Microreview

Lounging in a lysosome: the intracellular lifestyle of Coxiella burnetii

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Summary
Most intracellular parasites employ sophisticated mechanisms to direct biogenesis of a vacuolar replicative niche that circumvents default maturation through the endolysosomal cascade. However, this is not the case of the Q fever bacterium, *Coxiella burnetii*. This hardy, obligate intracellular pathogen has evolved to not only survive, but to thrive, in the harshest of intracellular compartments: the phagolysosome. Following internalization, the nascent *Coxiella* phagosome ultimately develops into a large and spacious parasitophorous vacuole (PV) that acquires lysosomal characteristics such as acidic pH, acid hydrolases and cationic peptides, defences designed to rid the host of intruders. However, transit of *Coxiella* to this environment is initially stalled, a process that is apparently modulated by interactions with the autophagic pathway. *Coxiella* actively participates in biogenesis of its PV by synthesizing proteins that mediate phagosome stalling, autophagic interactions, and development and maintenance of the mature vacuole. Among the potential mechanisms mediating these processes is deployment of a type IV secretion system to deliver effector proteins to the host cytosol. Here we summarize our current understanding of the cellular events that occur during parasitism of host cells by *Coxiella*.

Introduction
The obligate intracellular bacterium *Coxiella burnetii* is the causative agent of the zoonosis Q fever, a disease that generally manifests as an acute, debilitating flu-like illness (Maurin and Raoult, 1999). Unlike other obligate intracellular pathogens, *Coxiella* is highly resistant to environmental stresses such as high temperature, osmotic pressure and ultraviolet light (McCaul et al., 1981; Williams, 1991). These characteristics are attributed to a small cell variant (SCV) form of the organism that is part of a biphasic developmental cycle including a more metabolically and replicatively active large cell variant (LCV) form (McCaul and Williams, 1981; Heinzen et al., 1999; Coleman et al., 2004). Enhanced stability outside the host cell facilitates the primary mode of human infection which is inhalation of contaminated aerosols. The organism is highly infectious with an infectious dose approaching one organism (Moos and Hackstadt, 1987). Extracellular stability correlates with *Coxiella* replication in the most inhospitable compartment of the host cell – the phagolysosome. In fact, *Coxiella* is the only known example of a bacterial pathogen that replicates throughout its infectious cycle in a parasitophorous vacuole (PV) that is indistinguishable from a secondary lysosome (Burton et al., 1971; Akporiaye et al., 1983; Heinzen et al., 1996).

Lipopolysaccharide phase variation
The ability of *Coxiella* to prosper in a normally bacteriostatic/cidal vacuole is central to its pathogenesis. Unfortunately, our understanding of molecular mechanisms employed by *Coxiella* to persist in this environment is limited by the lack of a system to genetically manipulate the organism. Currently, lipopolysaccharide (LPS) is the only defined *Coxiella* virulence factor (Moos and Hackstadt, 1987). Virulent ‘phase I’ organisms isolated from natural sources and infections produce a full-length LPS. Serial *in vitro* passage of phase I *Coxiella* in embryonated eggs or tissue culture results in bacteria that produce LPS molecules of decreasing molecular weight, culminating in the severely truncated LPS of avirulent ‘phase II’ organisms (Hackstadt et al., 1985). A stable isogenic phase II LPS variant (Nine Mile, phase II, clone 4, RSA439) of the virulent Nine Mile phase I reference strain (RSA493) has been cloned that is exempt from US Centers for Disease Control and Prevention select agent regulations
D. E. Voth and R. A. Heinzen (http://www.cdc.gov/od/sap/sap/exclusion.htm) and is suited for work at biosafety level 2 (Hackstadt, 1996). All other Coxiella strains are considered biosafety level 3 organisms (Hackstadt, 1996). Coxiella RSA439 contains a chromosomal deletion that eliminates genes involved in O-antigen biosynthesis, a defect that is associated with attenuated virulence (Moos and Hackstadt, 1987; Hoover et al., 2002). However, the rough LPS chemotype and resultant avirulence of this strain is likely due to an additional point/frameshift mutation, small deletion or transposon insertion in a gene earlier in the LPS biosynthetic pathway (Beare et al., 2006; Denison et al., 2007). In addition to a lower bio-containment level, RSA439 has the advantage over phase I organisms for in vitro cellular biology studies by being -10-fold more infectious for cultured cells (Moos and Hackstadt, 1987). Moreover, with the exception of primary murine macrophages, most studies indicate that phase I and phase II organisms replicate with similar kinetics in phenotypically indistinguishable lysosome-like PV (discussed below).

The virulence properties of full-length Coxiella LPS are related to the molecule's ability to shield the outer membrane. For example, phase II, but not phase I, organisms are readily killed via the complement membrane attack complex (Vishwanath and Hackstadt, 1988). Additionally, antibodies to Coxiella surface proteins are sterically inhibited from binding phase I organisms, an effect that is reversed if LPS is first chemically extracted (Hackstadt, 1988). Phase I LPS also masks Coxiella toll-like receptor (TLR) ligands from innate immune recognition by dendritic cells (Shannon et al., 2005) and may also inhibit Coxiella interaction with the CR3 (α<sub>m</sub>β<sub>2</sub> integrin) receptor of macrophages (Capo et al., 1999). Phase I and phase II LPS lipid A moieties are chemically identical and the molecule not only fails to ligate TLR-4 (Zamboni et al., 2004; Shannon et al., 2005), but is antagonistic against TLR-4 signalling by other LPS (Zamboni et al., 2004).

Relative to phase I Coxiella, phase II organisms display growth defects in primary mouse macrophages (Zamboni et al., 2002; Brennan et al., 2004; Zamboni, 2004; Sauer et al., 2005). This growth restriction appears to be murine-specific as phase variants grow at similar rates in primary macrophages from guinea pigs (Kishimoto and Walker, 1976), non-human primates and humans (J. G. Shannon et al., manuscripts in preparation). Phase II Coxiella may specifically activate inhibitory innate immune mediators in primary mouse cells that limit growth (Shannon et al., 2005). Consistent with this idea is the observation that primary mouse macrophages treated with nitric oxide synthase inhibitors, or from TLR-2 knockout mice, are markedly more permissive for growth of phase II Coxiella than untreated or wild-type macrophages, respectively (Zamboni and Rabinovitch, 2003; Zamboni et al., 2004). Moreover, phase II Coxiella grows more moderately in macrophages from A/J and BALB/c mice, which have known deficiencies in innate immunity (Fortier et al., 2005), than those from immunocompetent C57BL/6 mice (Zamboni, 2004).

In vitro and in vivo model systems

In vitro, Coxiella promiscuously infects a wide variety of cell types, and many epithelial and fibroblast-like continuous cell lines have been used as models to study Coxiella–host interactions. Cell culture systems supporting Coxiella growth include Ora (African green monkey kidney epithelial) (Heinzen et al., 1996), BHK-21 (hamster kidney fibroblast) (Miller et al., 2004), L-929 (murine fibroblast) (Baca and Paretsky, 1983), HEL (human embryonic lung fibroblast) (Raoul et al., 1990), HeLa (human cervical epithelial) (Berger et al., 2002) and CHO (Chinese hamster ovary fibroblast) (Romano et al., 2007) cells. In natural infections, Coxiella has a tropism for cells of the mononuclear phagocyte system such as alveolar macrophages of the lung and Kupffer cells of the liver (Khaevkin and Tabibzadeh, 1988; Stein et al., 2005), with organisms also infrequently observed in pneumocytes, fibroblasts and endothelial cells (Khaevkin and Tabibzadeh, 1988). Consequently, monocyte/macrophage-like cell lines including J774A.1 (murine macrophage-like) (Brennan et al., 2004), P388D1 (murine macrophage-like) (Tjuljen et al., 1999) and THP-1 (human monocyte-like) (Ghigo et al., 2002) cells have been extensively employed to more accurately mimic the in vivo situation, with more recent studies focused on interactions with primary human monocytes/macrophages (Ghigo et al., 2004) and dendritic cells (Shannon et al., 2005). With a few notable exceptions (discussed below), the findings of these studies concur and have cumulatively allowed modelling of the Coxiella infectious cycle.

The most accurate rodent model of human Q fever is the guinea pig. Inoculation with as few as 10 phase I Coxiella organisms results in a symptomatic self-limiting infection that closely mimics clinical human acute Q fever with signs including sustained elevated fever (Moos and Hackstadt, 1987; La Scola et al., 1997; Russell-Lodrigue et al., 2006). However, a limitation of the guinea pig model is the lack of genetic and immunological tools that are the mainstay of mouse models of infectious disease. As determined by sero-conversion, inbred mouse strains are readily infected by low doses of Coxiella (Scott et al., 1987). However, higher doses (> 1 x 10<sup>6</sup> organisms) are required before strains that are both resistant (e.g. C57BL/6) and sensitive (e.g. A/J) to lethal Coxiella infection show clinical signs of infection such as pronounced splenomegaly. Nonetheless, splenomegaly is a reliable readout of the extent of Coxiella replication (Brennan et al., 2004; Zhang et al., 2004) and, among other applications, this system has been employed...
to test Q fever subunit vaccine candidates (Zhang et al., 2004). Moreover, in conjunction with infection of knockout, athymic and SCID mice, splenomegaly and other indicators of infection such as granuloma formation have been assessed to define important components of the innate and adaptive immune response to Coxiella (Andoh et al., 2003; Brennan et al., 2004; Honstetter et al., 2004; 2006; Meghari et al., 2005) and the pathogenic potential of different Coxiella isolates (Stein et al., 2005). Pulmonary infection of cynomolgus macaques is the established non-human primate model of Q fever (Waag et al., 1999); however, expense and biosafety level 3 animal care and use issues severely restrict its use.

Coxiella adherence and internalization

Coxiella internalization into host cells occurs by microfilament-dependent endocytosis (Baca et al., 1993; Meconi et al., 1998; Capo et al., 2003). Adherence of virulent phase I Coxiella to THP-1 human monocyte-like cells results in dramatic reorganization of the actin cytoskeleton that induces pronounced membrane protrusions at the site of bacterial attachment (Meconi et al., 1998). Similar cellular effects are not observed upon adherence of avirulent phase II organisms (Meconi et al., 1998). Adherence of phase I Coxiella also activates host protein tyrosine kinases with the resulting phosphorylated host proteins localizing to filamentous actin within the protrusions (Meconi et al., 2001). Inhibition of protein tyrosine kinase activity reduces membrane protrusion formation and enhances phase I phagocytosis (Meconi et al., 2001). Pathogen-induced actin reorganization resulting in membrane protrusions, such as the membrane ruffling associated with Salmonella spp. infection (Knodler et al., 2001), is generally considered a mechanism that increases the efficiency of pathogen uptake. However, membrane protrusion-inducing phase I Coxiella are internalized less efficiently than phase II organisms by both professional phagocytes (Capo et al., 1999) and fibroblast/epithelial cells (Moos and Hackstadt, 1987; Baca et al., 1993). In professional phagocytes, this discrepancy may reflect differential engagement of host cell receptors by phase variants. The THP-1 receptor for phase I organisms is the leukocyte response integrin αvβ3, whereas phase II organisms additionally engage the CR3 receptor (Capo et al., 1999). Membrane projections induced by phase I adherence are proposed to restrict engagement of the CR3 coreceptor, thereby lowering the efficiency of internalization (Meconi et al., 1998). Full-length LPS of phase I organisms may further restrict binding of CR3 by a critical Coxiella ligand as the molecule is known to sterically mask Coxiella surface proteins (Hackstadt, 1988; Shannon et al., 2005). Adherence and internalization of phase II organisms by non-phagocytic cells, such as Vero epithelial cells and L-929 fibroblasts, also occurs at much higher rates than phase I organisms. However, differential engagement of integrin αvβ3 and CR3 cannot account for increased phase II uptake as these cells lack these receptors. Phase II Coxiella produce a truncated LPS with a much lower carbohydrate content than phase I organisms, rendering phase II highly hydrophobic (Williams et al., 1981). Consequently, increased phagocytosis of phase II Coxiella by all host cell types is likely augmented by non-specific hydrophobic interactions that facilitate interactions with the host plasma membrane and cognate receptors (Baca et al., 1993). The Coxiella ligand(s) mediating uptake is unknown but is likely proteaceous as pretreatment of the organism with proteases dramatically inhibits internalization (Baca et al., 1993).

Coxiella phagosome maturation

Following internalization, the nascent Coxiella-containing phagosome transits through the default endocytic cascade to ultimately fuse with the lysosomal compartment (Heinzen et al., 1996). Sequential recruitment by the Coxiella phagosome is observed for the small GTPases Rab5 and Rab7, which are prototypic markers of early and late endosomes, respectively, that regulate membrane trafficking (Romano et al., 2007). However, fusion with lysosomes, as indicated by the presence of the lysosomal enzymes acid phosphatase (Howe and Mallavia, 2000) and cathepsin D (Romano et al., 2007), takes approximately 2 h, which is significantly slower than phagosomes harbouring inert particles such as latex beads that acquire lysosomal enzymes in about 15 min (Oh and Swanson, 1996). Exciting new studies from the laboratory of M. Colombo suggest that delayed lysosome interactions are due to early engagement between the Coxiella phagosome and the autophagic pathway (Beron et al., 2002; Gutierrez et al., 2005; Romano et al., 2007). Indeed, the Coxiella phagosomal membrane is decorated with the autophagosome marker microtubule-associated protein light-chain 3 (LC3) as early as 5 min post infection (Beron et al., 2002; Romano et al., 2007). Early autophagosome interactions and delayed lysosomal hydrolase delivery are reliant on Coxiella protein synthesis (Howe and Mallavia, 2000; Romano et al., 2007), indicating these are pathogen-directed processes.

Autophagy is normally employed to remove defective organelles and cytoplasmic material via trafficking of this material to autophagolysosomes (Kirkegaard et al., 2004), and pathogen interactions with autophagosomes can have either beneficial or detrimental effects (Pizarro-Cerda et al., 1998; Gutierrez et al., 2004). Coxiella clearly benefits from autophagy as induction of this pathway increases the number of infected cells, the size of the PV,
and the extent of Coxiella replication (Gutierrez et al., 2005). These effects are abolished when infected cells are treated with the autophagy inhibitors wortmannin and 3-methyladenine (Gutierrez et al., 2005).

The advantage to Coxiella of autophagy-mediated delay of phagosome maturation is unclear as the organism prospers in the normally toxic lysosomal environment. Phagosome stalling has been speculated to allow morphological differentiation of the resistant, metabolically sluggish SCV form of Coxiella to the metabolically and replicatively active LCV developmental form (Howe and Mallavia, 2000; Swanson et al., 2002). A similar ‘pregnant pause’ in phagosome maturation is associated with the vacuoles of Leishmania spp. and Legionella and is speculated to provide these organisms adequate time to differentiate into replicative forms that thrive in lysosomes (Swanson et al., 2002). However, differentiation of SCV to LCV takes 1–2 days to occur (Coleman et al., 2004), which is considerably longer than the few hours of phagosome stalling. Other possible benefits of Coxiella interactions with the autophagic pathway include delivery of nutrients and membrane. Because the Coxiella PV is not passively permeable to small molecules (Heinzen and Hackstadt, 1997), early interactions with autophagosomes may deliver a critical pulse of peptides/amino acids and saccharides that upregulate Coxiella metabolism and initiate SCV to LCV differentiation. Later interactions likely provide sustained metabolites for LCV exponential replication (Coleman et al., 2004; Gutierrez et al., 2005). Moreover, Coxiella does not synthesize its own PV membrane; thus, multiple fusion events with autophagosomes along with endolysosomal vacuoles are likely essential to provide sufficient membrane for the enlarging PV.

Type IV secretion

As mentioned earlier, Coxiella protein synthesis is required for early autophagosome interactions that promote stalling of PV maturation. Protein effectors of this process presumably target host factors that regulate vesicular trafficking and are likely delivered to the cytosol via the activity of a type IV secretion system (T4SS) that has homology to the Dot/Icm T4SS of Legionella (Sexton and Vogel, 2002). Dot/Icm T4SS function is essential for establishment of the replication vacuole of Legionella (Segal et al., 1998; Vogel et al., 1998), and T4SSs are employed by a number of Gram-negative pathogens to translocate proteins that modulate specific host processes for the pathogen’s benefit (Christie and Vogel, 2000).

Conservation of the Dot/Icm T4SS between Coxiella and Legionella is consistent with the recently established close phylogenetic relatedness of the two organisms (Roux et al., 1997). The Coxiella genome contains 23 of the 26 Legionella dot/icm genes with the exception of icmR, dotJ and dotV (Vogel, 2004). Coxiella dotB, icmS, icmW and icmT complement corresponding mutants in Legionella. However, Coxiella icmX, icmQ, dotM, dotL, dotN and dotO do not complement, suggesting there are interactions between functional Coxiella Dot/Icm T4SS components that are not conserved in Legionella (Zamboni et al., 2003; Zusman et al., 2003). The lack of complementation by Coxiella icmQ was initially attributed to failure of the encoded protein to interact with IcmR, a chaperone of Legionella IcmQ that is absent in Coxiella (Zamboni et al., 2003). However, a recent study shows that both Coxiella and Legionella produce proteins that are non-homologous, but functionally similar, to IcmR (Feldman et al., 2005).

To date, no secretion substrates have been described for the Coxiella Dot/Icm T4SS. Moreover, Coxiella does not contain homologues of Legionella type IV substrates including RalF (Nagai et al., 2002) and SidM/DrrA (Machner and Isberg, 2006) (guanine exchange factors), VipD, VpdA and VpdB (phospholipases) (VanRheenen et al., 2006), and the paralogue Sde (SdeA-C) (Bardill et al., 2005) and Sid (SidA-H) (Luo and Isberg, 2004) protein families. A consistent theme among secreted bacterial effector molecules is the presence of eukaryotic-like motifs that functionally mimic the activity of host cell proteins (Stebbins and Galan, 2001). Exploiting this property, a bioinformatic screen recently identified new Legionella T4SS substrates that were likely acquired by interdomain horizontal gene transfer (de Felipe et al., 2005). Novel effector molecules include protein families possessing coiled-coil domains (CCD), tetratricopeptide repeats (TPR), leucine-rich repeats and ankyrin repeats (Anks). A common property of these protein motifs is their involvement in multiple types of protein–protein interactions and signalling pathways. A similar in silico analysis reveals that the sequenced Nine Mile I Coxiella genome also encodes a large number of proteins containing eukaryotic-like motifs that may be potential T4SS effectors. These include at least eight TPR proteins (e.g. CBU1364) and 10 CCD proteins (e.g. CBU0891). Moreover, 20 Anks were revealed (e.g. CBU0781), which is seven more than originally highlighted in the Coxiella genome study (Seshadri et al., 2003). These candidate T4SS effector molecules await testing in heterologous systems and/or gene knockout experiments upon development of Coxiella genetics.

Legionella expression of the Dot/Icm T4SS coincident with, and immediately following, infection of macrophages is critical for biogenesis of a PV that supports growth (Roy et al., 1998; Coers et al., 1999). In vivo, Coxiella is metabolically quiescent outside of a moderately acidic host cell vacuole (Hackstadt and Williams, 1981); thus, it is unclear how type IV secretion, an energy-dependent process...
(Segal et al., 2005), could be involved in cellular uptake. However, secretion of a preloaded type III effector molecule by metabolically inert elementary bodies of Chlamydia trachomatis that induces their uptake has recently been reported (Clifton et al., 2004), and a similar event may occur during Coxiella internalization.

Early in the infectious process, Coxiella protein synthesis is clearly required for early autophagosome interactions, phagosome stalling (Howe et al., 2003; Romano et al., 2007), and homotypic fusion of individual Coxiella phagosomes in multiply infected cells (Howe et al., 2003). Thus, the organism must be metabolically activated early during transit through the endocytic pathway, with the potential to synthesize de novo T4SS effectors that modulate these processes. Coxiella Dot/Icm T4SS may also be required late in the pathogen’s infectious cycle as the PV collapses and fuses less efficiently with latex bead-containing phagosomes in the absence of Coxiella protein synthesis (Howe et al., 2003). Consistent with this idea is the observation that dotA is expressed during Coxiella’s stationary growth phase (Coleman et al., 2004). A late role for Legionella Dot/Icm secretion in delaying macrophage apoptosis has recently been defined (Losick and Isberg, 2006; Abu-Zant et al., 2007), with one effector (SdhA) identified that appears to act in part by suppressing caspase activity (Laguna et al., 2006). Anti-apoptotic effects may also be mediated by the Coxiella Dot/Icm T4SS as persistently infected macrophages show little cytopathic effect (Roman et al., 1986).

Features of the mature Coxiella PV

Based on cytochemical localization of the lysosomal enzymes acid phosphatase and 5'-nucleotidase in infected mouse L-929 cells, Burton and coworkers (Burton et al., 1971) were the first to suggest Coxiella replicates in a phagolysosome. This hypothesis was later buttressed by the results of Akporiaye et al. (Akporiaye et al., 1983) who demonstrated in infected J774A.1 mouse macrophages that the Coxiella vacuole acidifies and acquires thiom dioxide from secondary lysosomes. The membrane of the mature PV (> 2 days post infection) has subsequently shown to decorate with the vacuolar type H+ ATPase (Heinzen et al., 1996), Rab7 (Ghigo et al., 2002; Romano et al., 2007), three lysosomal glycoproteins [LAMP-1 and LAMP-2 and LAMP-3 (CD63)] (Heinzen et al., 1996; Ghigo et al., 2002; Sauer et al., 2005; Shannon et al., 2005), flotillin 1 and 2 (Howe and Heinzen, 2006), and the autophagosome markers LC3 (Romano et al., 2007) and Rab24 (Gutierrez et al., 2005).

Differential trafficking has been proposed for Coxiella phase variants in resting primary human monocytes and THP-1 human monocyte-like cells (Ghigo et al., 2002). Based on the presence of active cathepsin D, this study suggests maturation of phagosomes containing phase I Coxiella is impaired and stops at a growth-permissive late endosomal stage. Conversely, phagosomes sheltering phase II Coxiella are thought to fully mature to a microbicidal lysosomal compartment, a process presumably directed by the strain’s selective engagement and activation of the CR3 receptor (Capo et al., 1999; 2003). The results of other studies conflict with this proposal. First, phase II variants grow robustly in a number of CR3-expressing murine macrophage-like cells (Baca et al., 1981). Second, phase II Coxiella replicate in cathepsin D-positive vacuoles in CHO cells (Romano et al., 2007). Third, vacuoles harbouring replicating phase I bacteria in murine L-929 and J774A.1 cells contain lysosomal acid hydrolases, indicating full endolysosomal maturation (Burton et al., 1971; Akporiaye et al., 1983; Howe and Mallavia, 2000). Finally, we find that phase I and phase II Coxiella grow with similar kinetics in human monocyte-derived dendritic cells (Shannon et al., 2005) and macrophages where they traffic to, and replicate within, the same vacuole (J. G. Shannon et al., manuscript in preparation). Species-specific trafficking behaviour and/or differences in primary macrophage isolation and cultivation may account for these disparate findings. Nonetheless, differential trafficking as a mechanism that contributes to the virulence properties of phase variants is an intriguing possibility that deserves further investigation.

The volume of the mature Coxiella PV is striking. The vacuole enlarges to occupy nearly the entire cytoplasm with a volume usually well in excess of the resident organisms (Fig. 1). During division of an infected cell, the PV segregates to one daughter cell, leaving the companion daughter cell parasite-free (Roman et al., 1986). This appears to be the case even for the Priscilla strain of Coxiella which resides in an unusual multilobed vacuole (Hechemy et al., 1993). Among other possibilities, generation of spacious PV by intracellular parasites is proposed to dilute toxic lysosomal compounds, thereby rendering them less effective (Alpuce-Aranda et al., 1994).

The remarkable redistribution of host lipid components to the PV in the absence of obvious cytopathic effects and perturbation of the host cell cycle (Baca et al., 1985; Roman et al., 1986) is an exquisite example of parasite-host coevolution. However, relative to protein constituents, the source and composition of lipids comprising Coxiella and other parasite PV are poorly defined. A recent study addressed this gap in knowledge by examining cholesterol metabolism in Coxiella-infected Vero cells (Howe and Heinzen, 2006). Filipin staining indicates the PV membrane has approximately the same cholesterol content as the cholesterol-rich plasma membrane (Lange et al., 1989). To compensate for biogenesis of the

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novel *Coxiella* compartment, infected cells produce 73% more cholesterol late in the pathogen’s infectious cycle (6 days post infection) than uninfected cells, indicating a dramatic increase in cholesterol metabolism as opposed to simple redistribution of normal cholesterol stores. Accordingly, transcription of host genes involved in both cholesterol uptake (e.g. LDL receptor) and biosynthesis (e.g. lanosterol synthase) increases in response to the growth cycle of *Coxiella*, with upregulation of these genes observed only during log phase (~4 days post infection), a time when the PV is dramatically expanding (Coleman *et al*., 2004). These data indicate the *Coxiella* PV is integrated into the cholesterol distribution machinery of the host cell and that cholesterol homeostatic mechanisms respond to the progression of a *Coxiella* infection. Pharmacological agents that block specific steps of cholesterol uptake or biosynthesis inhibit *Coxiella* growth and PV formation, further suggesting exploitation by the pathogen of both pathways (Howe and Heinzen, 2006).

PV cholesterol may serve both important structural and signalling roles during *Coxiella* infection. As a structural component, cholesterol increases the mechanical strength of phospholipid bilayers (Needham *et al*., 1988). Consistent with this property, the mature *Coxiella* PV is highly resistant to mechanical disruption despite its spaciousness (Heinzen and Hackstadt, 1997). Cholesterol also decreases the ionic permeability of membranes (Corvera *et al*., 1992) and thereby may help the PV maintain a luminal acidic pH by minimizing the leakage of protons (Maurin *et al*., 1992). In a signalling role, cholesterol, in conjunction with sphingolipids, may form lipid raft microdomains in the PV membrane. Indeed, the PV membrane decorates with the lipid raft proteins flotillin-1 and flotillin-2 (Li *et al*., 2003; Howe and Heinzen, 2006). Lipid rafts are generally enriched in the plasma membrane where they partition signalling proteins that modulate a number of cellular functions including membrane fusion (Simons and Gruenberg, 2000). Thus, lipid raft-based signalling may mediate PV fusion with the endolysosomal compartment. (A model depicting the *Coxiella* infection process including steps in internalization, phagosome maturation and features of mature PV, is depicted in Fig. 2).

**Insight from coinfection of cultured cells with *Coxiella* and other intracellular pathogens**

Information on *Coxiella* PV biogenesis and composition has been acquired by coinfecting *Coxiella*-infected cells with other intravacuolar pathogens. This approach probes the dominance of pathogen signals directing PV maturation and the permissiveness of coinhabited vacuoles for pathogen replication (Rabinovitch and Veras, 1996). The *Coxiella* PV does not fuse with vacuoles harbouring...
Toxoplasma gondii (Sinai et al., 2000), C. trachomatis (Heinzen et al., 1996), or Legionella (Sauer et al., 2005). A common theme of these three pathogens is that their respective nascent phagosomes disconnect from the endocytic cascade soon after infection (Heinzen et al., 1996; Sinai et al., 2000; Roy et al., 2006). In contrast, mature Coxiella PV readily fuse with parasite vacuoles that remain in the endocytic pathway, including those of Mycobacterium avium (de Chastellier et al., 1999; Gomes et al., 1999), Mycobacterium tuberculosis (Gomes et al., 1999), Salmonella enterica (Drecktrah et al., 2007), Leishmania amazonensis (Veras et al., 1995) and Trypanosoma cruzi.
(Andreoli et al., 2006). The cohabitation of L. amazonensis, T. cruzi and S. enterica with Coxiella is not surprising as these pathogens normally traffic to vacuoles with lysosomal characteristics (Veras et al., 1995; Andreoli et al., 2006; Drecktrah et al., 2007). More striking is the trafficking of M. avium and M. tuberculosis to the Coxiella PV as maturation of their respective vacuoles normally stalls at an early endosomal stage (Clemens and Horwitz, 1995; Sturgill-Koszycki et al., 1996). Coxiella impedance of signals that promote stalling of the mycobacterial phagosome might be mediated by a type IV effector(s) that alters Rab GTPase(s) or vesicle/target SNARE(s) function (Vieira et al., 2002). Consistent with this general mechanism, a Legionella type IV effector (SidM/DrrA) that modulates Rab1 activity has recently been identified (Machner and Isberg, 2006; Murata et al., 2006).

Similar Dot/Icm T4SSs make Legionella and Coxiella coinfections particularly intriguing. In both epithelial and professional phagocytic cells, pre-existing Coxiella PV do not inhibit development of vacuoles harbouring Legionella. Similarly, infection by Legionella infection does not disrupt the acidification or structural integrity of an existing Coxiella PV (Sauer et al., 2005). It is interesting to note that these pathogens do not traffic to a common vacuole even in A/J mouse bone marrow-derived macrophages where the Legionella vacuole eventually acquires lysosomal characteristics (acidic and LAMP-2 positive) (Sauer et al., 2005). The Coxiella PV is promiscuously fusogenic with compartments possessing this phenotype (Howe et al., 2003). The exquisite specificity of Coxiella and Legionella vacuole development likely reflects the deployment of unique Dot/Icm effectors with non-overlapping functions.

Coinfection studies also provide information regarding the nutritional character and toxicity of PVs. M. avium, L. amazonensis and T. cruzi efficiently replicate in the Coxiella PV (Veras et al., 1995; Gomes et al., 1999; Andreoli et al., 2006), while M. tuberculosis does not. Interestingly, type IV secretion-deficient Legionella dotA mutants traffic to the Coxiella PV where they survive but fail to replicate (Sauer et al., 2005). Lack of replication is not explained solely by the acidity of the PV as wild-type Legionella replicate in acidic vacuoles in murine macrophages (Sturgill-Koszycki and Swanson, 2000; Sauer et al., 2005). The Coxiella PV may be nutritionally deficient for Legionella; alternatively, the dotA mutant may be incapable of differentiating into a replicative form in the absence of required T4SS signals (Swanson et al., 2002). Nonetheless, wild-type Legionella replicate in distinct vacuoles in singly and Coxiella-infected cells at approximately the same rate (Sauer et al., 2005), indicating that each pathogen utilizes a non-competitive nutrient source or that the host can metabolically adapt to provide the necessary nutrients via a common pathway.

**Strategies for phagolysosomal survival**

How Coxiella resists the bactericidal/static elements of its mature PV is a mystery. In this environment the organism must withstand acidic conditions and an onslaught of cat-ionic peptides and lysosomal hydrolases. The Coxiella surface must be intrinsically resistant to these insults as non-metabolizing organisms retain viability for several days in a lysosome-like, tight-fitting vacuole (Howe et al., 2003). A biochemically unusual peptidoglycan with associated protease-resistant proteins may provide protection from lysosomal digestion (Amano and Williams, 1984). In professional phagocytes, noxious reactive oxygen and nitrogen species are also produced that aid in disposal of invading pathogens. However, infection of resting macrophages by opsonized or non-opsonized Coxiella does not elicit a respiratory burst or superoxide production (Baca et al., 1994), a behaviour that may be mediated by a secreted protein tyrosine acid phosphatase that inhibits NADPH oxidase activity (Li et al., 1996). Indeed, Coxiella translocates several proteins in vitro during metabolic acid activation in a defined medium (Redd and Thompson, 1995), and delayed phagosome maturation may allow synthesis of protective molecules prior to delivery of toxic substances (Romano et al., 2007).

**Concluding remarks**

The cellular biology of Coxiella has been challenging to dissect because of difficulties inherent in working with a highly infectious obligate intracellular parasite that lacks genetic systems. However, progress has been made in revealing mechanisms behind the amazing ability of Coxiella to thrive in an environment that other intracellular parasites actively avoid. It is only in a lysosome-like compartment that Coxiella is metabolically active, morphologically differentiates, and secretes virulence factors that potentially modulate PV biogenesis and lysosomal resistance. While the nature of Coxiella interaction with the host cell is becoming clearer, important questions remain unanswered. First, generation of a stable SCV is obviously important for extracellular survival of Coxiella; however, what is the biological relevance of Coxiella morphological differentiation during the infectious process? Second, how does autophagy-mediated phagosome stalling benefit Coxiella? Finally, what is the nature of Coxiella T4SS effectors and what host cell functions do they target? Elucidation of these questions and other unresolved issues will dramatically aid our ability to model cellular interactions of Coxiella and to better understand

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virulence mechanisms employed by the pathogen to cause animal and human disease.

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References

Abu-Zant, A., Jones, S., Asare, R., Suttles, J., Price, C., Graham, J., and Kwaik, Y.A. (2007) Anti-apoptotic signaling by the Dot/Icm secretion system of L. pneumophila. Cell Microbiol 9: 246–264.

Akporiaye, E.T., Rowatt, J.D., Aragon, A.A., and Baca, O.G. (1983) Lysosomal response of a murine macrophage-like cell line persistently infected with Coxella burnetii. Infect Immun 40: 1155–1162.

Alpuche-Aranda, C.M., Racoosin, E.L., Swanson, J.A., and Miller, S.I. (1994) Salmonella stimulate macrophage m sign in tons inocytosis and persist within spacious phagosomes. J Exp Med 179: 601–608.

Amano, K., and Williams, J.C. (1984) Sensitivity of Coxella burnetii peptidoglycan to lysozyme hydrolysis and correlation of saccus rigidity with peptidoglycan-associated proteins. J Bacteriol 160: 989–993.

Andoh, M., Naganawa, T., Hotta, A., Yamaguchi, T., Fukushi, A., Amano, K., and Williams, J.C. (1984) Sensitivity of Coxella burnetii peptidoglycan to lysozyme hydrolysis and correlation of saccus rigidity with peptidoglycan-associated proteins. J Bacteriol 160: 989–993.

Andoh, M., Naganawa, T., Hotta, A., Yamaguchi, T., Fukushi, A., Masagi, T., and Hirai, K. (2003) SCID mouse model for survival of Coxella burnetii. Infect Immun 71: 4717–4723.

Andreu, W.K., Taniwaki, N.N., and Mortara, R.A. (2006) Survival of Trypanosoma cruzi metacyclic trypomastigotes within Coxella burnetii vacuoles: differentiation and replication within an acidic milieu. Microbes Infect 8: 172–182.

Baca, O.G., and Paretsky, D. (1983) Q fever and Coxella burnetii: a model for host–parasite interactions. Microbiol Rev 47: 127–149.

Baca, O.G., Akporiaye, E.T., Aragon, A.S., Martinez, I.L., Robles, M.V., and Warner, N.L. (1981) Fate of phase I and phase II Coxella burnetii in several macrophage-like tumor cell lines. Infect Immun 33: 258–266.

Baca, O.G., Scott, T.O., Akporiaye, E.T., DeBlassie, R., and Crissman, H.A. (1985) Cell cycle distribution patterns and generation times of L929 fibroblast cells persistently infected with Coxella burnetii. Infect Immun 47: 366–369.

Baca, O.G., Klassen, D.A., and Aragon, A.S. (1993) Entry of Coxella burnetii into host cells. Acta Virol 37: 143–155.

Baca, O.G., Li, Y.P., and Kumar, H. (1994) Survival of the Q fever agent Coxella burnetii in the phagolysosome. Trends Microbiol 2: 476–480.

Bardill, J.P., Miller, J.L., and Vogel, J.P. (2005) IcmS-dependent translocation of SdeA into macrophages by the Legionella pneumophila type IV secretion system. Mol Microbiol 58: 90–103.

Beare, P.A., Samuel, J.E., Howe, D., Virtaneva, K., Porcella, S.F., and Heinzen, R.A. (2006) Genetic diversity of the Q fever agent, Coxella burnetii, assessed by microarray-based whole-genome comparisons. J Bacteriol 188: 2309–2324.

Beron, W., Gutierrez, M.G., Rabinovitch, M., and Colomb, M.I. (2002) Coxella burnetii localizes in a Rab7-labelled compartment with autopagic characteristics. Infect Immun 70: 5816–5821.

Brennan, R.E., Russell, K., Zhang, G., and Samuel, J.E. (2004) Both inducible nitric oxide synthase and NADPH oxidase contribute to the control of virulent phase I Coxella burnetii infections. Infect Immun 72: 6666–6675.

Burton, P.R., Kordova, N., and Paretsky, D. (1971) Electron microscopic studies of the rickettsia Coxella burnetii: entry, lysosomal response, and fate of rickettsial DNA in 1-cells. Can J Microbiol 17: 143–150.

Capo, C., Lindberg, F.P., Meconi, S., Zaffran, Y., Tardei, G., Brown, E.J., et al. (1999) Subversion of monocyte functions by Coxella burnetii: impairment of the cross-talk between ab integrin and CR3. J Immunol 163: 6078–6085.

Capo, C., Moynauet, A., Collette, Y., Olive, D., Brown, E.J., Raoul, D., and Mege, J.L. (2003) Coxella burnetii avoids macrophage phagocytosis by interfering with spatial distribution of complement receptor 3. J Immunol 170: 4217–4225.

de Chastellier, C., Thibon, M., and Rabinovitch, M. (1999) Construction of chimeric phagosomes that shelter Mycobacterium avium and Coxella burnetii (phase II) in doubly infected mouse macrophages: an ultrastructural study. Eur J Cell Biol 78: 580–592.

Christie, P.J., and Vogel, J.P. (2000) Bacterial type IV secretion: conjugation systems adapted to deliver effector molecules to host cells. Trends Microbiol 8: 354–360.

Clemens, D.L., and Horwitz, M.A. (1995) Characterization of the Mycobacterium tuberculosis phagosome and evidence that phagosomal maturation is inhibited. J Exp Med 181: 257–270.

Clifton, D.R., Fields, K.A., Grieshaber, S.S., Dooley, C.A., Fischer, E.R., Mead, D.J., et al. (2004) A chlamydial type III translocated protein is tyrosine-phosphorylated at the site of entry and associated with recruitment of actin. Proc Natl Acad Sci USA 101: 10166–10171.

Coers, J., Monahan, J.J., and Roy, C.R. (1999) Modulation of phagosome biogenesis by Legionella pneumophila creates an organelle permissive for intracellular growth. Nat Cell Biol 1: 183–188.

Coleman, S.A., Fischer, E.R., Howe, D., Mead, D.J., and Heinzen, R.A. (2004) Temporal analysis of Coxella burnetii morphology: infection. Mol Microbiol 267: 261–270.

Corvera, E., Mouri, O.G., Singer, M.A., and Zuckermann, M.J. (1992) The permeability and the effect of acyl-chain length for phospholipid bilayers containing cholesterol: theory and experiment. Biochim Biophys Acta 1107: 261–270.

Denison, A.M., Massung, R.F., and Thompson, H.A. (2007) Analysis of the O-antigen biosynthesis regions of phase II isolates of Coxella burnetii. FEMS Microbiol Lett 267: 102–107.

Drecktrah, D., Knodler, L.A., Howe, D., and Steele-Mortimer, O. (2007) Salmonella trafficking is defined by continuous dynamic interactions with the endolysosomal system. Traffic 7 (in press).
Feldman, M., Zusman, T., Hagag, S., and Segal, G. (2005) Coevolution between nonhomologous but functionally similar proteins and their conserved partners in the Legionella pathogenesis system. 

Proc Natl Acad Sci USA 102: 12206–12211.

de Felice, K.S., Pampou, S., Jovanovic, O.S., Pericone, C.D., Ye, S.F., Kalachikov, S., and Shuman, H.A. (2005) Evidence for acquisition of Legionella type IV secretion substrates via interdomain horizontal gene transfer. 

J Bacteriol 187: 7716–7726.

Fortier, A., Min-Oo, G., Forbes, J., Lam-Yuk-Tseung, S., and Gros, P. (2005) Single gene effects in mouse models of host: pathogen interactions. 

J Leukoc Biol 77: 868–877.

Ghigo, E., Capo, C., Tung, C.H., Raoult, D., Gorvel, J.P., and Mege, J.L. (2002) Coxiella burnetii survival in THP-1 monocytes involves the impairment of phagosome maturation: IFN-gamma mediates its restoration and bacterial killing. 

J Immunol 169: 4488–4495.

Ghigo, E., Honstetttre, A., Capo, C., Gorvel, J.P., Raoult, D., and Mege, J.L. (2004) Link between impaired maturation of phagosomes and defective Coxiella burnetii killing in patients with chronic Q fever. 

J Infect Dis 190: 1767–1772.

Gomes, M.S., Paul, S., Moreira, A.L., Appelberg, R., Rabinovich, M., and Kaplan, G. (1999) Survival of Mycobacterium avium and Mycobacterium tuberculosis in acidified vacuoles of murine macrophages. 

Infect Immun 67: 3199–3206.

Gutierrez, M.G., Master, S.S., Singh, S.B., Taylor, G.A., Colombo, M.I., and Deretic, V. (2004) Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. 

Cell 119: 753–766.

Gutierrez, M.G., Vazquez, C.L., Munafö, D.B., Zoppino, F.C., Beron, W., Rabino-vitch, M., and Colombo, M.I. (2005) Autophagy induction favours the generation and maturation of the Coxiella-replicative vacuoles. 

Cell Microbiol 7: 981–993.

Hackstadt, T. (1988) Steric hindrance of antibody binding to surface proteins of Coxiella burnetii by phase I lipopolysaccharide. 

Infect Immun 56: 802–807.

Hackstadt, T. (1996) Biosafety concerns and Coxiella burnetii. 

Trends Microbiol 4: 341–342.

Hackstadt, T., and Williams, J.C. (1981) Biochemical strategies for obligate parasitism of eukaryotic cells by Coxiella burnetii. 

Proc Natl Acad Sci USA 78: 3240–3244.

Hackstadt, T., Peacock, M.G., Hitchcock, P.J., and Cole, R.L. (1985) Lipopolysaccharide variation in Coxiella burnetii: intrasubstrate heterogeneity in structure and antigenicity. 

Infect Immun 48: 359–365.

Hechamy, K.E., McKee, M., Marko, M., Samsonoff, W.A., Roman, M., and Baca, O. (1993) Three-dimensional reconstruction of Coxiella burnetii-infected L929 cells by high-voltage electron microscopy. 

Infect Immun 61: 4485–4488.

Heinzen, R.A., and Hackstadt, T. (1997) The Chlamydia trachomatis parasitophorous vacuolar membrane is not passively permeable to low-molecular-weight compounds. 

Infect Immun 65: 1088–1094.

Heinzen, R.A., Scidmore, M.A., Rockey, D.D., and Hackstadt, T. (1996) Differential interaction with endocytic and exocytic pathways distinguish parasitophorous vacuoles of Coxiella burnetii and Chlamydia trachomatis. 

Infect Immun 64: 796–809.

Heinzen, R.A., Hackstadt, T., and Samuel, J.E. (1999) Developmental biology of Coxiella burnetii. 

Trends Microbiol 7: 149–154.

Honstetttre, A., Ghigo, E., Moynault, A., Capo, C., Toman, R., Akira, S., et al. (2004) Lipopolysaccharide from Coxiella burnetii is involved in bacterial phagocytosis, filamentous actin reorganization, and inflammatory responses through Toll-like receptor 4. 

J Immunol 172: 3695–3703.

Honstetttre, A., Meghari, S., Nunes, J.A., Lepidi, H., Raoult, D., Olive, D., and Mege, J.L. (2006) Role for the CD28 molecule in the control of Coxiella burnetii infection. 

Infect Immun 74: 1800–1808.

Hoover, T.A., Culp, D.W., Vodkin, M.H., Williams, J.C., and Thompson, H.A. (2002) Chromosomal DNA deletions explain phenotypic characteristics of two antigen variants, phase II and RSA 514 (crazy), of the Coxiella burnetii nine mile strain. 

Infect Immun 70: 6726–6733.

Howe, D., and Heinzen, R.A. (2006) Coxiella burnetii inhabits a cholesterol-rich vacuole and influences cellular cholesterol metabolism. 

Cell Microbiol 8: 496–507.

Howe, D., and Mallavia, L.P. (2000) Coxiella burnetii exhibits morphological change and delays phagolysosomal fusion after internalization by J774A.1 cells. 

Infect Immun 68: 3815–3821.

Howe, D., Melnicakova, J., Barak, I., and Heinzen, R.A. (2003) Maturation of the Coxiella burnetii parasitophorous vacuole requires bacterial protein synthesis but not replication. 

Cell Microbiol 5: 469–480.

Khavkin, T., and Tabibzadeh, S.S. (1988) Histologic, immunostaining, and electron microscopic study of infectious process in mouse lung after intranasal challenge with Coxiella burnetii. 

Infect Immun 56: 1792–1799.

Kirkegaard, K., Taylor, M.P., and Jackson, W.T. (2004) Cellular autophagy: surrender, avoidance and subversion by microorganisms. 

Nat Rev Microbiol 2: 301–314.

Kishimoto, R.A., and Walker, J.S. (1976) Interaction between Coxiella burnetii and guinea pig peritoneal macrophages. 

Infect Immun 14: 416–421.

Knodler, L.A., Celli, J., and Finlay, B.B. (2001) Pathogenic trickery: deception of host cell processes. 

Nat Rev Mol Cell Biol 2: 578–588.

Laguna, R.K., Creasey, E.A., Li, Z., Valtz, N., and Isberg, R.R. (2006) A Legionella pneumophila-translocated substrate that is required for growth within macrophages and protection from host cell death. 

Proc Natl Acad Sci USA 103: 18745–18750.

Lange, Y., Swaisgood, M.H., Ramos, B.V., and Steck, T.L. (1989) Plasma membranes contain half the phospholipid and 90% of the cholesterol and sphingomyelin in cultured human fibroblasts. 

J Biol Chem 264: 3786–3793.

La Scala, B., Lepidi, H., and Raoult, D. (1997) Pathologic changes during acute Q fever: influence of the route of infection and inoculum size in infected guinea pigs. 

Infect Immun 65: 2443–2447.

Li, N., Mak, A., Richards, D.P., Naber, C., Keller, B.O., Li, L., and Shaw, A.R. (2003) Monocyte lipid rafts contain proteins implicated in vesicular trafficking and phagosome formation. 

Proteomics 3: 536–548.
Li, Y.P., Curley, G., Lopez, M., Chavez, M., Giew, R., Aragon, A., et al. (1996) Protein-tyrosine phosphatase activity of Coxiella burnetii that inhibits human neutrophils. Acta Virol 40: 263–272.

Losick, V.P., and Isberg, R.R. (2006) NF-kappaB translocation prevents host cell death after low-dose challenge by Legionella pneumophila. J Exp Med 203: 2177–2189.

Luo, Z.Q., and Isberg, R.R. (2004) Multiple substrates of the Legionella pneumophila Dot/Icm system identified by interbacterial protein transfer. Proc Natl Acad Sci USA 101: 841–846.

McCaul, T.F., and Williams, J.C. (1981) Developmental cycle of Coxiella burnetii: structure and morphogenesis of vegetative and sporogenic differentiations. J Bacteriol 147: 1063–1076.

Machner, M.P., and Isberg, R.R. (2006) Targeting of host Rab GTPase function by the intravacuolar pathogen Legionella pneumophila. Dev Cell 11: 47–56.

Maurin, M., and Raoult, D. (1999) Q fever. Clin Microbiol Rev 12: 518–553.

Maurin, M., Benoïlet, A.M., Bongrand, P., and Raoult, D. (1992) Phagolysosomes of Coxiella burnetii-infected cell lines maintain an acidic pH during persistent infection. Infect Immun 60: 5013–5016.

Meconi, S., Jacomo, V., Boquet, P., Raoult, D., Mege, J.L., and Capo, C. (1998) Coxiella burnetii induces reorganization of the actin cytoskeleton in human monocytes. Infect Immun 66: 5527–5533.

Meconi, S., Capo, C., Remacle-Bonnet, M., Pommier, G., Raoult, D., and Mege, J.L. (2001) Activation of protein tyrosine kinases by Coxiella burnetii: role in actin cytoskeleton reorganization and bacterial phagocytosis. Infect Immun 69: 2520–2526.

Meghani, S., Honstetter, A., Lepidi, H., Ryffel, B., Raoult, D., and Mege, J.L. (2005) TLR2 is necessary to inflammatory response in Coxiella burnetii infection. Ann NY Acad Sci 1063: 161–166.

Miller, J.D., Curns, A.T., and Thompson, H.A. (2004) A growth study of Coxiella burnetii Nine Mile Phase I and Phase II in fibroblasts. FEMS Immunol Med Microbiol 42: 291–297.

Moos, A., and Hackstadt, T. (1987) Comparative virulence of intra- and interstrain lipopolysaccharide variants of Coxiella burnetii in the guinea pig model. Infect Immun 55: 1144–1150.

Murata, T., Delprato, A., Ingumnadon, A., Toomre, D.K., Lambright, D.G., and Roy, C.R. (2006) The Legionella pneumophila effector protein DrtA is a Rab1 guanine nucleotide-exchange factor. Nat Cell Biol 8: 971–977.

Nagai, H., Kagan, J.C., Zhu, X., Kahn, R.A., and Roy, C.R. (2002) A bacterial guanine nucleotide exchange factor activates ARF on Legionella phagosomes. Science 295: 679–682.

Needham, D., McIntosh, T.J., and Evans, E. (1988) Thermomechanical and transition properties of dimyristoylphosphatidylcholine/cholesterol bilayers. Biochemistry 27: 4668–4673.

Oh, Y.K., and Swanson, J.A. (1996) Different fates of phagocytosed particles after delivery into macrophage lysosomes. J Cell Biol 132: 585–593.

Pizarro-Cerda, J., Mieres, S., Parton, R.G., van der Goot, G., Sola-Landa, A., Lopez-Goni, I., et al. (1998) Brucella abortus transits through the autophagic pathway and replicates in the endoplasmic reticulum of nonprofessional phagocytes. Infect Immun 66: 5711–5724.

Rabinovitch, M., and Vera, P.S. (1996) Cohabitation of Leishmania amazonensis and Coxiella burnetii. Trends Microbiol 4: 158–161.

Raoult, D., Vestris, G., and 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.

Redd, T., and Thompson, H.A. (1995) Secretion of proteins by Coxiella burnetii. Microbiology 141: 363–369.

Roman, M.J., Coriz, P.D., and Baca, O.G. (1986) A proposed model to explain persistent infection of host cells with Coxiella burnetii. J Gen Microbiol 132: 1415–1422.

Romano, P.S., Gutierrez, M.G., Beron, W., Rabinovitch, M., and Colombo, M.I. (2007) The autophagic pathway is actively modulated by phase II Coxiella burnetii to efficiently replicate in the host cell. Cell Microbiol (in press). doi: 10.1111/j.1462-5822.2007.00901.x.

Roux, V., Bergoin, M., Lamaze, N., and Raoult, D. (1997) reassessment of the taxonomic position of Rickettsiella grylli. Int J Syst Bacteriol 47: 1255–1257.

Roy, C.R., Berger, K.H., and Isberg, R.R. (1998) Legionella pneumophila DotA protein is required for early phagosome trafficking decisions that occur within minutes of bacterial uptake. Mol Microbiol 28: 663–674.

Roy, C.R., Salcedo, S.P., and Gorvel, J.P. (2006) Pathogen—endoplasmic—reticulum interactions: in through the out door. Nat Rev Immunol 6: 136–147.

Russell-Lodrigue, K.E., Zhang, G.Q., McMurray, D.N., and Samuel, J.E. (2006) Clinical and pathologic changes in a guinea pig aerosol challenge model of acute Q fever. Infect Immun 74: 6085–6091.

Sauer, J.D., Shannon, J.G., Howe, D., Hayes, S.F., Swanson, M.S., and Heinzen, R.A. (2005) Specificity of Legionella pneumophila and Coxiella burnetii vacuoles and versatility of Legionella pneumophila revealed by coinfection. Infect Immun 73: 4494–4504.

Scott, G.H., Williams, J.C., and Stephenson, E.H. (1987) Animal models in Q fever: pathological responses of inbred mice to phase I Coxiella burnetii. J Gen Microbiol 133: 691–700.

Segal, G., Purcell, M., and Shuman, H.A. (1998) Host cell killing and bacterial conjugation require overlapping sets of genes within a 22-kb region of the Legionella pneumophila genome. Proc Natl Acad Sci USA 95: 1669–1674.

Segal, G., Feldman, M., and Zusman, T. (2005) The icm/Dot type-IV secretion systems of Legionella pneumophila and Coxiella burnetii. FEMS Microbiol Rev 29: 65–81.

Seshadri, R., Paulsen, I.T., Eisen, J.A., Read, T.D., Nelson, K.E., Nelson, W.C., et al. (2003) Complete genome sequence of the Q-fever pathogen Coxiella burnetii. Proc Natl Acad Sci USA 100: 5455–5460.
Sexton, J.A., and Vogel, J.P. (2002) Type IVB secretion by intracellular pathogens. *Traffic* 3: 178–185.

Shannon, J.G., Howe, D., and Heinzen, R.A. (2005) Virulent *Coxiella burnetii* does not activate human dendritic cells: role of lipopolysaccharide as a shielding molecule. *Proc Natl Acad Sci USA* 102: 8722–8727.

Simons, K., and Gruenberg, J. (2000) Jamming the endosomal system: lipid rafts and lysosomal storage diseases. *Trends Cell Biol* 10: 459–462.

Simons, K.A., Paul, S., Rabinovitch, M., Kaplan, G., and Joiner, S. (2000) Coinfection of fibroblasts with *Coxiella burnetti* and *Toxoplasma gondii* to each their own. *Microbes Infect* 2: 727–736.

Stebbins, C.E., and Galan, J.E. (2001) Structural mimicry in bacterial virulence. *Nature* 412: 701–705.

Stein, A., Louveau, C., Lepidi, H., Ricci, F., Baylac, P., Davoust, B., and Raault, D. (2005) Q fever pneumonia: virulence of *Coxiella burnetii* pathovars in a murine model of aerosol infection. *Infect Immun* 73: 2469–2477.

Sturgill-Koszycki, S., and Swanson, M.S. (2000) *Legionella pneumophila* replication vacuoles mature into acidic, endocytic organelles. *J Exp Med* 192: 1261–1272.

Sturgill-Koszycki, S., Schaible, U.E., and Russell, D.G. (1996) *Mycobacterium*-containing phagosomes are accessible to early endosomes and reflect a transitional state in normal phagosome biogenesis. *EMBO J* 15: 6960–6968.

Swanson, M.S., Fernandez-Moreira, E., and Fernandez-Moreira, E. (2002) A microbial strategy to multiply in macrophages: the pregnant pause. *Traffic* 3: 170–177.

Tujulin, E., Lilliehook, B., Macellaro, A., Sjostedt, A., and Norlander, L. (1999) Early cytokine induction in mouse P88D1 macrophages infected by *Coxiella burnetii*. *Vet Immunol Immunopathol* 68: 159–168.

Van Rheezen, S.M., Luo, Z.Q., O’Connor, T., and Isberg, R.R. (2006) Members of a *Legionella pneumophila* family of proteins with ExoU (phospholipase A) active sites are translocated to target cells. *Infect Immun* 74: 3597–3606.

Veras, P.S., Moula, C., Dauguet, C., Tunis, C.T., Thibon, M., and Rabinovitch, M. (1995) Entry and survival of *Leishmania amazonensis* amastigotes within phagolysosome-like vacuoles that shelter *Coxiella burnetti* in Chinese hamster ovary cells. *Infect Immun* 63: 3502–3506.

Vieira, O.V., Botelho, R.J., and Grinstein, S. (2002) Phagosome maturation: aging gracefully. *Biochem J* 366: 689–704.

Vishwanath, S., and Hackstadt, T. (1988) Lipopolysaccharide phase variation determines the complement-mediated serum susceptibility of *Coxiella burnetii*. *Infect Immun* 56: 40–44.

Vogel, J.P. (2004) Turning a tiger into a house cat: using *Legionella pneumophila* to study *Coxiella burnetti*. *Trends Microbiol* 12: 103–105.

Vogel, J.P., Andrews, H.L., Wong, S.K., and Isberg, R.R. (1998) Conjugative transfer by the virulence system of *Legionella pneumophila*. *Science* 279: 873–876.

Waag, D.M., Byrne, W.R., Estep, J., Gibbs, P., Pitt, M.L., and Banfield, C.M. (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 burnetti*. *Lab Anim Sci* 49: 634–638.

Williams, J.C. (1991) Infectivity, virulence, and pathogenicity of *Coxiella burnetti* for various hosts. In *Q Fever: the Biology of Coxiella Burnetii*. Williams, J.C., Thompson, H.A. (eds). Boca Raton, Fl: CRC Press, pp. 21–71.

Williams, J.C., Peacock, M.G., and McCaul, T.F. (1981) Determinants of aerosol infection. *Proc Natl Acad Sci USA* 78: 8722–8727.

Zamboni, D.S. (2004) Genetic control of natural resistance of mouse macrophages to *Coxiella burnetii* infection *in vitro*: macrophages from restrictive strains control parasitophorous vacuole maturation. *Infect Immun* 72: 2395–2399.

Zamboni, D.S., and Rabinovitch, M. (2003) Nitric oxide partially controls *Coxiella burnetti* phase II infection in mouse primary macrophages. *Infect Immun* 71: 1225–1233.

Zamboni, D.S., Mortara, R.A., Freymuller, E., and Rabinovitch, M. (2002) Mouse resident peritoneal macrophages partially control *Coxiella burnetii* phase II infection *in vitro*. *Microbes Infect* 4: 591–598.

Zamboni, D.S., McGrath, S., Rabinovitch, M., and Roy, C.R. (2003) *Coxiella burnetii* express type IV secretion system proteins that function similarly to components of the *Legionella pneumophila* Dot/Icm system. *Mol Microbiol* 49: 965–976.

Zamboni, D.S., Campos, M.A., Torrecilhas, A.C., Kiss, K., Samuel, J.E., Golenbock, D.T., *et al.* (2004) Stimulation of toll-like receptor 2 by *Coxiella burnetti* is required for macrophage production of pro-inflammatory cytokines and resistance to infection. *J Biol Chem* 279: 54405–54415.

Zhang, G., Kiss, K., Seshadri, R., Hendrix, L.R., and Samuel, J.E. (2004) Identification and cloning of immunodominant antigens of *Coxiella burnetii*. *Infect Immun* 72: 844–852.

Zusman, T., Yerushalmi, G., and Segal, G. (2003) Functional similarities between the *icm/dot* pathogenesis systems of *Coxiella burnetii* and *Legionella pneumophila*. *Infect Immun* 71: 3714–3723.