutaneous inoculation with B. burgdorferi strain ZS7. Inflamed synovial membrane with exudation of mononuclear leukocytes into the synovial space. Mononuclear leukocyte infiltrates are also seen in the muscle (periartthritis). HE stain, x400. (d) Tibiotarsal joint from a scid mouse, 23 d after subcutaneous inoculation with B. burgdorferi strain ZS7. Hyperplastic inflamed synovium, exfoliated cellular debris in the lumen consisting of inflammatory and synovial cells and obliterating fibrous connective tissue. HE stain, x400. (e) Heart from a scid mouse, 87 d after subcutaneous inoculation with B. burgdorferi strain ZS7. Infiltrations of the myocard with mononuclear leukocytes. HE stain, x400. (f) Kidney from a scid mouse, 16 d after subcutaneous inoculation with B. burgdorferi strain ZS7. Perivascular infiltrations with monocytes. HE stain, x400.

(Fig. 1f), lung, and liver. The majority of mononuclear leukocytes infiltrating the synovial space (data not shown), the periarticular tissues, the heart, and other tissues stained with MAC-1 mAb, a marker for mature macrophages (5), but neither with L3T4 nor Ly-2 mAb (data not shown).
Discussion

The major findings reported herein are that the spirochete *B. burgdorferi* induces a multisystemic infection with a prominent and persistent polyarthritis and carditis as well as nephritis and hepatitis in immunodeficient *scid* mice but not in control C.B-17 mice. Viable infective low-passage organisms are required for the development of this disease.

The fact that *B. burgdorferi ZS7* organisms reisolated from *scid* mice showed protein and plasmid profiles indistinguishable from that of the original infective strain suggests retention of their pathogenicity and opens the possibility of maintaining virulent *B. burgdorferi* organisms in vivo. This is of importance because the high-passage tick isolate B31 did not induce arthritic lesions in *scid* mice, indicating a loss of virulence of *B. burgdorferi* during passage in artificial culture medium, which is in line with a recent study describing changes of infectivity and plasmid profile of the spirochete *B. burgdorferi* as a result of in vitro cultivation (6).

The acceleration and persistence of arthritis as observed in *B. burgdorferi*-infected *scid* mice, which is not found in normal C.B-17 mice, is indicative of immunological control of the pathogenic effect of this organism. This is supported by experiments in neonatal rats, in which *B. burgdorferi*-induced arthritis was found to decrease with the age of the animal (7), and presumably with the maturation of the immune system. This assumption is also indicated by the finding that a more severe arthritis is observed in irradiated, as compared with nonirradiated, hamsters upon inoculation with *B. burgdorferi* (8). Studies addressing this issue are presently being investigated.

At present the mechanism(s) by which *B. burgdorferi* induces the severe pathological alterations in tissues of *scid* mice are not known. The possibilities that arthritis was either elicited by inflammatory cell wall products of *B. burgdorferi*, similar to those described for group A, B, or C streptococci (9), or was caused by circulating immune complexes or antigen-specific T cells are unlikely since (a) inactivated organisms were not capable of inducing disease; and since (b) the *scid* mice lacked mature lymphocytes. The latter argument also holds true for the development of carditis and lesions in other organs. The presence of viable *B. burgdorferi* organisms within synovial tissue, rather, indicates a direct effect of the spirochetes on the permeability of vessel walls as well as on the underlying tissues. It is possible that *B. burgdorferi* itself breaches vessel integrity after attaching to endothelial cells and/or to basal lamina and extracellular matrix structures by enzymatic activity(ies), a process known to be operative in the pathogenesis of *Treponema pallidum* infection (10). On the other hand, spirochetes may also bind to and activate macrophages, the major constituents of cellular infiltrates in the inflamed tissues described herein, as well as other monocytes leading to the release of various mediators, such as IL-1, IFN-γ, and TNF, that induce and maintain inflammation, as recently shown for *Listeria monocytogenes* infection in *scid* mice (11).

In conclusion, the data presented indicate that generation of a persistent severe polyarthritis and carditis in *B. burgdorferi*-infected *scid* mice is the consequence of their immunodeficiency and emphasize the potential use of this laboratory animal as a model to study the role of B and T lymphocytes in the pathogenesis of Lyme disease.
Summary

We report that the spirochete *B. burgdorferi* induces progressive polyarthritis and carditis in mice with severe combined immunodeficiency syndrome (scid) but not in normal C.B-17 mice. The onset and severity of the disease were dependent on (a) the viability; (b) the infectivity; and (c) the dose of inoculated *B. burgdorferi* organisms. Infective spirochetes were isolated from both blood and joints of inoculated scid mice. These findings suggest that *B. burgdorferi*-induced chronic arthritis and carditis in mice develops independently of lymphocyte function and makes the scid mouse an attractive laboratory model to study the role of the immune system in experimental Lyme Borreliosis.

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