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SCID Mouse Model for Lethal Q Fever

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Q fever, a worldwide zoonosis caused by *Coxiella burnetii*, has many manifestations in humans. Endocarditis is the most serious complication of Q fever. Animal models are limited to acute pulmonary or hepatic disease and reproductive disorders. An appropriate experimental animal model for Q fever endocarditis does not yet exist. In this study, severe combined immunodeficient (SCID) mice infected with *C. burnetii* showed persistent clinical symptoms and died, whereas immunocompetent mice similarly infected became asymptomatic and survived. The SCID mice examined in this study had severe chronic lesions in their primary organs: the heart, lung, spleen, liver, and kidney. The heart lesions of the SCID mice were similar to those in humans with chronic Q fever endocarditis: they had focal calcification and expanded macrophages containing *C. burnetii*. The 50% lethal dose of *C. burnetii* in SCID mice was at least 10^8 times less than that in immunocompetent mice. The SCID mouse is highly susceptible to *C. burnetii*, and the immunodeficiency of the host enhances the severity of Q fever. This animal model could provide a new tool for the study of chronic Q fever and Q fever in immunodeficient hosts.

Q fever is a worldwide zoonosis caused by an obligate intracellular bacterium, *Coxiella burnetii*. The disease in humans typically has both an acute form and a chronic form. Acute Q fever is a flu-like illness which is self-limiting and easily treated with antibiotics when an appropriate diagnosis is made. Chronic Q fever is usually manifested as endocarditis or vascular infection. Endocarditis is the most serious complication of Q fever because the treatment is difficult and the mortality is high (9). Naturally infected animals rarely demonstrate illness except for reproductive disorders, such as abortion. Guinea pigs and mice are used as laboratory animal models for acute Q fever, but they require a high dose of inoculum. The A/J strain of mice, which is immunocompetent, is the strain that is most susceptible to *C. burnetii* (25). Immunosuppressive treatments of various experimental animals have been reported to raise their susceptibility to *C. burnetii* (1, 11, 26, 27).

The pathogenesis of the disease is little known because research on chronic Q fever has been limited to clinical case studies. An appropriate animal model for the study of chronic Q fever and its antibiotic therapy does not yet exist. Patients with Q fever endocarditis are known to have histories of heart valve damage or to lack an appropriate immune response. Several animal models for Q fever endocarditis have been proposed, but the clinical signs differ from those in human cases. Endocarditis in guinea pigs with previous valvular damage after *C. burnetii* infection was only transient (15). BALB/c mice that were physiologically immunosuppressed by repeated pregnancy for 2 years after *C. burnetii* infection (28) developed endocarditis with fibrin deposits, a generic sign of chronic lesions, but the incidence of endocarditis was low (2 out of 13 mice). BALB/c mice that underwent cyclophosphamide treatment after *C. burnetii* infection developed endocarditis, but the cases were transient (1). A suitable, more sensitive animal model is needed to clarify the pathogenicity of chronic Q fever.

The severe combined immunodeficient (SCID) mouse has no functional T and B cells (6, 8). It is highly susceptible to various pathogens that have low pathogenicity for immunocompetent animals. In the present study, we compared the clinical symptoms, the histopathology, and the survival rates of *C. burnetii* infection in SCID mice and immunocompetent mice to determine whether the SCID mouse could be used as an animal model for chronic Q fever. This is the first report of persistent *C. burnetii* infection in an animal that resulted in severe chronic lesions and death.

MATERIALS AND METHODS

Mice. SCID (C.B-17/Icr-scid/scid) mice and immunocompetent C.B-17 (C.B-17/Icr-+/+) mice were obtained from Japan CLEA Inc. (Tokyo, Japan). A/J mice were obtained from Japan SLC Inc. (Shizuoka, Japan). Five- to 6-week-old female mice were used in the experiments. They were housed under sterile conditions at all times. All procedures were done under the guidelines for animal experiments at Gifu University.

Microorganism. The Nine Mile I strain of *C. burnetii* was maintained in mice by passage in spleen homogenates. The spleen homogenates were prepared in sucrose phosphate glutamate, kept at −80°C, and diluted with phosphate-buffered saline (PBS). *C. burnetii* in the homogenate was titrated to the 50% tissue culture infectious dose ($TCID_{50}$) in buffalo green monkey (BGM) cells by the indirect immunoperoxidase method (24).

Inoculation of mice and clinical studies. To compare the pathogenicities of *C. burnetii* in immunodeficient and immunocompetent mice, SCID mice ($n = 11$), C.B-17 mice ($n = 6$), and A/J mice ($n = 6$) were inoculated intraperitoneally with 10 $TCID_{50}$ of *C. burnetii*. As controls, SCID mice ($n = 10$), C.B-17 mice ($n = 6$), and A/J mice ($n = 6$) were mock inoculated with PBS. The mice were observed for 37 days, which is the time at which the last *C. burnetii*-infected mouse died. To compare the dose responses of the immunodeficient and immunocompetent mice, SCID mice ($n = 6$) were inoculated intraperitoneally with 0.5 ml of serial 10-fold dilutions (10^4 to 10^{-5} $TCID_{50}$) of *C. burnetii*. C.B-17 mice ($n = 6$) were similarly inoculated with 10-fold dilutions (10^4 to 10^{-3} $TCID_{50}$) of *C. burnetii*. As controls, SCID mice ($n = 6$) and C.B-17 mice ($n = 6$) were mock inoculated with PBS. The SCID mice were observed for 60 days, and the C.B-17 mice were observed for 30 days. The 50% lethal dose (LD_{50}) was calculated by the Behrens-

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Kärber method (5). Clinical signs and body weight were recorded daily. Relative body weight is the weight on a given day divided by the body weight on the day of inoculation. Blood samples were obtained by puncture of the heart under anesthesia before euthanasia. At necropsy, the spleen and liver were weighed, and a part of each organ was stored at −80°C. The rest of the spleen and liver and the heart, lungs, and kidneys were preserved in 10% formalin PBS.

Histopathology and immunocytochemistry. The organs of the mice that received 10 TCID$_{50}$ of *C. burnetii* and the control mice were examined. Sections of paraffin-embedded organs were prepared and stained with hematoxylin and eosin. The distribution of *C. burnetii* was examined by immunocytochemistry, using an anti-*C. burnetii* rabbit antiserum, goat anti-rabbit immunoglobulins (DAKO Japan, Kyoto, Japan), and avidin-biotin complex (ABC; Vector Laboratories, Burlingame, Calif.), as described elsewhere (4). The number of *C. burnetii*-positive cells in a section was scored as follows: none, −; a few cells in a separate part, +; several cells assembled in a specific part, ++; and cells throughout the section, +++.

Serology. Immunoglobulin G antibodies to phase I and II *C. burnetii* from C.B-17 and A/J mice were detected by an indirect immunofluorescence test (K. K. Htwe, K. Amano, Y. Sugiyama, K. Yagami, N. Minamoto, A. Hashimoto, T. Yamaguchi, H. Fukushi, and K. Hirai, Vet. Rec. **131**:490, 1992) to determine whether the mice were infected. The serology of the SCID mice was not examined.

PCR. DNA was extracted from liver and spleen homogenates using a DNA extraction kit, SepaGene (Sanko Junyaku Co., Tokyo, Japan). The *com1* gene fragment of *C. burnetii* was amplified by nested PCR using the primers OMP1-OMP2 and OMP3-OMP4 (30). To avoid false positives, DNA extraction and PCR were performed carefully according to guidelines described previously (14).

Statistical analysis. Differences between organ weights in control and infected mice were determined by Student's *t* test or Welch's *t* test following an F test. *P* values of <0.05 were regarded as significant.

RESULTS

Clinical signs and gross findings in SCID and immunocompetent mice. All the SCID mice infected with 10 TCID$_{50}$ of *C. burnetii* showed the same symptoms and died. The onset and duration of the clinical signs were as follows: 7 days postinoculation (p.i.), ruffled fur; 9 to 13 days p.i., hunchback appearance and inactivity; 27 to 31 days p.i., lethargy. The onset of a hunchback appearance and inactivity correlated with loss of body weight. Body weight continued to decline until death (Fig. 1). The survival period was 33 ± 2.5 days. None of the C.B-17 or A/J mice showed any clinical signs or died. The relative body weights of C.B-17 mice and A/J mice that were infected with 10 TCID$_{50}$ of *C. burnetii* were similar to those of the control mice.

Hepatosplenomegaly was prominent in the SCID mice (Table 1), and the spleen had a pale color while the liver had necrotic foci. The A/J mice showed mild hepatosplenomegaly, whereas the C.B-17 mice showed only mild splenomegaly. In the C.B-17 and A/J mice, the liver did not have necrotic foci and the color of the spleen was normal.

None of the control mice showed any clinical signs or died, and no gross findings were detected.

Characteristic lesions with *C. burnetii* organisms in SCID mice. The lesions in SCID mice infected with 10 TCID$_{50}$ of *C. burnetii* were much more severe than the lesions in immunocompetent mice infected with 10 TCID$_{50}$ of *C. burnetii* (Table 2). The most characteristic and common feature observed in the lesions of SCID mice was severe cell infiltration. Almost all infiltrated cells were morphologically macrophages, and some were neutrophils. These macrophages were dilated, giving a vacuolated appearance, and were densely packed with basophilic granules, which varied from coarse to fine and were present in several extra- and intracellular forms. Immunocyto-
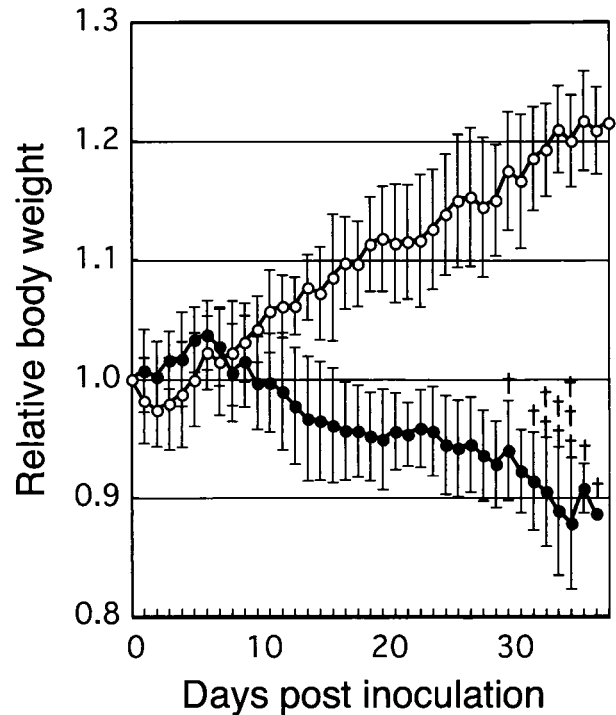

FIG. 1. Weight changes of SCID mice inoculated with *C. burnetii* (solid circles) and PBS (open circles). A significant difference (*P* < 0.005) between infected and control mice was found 10 days p.i. Relative body weight is the body weight on a particular day divided by the body weight on the day of inoculation. Points represent means, error bars indicate standard deviations, and † indicates death of the mouse.

chemistry revealed that these granules were *C. burnetii* organisms.

The hearts had severe infiltration of vacuolated macrophages in the epicardium and endocardium, including the valvular part. Focal calcification, evidence of chronic lesions, was observed in the epicardium and myocardium (Fig. 2A). Vacuolated macrophages were also found in the myocardium (Fig. 2B). In the kidney, the glomeruli were typically infiltrated with vacuolated macrophages (Fig. 2C), and the proximal tubules exhibited hyaline degeneration.

The lungs, spleen, and liver, which are the general target organs of *C. burnetii* in experimental animals, had severe lesions and many *C. burnetii* organisms. In the lungs, cells accumulated in intra-alveolar septi, stroma adjacent to airways, or venules (Fig. 3, top). The lumens contained no exudate. The spleen and liver did not retain their original structures due to bursting with macrophages and numerous vacuoles, which were probably the remains of necrotic cells. The margins of vacuoles were strongly immunopositive (Fig. 3, middle and bottom). *C. burnetii* organisms were also present within the hepatocytes (Fig. 3, middle). The liver also had some sporadic microabscesses, mainly containing macrophages, neutrophils, or lymphocytes. Additionally, the liver exhibited mild extramedullary hematopoiesis and contained a few megakaryocytes. Neither granulomas nor fibroblasts were found in any organs of the SCID mice.

The spleens of the control SCID mice, which originally

TABLE 1. Liver and spleen weights in mice inoculated with 10 $TCID_{50}$ of *C. burnetii* or PBS

| Organ | Weight[a] | | | | | |
| | SCID mice | | C.B-17 mice | | A/J mice | |
	C. burnetii	PBS	*C. burnetii*	PBS	*C. burnetii*	PBS
Liver	1.81 ± 0.14^b	0.85 ± 0.04	1.06 ± 0.12	1.16 ± 0.10	1.03 ± 0.11^b	0.83 ± 0.06
Spleen	1.01 ± 0.09^b	0.03 ± 0.01	0.17 ± 0.03^b	0.10 ± 0.02	0.17 ± 0.05^b	0.07 ± 0.02

[a] Mean ± standard deviation (grams).
[b] Significantly different ($P < 0.05$) from control mice.

lacked characteristic lymphoid follicles due to the absence of T and B lymphocytes, were somewhat smaller than those of the immunocompetent mice. No significant lesions were found in the control SCID mice.

General lesions in immunocompetent mice. The lesions of immunocompetent C.B-17 and A/J mice infected with 10 $TCID_{50}$ of *C. burnetii* were mild (Table 2) and were probably residual lesions, as reported elsewhere (3). The lesions of these two mouse strains were not noticeably different. A few small granulomas characterized by unexpanded macrophages, lymphocytes, or neutrophils were observed in the spleen, liver, and lungs. No lesions were observed in the heart or kidney. No significant lesions were found in control immunocompetent mice. Sections from both *C. burnetii*-inoculated and control immunocompetent mice were immunocytochemically negative and did not show nonspecific binding of ABC.

In the mice infected with 10 $TCID_{50}$ of *C. burnetii*, the antibody titers to phase I and II *C. burnetii* ranged from 1:256 to 1:1,024 and from 1:512 to 1:1,024, respectively, and were equivalent in the two mouse strains. The livers and spleens of the mice were PCR positive. No *C. burnetii*-specific antibodies or *C. burnetii* DNA was detected in the control mice.

Dose responses in SCID and immunocompetent mice. To determine the LD_{50} and to observe the dose response in SCID mice, graded doses of *C. burnetii* (10^4 to 10^{-5} $TCID_{50}$) were administered. All SCID mice that died in this experiment demonstrated clinical symptoms in the same progression and died within 60 days. The mice that died included all the ones that

were given 10^5 to 10^{-2} $TCID_{50}$, four of six that were given 10^{-3} to 10^{-4} $TCID_{50}$, and one of six that were given 10^{-5} $TCID_{50}$ (Table 3). None of the diseased mice recovered. The LD_{50} of *C. burnetii* in the SCID mouse was $<10^{-4}$ $TCID_{50}$. Both the latent period (data not shown) and the survival period were inversely proportional to the inoculum size. Hepatosplenomegaly was observed in the dead SCID mice and was proportional to the survival period, but not to the inoculum size. Among the C.B-17 mice, only those receiving 10^4 and 10^3 $TCID_{50}$ showed ruffled fur, hunchback appearance, inactivity, and body weight loss, but they recovered within 15 days p.i. None of the C.B-17 mice died. Splenomegaly, which was proportional to the inoculum size, was observed in mice given 10^4 to 10^{-3} $TCID_{50}$. Hepatomegaly was observed only in mice administered 10^4 and 10^3 $TCID_{50}$.

DISCUSSION

Given equivalent inocula of *C. burnetii*, SCID mice became persistently ill until death, whereas immunocompetent mice became transiently ill or symptomless. The SCID mice had severe chronic lesions. These results suggest that the SCID mouse is highly susceptible to *C. burnetii*, and the immunodeficiency of the host enhances the severity of Q fever. This is the first report of persistent *C. burnetii* infection in an animal that results in severe chronic lesions and death.

The higher susceptibility of immunocompromised animals to *C. burnetii* has been acknowledged (26, 27), and immunosup-

TABLE 2. Lesions and immunolocalization in mice inoculated with 10 $TCID_{50}$ of *C. burnetii*

| Organ | SCID mice | | C.B-17 and A/J mice[a] | |
	Lesion	*C. burnetii*[b]	Lesion	*C. burnetii*
Heart	Pancarditis; vacuolated macrophage infiltration, particularly severe in pericardium; focal calcification	++	None	−
Kidney	Glomerulonephritis; confluence of macrophages in glomeruli and pelvis	++	None	−
Spleen	Severe splenitis; diffuse replacement by macrophages; many vacuoles	+++	Focal granulomas	−
Liver	Severe hepatitis; diffuse replacement by macrophages; denatured and withered hepatocytes; many vacuoles; megakaryocytes	+++	Focal granulomas	−
Lung	Severe interstitial pneumonia; macrophage infiltration, particularly around the vessels	+++	Minimal interstitial pneumonia[c]	−

[a] There was no noticeable difference between the two mouse strains.
[b] *C. burnetii* detection by immunocytochemistry. −, no immunopositive cells in the section; ++, immunopositive cells in a certain part of the section; +++, immunopositive cells throughout section.
[c] 3 of 6 C.B-17 mice and 3 of 5 A/J mice.
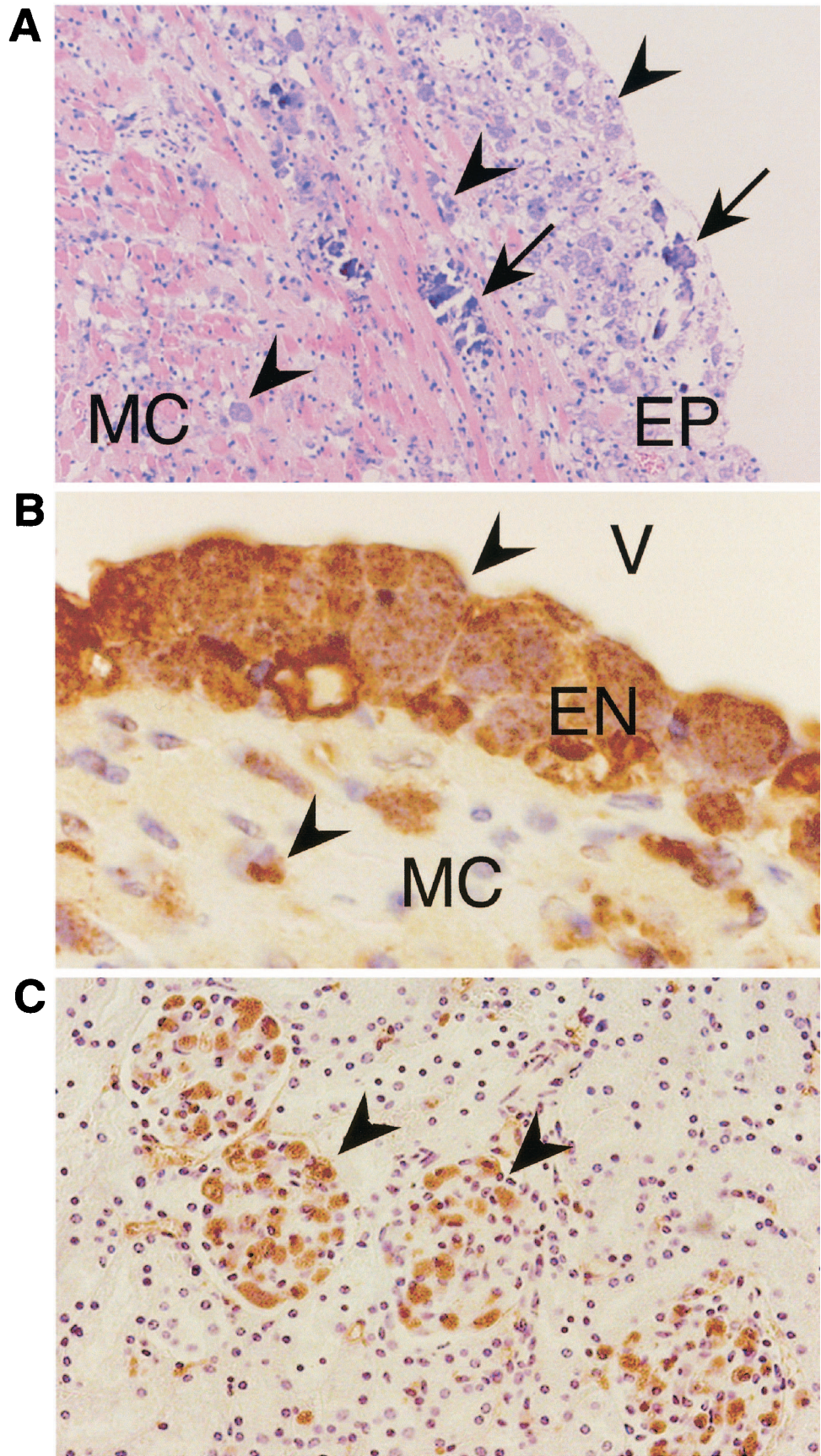
pressed mice have been used for the study of Q fever (1, 28) and the isolation of *C. burnetii* (11). However, the immune state of an immunosuppressed animal is unstable, which makes it difficult to study immune reactions. Immunodeficiency has been indicated as a host factor in chronic Q fever (19, 21), and Q fever endocarditis patients have been reported to be in an unbalanced immune state (7, 23). Q fever has also been observed in patients with cancer or human immunodeficiency virus infection or undergoing immunosuppressive therapy (13, 16, 20, 21). The SCID mouse has a clear and stable immunodeficient state, so it can be an animal model for the study of Q fever in an immunodeficient host.

Infection with 10 $TCID_{50}$ of *C. burnetii* caused death in the SCID mice, but the immunocompetent mice were asymptomatic. We concluded that the deaths of the SCID mice were due to chronic disease, as we observed that the survivability or death of mice infected with *C. burnetii* is determined within 2 weeks postinfection (references 3 and 25 and our unpublished data). The lesions in the SCID mice were more severe than those in the immunocompetent mice. *C. burnetii* replicated abundantly within macrophages in SCID mice, as revealed by immunocytochemistry. This result agrees with those of previous studies (2, 17) indicating that during infection, *C. burnetii* is concentrated in cells with macrophage activity. Because the SCID mouse is the key animal for the study of macrophage-dependent resistance (6), our new animal model should also help to elucidate the unexplained pathogenesis of Q fever.

The most characteristic lesions of SCID mice were observed in the heart and kidney. To our knowledge, these are the most severe chronic lesions to be reported in animals. Our present results reinforce the hypothesis that an immunocompromised state is an important factor in Q fever endocarditis (19). Heart disease developed in the SCID mouse within 2 months, whereas human chronic Q fever may take years to develop. Nevertheless, the hearts of SCID mice infected with *C. burnetii* share some characteristics with the hearts of humans with Q fever endocarditis: focal calcification and large macrophages containing *C. burnetii* organisms (22). Because the SCID mouse shows a high incidence of endocarditis without any treatment, it is a promising new animal model for Q fever endocarditis.

Glomerulonephritis has been reported as a manifestation of Q fever in humans (18, 29; M. Morovic, B. Dzelalija, S. Novakovic, S. Stankovic, and J. Dujella, Letter, Nephron **64:**335, 1993). In animals, renal disease caused by *C. burnetii* is only a transient lesion as part of a disseminated *C. burnetii* infection (3). However, the pathogenesis of renal disease in SCID mice may be different from that in human Q fever cases, because renal disease in human Q fever is due to immune complexes, while the SCID mouse is unable to produce immunoglobulins. However, the SCID mouse model suggests that there is a risk of renal disease associated with *C. burnetii* infection in immunocompromised hosts.
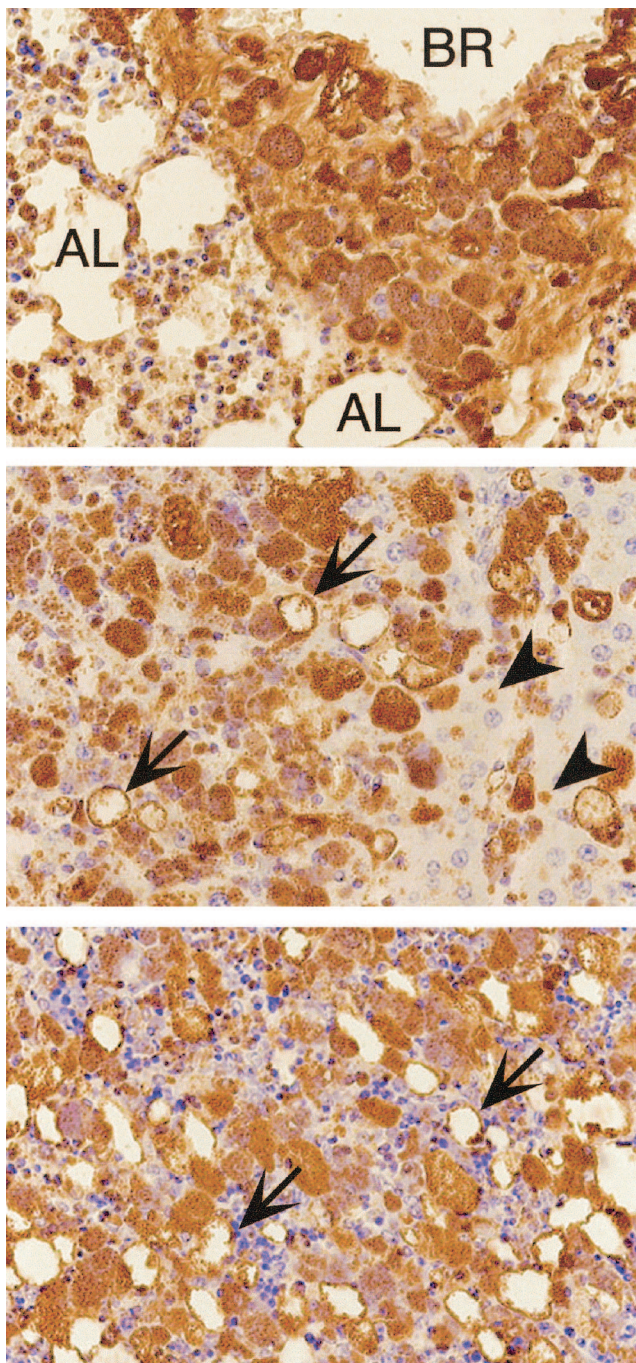

FIG. 3. Immunocytochemistry of the lung (top), liver (middle), and spleen (bottom) of SCID mouse infected with *C. burnetii*. *C. burnetii* antigens were detected as brown granules. (Top) *C. burnetii* antigen-positive cells infiltrated particularly in the stroma adjacent to a bronchiole (BR). AL, alveolus. (Middle) *C. burnetii* antigens were also found in hepatocytes (arrowheads). (Middle and bottom) The margins of vacuoles were immunopositive (arrows). ABC method; magnification, ×200.

FIG. 2. Heart (A and B) and kidney (C) sections from SCID mouse infected with *C. burnetii*. (A) Calcifications were observed in severely infiltrated lesions (arrows). There was macrophage infiltration in the epicardium (EP) and myocardium (MC). The macrophages were expanded and packed with granules (arrowheads). Hematoxylin and eosin staining; magnification, ×200. (B) *C. burnetii* antigens were detected as brown granules (arrowheads). ABC method; magnification, ×400. EN, endocardium; V, ventricle. (C) *C. burnetii* antigen-positive cells were characteristic in glomeruli (arrowheads). ABC method; magnification, ×200.

TABLE 3. Survival, relative body weights, and organ weights of SCID mice infected with *C. burnetii*

Inoculum size ($TCID_{50}/0.5$ ml)	Mouse mortality (%)	Survival period (days)[a]	Relative body weight of dead mice[a]	Organ weight (mg) of dead mice[a]	
				Liver	Spleen
10^4	6/6 (100)	25.8 ± 1.7	0.73 ± 0.03	$1,606 \pm 250$	467 ± 111
10^3	5/5 (100)	28.8 ± 2.6	0.78 ± 0.06	$1,457 \pm 165$	629 ± 165
10^2	6/6 (100)	32.3 ± 0.5	0.81 ± 0.03	$1,586 \pm 135$	806 ± 118
10	6/6 (100)	34.8 ± 0.8	0.85 ± 0.04	$1,781 \pm 188$	999 ± 950
1	6/6 (100)	37.3 ± 2.3	0.86 ± 0.05	$1,696 \pm 293$	$1,018 \pm 144$
10^{-1}	6/6 (100)	36.3 ± 1.6	0.90 ± 0.04	$2,000 \pm 144$	$1,150 \pm 211$
10^{-2}	6/6 (100)	37.6 ± 0.8	0.89 ± 0.04	$1,783 \pm 132$	$1,107 \pm 238$
10^{-3}	4/6 (66.6)	41.0 ± 2.9	0.91 ± 0.05	$2,131 \pm 276$	$1,151 \pm 163$
10^{-4}	4/6 (66.6)	43.0 ± 2.5	0.94 ± 0.02	$2,144 \pm 193$	$1,251 \pm 290$
10^{-5}	1/6 (16.6)	47^b	0.89^b	$1,424^b$	$1,306^b$
PBS (control)	0/6 (0)	60^c	1.29^c	$1,185 \pm 87^c$	39 ± 9^c

[a] Mean $\pm$ standard deviation
[b] Data for one mouse.
[c] Data from euthanized mice.

The LD_{50} of *C. burnetii* in the SCID mice was at least 10^8 times less than that in the C.B-17 mice, none of which died after *C. burnetii* infection in this study. Despite the inoculum size, the SCID mice could not recover from the disease and died. The finding that hepatosplenomegaly was proportional to the survival period suggests that *C. burnetii* continued to proliferate in the bodies of the SCID mice. (The amount of *C. burnetii* in the spleen was ~10^7 $TCID_{50}/g$; other organs were not examined.) It has been suggested that immunosuppression can amplify the severity of Q fever (1, 21, 26, 27, 28). Our findings agree with this theory. Because the number of immunocompromised hosts is increasing with greater use of immunosuppression in modern medicine and with the spread of human immunodeficiency virus infection, an increasing number of people are at risk of acquiring Q fever.

The SCID mice in this study were probably acutely infected with *C. burnetii* and then experienced chronic disease. Our results support the importance of host factors in Q fever and contradict the hypothesis that certain strains of *C. burnetii* specifically cause acute or chronic disease. However, we cannot assume that all strains of *C. burnetii* have the same pathogenicity (10). We are presently investigating in more detail the distribution of *C. burnetii* in SCID mice and the pathogenicities of other *C. burnetii* isolates, which have different plasmid or gene patterns. We are also investigating the pathogenicity of *C. burnetii* associated with lipopolysaccharide variants during the phase variation reported earlier (12).

ACKNOWLEDGMENTS

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