Mouse Model of *Coxiella burnetii* Aerosolization

Cléa Melenotte,^{a,b} Hubert Lepidi,^a Claude Nappes,^a Yassina Bechah,^a Gilles Audoly,^a Jérôme Terras,^a Didier Raoult,^{a,b} Fabienne Brégeon^{a,c}

Aix Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Marseille, France^a; Pôle des Maladies Infectieuses et Tropicales Clinique et Biologique, Fédération de Bactériologie-Hygiène-Virologie, Centre Hospitalo-Universitaire Timone, Assistance Publique des Hôpitaux de Marseille, Marseille, France^b; Service des Explorations Fonctionnelles Respiratoires Centre Hospitalo-Universitaire Nord, Pôle Cardio-Vasculaire et Thoracique, Assistance Publique Hôpitaux de Marseille, Marseille, France^c

Coxiella burnetii is mainly transmitted by aerosols and is responsible for multiple-organ lesions. Animal models have shown *C. burnetii* pathogenicity, but long-term outcomes still need to be clarified. We used a whole-body aerosol inhalation exposure system to mimic the natural route of infection in immunocompetent (BALB/c) and severe combined immunodeficient (SCID) mice. After an initial lung inoculum of 10^4 *C. burnetii* cells/lung, the outcome, serological response, hematological disorders, and deep organ lesions were described up to 3 months postinfection. *C. burnetii*-specific PCR, anti-*C. burnetii* immunohistochemistry, and fluorescent *in situ* hybridization (FISH) targeting *C. burnetii*-specific 16S rRNA completed the detection of the bacterium in the tissues. In BALB/c mice, a thrombocytopenia and lymphopenia were first observed, prior to evidence of *C. burnetii* replication. In all SCID mouse organs, DNA copies increased to higher levels over time than in BALB/c ones. Clinical signs of discomfort appeared in SCID mice, so follow-up had to be shortened to 2 months in this group. At this stage, all animals presented bone, cervical, and heart lesions. The presence of *C. burnetii* could be attested *in situ* for all organs sampled using immunohistochemistry and FISH. This mouse model described *C. burnetii* Nine Mile strain spread using aerosolization in a way that corroborates the pathogenicity of Q fever described in humans and completes previously published data in mouse models. *C. burnetii* infection occurring after aerosolization in mice thus seems to be a useful tool to compare the pathogenicity of different strains of *C. burnetii*.

Q fever is a worldwide zoonosis caused by *Coxiella burnetii*, an intracellular bacterium. Cattle, goat, and sheep constitute the main reservoir for the bacteria (1). Humans contract the infection through the aerosol route, either from dust contaminated with the parturient fluid of the infected animal, or after ingestion of dairy products (1). In most cases, human contact with *C. burnetii* is poorly symptomatic and usually resolves spontaneously within a few weeks. Clinical manifestations of symptomatic primary infection are dominated by flu-like symptoms and/or pneumonia and hepatitis. In <5% of cases, persistent infection develops mainly as endocarditis and vascular infection (2).

Based on animal studies, the severity and pathogenicity of the infection seem to be related to the route of contamination and to the strain of *C. burnetii* (3). At the end of the 1930s, the “filter-passing agent” responsible for human Q fever was identified as a rickettsial pathogen by Burnet and Freeman after inoculation of infected material in mice, guinea pigs, and monkeys (1, 4). Soon after the isolation of the bacterium by Cox and Burnet, the pathogenicity of *C. burnetii* was studied in mice and guinea pigs, and this investigation was completed and enriched by Sidwell’s work in the 1960s (5–8). Since then, several animal models have been described using intraperitoneal, intranasal, or intratracheal routes and, more recently, using aerosolization (6–12). Intraperitoneal inoculation results in a short incubation time and a systemic distribution of the bacterium involving many deep organs such as heart, lung, lymph nodes, and bone marrow (9–11, 13, 14). Intratracheal or intranasal routes are, however, the models of choice in order to obtain pneumonia (15) but may be associated with a lower rate of systemic dissemination (14). The description of *C. burnetii* aerosolization models is recent (12) and lacks a standardized protocol and precision about initial lung bacterial burden. In addition, follow-up usually focuses on 15 days postinfection (p.i.).

Hematological response and the spread of infection in the lymphoid organs such as the lymph nodes and bone marrow are usually not targeted (11, 12, 16, 17).

Our aim was therefore to increase experimental data on *C. burnetii* infection by assessing long-term follow-up and describing broad organ damage in a rodent model mimicking the natural route of infection. To do so, we followed animals for 3 months after *C. burnetii* infection via the aerosol route and described its pathogenesis at the serological, hematological, and histological levels, including detection of the bacterium in tissues using immunohistochemistry, PCR, and fluorescent *in situ* hybridization (FISH).

MATERIALS AND METHODS

Ethics statement. The experimental protocol, registered by the French Ministry for Higher Education and Research under reference number 01085.01, had an obtained institutional ethics approval number (C2EA-14). All procedures were performed in accordance with European law and complied with Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines (<http://www.nc3rs.org.uk>). Animals were euthanized using blood removal by cardiac puncture under full general anes-

Received 16 February 2016 Returned for modification 18 March 2016
Accepted 28 April 2016

Accepted manuscript posted online 9 May 2016

Citation Melenotte C, Lepidi H, Nappes C, Bechah Y, Audoly G, Terras J, Raoult D, Brégeon F. 2016. Mouse model of *Coxiella burnetii* aerosolization. Infect Immun 84:2116–2123. doi:10.1128/IAI.00108-16.

Editor: C. R. Roy, Yale University School of Medicine

Address correspondence to Fabienne Brégeon, fabienne.bregeon@ap-hm.fr.

Copyright © 2016, American Society for Microbiology. All Rights Reserved.

thetia (Sevoflurane), followed by cervical dislocation. All of these experiments were performed in a biosafety level 3 laboratory of the Faculty of Medicine at Aix-Marseille University.

Strains, culture conditions, and the preparation of infective inocula.

C. burnetii Nine Mile phase I strain (RSA493 Nine Mile strain) was cultured in L929 cells. To produce phase I bacteria, $\sim 10^8$ CFU was inoculated intraperitoneally into BALB/cByJ mice. One week later, mice were euthanized, and their spleens were homogenized and inoculated onto L929 cells incubated in medium M4+2 at 35°C as described previously (18, 19). To maintain the bacteria in phase I and to produce a stock solution, three passages on L929 cells were performed. After 14 to 21 days of incubation, the percentage of infected cells (>90%) was determined. Monolayer cells were harvested and purified through three sonications and low-speed centrifugations. To eliminate the last cellular debris, two filtrations were performed (5- and 0.8- μ m-pore-size filters; Millipore, France). Aliquots (2 ml) were stored at -80°C .

C. burnetii phase II was obtained by subculturing the strain on L929 monolayers at 35°C. Once a week, the medium containing the infected cells was centrifuged. The pellet was discarded from the medium, and the medium containing the infected cells was stored at -80°C in 2-ml fractions before purification. The infected cells were then harvested, and the bacteria were purified using the technique described above. *C. burnetii* phase II was used for phase 2 antigen preparation.

For *in vivo* experiments, aliquots containing bacteria were thawed and the bacterial levels were adjusted to 10^7 *C. burnetii* phase I per ml. Serial dilutions were performed in Hanks balanced salt solution (HBSS) and 10 μ l was applied to an 18-well microscope slide (Thermo Cell-Line Diagnostic 6-mm-diameter well), fixed by heat for 15 min at 100°C , and stained using the Gimenez method (20). The precise concentrations were confirmed by visual counting after Gimenez staining.

In vivo experiments: general procedures and infection. Six-week-old BALB/c and SCID mice (Charles River Laboratories, l'Arbresle, France) weighing between 25 and 30 g were housed in individual plastic cages (five per cage) in a ventilated pressurized cabinet (A-BOX 160; Noroit, Rezé, France) with free access to water and standard diet food until the experiment took place. Aerosols were delivered using the whole-body aerosol inhalation exposure system (A4224 IES; Glas-Col LLC, Terre Haute, IN). To do this, animals ($n = 50$ BALB/c and $n = 50$ SCID mice) were gently placed and randomly dispatched into four steel baskets and then transferred into the nebulizing chamber. A total of 10^7 phase I bacteria were suspended in 5 ml of phosphate-buffered saline (PBS) and placed into a glass vial for liquid Venturi flow aerosol generation according to the manufacturer's recommendations. The total time exposure was 2 h, the cloud decay time was 30 min, and the UV decontamination time was 30 min. Control animals were aerosolized with PBS. Two animals per group were euthanized immediately after aerosolization to assess their initial lung bacterial burden. The others were transferred in a safety cabinet with food and water *ad libitum* until euthanasia.

Animal follow-up and tissue sampling. The animals were observed daily until 3 months p.i. to check their conditions and clinical status. Ten BALB/c and 10 SCID mice were euthanized at days 3, 7, 14, and 28 days p.i., and subsequently four additional animals of both groups were euthanized at 8 and 12 weeks p.i. Blood, lungs, spleens, livers, lymph nodes, and bone marrow were sampled at each period. Hearts were removed at a later stage (at 1, 2, and 3 months). Spleens were immediately weighed; blood was divided into aliquots for complete blood cell count (Horiba Medical, Irvine, CA), PCR, and serology assays. The organs removed from 6 of the 10 animals in each group were stored at -80°C for further PCR analysis, while those of the remaining 4 animals were fixed in 4% formalin.

Immunofluorescence assay. Purified bacterial phases I and II of *C. burnetii* used as antigens for antibody detection were inactivated by 5% formalin. Then, by using a pen nib, a droplet was deposited into each of 18 wells on glass slides and left to dry at room temperature. The slides were fixed in a methanol bath for 10 min, dried under a chemical safety cabinet, and then stored at 4°C until use. At starting serum dilutions of 1:25 for

IgM and 1:50 for IgG, sera were deposited onto the slides, followed by incubation at 37°C for 30 min in a wet chamber. Excess sera was removed using two 8-min consecutive baths with PBS–0.5% Tween and one 8-min bath with distilled water. The slides were dried at room temperature, and fixed antibodies were detected with a fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Immunotech, Marseille, France) or anti-mouse IgM (Jackson ImmunoResearch Laboratories, West Grove, PA) at a 1:400 dilution in PBS. Slides were observed using a Leica DM 2500 epifluorescence microscope (Leica, Wetzlar, Germany) at 488-nm wavelengths (21). As positive and negative controls, sera from immunized and nonimmunized mice were included in each run.

Molecular detection. Whole lungs and whole spleens were homogenized in PBS, and DNA from lung suspensions (200 μ l), spleen suspensions (100 μ l), and blood (200 μ l) was extracted using a QIAamp tissue kit (Qiagen) in a 100- μ l final volume. Quantitative real-time PCR (qPCR) was performed using the CFX96 qPCR detection system (Bio-Rad, France), and carried out with 5 μ l of DNA extract and specific primers and a probe targeting a fragment of the *C. burnetii* 16S DNA gene, as previously described (22), and IS1111 (23, 24). Negative controls consisted of DNA extracted from the organs and blood of PBS-challenged mice. In each qPCR run, a standard curve was generated using 10-fold serial dilution of a known concentration of *C. burnetii* DNA.

Organ sample processing for pathological examination. Each organ that was removed was fixed with buffered formalin 4% and embedded in paraffin. Serial sections (3- μ m) of these specimens were obtained for routine hematoxylin-eosin-saffron staining, immunohistochemistry investigations (rabbit anti-*C. burnetii* antibody at a 1:2,000 dilution) (12, 25), and FISH. For each tissue section, a negative-control experiment was performed with normal rabbit serum.

FISH. We used two specific probes targeting *C. burnetii* 16S rRNA, CB-440 and CB-1348, and the probe EUB-338, which is specific for most eubacteria (26). Non-EUB-338 was used to exclude nonsense hybridization. The *in situ* hybridization assays were performed based on previous reports (27, 28). To minimize nonspecific hybridization, a prehybridization step ($2\times$ SSC [$1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 10% formamide) at room temperature for 15 min was performed. The slides were rinsed in 0.1% PBS and then incubated at 37°C overnight with 15 to 30 μ l of hybridization solution ($2\times$ hybridization buffer [final, $1\times$], 10% formamide, 200 mM H_2O , Ribo-Vanadyl complex [final, 10 mM], salmon sperm, and probes). The slides were washed after hybridization with three baths using $2\times$ SSCT (saline sodium citrate, 0.1% Tween 20), one bath using $1\times$ SCCT, and one bath using $0.5\times$ SCCT for 5 min each and then rinsed with distilled water. Sudan black B staining dye was applied before nucleic acid staining with DAPI (4',6'-diamidino-2-phenylindole) from a ready-to-use solution (ProLong Gold antifade reagent; Molecular Probes, Cergy-Pontoise, France) (29, 30). The imaging system was driven by Leica MetaMorph (version 1.6.0; Molecular Devices, Sunnyvale, CA). L929 cells infected with *C. burnetii* and tissues from PBS-treated mice were used as positive and negative controls, respectively, as shown in Fig. 1.

Statistical analysis. The variables are expressed as means $\pm$ the standard deviations when the distribution was normal or as medians when the distribution was not normal. To compare BALB/c mice to SCID mice when the distribution was normal, a Student *t* test was performed; in other cases, we used a Mann-Whitney test. Rates and proportions were compared using a chi-squared test, applying the appropriate Yates correction.

RESULTS

Signs of *C. burnetii* infection. Tolerance of the infection appeared to be good during the first month, with no spontaneous deaths or symptoms of discomfort in either BALB/c or SCID mice. Thereafter, SCIDs exhibited progressive asthenia. At 2 months, one SCID mouse died, and the other three presented ruffled fur and lethargy. All were therefore euthanized according to the ethical limitations. The BALB/c mice appeared to remain healthy

FIG 1 FISH targeting *C. burnetii* 16S rRNA. (A1) Controls assayed: L929 cells infected with *C. burnetii*. Blue indicates the DAPI-stained nuclei of L929 cells. Yellow signal indicates colocalization of the *C. burnetii*-specific 16S rRNA probe (green) and the universal 16S probe EUB (red). *C. burnetii* appears as a perinuclear, rounded, and vacuolar signal. Magnification, $\times 100$. (A2) Negative control: SCID mice aerosolized with PBS in the liver. Magnification, $\times 100$. (A3) Negative control: SCID mice aerosolized with PBS in the lung. Magnification, $\times 100$. (B to D) Infected tissues of a SCID mouse at 2 months. DAPI-stained nuclei appear in blue. A positive signal appears in yellow, reflecting colocalization of the *C. burnetii*-specific 16S rRNA probe (green) and the universal EUB probe (red). (B1) Lung. Alveoli are recognized, as defined by the pneumocytes. (B2) Lung. On the right, *C. burnetii* can be positively identified, presumably in an alveolar macrophage. A positive signal appears as a rounded perinuclear intracytoplasmic and vacuolar signal. (C1 and C2) Liver. Multiple and diffuse rounded signals are evident. (D1 to D3) Heart. A positive signal can be seen in the pericardium (D1 and D2) and myocardium (D1 and D3).

FIG 2 Changes in spleen weight over time in BALB/c and SCID mice. The asterisk indicates a significant intragroup difference ($P < 0.05$) from day 3.

until the end of the follow-up and had antibodies to *C. burnetii* phase I and II antigens detectable from day 14 p.i. (titers from 1:200 to 1:800). The sera were negative for *C. burnetii* antibody detection in all controls and in SCID mice.

Baseline spleen weight of SCID mice tended to be lower than that of BALB/c mice (means $\pm$ the standard deviations: 0.10 ± 0.01 g for BALB/c mice versus 0.05 ± 0.00 g for SCID mice; $P =$ not significant). After infection, spleen weight remained stable throughout the experiment in BALB/c mice, whereas a splenomegaly appeared in the SCID mice, reaching 1.6 ± 0.5 g at 2 months ($P < 0.0001$ versus the other times) (Fig. 2).

Blood cell count. In BALB/c mice, we observed a transient thrombocytopenia at day 3 (from $1,000 \times 10^9$ cells/liter at baseline to $<500 \times 10^9$ cells/liter) and then a return to the baseline value at day 28, whereas the hemoglobin and hematocrit levels remained

FIG 3 PCR results in different organs over time in BALB/c and SCID mice. Asterisks indicate significant intragroup differences ($P < 0.05$) from day 3.

FIG 4 Representative pathological and immunohistological findings for organs from *C. burnetii*-infected SCID mice at 2 months p.i. *C. burnetii* infection via aerosol induced an important macrophage infiltration in tissues, as shown by hematoxylin-eosin-saffron staining (figures on the left). Bacteria were visualized by immunostaining with a polyclonal rabbit anti-*C. burnetii* antibody used at a dilution of 1:2,000, along with hemalun counterstain (figures on the right). Macrophages were packed with coarse granular immunopositive material. (Lung images) Note the macrophage infiltration of the alveolar walls with several intra-alveolar macrophages easily identified by immunostaining. (Liver images) Macrophage infiltration is randomly distributed in liver parenchyma and mixed with necrotic areas. Note the great number of immuno-

stable at all endpoints. A lymphopenia and a monocytopenia were observed at day 7 and regressed completely at day 14. In SCID mice, the occurrence of thrombocytopenia was not significant, but a monocytosis was observed between days 14 and 28.

Molecular detection of *C. burnetii*. This model of aerosol delivery resulted in a fairly standardized lung inoculation since the initial lung bacterial burdens, immediately after aerosol delivery, were similar in the four animals, ranging from 3.85×10^3 to 1.74×10^4 DNA copies/lung [means $\pm$ the standard deviations = $(1.15 \times 10^4) \pm (5.63 \times 10^3)$ DNA copies/lung].

Globally, during the first week, the initial kinetics of *C. burnetii* detection by qPCR were quite similar in SCID and BALB/c mice. A more pronounced response occurred in SCID mice from the second week until the end of the protocol. For both groups, we detected a systemic dissemination with positive PCR results in the spleen and blood, which occurred more frequently (all animals at day 28) and was more intense in SCID mice. Figure 3 shows the changes in DNA copies over time in various organs in BALB/c and SCID mice. By day 3 after infection, the lung bacterial burden tended to increase from the baseline level in SCID mice, whereas it decreased in BALB/c mice. At this early time, some animals of both groups yielded positive PCR results in the blood, spleen, liver, bone marrow, and lymph nodes. No positive PCR detection was obtained in any control sample.

Histopathological findings. Granulomas, as an inflammatory response against *C. burnetii* infections, mainly consisted of macrophages. Granuloma formation is indicative of a protective immune response to *C. burnetii* and is defective in SCID mice. In BALB/c mice, all of the removed organs were globally preserved, since only one mouse presented a few granulomas at day 14 p.i. These granulomas were seen only in the livers and spleens and were paucibacillary with the immunohistological detection of *C. burnetii* antigens. In SCID mice, organ lesions were delayed compared to PCR detection, and began to be detected from day 14 p.i. in the lung, liver, and spleen (Fig. 4). From day 28 to 2 months p.i., all of the SCID mice analyzed presented a significant macrophage infiltration in the lung, liver, and spleen and necrotic areas. In contrast to BALB/c mice, the immunohistological detection of *C. burnetii* in the lung, liver and spleen of SCID mice showed a multibacillary aspect, with a high bacterial load in cells with macrophage morphology (Fig. 4, lung, liver, and spleen). At 2 months p.i., the hearts from four SCID mice showed histological lesions compatible with pericarditis, myocarditis and endocarditis. One of these animals died before planned euthanasia and presented a splenic infarct very likely caused by splenic emboli (Fig. 4). The immunohistological detection of *C. burnetii* in cells infiltrating the heart tissue was strongly positive (Fig. 4, heart). Finally, the bone marrow and lymph nodes were strongly infiltrated by immunopositive macrophages from 2 months p.i. only in SCID mice (Fig. 4, lymph node and bone).

FISH. No positive signal was detected in controls. Presumably, viable *C. burnetii* was identified in abnormal organs of infected

positive macrophages. (Spleen images) Macrophage infiltration is seen mainly in the red pulp. Note the great number of immunopositive macrophages. (Heart images) Note the areas of macrophage infiltration in the pericardium (arrowhead), the myocardium (star), and a cardiac valve (arrow). (Lymph node and bone marrow images) Note the significant infiltration by numerous and highly immunopositive macrophages.

animals (Fig. 1): two colocalized signals, one targeting the *C. burnetii* 16SrRNA (green) and the other targeting the universal 16S probe (red), could be observed.

C. burnetii appeared as multiple rounded intracytoplasmic structures around the DAPI-stained nuclei. These signals were observed in the lungs, concentrated near the vessels, in the interstitial infiltrate or within an alveolar macrophage. In the liver, signals were observed within granulomas and in the sinusoid vessels; in the spleen, signals were observed in the white pulp; in the bones and lymph nodes, signals were diffuse.

DISCUSSION

We developed a mouse model to induce *C. burnetii* infection via the aerosol route and described broad dissemination of the infection leading to a fatal outcome in SCID mice, whereas nonimmunosuppressed animals were able to recover. Our study provides additional data on the systemic pathogenicity of *C. burnetii* and completes the description with long-time follow-up after the natural route of infection.

Immediate lung bacterial load was $\sim 10^4$ bacteria per animal lung, a value is close to that obtained in the aerosol model described by Schoffelen et al. (17). Previously published studies using aerosols stopped their investigations at day 14 p.i., whereas those using the intraperitoneal route continued for up to 730 days (11–13). Here, follow-up was conducted up to 3 months, which could be reached by BALB/c mice only.

In line with previous reports involving aerosolization of Nine Mile strain in BALB/c mice, our results exhibited *C. burnetii* lesions in the lungs and deep organs but fairly stable spleen weight (12). In contrast, Schoffelen et al. obtained clinical signs of severe infection and splenomegaly in BALB/c mice infected with aerosols of the Nine Mile *C. burnetii* strain (17). The animal laboratory providers differed between Schoffelen's study and ours. Because several substrains of BALB/c mice are available and are known to differ in their susceptibilities to infection and host-pathogen responses, we think it likely that the substrain of BALB/c mice used by Schoffelen et al. was more susceptible to *C. burnetii* infection than the ones from the present work, thus possibly explaining the discrepancies between the two reports. Interestingly, the first manifestation in BALB/c mice was a hematological response, such as thrombocytopenia, lymphocytopenia, and monocytopenia from day 3, correlated to *C. burnetii* DNA detection in deep organs and attesting to systemic spread. In SCID mice, thrombocytopenia was less severe, and monocytes increased significantly from day 0 to day 28. Lymphopenia and thrombocytopenia were previously described in mice using the intraperitoneal route and in cynomolgus monkeys, which developed a *C. burnetii* infection very close to that observed in humans (10, 31).

As expected, SCIDs had earlier and more intense lesions in line with larger amounts of *C. burnetii* DNA copies. Moreover, the kinetics of the infection showed progressive impairment until the ethical limit was reached (at 2 months). Immunohistological detection of *C. burnetii* in cells infiltrating the tissues was strongly positive, indicating a broad dissemination of the bacterium. Bone marrow and lymph node involvement in *C. burnetii* pathogenicity was first described in 1993 by Baumgärtner et al. using immunohistochemistry in BALB/c mice 3 days after intraperitoneal infection (10): bone marrow lesions were characterized by granulomas and the depletion of hematopoietic

cells. In our aerosolization model, only SCID mice presented bone marrow lesions with diffuse macrophagic infiltrates, observed only from day 28. The bone marrow tropism of *C. burnetii* observed experimentally could fit with the hemophagocytic syndrome, ring granuloma, and hairy cell leukemia observed in humans (32–35). Lymph node involvement during Q fever has recently been described in humans as lymphadenitis and lymphoma (36). In the present study, we report early and persistent lymph node *C. burnetii* detection (qPCR) in both BALB/c and SCID mice. Taken together, the data obtained in mice and from clinical cases should drive forward a new way of investigation to study lymphomagenesis during *C. burnetii* infection.

Endocarditis was diagnosed in all SCID animals at 2 months p.i. In line with clinical patterns (37), our model was able to produce cardiac infections compatible with the valvular affinity of *C. burnetii* in immunosuppressed animals, even in the absence of preexisting valvular disease. Cardiac involvement has been well described in guinea pigs (38) and after intraperitoneal infection in mice (39). Originally, we described *C. burnetii* endocarditis 2 months after aerosol infection in mice (13). Atzpodien et al. described myocardium, endocardium, and valvular inflammatory infiltrates 10 days after peritoneal infection, resolving at day 150 (13, 39, 42). Interestingly, *C. burnetii*-related transient endocarditis has recently been published in humans combined with antiphospholipid syndrome (39). Here, nonimmunosuppressed animals appeared to clear the bacterial infection and develop self-limited disease, whereas immunosuppressed animals reached the limit point at 2 months p.i. The life span of SCID mice is known not to exceed 8.5 months (34 weeks) under specific-pathogen-free conditions and, in the present study, SCID mice were 14 weeks old at the last time of evaluation. Reducing the bacterial inoculum in this group may have permitted a longer follow-up time to assess the chronic infection condition. However, given the large diffusion of the infection, including bone and heart sites in SCID animals infected for a duration of about one-quarter of their expected life spans, we think we were not far from the chronic picture.

FISH has been used in veterinary reports to detect *C. burnetii* in ruminant placentas using both IS1111 and 16S rRNA probes (40, 41). Its use to detect *C. burnetii* in human lymph nodes has only recently been published (34). Originally, we reported *C. burnetii*-specific 16S rRNA detection by FISH, reflecting the bacterium replication in a mouse model and presumably viable *C. burnetii* in the lung, liver, spleen, bone, and lymph nodes, enhancing immunohistochemistry findings.

The whole-body aerosol inhalation procedure may also have favored digestive inoculation. Since we did not focus on the digestive organs, the question of whether the systemic dissemination of the infection only comes from the lung remains uncertain. However, our model showed reproducible lung bacterial burden and appears to be a reliable way to study *C. burnetii* pathogenicity. Thus, it appears to be a useful tool for investigating the comparative pathogenicities of different strains of *C. burnetii*, as well as for studying *C. burnetii* lymphomagenesis.

ACKNOWLEDGMENTS

We thank Muriel Milittello and Nathalie Wurtz for the NSB3 maintenance. We thank Elsa Prudent for cutting the histological slides used for FISH.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

- Tissot-Dupont H, Raoult D. 2008. Q fever. *Infect Dis Clin North Am* 22:505–514. <http://dx.doi.org/10.1016/j.idc.2008.03.002>.
- Maurin M, Raoult D. 1999. Q fever. *Clin Microbiol Rev* 12:518–553.
- Glazunova O, Roux V, Freylikman O, Sekeyova Z, Fournous G, Tyczka J, Tokarevich N, Kovacava E, Marrie TJ, Raoult D. 2005. *Coxiella burnetii* genotyping. *Emerg Infect Dis* 11:1211–1217.
- Burnet FM, Freeman M. 1937. Experimental studies on the virus of “Q” fever. *Med J Austral* 1937:299–305.
- Burnet FM, Freeman M. 1938. The rickettsia of “Q” fever: further experimental studies. *Med J Austral* 1938:296–298.
- Sidwell RW, Thorpe BD, Gebhardt LP. 1964. Studies on latent Q fever infections. I. Effects of whole body x-irradiation upon latently infected guinea pigs, white mice, and deer mice. *Am J Hyg* 1964:113–124.
- Sidwell RW, Thorpe BD, Gebhardt LP. 1964. Studies of latent Q fever infections. II. Effects of multiple cortisone injections. *Am J Hyg* 1964:320–327.
- Sidwell RW, Gebhardt LP. 1950. Q fever antibody response in experimentally infected wild rodents and laboratory animals. *J Immunol* 89:318–322.
- Meghari S, Bechah Y, Capo C, Lepidi H, Raoult D, Murray PJ, Mege JL. 2008. Persistent *Coxiella burnetii* infection in mice overexpressing IL-10: an efficient model for chronic Q fever pathogenesis. *PLoS Pathog* 4:e23. <http://dx.doi.org/10.1371/journal.ppat.0040023>.
- Baumgärtner W, Dettinger H, Schmeer N. 1993. Spread and distribution of *Coxiella burnetii* in C57BL/6J(H-2b) and BALB/c(H-2d) mice after intraperitoneal infection. *J Comp Pathol* 108:165–184. [http://dx.doi.org/10.1016/S0021-9975\(08\)80219-8](http://dx.doi.org/10.1016/S0021-9975(08)80219-8).
- Elliott A, Peng Y, Zhang G. 2013. *Coxiella burnetii* interaction with neutrophils and macrophages in vitro and in SCID mice following aerosol infection. *Infect Immun* 81:4604–4614. <http://dx.doi.org/10.1128/IAI.00973-13>.
- Stein A, Louveau C, Lepidi H, Ricci F, Baylac P, Davoust B, Raoult D. 2005. Q fever pneumonia: virulence of *Coxiella burnetii* pathovars in a murine model of aerosol infection. *Infect Immun* 73:2469–2477. <http://dx.doi.org/10.1128/IAI.73.4.2469-2477.2005>.
- Stein A, Lepidi H, Mege JL, Marrie TJ, Raoult D. 2000. Repeated pregnancies in BALB/c mice infected with *Coxiella burnetii* cause disseminated infection, resulting in stillbirth and endocarditis. *J Infect Dis* 181:188–194. <http://dx.doi.org/10.1086/315166>.
- Marrie TJ, Stein A, Janigan D, Raoult D. 1996. Route of infection determines the clinical manifestations of acute Q fever. *J Infect Dis* 173:484–487. <http://dx.doi.org/10.1093/infdis/173.2.484>.
- Khavkin T, Tabibzadeh SS. 1988. Histologic, immunofluorescence, and electron microscopic study of infectious process in mouse lung after intranasal challenge with *Coxiella burnetii*. *Infect Immun* 56:1792–1799.
- Russell-Lodrigue KE, Andoh M, Poels MWJ, Shive HR, Weeks BR, Zhang GQ, Tersteeg C, Masegi T, Hotta A, Yamaguchi T, Fukushi H, Hirai K, McMurray DN, Samuel JE. 2009. *Coxiella burnetii* isolates cause genogroup-specific virulence in mouse and guinea pig models of acute Q fever. *Infect Immun* 77:5640–5650. <http://dx.doi.org/10.1128/IAI.00851-09>.
- Schoffelen T, Self JS, Fitzpatrick KA, Netea MG, van Deuren M, Joosten LAB, Kersh GJ. 2015. Early cytokine and antibody responses against *Coxiella burnetii* in aerosol infection of BALB/c mice. *Diagn Microbiol Infect Dis* 81:234–239. <http://dx.doi.org/10.1016/j.diagmicrobio.2014.12.008>.
- Fournier PE, Marrie TJ, Raoult D. 1998. Diagnosis of Q fever. *J Clin Microbiol* 36:1823–1834.
- Raoult D, Vestris G, Enea M. 1990. Isolation of 16 strains of *Coxiella burnetii* from patients by using a sensitive centrifugation cell culture system and establishment of the strains in HEL cells. *J Clin Microbiol* 28:2482–2484.
- Gimenez DF. 1964. Staining rickettsiae in yolk-sac cultures. *Stain Technol* 39:135–140. <http://dx.doi.org/10.3109/10520296409061219>.
- Dupont HT, Thirion X, Raoult D. 1994. Q fever serology: cutoff determination for microimmunofluorescence. *Clin Diagn Lab Immunol* 1:189–196.
- Bechah Y, Verneau J, Ben Amara A, Barry AO, Lépolard C, Achard V, Panicot-Dubois L, Textoris J, Capo C, Ghigo E, Mege JL. 2014. Persistence of *Coxiella burnetii*, the agent of Q fever, in murine adipose tissue. *PLoS One* 9:e97503. <http://dx.doi.org/10.1371/journal.pone.0097503>.
- Edouard S, Mahamat A, Demar M, Abboud P, Djossou F, Raoult D. 2014. Comparison between emerging Q fever in French Guiana and endemic Q fever in Marseille, France. *Am J Trop Med Hyg* 90:915–919. <http://dx.doi.org/10.4269/ajtmh.13-0164>.
- Eldin C, Angelakis E, Renvoisé A, Raoult D. 2013. *Coxiella burnetii* DNA, but not viable bacteria, in dairy products in France. *Am J Trop Med Hyg* 88:765–769. <http://dx.doi.org/10.4269/ajtmh.12-0212>.
- Lepidi H, Houpihan P, Liang Z, Raoult D. 2003. Cardiac valves in patients with Q fever endocarditis: microbiological, molecular, and histologic studies. *J Infect Dis* 187:1097–1106. <http://dx.doi.org/10.1086/368219>.
- Amann RI, Krumholz L, Stahl DA. 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *J Bacteriol* 172:762–770.
- Atieh T, Audoly G, Hraiech S, Lepidi H, Roch A, Rolain J-M, Raoult D, Papazian L, Brégeon F. 2013. Evaluation of the diagnostic value of fluorescent *in situ* hybridization in a rat model of bacterial pneumonia. *Diagn Microbiol Infect Dis* 76:425–431. <http://dx.doi.org/10.1016/j.diagmicrobio.2013.04.028>.
- Audoly G, Vincentelli R, Edouard S, Georgiades K, Mediannikov O, Gimenez G, Socolovschi C, Mège JL, Cambillau C, Raoult D. 2011. Effect of rickettsial toxin VapC on its eukaryotic host. *PLoS One* 6:e26528. <http://dx.doi.org/10.1371/journal.pone.0026528>.
- Corrêa FM, Innis RB, Rouot B, Pasternak GW, Snyder SH. 1980. Fluorescent probes of alpha- and beta-adrenergic and opiate receptors: biochemical and histochemical evaluation. *Neurosci Lett* 16:47–53. [http://dx.doi.org/10.1016/0304-3940\(80\)90099-3](http://dx.doi.org/10.1016/0304-3940(80)90099-3).
- Schnell SA, Staines WA, Wessendorf MW. 1999. Reduction of lipofuscin-like autofluorescence in fluorescently labeled tissue. *J Histochem Cytochem* 47:719–730. <http://dx.doi.org/10.1177/002215549904700601>.
- Waag DM, Byrne WR, Estep J, Gibbs P, Pitt ML, Banfield CM. 1999. Evaluation of cynomolgus (*Macaca fascicularis*) and rhesus (*Macaca mulatta*) monkeys as experimental models of acute Q fever after aerosol exposure to phase-I *Coxiella burnetii*. *Lab Anim Sci* 49:634–638.
- Dugdale C, Chow B, Yakirevich E, Kojic E, Knoll B. 2014. Prolonged pyrexia and hepatitis: Q fever. *Am J Med* 127:928–930. <http://dx.doi.org/10.1016/j.amjmed.2014.06.003>.
- Herndon G, Rogers HJ. 2013. Multiple “doughnut” granulomas in *Coxiella burnetii* infection (Q fever). *Blood* 122:3099. <http://dx.doi.org/10.1182/blood-2013-06-511063>.
- Alwis L, Balan K, Wright P, Lever A, Carmichael A. 2009. Bone marrow involvement in Q fever detection by fluorine-18-labeled fluorodeoxyglucose PET. *Lancet Infect Dis* 9:718. [http://dx.doi.org/10.1016/S1473-3099\(09\)70113-6](http://dx.doi.org/10.1016/S1473-3099(09)70113-6).
- Lee WY, Lee JM, Park KH, Park C, Chang M, Hong WP, Park IY. 1995. *Coxiella burnetii* in polymorphic lymphocytes in tissue and blood of patients with polymorphic reticulosis. *Acta Virol* 39:269–274.
- Melenotte C, Million M, Audoly G, Gorse A, Dutronc H, Roland G, Dekel M, Moreno A, Cammilleri S, Carrieri MP, Protopenescu C, Ruminy P, Lepidi H, Nadel B, Mege JL, Xerri L, Raoult D. 2016. B-cell non-Hodgkin lymphoma linked to *Coxiella burnetii*. *Blood* 127:113–121. <http://dx.doi.org/10.1182/blood-2015-04-639617>.
- Million M, Walter G, Thuny F, Habib G, Raoult D. 2013. Evolution from acute Q fever to endocarditis is associated with underlying valvulopathy and age and can be prevented by prolonged antibiotic treatment. *Clin Infect Dis* 57:836–844. <http://dx.doi.org/10.1093/cid/cit419>.
- La Scola B, Lepidi H, Maurin M, Raoult D. 1998. A guinea pig model for Q fever endocarditis. *J Infect Dis* 178:278–281. <http://dx.doi.org/10.1086/517453>.
- Million M, Thuny F, Bardin N, Angelakis E, Edouard S, Bessis S, Guimard T, Weitten T, Martin-Barbaz F, Texereau M, Ayouz K, Protopenescu C, Carrieri P, Habib G, Raoult D. 2016. Antiphospholipid antibody syndrome with valvular vegetations in acute Q fever. *Clin Infect Dis* 62:537–544. <http://dx.doi.org/10.1093/cid/civ956>.

40. Hansen MS, Rodolakis A, Cochonneau D, Agger JF, Christoffersen A-B, Jensen TK, Agerholm JS. 2011. *Coxiella burnetii* associated placental lesions and infection level in parturient cows. Vet J 190:e135–e139. <http://dx.doi.org/10.1016/j.tvjl.2010.12.021>.
41. Jensen TK, Montgomery DL, Jaeger PT, Lindhardt T, Agerholm JS, Bille-Hansen V, Boye M. 2007. Application of fluorescent in situ hybridisation for demonstration of *Coxiella burnetii* in placentas from ruminant abortions. APMIS 115:347–353. http://dx.doi.org/10.1111/j.1600-0463.2007.apm_591.x.
42. Atzpödien E, Baumgärtner W, Artelt A, Thiele D. 1994. Valvular endocarditis occurs as a part of a disseminated *Coxiella burnetii* infection in immunocompromised BALB/cJ (*H-2d*) mice infected with the nine mile isolate of *C. burnetii*. J Infect Dis 170:223–226. <http://dx.doi.org/10.1093/infdis/170.1.223>.