Intracellular Development of *Coxiella burnetii*

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1. INTRODUCTION

The causative agent of human Q (query) fever, *Coxiella burnetii*, is a bacterial obligate intracellular parasite.¹ The pathogen has a worldwide distribution with a broad host range that includes humans, arthropods, fish, birds, rodents, and livestock.² In most wild or domestic animals, *C. burnetii* does not appear to cause overt disease. However, in goats and sheep it can cause abortions.¹ Humans become infected primarily by inhaling contaminated aerosols generated by domestic livestock operations.² In humans, the disease usually manifests as an acute flulike illness (Q fever) with symptoms that include high fever, severe preorbital headache, and hepatitis.¹ This acute form of the disease is treatable with antibiotics and is generally self-limiting. However, in rare instances chronic cases can occur that usually manifest as endocarditis. Q fever endocarditis is refractory to antibiotics and results in significant mortality.³

Although *C. burnetii* is included within the *Rickettsieae*, 16S rRNA sequences reveal that the organism is phylogenetically unrelated to the other genera within this tribe, namely, the *Rickettsia* and *Rochalminaea*.⁴ *C. burnetii* is most closely related to the facultative intracellular bacterium, *Legionella pneumophila*.⁴ Many phenotypic differences are observed between *C. burnetii* and other rickettsiae. For example, *C. burnetii* proliferates intracellularly within a membrane-bound vacuole with lysosomal characteristics⁵⁻⁶ whereas members of the genus *Rickettsia* rapidly escape the
early endosome and replicate within the host cell cytoplasm. A hallmark that further distinguishes *C. burnetii* from other rickettsiae is its impressive long-lived extracellular stability and insensitivity to physical and chemical disruption. The heat resistance of the pathogen is shown by survival in milk to temperatures of 63°C for 30 min. The organism survives in dried guinea pig blood or in a 10% salt solution for at least 180 days at room temperature. Resistances to 1% phenol or 0.5% formalin for 24 hr have also been reported. *C. burnetii* remains highly infectious even after sonication of purified organisms in distilled water for over 30 min at 4°C (R. A. Heinzen and T. Hackstadt, unpublished data, 1996).

2. HOST–PARASITE INTERACTIONS

*C. burnetii* proliferates within a wide variety of epithelial, fibroblast, and macrophagelike cell lines. The organism plays a passive role in adherence and entry into host cells. *C. burnetii* that are inactivated by heat or glutaraldehyde are endocytosed via a microfilament-dependent process at rates equal to those observed for viable bacteria. The early parasite-containing phagosome proceeds through the endocytic pathway eventually acidifying to a pH of approximately 4.8. *C. burnetii* has an absolute requirement for this moderately acidic pH to activate metabolism and growth in *vivo*, as well as metabolic activation in *vitro*. The *C. burnetii*-containing vacuole fuses with lysosomes as demonstrated by the colocalization of the lysosomal enzymes 5′-nucleotidase, acid phosphatase, cathepsin D, and two predominant lysosomal glycoproteins. The organism undergoes luxurious growth within this vacuole, despite the presence of factors normally considered bactericidal. These can include reactive oxygen species generated during the phagocyte oxidative burst, and antimicrobial agents present in lysosomes such as acid hydrolases and defensins. The *C. burnetii*-containing phagosome may be somewhat atypical as ingestion of the agent by macrophagelike cell lines results in a greatly diminished respiratory burst with little production of superoxide anion. This phenomenon has also been observed during phagocytosis of *Leishmania*, *Histoplasma*, and some pathogenic *Mycobacteria*.

3. SMALL-CELL AND LARGE-CELL VARIANTS

The remarkable extracellular stability of *C. burnetii* has been attributed to the existence of a small, resistant cell form that is part of a poorly defined developmental cycle. Davis and Cox first described the pleomorphic nature and filterability of *C. burnetii*. Using light microscopy, they characterized round particles that were filterable through 0.4-μm pores and fully infectious for guinea pigs.