Pathogenic Properties of Epidemic Typhus-Causing Bacteria *Rickettsia prowazekii*: A Review

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Rickettsia prowazekii is the pathogenic bacterium responsible for epidemic typhus fever, an infectious disease that has plagued humans ever since it was first recorded in Europe nearly 1000 years ago (Szybalski, 1999). More recently, *R. prowazekii* is estimated to have infected nearly 30 million humans following the First and Second World War (Andersson *et al.*, 1998), and continues to pose a major health threat in certain parts of the world, despite the availability of antibiotics. *R. prowazekii* is typically transmitted to humans by the body louse – an obligate ectoparasitic arthropod (Figure 1) (Baxter, 1996). In its vegetative state, the pleomorphic *R. prowazekii* is 0.3 μm to 0.5 μm by 0.8 μm to 2.0 μm in size (Walker, 1998), which makes this species too small to be clearly seen under an ordinary light microscope. *R. prowazekii* belongs to a large group of Gram-negative bacteria known as α-proteobacteria that multiply in eukaryotic cells. This suggests that they can only grow and reproduce within the living cells of their host. Although classified as Gram-negative bacteria, *R. prowazekii* are poorly stained by the Gram method and are better visualized using the Giemsa or Giménez stains (Raoult *et al.*, 2004).

Figure 1. This image shows an adult female human body louse (*Pediculus humanus humanus*) along with two larval young. Obtained from WHO: World Health Organization (1976).

Intracellular *R. prowazekii* often appear as short, paired or single, lanceolate-to-ovoid coccobacilli (Raoult *et al.*, 2004). To undergo cellular respiration, this species requires oxygen as a terminal electron acceptor. Its envelope consists of three major layers: an innermost cytoplasmic membrane, a thin electron-dense rigid cell wall containing peptidoglycan, and an outer layer, which contains lipopolysaccharide endotoxin (Yu & Walker, 2005) and immunodominant surface-exposed proteins that provide structure or potentially contribute to host cell adhesion or other host cell interactions (Figure 2) (Raoult *et al.*, 2004). Cells of *R. prowazekii* also possess intracytoplasmic invaginations of the plasma membrane – a morphological feature that resembles cristae found in the mitochondria. Unlike the mitochondria, however, members of this species possess a flagellum for motility – a feature not common among the family Rickettsiaceae (Yu & Walker, 2005).
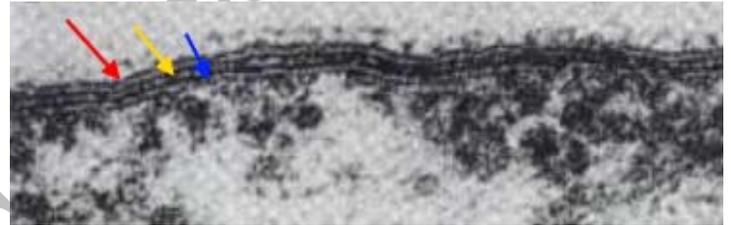

Figure 2. A transmission electron micrograph revealing the cell envelop of *R. prowazekii*. The red arrow points to the outer layer, the yellow arrow points to the thin electron-dense rigid cell wall, and the blue arrow points to the innermost cytoplasmic membrane [X 196 000]. Obtained from Yu & Walker (2005).

Since *R. prowazekii* requires an intracellular habitat, cells must be co-cultivated in tissue culture or yolk sac of developing chicken embryos (Figure 3); hence, a characteristic colonial morphology is inexistent. Under poor nutritional conditions, cells of *R. prowazekii* grow into long, 4 μm filaments and cease to multiply via transverse binary fission (Yu & Walker, 2005). However, when proper conditions are restored, they immediately divide into their ordinary short rod form and engage in extensive movements until released by the disruption of massively infected cells (Tritz, 2000).

The nutritional requirements of *R. prowazekii*, outside their host cell, are not known (Yu & Walker, 2005). What is known is that *R. prowazekii* acquires most of its amino acids from its host cell because only the genes associated with the biosynthesis of lysine and serine are present in its DNA (Yu & Walker, 2005). In addition, maximal growth of this species can only occur if there is a sufficient amount of host cell proline and serine or glycine (Yu &

Walker, 2005). This implies that *R. prowazekii* has a highly permeable cytoplasmic membrane. Likewise, glycolytic intermediates and products, such as acetyl-CoA, must also be obtained by its host. Similar to the mitochondrion found in eukaryotic cells, this species produces its own physiological energy supply of ATP via the enzyme ATPase (Yu & Walker, 2005). This reason, along with the evidence suggesting that *R. prowazekii* shares a phylogenetic relationship with the mitochondria, is why some biologists speculate that this species may have given rise to the modern-day mitochondria. Altogether, to be able to identify this species for diagnostic purposes, samples containing bacterial cells must be cultivated in tissue culture at 35°C. Once nutrients are depleted, longer, more filamentous-like cells with prominent vacuoles will develop (Figure 4). Infected tissues stained with Giemsa should show bluish-purple organisms, whereas tissue stained with Giménez should show bright red organisms, with a decolorized background stain of pale greenish blue (Yu & Walker, 2005). Due to the inability to grow *R. prowazekii* on ordinary microbiological media, such as nutrient broth, diagnosis is often difficult (Ge *et al.*, 2004).
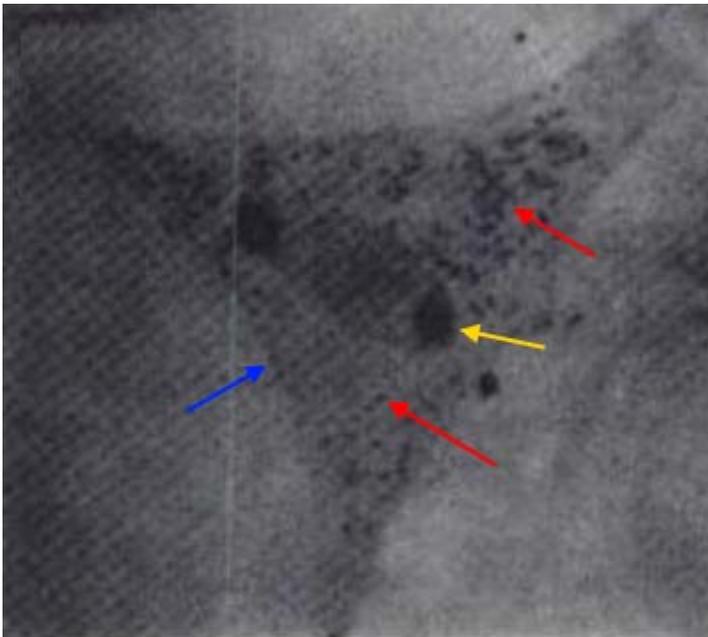

Figure 4. This transmission electron micrograph shows a large number of free cytoplasmic *R. prowazekii* late in chicken embryo fibroblast infection. The red arrow shows an example of a single vacuole-like structure that appears when nutrients are low or depleted, and the yellow arrow points to bacteria dividing by binary fission [X 13 600]. Obtained from Yu & Walker (2005).

Figure 3. This transmission electron micrograph depicts a chicken embryo fibroblast infected with a large mass of *R. prowazekii*. The red arrows point to the bacteria, the blue arrow points to the fibroblast cell membrane, and the yellow arrow points to the nucleus of the fibroblast [X 1200]. Obtained from Yu & Walker (2005).

The entry of *R. prowazekii* into a human host cell (typically a phagocyte) involves three steps: attachment (via OmpA protein and a host cell receptor), internalization and escape from the phagosome, prior to lysosomal
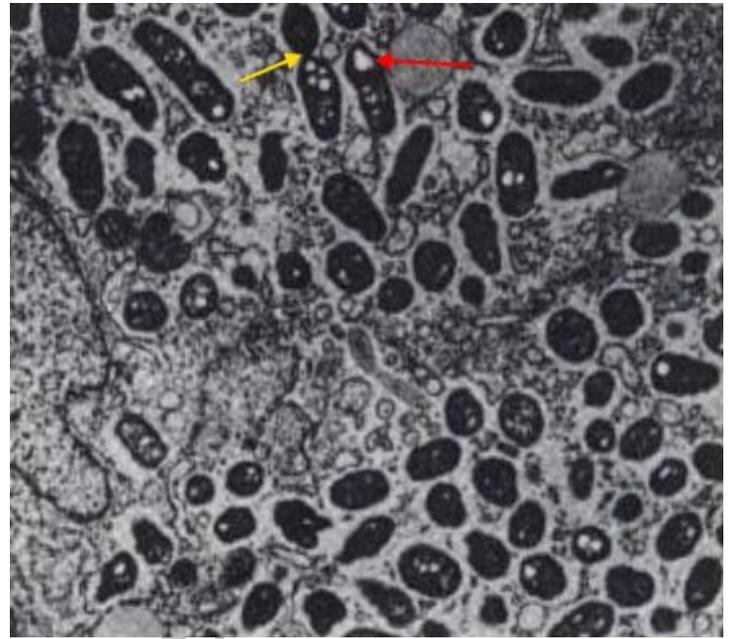
fusion (Figure 5) (Yu & Walker, 2005). By escaping phagosomal digestion, this evasion mechanism allows the pathogen to reside in the cytoplasm and escape class II MHC presentation. However, the pathogenic mechanism used particularly by *R. prowazekii* as a means to escape phagosomal entrapment is unclear, but may involve potential virulence factors, such as phospholipase A (Winkler and Miller, 1982), antigenic surface proteins, hemolysin, type IV secretion systems, or hydrolase enzymes (Ge *et al.*, 2004).
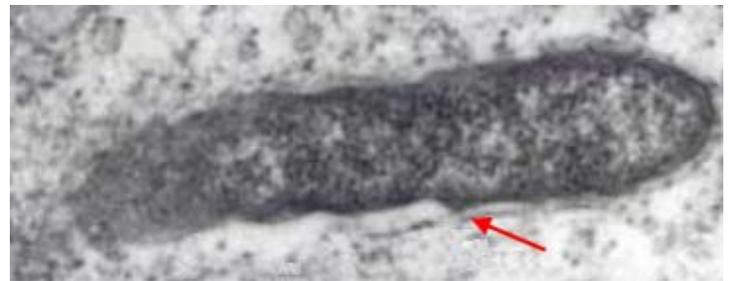

Figure 5. This micrograph shows *R. prowazekii* in the process of escaping phagosomal entrapment. The red arrow points to a break in the phagosome membrane [X 31 350]. Obtained from Yu & Walker (2005).

Body louse (*Pediculus humanus humanus*), the flying squirrel flea (*Orchopeas howardii*), and louse (*Neohematopinus sciuropteri*) are the most effective vectors in the spread of *R. prowazekii*-induced epidemic typhus fever. Once transmitted to the mammalian host through a bite via arthropod saliva, the bacteria are found principally in the endothelium of blood vessels, particularly in those of the brain, skin and heart (Tritz, 2000). This, in turn, causes hyperplasia of endothelial cells and localized thrombus formation, resulting in petechial rash, fever – which may reach 39°C – and terminal shock (Tritz, 2000). Finally, areas of poor hygiene, such as refugee camps during a major famine or natural disaster, are places where diseases such as typhus fever tend to flourish. In fact, due to recent outbreaks in parts of Europe, Africa, and South America, some experts support the notion that epidemic typhus is a reemerging public health problem (Ge *et al.*, 2004).

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