Brief Definitive Report

THE SEVERE COMBINED IMMUNODEFICIENCY (*scid*) MOUSE

A Laboratory Model for the Analysis of Lyme Arthritis and Carditis

By U. E. SCHAIBLE, M. D. KRAMER,* C. MUSETEANU, G. ZIMMER,‡ H. MOSSMANN, AND M. M. SIMON

*From the Max-Planck-Institut für Immunbiologie, Freiburg; the *Universität-Hautklinik, Heidelberg; and the ‡Institut für Pathologie, Heidelberg, Federal Republic of Germany*

Lyme borreliosis, 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

Mice 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, 5×10^5, 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 rizinus* ticks collected by flagging in the Freiburg area. All *B. burgdorferi* strains were grown in modified Kelly's medium as described (2).

This work was supported in part by the Boehringer Ingelheim Fonds (U. E. Schaible) and a Postdoktoranden-Stipendium by the Deutsche Forschungsgemeinschaft (M. D. Kramer). Address correspondence to M. M. Simon, Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-7800 Freiburg, FRG.

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 hematocrit tube (Becton Dickinson & Co., Heidelberg, FRG) and centrifuged at 5,000 *g* in a hematocrit 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 Mergentheim, FRG).

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-

TABLE I

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

Mouse strain	*B. burgdorferi* strain	Number inoculated	Days after inoculation	Detection in blood*	Isolation‡	Arthritis		Antibodies‖			
						Clinical§	Histopathological	Total Ig		Specific Ig	
								μ	γ	μ	γ
									μg/ml		
C.B-17	ZS7	5×10^5	7	+	–	–.	–	–	20	–	–
scid			36	+	b	+	+	–	21	–	–
(n = 1)			49	+	b	+	+	–	26	–	–
			59	+	j	+	+	–	396	–	–
	ZS7	10^8	7	+	–	+	+	–	108	–	–
			23	+	b	+	+	–	54	–	–
	ZS7	10^8	22	+	–	+	+	–	41	–	–
			29	+	j	+	+	–	–	–	–
			87	+	b/j	+	+	–	–	–	–
	ZS7	10^5	–	ND	ND	+	ND	ND		ND	
		10^6	–	ND	ND	+	ND	ND		ND	
		10^8	16	+	–	+	+	–	–	–	–
	$ZS7uv_{tr}$	10^8	16	–	–	–	–	–	–	–	–
	ZQ1	10^8	57	+	j	+	ND	–	–	–	–
			57	+	b/j	+	ND	–	–	–	–
	B31	10^8	22	+	–	–	–	26	37	–	–
			29	+	–	–	–	–	–	–	–
	–		22	–	–	–	–	–	47	–	–
	–		29	–	–	–	–	54	364	–	–
C.B-17	ZS7	10^8	16	–	–	–	–	2,515	5,963	438	56
(n = 7)			24	–	–	–	–	2,145	6,374	506	94
	–	–	–	–	–	–	–	304	3,804	–	–
	–	–	–	–	–	–	–	216	1,952	–	–

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

tion, a severe periarthritis involving connective and muscular tissue developed (Fig. 1, *c* and *d*). Later during the disease, additional joints, i.e., ulnacarpal and metatarsal, also showed similar clinical and microscopical symptoms (data not shown). In contrast, no macroscopical evidence for arthritis or histopathological alterations in the joints were seen at any time tested in (*a*) *scid* mice inoculated either with UV-irradiated *B. burgdorferi* ZS7 or with viable, nonvirulent *B. burgdorferi* B31 organisms; or in (*b*) normal C.B-17 mice inoculated with viable *B. burgdorferi* ZS7 organisms (Table I).

B. burgdorferi-induced pathological alterations of the heart, including prominent infiltrations of the myo- and pericard, were also more pronounced in *scid* (Fig. 1 *e*) as compared with normal C.B-17 mice. Similar observations were also made comparing other organs of *B. burgdorferi*-inoculated *scid* and C.B-17 mice, such as kidney

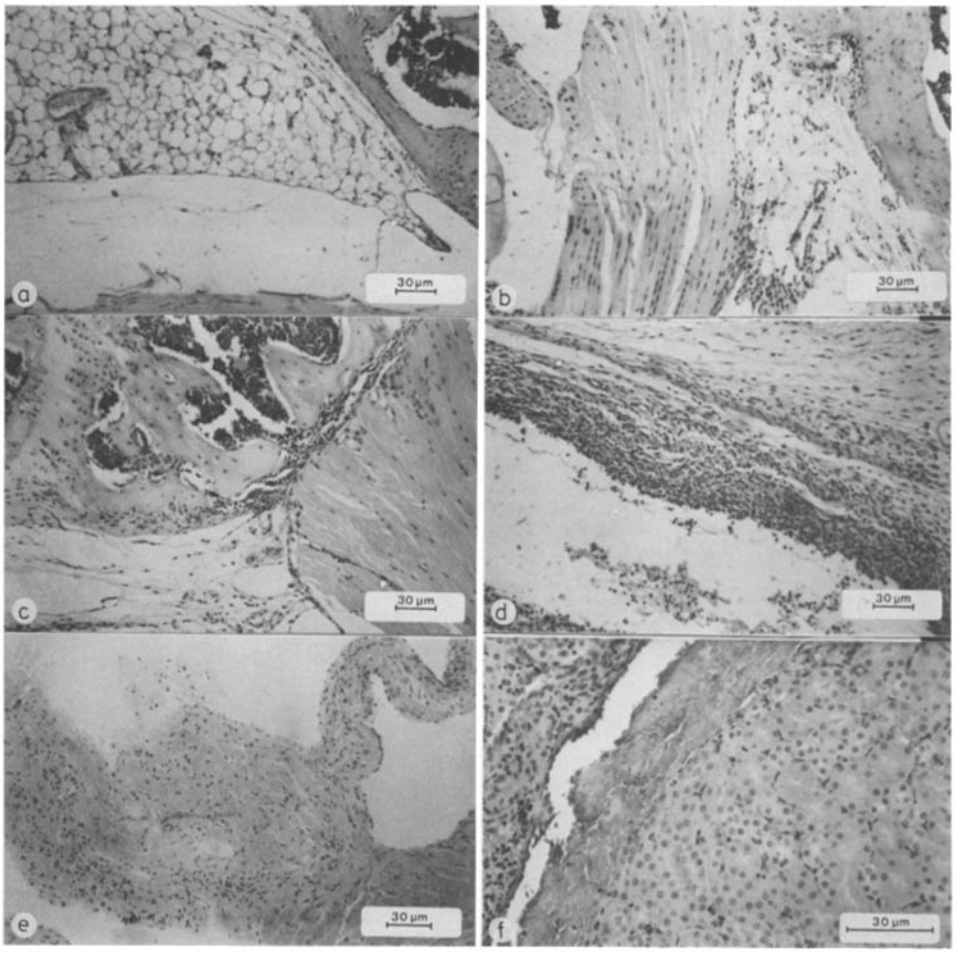

FIGURE 1. Paraffine sections of tibiotarsal joints from noninfected and *B. burgdorferi*–infected *scid* mice. (*a*) Tibiotarsal joint from a noninfected *scid* mouse. HE stain, ×400. (*b*) Tibiotarsal joint from a *scid* mouse, 7 d after subcutaneous inoculation with *B. burgdorferi* strain ZS7. There is an initial phase of pannus formation with infiltrating mononuclear leukocytes in the inflamed synovium. HE stain, ×400. (*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 (periarthritis). HE stain, ×400. (*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, ×400. (*e*) Heart from a *scid* mouse, 87 d after subcutaneous inoculation with *B. burgdorferi* strain ZS7. Infiltrations of the myocard with mononuclear leucocytes. HE stain, ×400. (*f*) Kidney from a *scid* mouse, 16 d after subcutaneous inoculation with *B. burgdorferi* strain ZS7. Perivascular infiltrations with monocytes. HE stain, ×400.

(Fig. 1 *f*), 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.

We thank Ms. U. Museteanu, G. Nerz, and M. Prester for expert technical assistance, Ms. R. Schneider and R. Brugger for patient secretarial assistance, and Dr. J. Langhorne for critically reading the manuscript.

Received for publication 3 April 1989 and in revised form 3 July 1989.

References

1. Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmidt, E. Johnson, and S. E. Malawista. 1983. The spirochetal etiology of lyme disease. *N. Engl. J. Med.* 308:733.
2. Schaible, U. E., M. D. Kramer, C. W. E. Justus, C. Museteanu, and M. M. Simon. 1988. Demonstration of antigen specific T cells and histopathological alterations in mice experimentally inoculated with *Borrelia burgdorferi*. *Infect. Immun.* 1:41.
3. Bosma, G. C., M. Fried, R. P. Custer, A. Carroll, D. M. Gibson, and M. J. Bosma. 1988. Evidence of functional lymphocytes in some (leaky) scid mice. *J. Exp. Med.* 167:1016.
4. Kramer, M. D., U. Fruth, H.-G. Simon, and M. M. Simon. 1989. Expression of cytoplasmic granules with T cell associated serine proteinase-1 activity in Ly-2$^+$ (CD8$^+$) T lymphocytes responding to lymphocytic choriomeningitis virus in vivo. *Eur. J. Immunol.* 19:151.
5. Springer, T., G. Galfre, D. S. Secher, and C. Milstein. 1979. MAC-1: a macrophage differentiation antigen identified by monoclonal antibody. *Eur. J. Immun.* 9:301.
6. Schwann, T. G., W. Burgdorfer, and C. F. Garon. 1988. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of in vitro cultivation. *Infect. Immun.* 56:1831.
7. Barthold, S. W., K. D. Moody, G. A. Terwilliger, R. O. Jacoby, and A. C. Steeve. 1988. An animal model for lyme arthritis. *Ann. NY Acad. Sci.* 539:264.
8. Schmitz, J. L., R. F. Schell, A. Hejka, D. M. England, and L. Komick. 1988. Induction of Lyme arthritis in LSH hamsters. *Infect. Immun.* 9:2336.
9. Wilder, R. L., J. B. Allen, L. M. Wahl, G. B. Calandra, and S. M. Wall. 1983. The pathogenesis of group A streptococcal cell wall-induced polyarthritis in the rat. Comparative studies in arthritis resistant and susceptible inbred rat strains. *Arthritis Rheum.* 26:1442.
10. Fitzgerald, J. 1981. Pathogenesis and immunology of Treponema pallidum. *Annu. Rev. Microbiol.* 35:29.
11. Deschryver-Kecskemeti, K., G. J. Bancroft, G. C. Bosma, M. J. Bosma, and E. R. Unanue. 1988. Pathology of Listeria infection in murine severe combined immunodeficiency. A study by immuno-histochemistry and electronmicroscopy. *Lab. Invest.* 6:698.