

Overview

Animal Models of Q Fever (*Coxiella burnetii*)

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Q fever, caused by the pathogen *Coxiella burnetii*, is an acute disease that can progress to become a serious chronic illness. The organism leads an obligate, intracellular lifecycle, during which it multiplies in the phagolytic compartments of the phagocytic cells of the immune system of its hosts. This characteristic makes study of the organism particularly difficult and is perhaps one of the reasons why, more than 70 y after its discovery, much remains unknown about the organism and its pathogenesis. A variety of animal species have been used to study both the acute and chronic forms of the disease. Although none of the models perfectly mimics the disease process in humans, each opens a window onto an important aspect of the pathology of the disease. We have learned that immunosuppression, overexpression of IL10, or physical damage to the heart muscle in mice and guinea pigs can induce disease that is similar to the chronic disease seen in humans, suggesting that this aspect of disease may eventually be fully understood. Models using species from mice to nonhuman primates have been used to evaluate and characterize vaccines to protect against the disease and may ultimately yield safer, less expensive vaccines.

Coxiella burnetii is the causative agent of human Q fever. Infection can take several forms and has been described as clinically polymorphic.⁶ In humans, presentation ranges from asymptomatic, through acute disease, to chronic illness. In the majority of cases, acute disease presents as a self-limiting febrile illness, with half of cases also having severe headaches.⁸⁸ In severe cases of acute disease, atypical pneumonia is often found.⁸⁸ A small proportion (2% to 4%) of subjects with symptomatic acute Q fever are admitted to hospital.^{70,88} Chronic disease may develop in approximately 5% of those infected;¹⁶ the vast majority of these cases will present as a bacterial culture-negative endocarditis^{16,22} often in those with predisposing heart-damage¹⁹ or immunosuppression.¹⁶ Without effective treatment, Q fever endocarditis is generally fatal, but early diagnosis coupled with novel treatment strategies has brought the death rate down to less than 5%.⁶⁹ The 2009 outbreak in the Netherlands involved 2357 human cases, of which more than 400 required hospitalization.⁹⁰ The animal cost in the Netherlands was far higher, with more than 50,000 pregnant goats culled in an attempt to control the epidemic.⁸²

Two other clinical manifestations of Q fever are worthy of mention owing to their less-than-satisfactory outcomes with current treatment strategies. These are Q fever during pregnancy and Q fever fatigue syndrome. *C. burnetii* infection during pregnancy results in premature delivery in almost half of those affected and spontaneous abortion in more than a quarter.¹⁴ There have been few studies in this area, but there are indications that among those infected during the first trimester and treated suboptimally, the abortion rate is 100%.⁶⁸ This effect is compounded by the fact that the frontline bactericidal drugs for treatment (doxycycline and hydroxychloroquine) are contraindicated for use during pregnancy.⁶⁸

A bacteriostatic regimen (cotrimoxazole) has therefore been proposed for use⁶⁸ until delivery. Without satisfactory treatment during and after pregnancy, there is also a high probability for infection to lead to chronic Q fever: an incidence of 70% was reported in a group of pregnant women in France.⁶⁸

Post-Q fever fatigue syndrome was first reported in 1996,⁵² but an association between Q fever and chronic fatigue had been observed as early as 1982.⁵² Between 10% and 15% of those who have had acute Q fever develop a chronic fatigue syndrome that can last between 5 and 10 y—and even longer in some cases.⁵³ Some of these patients have been found to have long-term persistence of *C. burnetii* cell components and LPS associated with traces of genomic DNA,⁵³ suggesting that Q fever fatigue syndrome may be immunologically mediated rather than caused by the organism directly.

Q fever is a zoonosis that has been described worldwide,⁵⁶ and human outbreaks are often associated with contact with the birth products of farm animals.⁵⁶ However, outbreaks associated with the birth products of domestic cats have also been reported.⁵⁴ Human infection primarily occurs via the inhalation of infectious aerosols.⁵⁶ Over the past 10 y, outbreaks have been reported in the Netherlands,⁷¹ Slovenia,²⁶ the United Kingdom,^{91,97,99} Israel,² Iraq,¹⁸ the United States,¹¹ Germany,²⁴ Bulgaria,⁶³ Croatia,⁵⁸ Spain,²³ Italy,⁸³ and France.⁸⁸

A very small number of *C. burnetii* organisms can cause infection by inhalation. Infection has been predicted to be possible after exposure to only a single organism.³³ This low dosage, coupled with the organism's ability to cause debilitating disease and high levels of resistance to various means of inactivation^{67,77,78} have resulted in it being listed as a category B biologic warfare and bioterrorism agent by the Centers for Disease Control.⁴⁹

Prevention of Q fever in man can be achieved by vaccination; the only vaccine available for general use is Q-Vax, which was licensed in Australia in 1989.⁵¹ This vaccine consists of formalin-inactivated

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C. burnetii whole cells, produced in chick embryos. Its use has been associated with severe local reactions in those with preexisting immunity. As a precaution, prevaccination screening (history, skin test, and serology) must therefore be performed prior to administration.³⁵ Despite this safeguard, severe local reactions to vaccination are reported.⁴⁴ The vaccine is also hazardous to produce, with the organism requiring culture in chick-embryos at biosafety level 3 prior to inactivation.⁵¹ There is, therefore, a need for a vaccine that is safer to produce and safer to use and that does not require prevaccination screening.

The organism displays antigenic phase variation often paralleled with the rough-smooth variation seen in *Enterobacteriaceae*. In *C. burnetii*, phase variation has been demonstrated to be due to differences in LPS. Phase I has been shown to contain a unique disaccharide galactosaminuronyl glucosamine and 9 unidentified components in addition to the components of phase II LPS.¹ Organisms with the phase I phenotype are the infectious and virulent form found in the environment. Organisms with the phase II phenotype are observed only during repeated subculture in laboratory chick embryo or cell culture systems;²⁷ they have a chemically simpler LPS¹ and several deletions in the genome.^{32,92} Phagocytosis of phase I, but not phase II, organisms by macrophages involves an interaction between the bacterial LPS and Toll-like receptor 4. This mechanism also stimulates F-actin reorganization of the host cells and stimulates the release of type 1 cytokines including IFN γ and TNF.³⁰ This interaction appears important in the initial priming of the immune response and could provide an explanation for the limited protection of vaccines based on potential virulence genes (*omp1*, *HspB*, *Pmm*, *Fbp*, *Orf 410*, *Crc*, *CbMip*, *MucZ*, *P28*) singly and in combinations but containing no LPS.^{47,89,102}

In addition to its antigenic phase variation, *C. burnetii* occurs in 2 morphologic forms, a large-cell variant and a small-cell variant. These forms differ antigenically due to differences in the proteins expressed on their surface. It has been suggested that the resistance of *C. burnetii* to host defense mechanisms may be enhanced by antigenic differences between the different developmental forms.^{57,94} The small-cell morphologic form is highly resistant to destruction by chemical and environmental factors and is likely the transmissible form of the pathogen.^{15,67} After infection, which generally occurs by inhalation of the small-cell form, the organisms are taken up by host alveolar macrophages.⁸¹ Morphogenesis from the small-cell to large-cell form then occurs, the large-cell variant being the replicative form of the organism.¹⁵ These organisms then replicate within parasitophorous vacuoles.⁵⁰ As the organisms enter the stationary phase of their growth within the cell, they condense back into the small-cell form.¹⁵ During replication within the host cell, the organism subverts cellular processes though active mechanisms to avoid and modify the host immune response.⁵⁰ *C. burnetii* possesses a type IV secretion system, and the proteins that cause this subversion are likely delivered to the host cell by this machinery.^{50,93}

Because *C. burnetii* is an obligate intracellular organism, it has only been possible to study the organism within living animal hosts. Host-cell-free growth of the organism has been reported recently,⁶² but the technique has yet to be exploited fully. Cell-culture-based in vitro systems remain limited in the study of *C. burnetii*, given that the organism soon reverts to the avirulent (at least in immunocompetent hosts) phase II form (characterized by the loss of the phase I LPS phenotype) in these systems.¹⁰ A key

problem in comparing models of *C. burnetii* infection is related to the organism's intracellular nature, which complicates attempts to count the organisms used for infection. The literature reflects this difficulty in the fact that there are many different methods used (including plaque assay in primary cell cultures, median infectious doses in chick eggs or mice, and median lethal dose in SCID mice) and no way to directly compare them.

Host Range

In nature, *C. burnetii* has been detected in species throughout the animal kingdom,⁹ including wild and livestock mammals as well as invertebrates, such as ticks. Animals are often asymptomatic despite chronic infection. Although *C. burnetii* can be shed into the environment via bodily fluids, the majority of shedding occurs via the birth products of domestic farm animals; more than 10⁹ bacteria can be released per gram of placenta from domestic ruminants during birth.⁹

A great deal of information is still missing regarding the immune response to the infection in humans and animal models. Of particular importance in understanding the pathogenesis of disease is elucidating the composition and production of cytokines in response to infection. Unfortunately, modern technologies such as multiplex bead arrays and mRNA expression microarrays have only recently been applied to the field of *Coxiella* research. The only comprehensive description of cytokine response in the literature is in a mouse model, in which levels of IL6, TNF α , IL12, IFN γ , IL3, IL4, IL10, G-GCF, MIP1 α , and RANTES were examined.⁷³ The authors suggested that the levels of some cytokines differed depending on the genogroup of the *Coxiella* strain used, thus adding weight to the argument that disease progression isn't a wholly a host-dependent feature. However, without similar descriptions of the cytokine profiles in human patients and other animal models, useful comparisons are difficult to make.

The remainder of this review will focus on the main small animal models of acute Q fever (mouse and guinea pig), chronic Q fever, and 'special cases' of Q fever, such as disease during pregnancy and in immunocompromised hosts (Table 1). In addition, an overview of nonhuman primate models that have been used to investigate both the pathology of acute Q fever and evaluate the protection afforded by various vaccine candidates will be provided.

Small Animal Models of Acute Q Fever

Mice. Mice have been used to study *C. burnetii* since the discovery of the organism.⁶⁶ Generally, mice do not display overt febrile disease to the degree seen in humans; in fact, often there are minimal signs of infection in laboratory mice.^{20,66} As in other models, pneumonia occurs after intranasal or aerosol exposure but not after intraperitoneal injection.^{20,66} Most reports suggest that splenomegaly and weight change, followed by confirmation of seroconversion, are the most reliable markers of infection in mice. In an evaluation of 47 strains of inbred laboratory mice, 33 were found to be resistant to infection with *C. burnetii*, 10 were partially susceptible, and 4 were susceptible.⁷⁹ The A/J strain of mouse was found to have the highest mortality (70%) but could still be protected by vaccination with formalin-inactivated *C. burnetii* whole cells.⁷⁹ The BALB/c strain of mouse was reliably infected and displayed overt signs of illness, including ruffled fur and lethargy in all members of the group, but with no mortality.⁷⁹

Table 1. Summary of use of animal models for Q fever

Species	Route	Study focus (reference citation numbers)				
		Pathology (acute)	Pathology (special or chronic)	Virulence assessment	Vaccine development	Adverse reaction to vaccine
Guinea pig	Intraperitoneal	28, 48, 64, 65	42	34, 73	39	7, 72
	Aerosol	37, 43, 74		34, 76		
Mouse	Intraperitoneal	5, 20, 45, 46, 55, 66, 79, 80, 86	12, 84	73	17, 21, 89, 98, 100, 101	
	Aerosol	36, 55, 66, 85				
Immunocompromised and genetically manipulated mice	Intraperitoneal		3, 4, 8, 40, 41, 53, 59, 61	73		
	Aerosol	85	41			
Nonhuman primates	Aerosol	25, 95			38, 96	

In mouse models, as in humans, the male sex is associated with more severe signs of infection.^{20,46} One study established a protective role of 17 β -estradiol⁴⁵ that could partially explain why males experience more severe clinical disease than females despite similar seropositivity rates. The presence of 17 β -estradiol was correlated with lower bacterial loads and less granuloma formation.⁴⁵ Recent studies using microarray analysis found that 86% of the genes probed were differentially expressed in infected male and female mice.⁸⁶ Of those genes, 60% were due to sex hormones. Perhaps surprisingly, many of the genes that appeared to play a role in protection of female mice from severe disease were involved in the circadian rhythm clock.⁸⁶

For experiments requiring the titration of *C. burnetii* infectivity (generally presented as a median infectious dose), an assay consisting of mouse infection followed by testing for seroconversion has been recognized as less labor-intensive and more economical than is the guinea pig assay. The titers given were reported as being reasonably comparable between the mouse and guinea pig assays.⁷⁵

The mouse model, predominantly using intraperitoneal exposure, has also been used extensively to evaluate the efficacy of vaccines to protect against Q fever.^{17,89,98,101} These models have consistently found that the phase I formalin-inactivated whole-cell vaccine (similar to the commercially available vaccine) is more effective at protection than are various chloroform:methanol extracts of *C. burnetii*, the phase II antigen-based vaccines,¹⁰¹ and a range of recombinant antigens.⁸⁹ Mice have become the model of choice for research investigating the immunology of Q fever, because of the availability of mouse strains with specific immunologic lesions, coupled with the availability of reagents and antibodies for the characterization of their immune responses.^{4,5,40,61}

Guinea pigs. Guinea pigs were recognized early in the study of *C. burnetii* as being an effective model for the disease course of Q fever in humans.⁴⁸ With the exception of immunocompromised strains, guinea pigs are more susceptible to infection than are mice.⁷⁶ Early studies concentrated on intraperitoneal challenge as the route of infection.^{28,29,48,64,65,87} As in humans, pronounced fever (with some strains achieving temperatures greater than 40 °C) is produced.^{29,48,64} Granulomas occur in the liver, spleen, and bone marrow.^{29,48} Biochemical changes include increases in liver glycogenolysis and lipogenesis,^{64,65} biochemical markers in the blood including glucose, serum ALP, serum AST, serum α -hydroxybutyrate dehydrogenase,

and serum creatine phosphokinase all were elevated.²⁹ The animals also demonstrated marked weight loss.⁶⁴

The model was later refined to use aerosol delivery, to better replicate the natural mode of infection.^{39,43,74} This method of delivery causes greater lung changes and less pronounced liver changes than does the intraperitoneal route.⁴³ In addition to fever and weight loss, guinea pigs display a marked pneumonia when exposed by this route.⁷⁴ As few as 20 organisms can cause acute Q fever in this model.⁷⁴ Among the small animal models for acute Q fever, the guinea pig model with aerosol delivery of the organism can be regarded as the model of choice.

Small Animal Models of Chronic Q fever

Mice. In a model of *C. burnetii* infection during pregnancy using BALB/cj mice, a large accumulation of organisms in the placenta was present,¹² mimicking findings in domestic livestock.⁹ Infection was associated with clinical signs including high levels of abortion, still birth, and perinatal death. The birthrate fell to 7.1% in infected mice, compared with 100% in the control group.¹² Another study investigating Q fever in pregnant BALB/c mice had similar findings but also reported endocarditis in 20% of the mice.⁸⁵ This pattern is consistent with the observation that Q fever in pregnancy can lead to chronic infection in humans (as many as 12 of 14 women infected in the first 2 trimesters in one report⁶⁸). The current recommended therapy for women infected during pregnancy is treatment with cotrimoxazole until delivery.¹⁴ In a study of women treated with cotrimoxazole, although the outcome of pregnancy was good, the mother still became chronically infected in 2 of 4 cases.⁶⁸ The pregnant mouse model could be a useful and important tool, by allowing detailed examination of the pathology of Q fever in pregnancy as well as investigation of different antimicrobial therapies.

Using cyclophosphamide to immunosuppress the BALB/cj strain of mouse revealed that intraperitoneal infection could lead to Q fever endocarditis without the need to predamage the heart,⁸ as had been required in a rabbit model.²⁷ There was no report of focal calcification in the heart in the BALB/cj mouse model, as would be expected in human chronic Q fever endocarditis. However, the mortality rate was very high (53%) and was associated with *C. burnetii* antigen deposition.⁸ This model is perhaps

a model of acute Q fever endocarditis, rather than of the chronic form of the disease seen in humans. Chronic Q fever infection was established in another model using mice with severe combined immunodeficiency, in which all infected mice eventually died, with a mean time to death of 33 d.³ In this model, endocarditis was associated with focal calcification in the heart, as occurs in human chronic disease.³ However, in a report by another group, an aerosol exposure model using SCID mice was not lethal⁸⁵—the fact that this experiment was terminated at 14 d postexposure probably explains this disparity between the reports.

Chronic infection with *C. burnetii* was achieved by using mice genetically modified to overproduce the cytokine IL10,⁵⁹ which is associated with eliciting an antibody-mediated (Th2) immune response. This chronic infection was characterized by high levels of antibody, impaired granuloma formation, and a sustained *C. burnetii* burden in the tissues.⁵⁹ These features, including an increased level of IL10, are similar to those reported in human chronic Q fever.³¹ This model provides clues about the host immunologic response that leads to some subjects becoming chronically infected and affords a model in which the pathology of the disease can be studied and treatment and prophylaxis options assessed.

Guinea Pigs. Although not strictly a manifestation of chronic *C. burnetii* infection, a common side effect of repeated vaccination is severe local skin reactions, including sterile abscess formation.³⁵ This adverse response has led to the requirement for prevaccination screening on potential vaccine recipients to test for preexisting immunity and reactivity.³⁵ It is desirable to assess new vaccines for hypersensitivity and other adverse reactions in an animal model during vaccine development. The initial model required injection of the vaccine under test into the footpads of Hartley guinea pigs.⁷ This model replicated the hypersensitivity reactions well but would have affected mobility of the animals in the case of severe reactions. A refinement of the model using hairless guinea pigs inoculated subcutaneously⁷² was found to be as effective for assessing vaccine reactions but was more humane than was the Hartley model.

For assessing the most common of the chronic diseases, endocarditis, a model has been proposed that uses electrocoagulation of the native aortic valve prior to exposure to *C. burnetii*.⁴² In this model, approximately half of the guinea pigs with previously damaged heart valves developed endocarditis, with a corresponding high level of mortality. The authors noted, however, that this model is one of acute *C. burnetii* endocarditis rather than of the chronic endocarditis seen in humans. However, this model could still be useful in assessing prophylaxis in those with preexisting heart conditions during outbreaks.

Nonhuman Primate Models of Q Fever

Although Q fever affects many large animals, such as domestic ruminants, most development of large animal models has focused on nonhuman primates. In the late 1970s, cynomolgus macaques (*Macaca fascicularis*) were exposed to aerosols of *C. burnetii*.²⁵ These primates displayed clinical signs of acute disease similar to those seen in humans, including anorexia, depression, and fever.²⁵ The duration of fever was 5 d, and a bacteremia was detected between days 7 and 14.²⁵ Hematology and clinical chemistry analyses showed significant increases in plasma ALP, AST, plasma fibrinogen, and total bilirubin.²⁵ Pathology examinations at necropsy revealed signs of subacute hepatitis, supporting the observations regarding bilirubin and AST observations.²⁵ Radiologically, signs of

pneumonia were seen from day 5 increasing in severity until day 12.²⁵ Serologically, the antibody response to phase II antigens rose first and reached higher titers than did the later phase I antibody response. The authors concluded that the disease in these primates closely resembled the acute Q fever seen in humans.

In the early 1980s, this model was used to evaluate a formalin-inactivated *C. burnetii* whole-cell vaccine.³⁸ The vaccine protected the majority of macaques, but some of those vaccinated developed clinical signs of illness after challenge. In the unvaccinated group, similar clinical symptoms to those found in the earlier study were described and included anorexia, depression, and fever.²⁵ The vaccine study³⁸ reported similar increases in ALP, AST, and total bilirubin to those of the previous work.²⁵ In infected animals, interstitial pneumonia was noted in radiographs and during pathologic examination.³⁸ In the group of macaques that were vaccinated, even those that displayed clinical signs had no changes in ALP, AST, total bilirubin, radiographs, or pathology.³⁸ Plasma fibrinogen was elevated in control and vaccinated animals that had an elevated temperature.³⁸ Vaccinated macaques displayed a shorter duration of bacteremia (2 d rather than 7 d) that also was of a smaller magnitude.³⁸ These results confirm the utility of the cynomolgus macaque model in assessing the protection afforded by vaccination.

A later study compared rhesus (*Macaca mulatta*) and cynomolgus macaques as models using aerosol challenge.⁹⁵ As in previous reports,^{25,38} pulmonary radiologic changes were seen in most animals⁹⁵ and more pronounced in cynomolgus macaques. More rhesus macaques were bacteremic and for longer periods than were cynomolgus macaques.⁹⁵ Bacteremia was assessed only by whether samples of blood from macaques infected mice, and no attempt was made to quantify the levels of bacteria present in the circulation (for example, by real-time quantitative PCR analysis).⁹⁵ Typical antibody responses, as described previously, developed in both species of macaques, but duration of fever was slightly longer in rhesus macaques.⁹⁵ Clinical features of disease (decreases in ALP and total bilirubin concentration) were similar to those described in human cases of acute Q fever but contrasted with previous reports,^{25,38,95} but no explanation of this difference was offered. However, serum AST and fibrinogen levels were elevated in both macaque species, as in human disease and in the previously described primate models. In addition, LDH (another marker of liver disease) was significantly higher in infected macaques, which also had elevated AST values, providing further evidence of liver involvement. The comparison of the rhesus and cynomolgus models did not include pathologic examinations or necropsy, thus preventing comparison between these and previous models in regard to the presence of liver granulomas or other specific liver pathology.⁹⁵ The authors suggested that both rhesus and cynomolgus macaques would be suitable models for studies of vaccine efficacy but favored cynomolgus macaques because of the more significant radiologic findings and the more human-like changes in lactate dehydrogenase and AST in that species.⁹⁵

The cynomolgus model with aerosol exposure was used in 2002 to compare the formalin-inactivated whole-cell vaccine that was licensed for use in humans in Australia with a chloroform:methanol residue of *C. burnetii*.⁹⁶ In contrast to the previous study,³⁸ vaccinated macaques exhibited signs of illness when challenged 6 mo after vaccination.⁹⁶ However, the disease in vaccinated animals was of shorter duration and lower severity than that in unvaccinated controls.⁹⁶ Levels of ALP raised, as in

previous studies,^{25,38,96} but, in contrast to previous studies, AST and bilirubin values were not increased.⁹⁶ The study demonstrated that the chloroform:methanol residue vaccine gave equivalent protection to that of the whole-cell vaccine.⁹⁶ Additional studies to assess whether chloroform:methanol residue-based vaccines effectively immunize subjects without the need for prescreening for adverse reactions could be the first step toward licensure of a less expensive, safer Q fever vaccine.

Identification of Virulence Determinants

The virulence determinants of *C. burnetii* strains have not yet been determined fully. Early in the study of Q fever, it was believed that different strains were responsible for acute compared with chronic disease presentations. Differences in the abilities of different strains to elicit a febrile response in the guinea pig model are well described, with acute strains (for example, Nine Mile) inducing a greater febrile response than do chronic strains (for example, Priscilla);^{60,73} however, vaccines based on either a chronic or acute strain cross-protected against challenge from the other strain type.⁶⁰ Acute strains such as Nine Mile have been used to induce endocarditis in guinea pigs,⁴² rabbits,²⁷ and immunosuppressed mice.⁸ A comparison of chronic and acute strains in mouse and guinea pig models revealed that chronic strains infected hosts as well as did acute strains but subsequent signs of infection were less severe.³⁴ Differences in pathology and virulence between chronic and acute strains have been reported,^{73,85} with acute strains proving more virulent in the mouse model used. Perhaps the more aggressive systemic reaction to the acute strains allows the host to more effectively clear the infection, whereas the milder response to chronic strains leads to ineffective clearance and allows the organism to efficiently colonize the host, particularly those with preexisting heart defects and immunodeficiency. Clearly, although the strain of *C. burnetii* may be part of the explanation of disease development, host factors including sex, age, the presence of heart damage, and immune status all have roles to play.

Histopathologic Features

Intraperitoneal and respiratory inoculation of guinea pigs with *C. burnetii* has been described in detail in several articles.^{43,48,74} All of the organs examined (heart, lung, liver, spleen, kidney) develop specific pathomorphologic changes, although kidney involvement is present in some⁴⁸ but not other⁴³ reports. Liver and spleen typically have damage manifesting as multifocal granulomas consisting of mononuclear aggregates, composed mainly of lymphocytes and macrophages. These focal granulomas are seen throughout the liver lobes and red pulp of the spleen but are longer lasting in liver. The lungs demonstrate mononuclear cell infiltration into the alveoli. Lung involvement is more pronounced in respiratory exposure models than in those involving intraperitoneal routes.^{43,74} The heart typically contains foci consisting of interstitial mononuclear cell infiltrates within the myocardium. No valvular involvement is described, except in the guinea pigs whose cardiac valves had previously been electrocoagulated.⁴² In those animals, endocarditis was associated with a valvular inflammatory infiltrate that was composed mainly of macrophages of lymphocytes.⁴² When changes were described, the kidney showed occasional mineralization of the kidney tubules;⁷⁴ other reports mentioned focal lymphocyte infiltration of the pelvic mucosa and fat.^{43,48} Although the testes typically have been described as displaying only minor degenerative changes in guinea pigs

infected with *C. burnetii*, the epididymis has been reported to show interstitial and perivascular infiltration by lymphocytes.⁴⁸

In mouse models using intraperitoneal and respiratory exposure, features similar to those seen in guinea pigs are reported; however, lesions tend to be more widespread in mice than in guinea pigs.⁶⁶ The spleen typically has the most striking lesions in mouse models of *C. burnetii* infection. Primarily found in the red pulp, these lesions are nodular or patchy and are granulomatous in nature, consisting mainly of mononuclear cells and. Liver contains similar lesions, although they are less numerous than are those in spleen.⁶⁶ Unlike in the guinea pig, the epididymis has not been identified as a tissue of interest in mice with *C. burnetii*.⁶⁶ In direct comparisons of guinea pigs and mice that have been exposed through various routes, interstitial pneumonia has been noted in multiple animals of both species, although in one study,⁷⁶ all guinea pigs developed pneumonia, whereas mice demonstrated some interstrain variation in regard to this lesion. In addition, the comparison study⁷⁶ noted mild lymphocytic myocarditis, in contrast to earlier work.⁶⁶

Recent work compared the histopathologic features of SCID mice with those of CB17 and A/J mice.³ In this work, consistent with other studies using immunocompetent mice, focal granulomas were seen in spleen and liver. In addition, minimal interstitial pneumonitis and an absence of heart or kidney involvement occurred in immunocompetent mice. In SCID mice, all of these lesions were abundantly present, with the addition of marked macrophage infiltration, glomerulonephritis, and pericarditis featuring focal calcification of the epicardium and endocardium.³ A much less severely immunocompromised mouse model uses mice overexpressing IL10 to interfere with macrophage activation, mirroring the observation that human patients with chronic Q fever sufferers overexpress this cytokine.¹³ In the IL10 overexpression model compared with the SCID mouse model, fewer granulomas are present, and those that do form are larger, often merging with the surrounding lymphoid tissue in the spleen. This effect is coupled with less pronounced splenomegaly in the IL10-overexpressing mice. These features are "reminiscent" of chronic infection in humans.⁵⁹

The histopathology of nonhuman primate models has been described only cursorily, although it is clear that these animals also develop moderate to severe interstitial pneumonia and mild to moderate multifocal granulomatous hepatitis.^{25,38,95} These data likely are rarer due to the need to perform a serial-euthanasia experiment to obtain them and the associated expense of performing this type of study in primates.

Candidate Vaccines

The vaccine currently licensed for human use is a formalin-killed whole-cell product.⁵¹ It is effective in protecting those at risk from Q fever.⁵¹ However, it is associated with severe local reactions to such an extent that potential vaccinees must be prescreened in regard to adverse skin reactions (to a small quantity of vaccine) and blood antibody levels⁴⁴ in an effort to avoid these reactions. Despite prescreening, severe reactions do still occur in some subjects,⁴⁴ thus making the vaccine less appropriate for general use and limited to those in defined risk groups. Alternative vaccines based on chloroform:methanol residues of the formalin-inactivated whole-cell material have been developed and shown to have similar levels of protection in some animal models^{96,98} with potentially fewer side effects. However, vaccination with

chloroform:methanol residues that appeared to protect against disease after challenge failed to protect against splenomegaly, hepatomegaly, and lesions in the liver, whereas whole-cell vaccines did provide this protection.⁹⁸ Indeed, animals vaccinated with chloroform:methanol residues showed significant splenomegaly and hepatomegaly when either challenged with live *C. burnetii* or injected with whole-cell vaccine. In addition, both whole-cell and chloroform:methanol residue vaccines require the production of large quantities of virulent *C. burnetii* at high containment levels during their manufacture. Vaccines based on the phase II, avirulent form of the organism were investigated briefly but found to be nonprotective,¹⁰¹ as were recombinant vaccines based on immunodominant proteins of *C. burnetii*.⁸⁹ Considerable work remains in the effort to produce an easy-to-manufacture, safe-to-administer, effective vaccine against Q fever.

Summary

Several animal models have been described for the study of Q fever and its causative organism, *C. burnetii*. Although cell culture and other in vitro systems are useful in the study of *C. burnetii*, vast amounts of new knowledge cannot be gained without the use of robust animal models. These models have proven useful in the study of the complex pathogenesis of the broad-spectrum of clinical disease caused by *C. burnetii*. In addition, animal models continue to help define the correlates of protection from infection that an effective vaccine needs to achieve.

In terms of the similarity to acute disease seen in humans, cynomolgus macaque model with aerosol exposure provides an excellent model of *C. burnetii* disease in humans. However, this model is expensive in terms of animal use, husbandry, and space and, in many cases, might be difficult to justify on ethical grounds. In addition, the work on primates predates technologies such as multiplex bead arrays and microarrays, so it suffers from a lack of data on cytokine responses. The guinea pig aerosol exposure model is a much more convenient model. The clinical features of *C. burnetii* infection in guinea pigs are highly similar to those seen in human acute infections, in contrast to the lack of clinical signs in other models (for example, mice). In addition, the model involving electrocoagulation of the aortic valve is a shows promise for the study of chronic Q fever and endocarditis. Application of the IL10 overexpression concept, which was very successful in mice,⁵⁹ would be particularly interesting to evaluate in the guinea pig model. More effective use of the guinea pig aerosol models is impeded by the lack of tools to effectively dissect the immune response to infection. There are many more tools and reagents for monitoring the expression of immune system molecules in mice than in guinea pigs. This discrepancy is illustrated by direct comparisons between the 2 models: for mice, data on circulating cytokines are given (several interleukins, IFN γ , TNF α , and so forth), but no comparative data from guinea pigs are given.⁷³ For the guinea pig model to be maximally beneficial, species-specific tools such as whole-genome microarrays and reagents to measure levels of key immune system markers need to be developed. Finally, to derive the full benefit from data on the cytokine profiles of infected animals, corresponding data need to be collected from human patients.

Although an effective vaccine is available currently, there is great scope for improvement in terms of ease of production and the requirement for prevaccination skin and blood testing. For a pathogen that was first described more than 70 y ago, there is still

much that is not known about *C. burnetii*. The models described in this review are crucial tools in solving the mysteries of this organism.

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