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# *Coxiella burnetii*as a useful tool to investigate bacteria-friendly host cell compartments

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ABSTRACT

*Coxiella burnetii* is an obligate intracellular and airborne pathogen which can cause the zoonotic disease Q fever. After inhalation of contaminated aerosols alveolar macrophages are taking up *C. burnetii* into a phagosome. This phagosome matures to a very large vacuole called the *C. burnetii*-containing vacuole (CCV). Host endogenous and bacterial driven processes lead to the biogenesis of this unusual compartment, which resembles partially a phagolysosome. However, there are several important differences to the classical phagolysosome, which are crucial for the ability of *C. burnetii* to replicate intracellularly and depend on a functional type IV secretion system (T4SS). The T4SS delivers effector proteins into the host cell cytoplasm to redirect intracellular processes, leading to the establishment of a microenvironment allowing bacterial replication. This article summarizes the current knowledge of the microenvironment permissive for *C. burnetii* replication.

## 1. Introduction

Pathogens co-evolve with their hosts, including us humans. As pathogens represent threats to human health, our immune system has established an arsenal of defense mechanisms. Consequently, pathogens have developed virulence factors to prevent clearance by the host immune response. Additional virulence factors modulate host cell pathways to enable bacterial survival and replication. However, the microenvironment present at the site of infection is decisive for the host-pathogen-interaction. This microenvironment affects not only the ability of the host to combat infection, but also the activity of the pathogen. Depending on the conditions existing at the site of infection, the pathogen might escape the unfavorable micro-milieu, induce dormancy, adjust to the conditions at hand or modulate the microenvironment to its own benefit. For obligate intracellular pathogens this microenvironment is represented by the phagosome/vacuole surrounding the pathogen. Importantly, the conditions within the pathogen-containing vacuole can differ, dependent on the host cell type and the immune status of the host. In this review we will discuss our current understanding of a *Coxiella burnetii*-friendly microenvironment (Fig. 1).

## 2. The pathogen *C. burnetii*

*Coxiella burnetii* is a Gram-negative, small (0.4–1.0 µm length, 0.2–0.4 µm width) and pleomorphic coccoid to rod-shaped bacterium (Maurin and Raoult, 1999). Although *C. burnetii* is obligate intracellular in nature, a medium has been established that allows axenic culture in the laboratory (Omsland et al., 2011; Omsland et al., 2009). *C. burnetii* belongs to the gamma subdivision of Proteobacteria (Stein et al., 1993). Its closest relatives are the facultative intracellular human pathogen *Legionella pneumophila* and the intracellular arthrophod pathogen *Rickettsiella grylli* (Seshadri et al., 2003). Inside of its eukaryotic host cell, *C. burnetii* is able to replicate in high numbers in a parasitophorous vacuole (Burton et al., 1978). Depending on the host cell type and the bacterial strain, doubling times *in vitro* are estimated to range from 15 to 37 h (Boulos et al., 2004). *C. burnetii* has a biphasic lifestyle (McCaul and Williams, 1981). The small cell variant (SCV) represents the environmentally resistant and metabolically less active form. It allows survival of *C. burnetii* outside of a host cell (Maurin and Raoult, 1999). Upon infection, SCVs differentiate into the large cell variant (LCV), which is the intracellular and metabolically active form (Coleman et al., 2004). The genome sizes of different *C. burnetii* strains vary from 1.5 to 2.4 Mb (Willems et al., 1998). In 2003 the *C. burnetii* isolate Nine Mile I was sequenced. Its genome has a size of about 2 Mb with a GC content of roughly 43% and includes 2134 predicted coding sequences (Seshadri et al., 2003). One virulence determinant of *C. burnetii* is the lipopolysaccharide (LPS). The virulent form of *C. burnetii,* which is called phase I, expresses a full-length, smooth LPS with a typical core glycolipid and O-specific polysaccharide chain (Amano and Williams,

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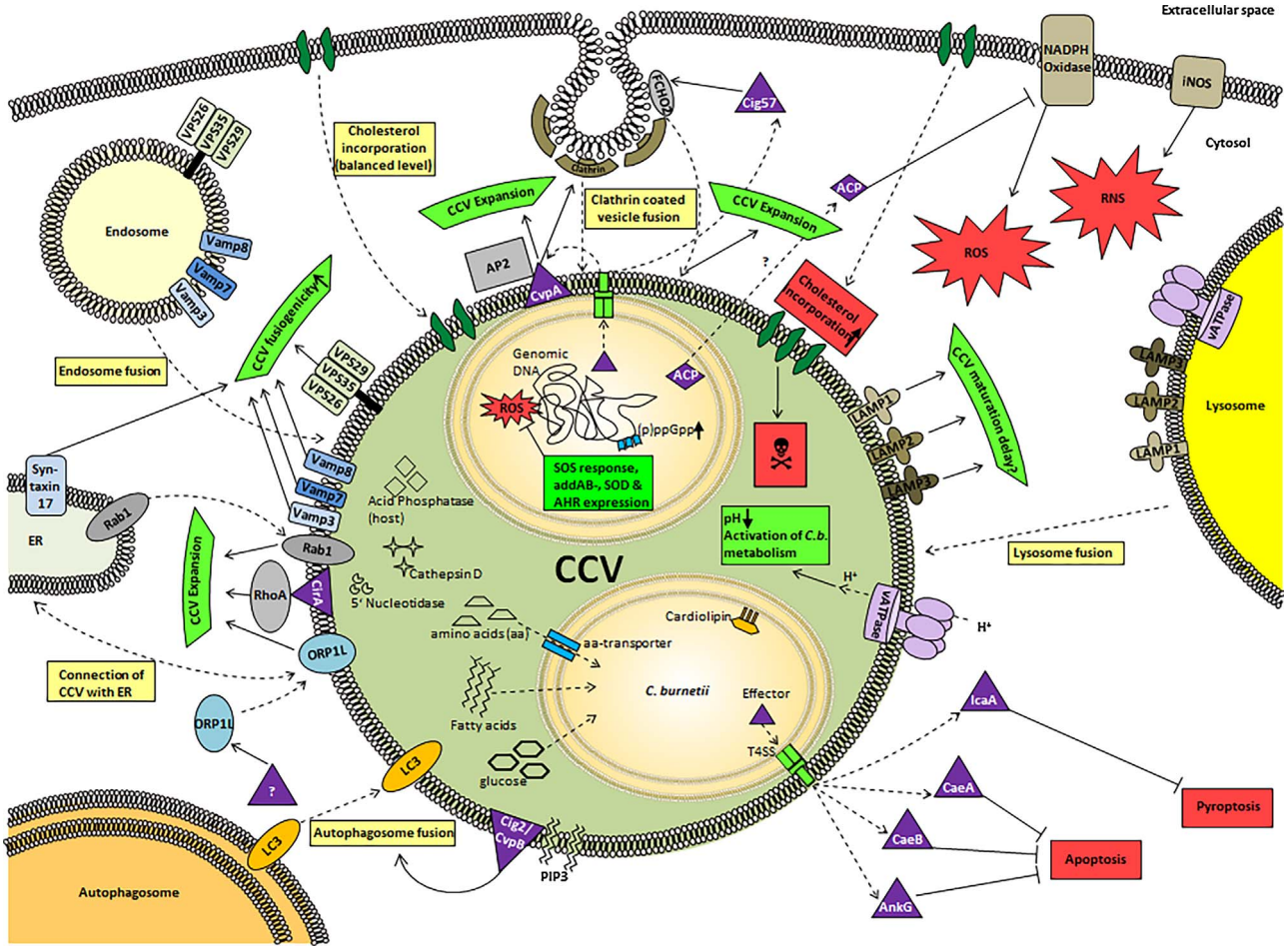


**Fig. 1.** Biogenesis and microenvironment of the replicative CCV. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Representative events that favor or hamper CCV biogenesis and *C. burnetii* replication are shown in this figure. Maturation of the CCV results from fusion events with endosomes, autophagosomes, lysosomes and secretory vesicles. These fusion processes are partially mediated by T4SS effector proteins and provide nutrients for *C. burnetii* biogenesis. Additionally, *C. burnetii* possesses several counter measures to prevent host cell mediated killing. The details are described and discussed in the main text.

Green boxes represent events in favor of CCV biogenesis and bacterial replication. Red boxes indicate detrimental effects on *C. burnetii* viability. Dotted lines represent translocation, transport- and fusion processes. Filled lines represent direct interaction or consequences of action. ACP: *C. burnetii* acid phosphatase; AP2: adapter protein complex 2; ROS: reactive oxygen species; RNS: reactive nitrogen species; iNOS: inducible nitric oxide synthase.

1984). After serial passage in cell culture, *C. burnetii* undergoes a phase variation to phase II (Fiset et al., 1956). *C. burnetii* phase II is less virulent and characterized by the expression of a truncated, rough LPS (Hoover et al., 2002). It has been suggested that this phase variation is due to several deletion events in the chromosome and intermediate forms have been described (Amano et al., 1987). While phase I is classified as a biosafety level 3 organism, the plaque-isolated *C. burnetii* Nine Mile phase II clone 4 can be used under biosafety level 2 (Amano and Williams, 1984). Importantly, both phase I and II *C. burnetii* Nine Mile variants replicate with similar kinetics in human macrophages (Howe et al., 2010). Therefore, *C. burnetii* Nine Mile phase II is a good and widely used model to investigate the host pathogen interaction. Recently, the sequence of *C. burnetii* Nine Mile phase II became available (Millar et al., 2017), which will allow to determine which genes are decisive for the pathogenic potential of the bacterium.

## 3. *C. burnetii* infection - Q fever

*Coxiella burnetii* is the causative agent of Q fever. With the exception of New Zealand this disease is found worldwide. In humans, Q fever is asymptomatic or might manifest as a mild and self-limited flu-like illness. However, acute Q fever can also develop into an interstitial pneumonia or hepatitis (Maurin and Raoult, 1999). While good treatment options exist for acute Q fever, these are missing for chronic Q fever. About 1–5% of the infected individuals will develop chronic Q fever years after the primary infection (Kazar, 2005), which is typically characterized by endocarditis and potentially fatal (Maurin and Raoult, 1999). The main source of human Q fever infections are infected ruminants and transmission occurs via aerosols of contaminated dust or of animal excretions like milk, urine, feces and parturient products from infected animals (Angelakis and Raoult, 2010). During parturition more than $10^9$ bacteria per gram of placenta are released into the environment (Maurin and Raoult, 1999). Because infection with less than ten bacteria can result in human disease (Benenson and Tigertt, 1956; Madariaga et al., 2003), birthing products are a tremendous biohazard. Interestingly, the infection routes as well as the clinical symptoms differ between humans and ruminants. Domestic livestock may be infected by tick bites or tick feces (Stoker and Marmion, 1955), while infected ticks do not seem to be involved in human infection. Infected animals perpetuate and maintain the infection within animal populations (Sting et al., 2013) and the infection is mainly unapparent. Interestingly, chronic infections do not result in endocarditis as observed in humans. Instead chronic *C. burnetii* infection in ruminants is mainly observed in mammary glands and uteri. As a result, chronic infection in ruminants might lead to abortion and reduced reproductive efficiency (Maurin and Raoult, 1999). Similarly, in pregnant woman the illness is likely