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Rickettsia and Rickettsial Diseases

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1. Introduction

Rickettsia prowazekii and *R. rickettsii* are HHS and USDA select agents (http://biosafety.utk.edu/pdfs/salist.pdf), and *R. prowazekii* is a category B bioterrorism agent as determined by the Centers for Disease Control and Prevention (CDC) (http://www.bt.cdc.gov/agent/agentlist-category.asp). The criteria for CDC category B bioterrorism agents are that the organisms are moderately easy to disseminate, cause diseases with moderate morbidity and low mortality and require specific enhancements of diagnostic capacity and enhanced disease surveillance. However, the case fatality ratios of both *R. prowazekii* and *R. rickettsii* may exceed the CDC bioterrorism agent category B level. Epidemic typhus caused by *R. prowazekii* and Rocky Mountain spotted fever (RMSF) caused by *R. rickettsii* can reach up to 60% fatalities without antibiotic treatment and 4% even with antibiotic treatment (Raoult et al., 2004). *Rickettsia* had been explored for biowarfare use. The former Soviet Union developed *R. prowazekii* as a biologic weapon in the 1930s (Alibek K and Handelman S, 2009). During World War II, the Japanese performed human experiments with rickettsial agents for purposes of biologic weapon development during their occupation of China (Harris S, 1992).

Epidemic typhus, also known as louse-borne typhus, has been distributed worldwide, was one of the man's major scourges and frequently played a decisive role in wars in Europe from the 15th through 20th centuries, thus affecting the course of European history (Conlon JM, 2007). It killed millions of people through this period. Although worldwide epidemics of typhus may not occur again, the threat of louse-borne typhus is still real as small scale epidemics or large scale epidemics in settings of extreme poverty and natural and manmade disasters. Louse-borne typhus occurs in epidemics when social, economic, or political systems are disrupted exposing a large population such as refugees to louse infestation due to lack of hygiene. This situation has been observed in recent outbreaks of typhus in Burundi, Algeria, Peru, and Russia. In 1997, it was estimated that as many as 100,000 cases of typhus occurred in the refugee camps of Burundi during a civil war (Raoult et al., 2004).

RMSF originated as an emerging infectious disease on the western *frontier* in the Rocky Mountains. Now the disease is found all over the United States, and over half of the cases occur in the southeastern and south-central regions of the United States and in South America (Center for Disease Control and prevention[CDC], 2010).

2. Etiologic agents

Rickettsia are small (0.3 – 0.5 x 0.8 – 1.0 μm) gram-negative obligately intracellular bacterial parasites of eukaryotic cells. The genus is subdivided into the typhus group (TG) and spotted fever group (SFG) based on lipopolysaccharide (LPS) antigens. The TG rickettsiae include louse-borne *R. prowazekii* that causes epidemic typhus and flea-borne *R. typhi* that causes murine typhus. The SFG rickettsiae consist of more than 20 named species, which are transmitted by tick bite except for *R. akari* (mite-borne) and *R. felis* (flea-borne). Antibodies to LPS antigens cross-react among organisms within the same biogroup, but do not cross-react between the two groups (Vishwanath, 1991). There are two major outer membrane proteins in *Rickettsia* OmpA (Sca 0) and OmpB (Sca5). OmpB exists in all *Rickettsia* and OmpA exists only in SFG rickettsiae. Genomic sequencing identified additional 14 surface cell antigens (Scas) among *Rickettsia (Blanc et al., 2005)*. However, most *sca* genes are degenerated in *Rickettsia,* and only two Scas (Sca4 and Sca5 also called OmpB) present in all *Rickettsia*. Scas of gram-negative bacteria belong to the autotransporter protein family which are usually associated with virulence functions.

2.1 Pathogenesis and pathophysiology

In vitro experiments showed that *Rickettsia* attaches to the host cell through its surface proteins, OmpB, OmpA, Sca1, and Sca2, and host cell receptors (Martinez et al., 2005). After attachment, *Rickettsia* induces non-phagocytic endothelial cells to engulf it. Once it enters the host cell, *Rickettsia immediately* lyses the phagosomal membrane and escapes into the cytoplasm. *Rickettsia* multiplies by binary fission with a doubling time of 8 hours. Both TG and SFG rickettsiae multiply in the cytoplasm of host cells, but SFG rickettsiae can also invade and multiply in the nucleus of host cells. TG rickettsiae accumulate in cytoplasm until the host cell bursts, but SFG rickettsiae seldom accumulate in host cells. The difference in the quantity of organisms that accumulate in host cells between TG and SFG rickettsiae is believed to be caused by the facts that SFG rickettsiae move by actin-based mobility inside the cytoplasm and can spread to adjacent cells, but TG rickettsiae do not move and cannot spread. SFG rickettsiae hijack the cell's actin which they stimulate to polymerize at one bacterial pole to facilitate their own movement (Teysseire et al., 1992). Due to the actin-based movement, SFG rickettsiae spread from cell to cell eventuating in cell death and thus the formation of large plaques in cell culture. In contrast, typhus group rickettsiae accumulate in the host cell and form very small plaques when the heavily infected cells burst.

The target cell of *Rickettsia in vivo* is microvascular endothelium. The crucial pathophysiologic effect of rickettsial endothelial infection is increased microvascular permeability resulting from discontinuities in interendothelial adherens junctions. The pathogenic mechanisms of endothelial injury include endothelial cell production of toxic reactive oxygen species, damage to the cell membrane upon rickettsial exit, and cytotoxic T lymphocyte-induced apoptosis of infected endothelial cells. Rickettsial infections cause a procoagulant state, but only very rarely disseminated intravascular coagulation. Thrombi comprise non-occlusive hemostatic plugs that are appropriately located at foci of severe endothelial damage to mitigate hemorrhage (Walker DH, 2011).

2.2 Epidemiology and ecology

A part or the entire life cycle of rickettsiae is usually associated with one species of arthropod, including lice, fleas, ticks and mites. Arthropods are the vectors and, in most cases, the reservoir of rickettsiae. The distribution of tick-borne SFG rickettsioses are restricted to areas where their tick reservoirs are present, such as Rocky Mountain spotted fever in the Americas, Mediterranean spotted fever in Europe, Africa and Asia, and Japanese spotted fever in Japan and eastern Asia (Table 1). Human louse-, flea-, and mouse

Disease	Rickettsial agent	Vector	Geographic Distribution
SFG rickettsiae			
African tick- bite fever	*Rickettsia africae*	Ticks	Sub-Saharan Africa, Caribbean islands (Mediannikov et al., 2010)
Far eastern spotted fever	*Rickettsia heilongjiangensis*	Ticks	Far East of Asia (Mediannikov et al., 2004)
Flinders Island spotted fever	*Rickettsia honei*	Ticks	Australia and southeastern Asia (Graves S & Stenos J, 2003)
Mediterranean spotted fever	*Rickettsia conorii*	Ticks	Southern Europe, southern and western Asia, and Africa (Rovery et al., 2008)
North Asian tick typhus Lymphangitis-associated rickettsiosis	*Rickettisa sibirica Rickettsia sibirica mongolotimonae*	Ticks	Asia, Europe, and Africa (Fournier et al., 2005)
Japanese spotted fever	*Rickettsia japonica*	Ticks	Japan and eastern Asia (Chung et al., 2006)
Queensland tick typhus	*Rickettsia australis*	Ticks	Australia (Sexton et al., 1991)
Rocky Mountain spotted fever	*Rickettsia rickettsii*	Ticks	North, Central and South America (Galvao MA et al., 2003)
Tick-borne lymphadenopathy	*Rickettsia slovaca*	Ticks	Europe (Selmi et al., 2008)
Unnamed	*Rickettsia parkeri*	Ticks	North and South America (Nava et al., 2008)
Unnamed	*Rickettsia massiliae*	Ticks	Europe and North and South America (Labruna MB, 2009)
Unnamed	*Rickettsia aeschlimannii*	Ticks	Europe and Africa (Raoult et al., 2002)
Unnamed	*Rickettsia monacensis*	Ticks	Europe (Jado et al., 2007)
Unnamed	*Rickettsia helvetica*	Ticks	Europe and Asia (Fournier et al., 2004)
Rickettsialpox	*R. akari*	Mite	Worldwide
Cat flea rickettsiosis	*R. felis*	Flea	Worldwide
TG rickettsiae			
Epidemic typhus	*R. prowazekii*	Louse	Worldwide
Murine typhus	*R. typhi*	Flea	Worldwide

Table 1. Distribution of rickettsioses

mite-borne *Rickettsia* such as *R. prowazekii, R. typhi, R. felis,* and *R. akari* are distributed worldwide with their hosts and vectors.

Non-virulent or low virulence SFG rickettsiae may be maintained in nature largely via transovarian transmission in the arthropod hosts. However, highly virulent SFG rickettsiae such as *R. rickettsii* and *R. conorii* are pathogenic for the *Dermacentor* and *Rhipicephalus* ticks, respectively (Niebylski et al., 1999; Santos et al., 2002). Virulent rickettsiae such as *R. rickettsii* need an animal host to amplify the organisms for establishing new lines of transovarian rickettsial maintenance (e.g., *D. variabilis* ticks acquire *R. rickettsii* while feeding on rickettsemic cotton rats) (Niebylski et al., 1999). Each species of pathogenic SFG rickettsiae may have one or multiple tick vector species. The vectors of Rocky Mountain spotted fever are *D. variabilis* (American dog tick) in the eastern two-thirds of the US and regions of the Pacific coast states, *D. andersoni* (wood tick) in the Rocky Mountain states, *Rhipicephalus sanguineus* (brown dog tick) in the southwestern US, northern Mexico, and South America and *Amblyomma cajennense* and *A. aureolatum* in South America. The seasonal and geographic distribution of each rickettsiosis reflects the months of activity of the vector and its contact with humans. Over 90% of cases with Rocky Mountain spotted fever occur during April through September. Approximately 250-2000 cases of Rocky Mountain spotted fever have been reported annually in the United States (CDC, 2010).

Epidemic typhus is transmitted primarily by the human body louse, *Pediculus humanus corporis*. However, the louse is only a vector and not a reservoir because infected lice die 5-7 days after they become infected with *R. prowazekii*. *Rickettsia prowazekii* multiplies in louse gut epithelium, which detaches, ruptures and releases rickettsiae into the feces. The louse feces containing rickettsiae are scratched into the skin, rubbed into mucous membranes such as the conjunctiva, or inhaled. Humans can develop latent infection after acute louse-borne typhus and serve as reservoirs of *R. prowazekii*. *R. prowazekii* can be reactivated causing recrudescent typhus fever (Brill-Zinsser disease) when latently infected persons' immunity wanes. *Rickettsia prowazekii* is also maintained in a zoonotic cycle involving flying squirrels (*Glaucomys volans*) and their specific flea and louse in the United States. Sporadic epidemic typhus occurring in the United States is transmitted by fleas of flying squirrels (Duma et al., 1981). Murine typhus is transmitted by fleas including rat fleas and cat fleas.

3. Virulence determinants of *Rickettsia*

Rickettsia has no exotoxin, and rickettsial LPS is not toxic and apparently is not associated with the pathogenesis of *Rickettsia* infections. Since *Rickettsia* are obligately intracellular bacteria, their survival mechanisms involve proteins related to the attachment, to entry into and exit from host cells, and enzymes for protein and DNA modification and obtaining nutrition from the host.

Adhesins: The first step for obligately intracellular *Rickettsia* to establish infection is to adhere to and invade the host endothelium. These processes require the interaction of rickettsial surface proteins with mammalian host cell receptors. Three outer membrane proteins of *Rickettsia* OmpA, OmpB, and Sca2 have been identified as adhesins of *Rickettsia*(Li & Walker, 1998; Martinez et al., 2005; Cardwell & Martinez, 2009). OmpB has been demonstrated to interact with its mammalian receptor, Ku70. However, in Ku70$^{-/-}$ mouse embryonic fibroblasts *R. conorii* invasion is reduced only 50 to 60%, suggesting that *Rickettsia* may use multiple adhesins and receptors.

Membranolytic enzymes: Internalized rickettsiae are initially bound within a phagosome (Teysseire et al., 1995). *Rickettsia* quickly (<10 min) lyse the phagosomal membrane to escape from phagosomal vacuoles before phagolysosomal fusion occurs, which would result in the death of the *Rickettsia* through the activity of the lysosomal enzymes (Teysseire et al., 1995;Hackstadt, 1996;Feng & Walker, 2000;Walker et al., 2001a). *Rickettsia* are also required to exit the host cell by lysis of the host cell membrane. The mechanism of lysis of the phagosomal membrane and the host cell membrane has been hypothesized to be mediated by a phospholipase enzyme (Radulovic et al., 1999;Renesto et al., 2003). The genomic sequences of *Rickettsia* have revealed four proteins with potential membranolytic activities: patatin B1 precursor (*pat-1* gene), hemolysin A (*tlyA*), hemolysin C (*tlyC*), and phospholipase D (*pld*) (Andersson et al., 1998;Ogata et al., 2001;McLeod et al., 2004). TlyC has been demonstrated to have hemolytic activity, (Radulovic et al., 1999) and can mediate escape by *S. enterica* serovar Typhimurium from phagosomes (Whitworth et al., 2005). Patatin B of *R. typhi* (RT0522) has been implicated as a phospholipase A2. However, knockout of *R. prowazekii pld* gene does not prevent *R. prowazekii* escape from the phagosome or exit from host cells. Patatin B is truncated by an ISRpe 1 transposon in *R. peacockii*, which apparently does not affect the release of *R. peacockii* from phagosomes (Felsheim RF). These observations suggest that the multiple membranolytic enzymes of *Rickettsia* may be functionally redundant.

Actin-based mobility: Like other intracytosolic bacteria such as *Listeria monocytogenes* and *Shigella flexneri*, SFG rickettsiae exploit the host cell actin cytoskeleton to promote intracellular mobility and cell-to-cell spread by assembling distinctive 'comet tails' that consist of long, unbranched actin filaments(Tilney and Portnoy, 1989;Bernardini et al., 1989;Teysseire et al., 1992;Heinzen et al., 1993). The molecular mechanisms of actin polymerization by *L. monocytogenes a*nd *S. flexneri* primarily involve activation of the Arp2/3 complex, an actin nucleator that can initiate the polymerization of new actin filaments and organize filaments into Y-branched arrays (Mullins et al., 1998;Welch et al., 1998;Blanchoin et al., 2000). In host cells, the Arp2/3 complex is activated by nucleation-promoting factors including members of the Wiskott–Aldrich syndrome protein (WASP) family of proteins(Higgs & Pollard, 2001;Welch & Mullins, 2002). The mechanisms of activation of the Arp2/3 complex by intracellular pathogens are that they express surface proteins that recruit host WASP family proteins (e.g., *S. flexneri* IcsA), or they express functional mimics of WASPs (e.g., *L. monocytogenes* ActA) (Goldberg, 2001). It was proposed that actin in *Rickettsia* comet tails is nucleated by the host Arp2/3 complex, and the bacterial protein RickA has been shown to assemble branched actin networks *in vitro*(Jeng et al., 2004;Gouin et al., 2004) . Coincidently, RickA is inactivated by an ISRpe1 transposon in *R. peacockii*, which does not have actin-based mobility(Simser et al., 2005). However, a new discovery suggests that besides RickA Sca2 is also involved in the actin-based mobility of *Rickettsia*. Knocking out the *sca2* gene completely aborts actin-based mobility of *R. rickettsii*. Sca2 mimics eukaryotic formins to determine the unique organization of actin filaments in *Rickettsia* tails and to drive bacterial mobility, independently of host nucleators. Actin-based mobility is important for the virulence of some rickettsiae such as *R. rickettsii*, but it is not important for the virulence of TG rickettsiae because TG rickettsiae do not have actin-based mobility or have only erratic mobility. Even SFG rickettsiae with actin-based mobility are not all virulent such as *R. bellii*, a tick symbiont, which has actin-based mobility but has not yet been shown to cause disease in humans or animals.

Methyltransferase: The patterns of methylation of lysine in the surface antigens of avirulent Madrid E (E) strain, virulent revertant Evir strain, and wild type Breinl strain of *R. prowazekii* are different. The major surface antigen of the virulent Breinl and Evir strains contains more N^{ϵ}-Me3-lysine and less N^{ϵ}-Me-lysine than the avirulent E strain (Rodionov et al., 1991;Turco & Winkler, 1994). Outer membrane protein B (OmpB) is heavily methylated in the virulent strains, while OmpB from the attenuated strain is hypomethylated(Ching et al., 1992). The methyltransferase gene (Rp028/Rp027) is inactivated by a frameshift mutation in E strain, but the mutation reverts to wild type in the virulent revertant Evir strain. A single nucleotide mutation in the methyltransferase gene is the only mutation in E strain compared to Evir strain (Yu, unpublished data). Taken together our results and the previous discovery that E strain is deficient in methylation of OmpB suggests strongly that the reversible mutation in the methyltransferase gene determines the virulence state of E strain, i.e., the organisms become avirulent when the gene is inactivated in E strain, and the organisms become virulent when the gene function is restored in Evir strain.

3.1 Clinical spectrum/treatment

Spotted fever: Rocky Mountain spotted fever caused by *R. rickettsii* is a very severe disease, and fatal cases occur in association with delayed or ineffective antibiotic treatment in as many as 4% of cases. RMSF occurs 1 -2 weeks after feeding by an infected tick. The disease is characterized by acute onset of fever that may be accompanied by headache, malaise, myalgia, nausea/vomiting, or neurologic signs. A macular or maculopapular rash appears 3-5 days following onset in most (~90%) patients, and the rash has a centripetal pattern of spread, meaning that it begins on the extremities and spreads towards the trunk. In severe disease, petechiae appear in the center of the maculopapules. However, 10% to 15% of persons with RMSF never develop a rash, a condition referred to as "Rocky Mountain *spotless* fever"(Sexton & Corey, 1992). The target of *Rickettsia* is endothelium of blood vessels. Inflammation and damage of endothelia of capillary blood vessels by *Rickettsia* results in increased vacular permeability, which causes rash, hypovolemic hypotension, pulmonary and cerebral edema and organ failure.

Other spotted fevers may have similar clinical symptoms as RMSF, but are less severe. Rash is less frequent in less severe rickettsioses such as African tick bite fever and *R. parkeri* infection. Focal skin necrosis with a dark scab (an eschar) at the site of tick feeding is a common feature of boutonneuse fever, African tick bite fever, North Asian tick typhus, Queensland tick typhus, Japanese spotted fever, Flinders Island spotted fever, rickettsialpox, tick-borne lymphadenopathy, and the recently described infections in the US caused by *R. parkeri* and a novel strain 364 D, but is rare in Rocky Mountain spotted fever.

Typhus: Epidemic typhus and murine typhus are caused by *R. prowazekii* and *R. typhi*, respectively. Historically murine typhus and epidemic typhus were difficult to differentiate due to similar clinical symptoms, but epidemic typhus is more severe than murine typhus. Murine typhus occurs predominantly in summer and fall, and epidemic typhus usually occurs in winter. Symptoms of typhus may include high fever (105 - 106 degrees Fahrenheit), which may last up to 2 weeks, severe headache, severe muscle pain (myalgia), dry cough, delirium, stupor, and a dull red rash that begins on the trunk and spreads peripherally.

Tetracyclines are first-line treatment, and doxycycline may be used to avoid tooth staining in children. Tetracyclines are rickettsiostatic, not rickettsicidal. Chloramphenicol is a second line, less effective treatment that can be used in the rare instance of contraindication to use of doxycycline. Ciprofloxacin, other fluoroquinolones, azithromycin, and clarinthromycin are effective against certain rickettsiae but are not recommended for the severe rickettsioses. Because diagnostic tests can take time and may be insensitive, antibiotics are usually begun presumptively to prevent significant deterioration, complications, death, sequelae, and prolonged recovery.

3.2 Immune mechanisms of *Rickettsia*

Most of our understanding of the immune response against *Rickettsia* is derived from *in vitro* studies as well as the murine models of rickettsioses. Proinflammatory cytokines such as IFN-γ and TNF-α are essential for primary defense against rickettsial infection. These cytokines act in concert to activate endothelial cells, the major target cells of rickettsial infections, as well as other minor target cells to kill intracellular organisms via nitric oxide synthesis-dependent and indoleamine 2,3-dioxygenase-dependent mechanisms. The sources of these protective cytokines are hypothesized to be the T lymphocytes and macrophages that infiltrate the perivascular space surrounding the vessels with infected endothelium.

Cell mediated immunity plays a critical role in host defenses against rickettsial infections(Walker et al., 2001b). There are two important effector components of the acquired immune response against *Rickettsia*, namely IFN- γ production by $CD4^+$ and $CD8^+$ type-1 cells, which activates intracellular bactericidal mechanisms of endothelial cells and macrophages, and the generation of *Rickettsia*-specific cytotoxic $CD8^+$ T cells that induce apoptosis in infected target cells via pathways involving perforin and/or granzymes. $CD8^+$ T cells are more important in clearance of rickettsial infection than $CD4^+$ T cells(Walker et al., 2001b). Although adoptive transfer of either CD4 or CD8 immune T lymphocytes controls the infection and leads to survival, only depletion of CD8 T lymphocytes alters the outcome of infection, and depletion of CD4 cells has no observed effect on the course or outcome of infection(Walker et al., 2001c).

The humoral response may play an important role in protection against infection, and antibodies against surface protein antigens are very likely critical effectors of vaccine–associated protective immunity. In animal experiments, antibodies to Rickettsia or rickettsial outer membrane proteins can neutralize rickettsia (Anacker et al., 1987;Li et al., 1988). However, natural infection does not result in the production of protective antibodies prior to clearance of rickettsiae. Thus, humoral immunity may be more important in preventing reinfection and in vaccine-induced immunity than in clearance of primary infection.

4. Experimental vaccines and other potential vaccine prospects

Currently no commercial vaccine is available for any rickettsial disease. Infection with *R. rickettsii* and *R. conorii* is believed to confer long lasting immunity against re-infection. Thus, it is feasible to develop a vaccine against rickettsial diseases. In theory, a subunit vaccine targeting a conserved rickettsial protein such as OmpB may be developed to prevent all rickettsial diseases. An attenuated organism that can multiply, but does not cause disease in the host, has been proved to be effective in protection against rickettsial infections in

humans and laboratory animals. Attenuated *Rickettsia* has been selected by passage in chicken egg yolk sacs in the past and was recently achieved by gene knockout technology.

Inactivated vaccine. The history of development of vaccines against rickettsial diseases contains numerous failures and limited success in preventing or ameliorating disease. Killed rickettsial vaccine was prepared from infected ticks in 1924, in yolk sac of embryonated chicken eggs in 1938 (Cox HR, 1939), and from cell culture in the 1970s (Gonder et al., 1979;Kenyon et al., 1979). The original tick-derived rickettsial vaccine produced severe local inoculation site reactions(Spencer RR and Parker RR, 1925). Evaluation of its protective effect in field use was impressive by the standards of the day. The fatality rate from Rocky Mountain spotted fever among vaccine recipients was reduced dramatically although illness and even death occurred in some vaccinated persons. The killed yolk sac-derived rickettsial vaccine was never field tested. When tested by challenge of human volunteers, neither the yolk sac vaccine nor the tick vaccine prevented the illness, which, of course, was treated to prevent severe illness or death. The yolk sac vaccine was withdrawn from the market in 1978. A subsequent challenge trial of a cell culture killed-*R. rickettsii* vaccine yielded protection of 25% of the volunteers who received it(Gonder et al., 1979).

Subunit vaccine for Rickettsia. Two surface protein antigens of *R. rickettsii*, OmpA and OmpB, have been identified as major protective antigens and are candidates for use as subunit vaccines. The first evidence that OmpA and OmpB contain protective epitopes came from the studies of monoclonal antibodies to heat sensitive epitopes of OmpA and OmpB, which neutralized *R. rickettsii* toxicity in mice and infection in guinea pigs (Anacker et al., 1987;Li et al., 1988). The *E. coli*-expressed OmpA N-terminal fragment partially protects guinea pigs against a lethal challenge dose of *R. rickettsii* (McDonald et al., 1988). A fragment from the N-terminus of *R. conorii* OmpA protects guinea pigs against experimental infection with *R. conorii* and partially protects guinea pigs from challenge with the heterologous *R. rickettsii* (Vishwanath et al., 1990). Fragments of the *ompA* and *ompB* genes have been tested as DNA vaccines. In a regimen of DNA immunization followed by boosters of the corresponding peptide, mice immunized with *R. rickettsii ompA* or *ompB* fragments are partially protected against a lethal challenge with heterologous *R. conorii*(Diaz-Montero et al., 2001). It is not known whether the incomplete protection of OmpA and OmpB against the heterologous *Rickettsia* species challenge in these experiments is caused by the antigenic differences between the rickettsial species, the immunization regimen, or the antigen composition.

Live vaccine. The attenuated Madrid E (E) strain of *R. prowazekii* was used in humans as an experimental vaccine from the 1950s to 1970s. E strain is a spontaneous laboratory variant of *R. prowazekii* that was isolated from a typhus patient in Madrid in 1941 and passed in rapid succession in embryonated chicken eggs 255 times. This strain has limited virulence for guinea pigs and low virulence for humans. E strain is protective and provides long term immunity against louse-borne typhus. Ninety-four percent (170/181) of immunized persons were protected from natural infection by epidemic typhus compared to the unvaccinated controls in a 14-month period after vaccination. Ninety-six percent (27/28) of volunteers who were vaccinated with E strain and subsequently challenged with Breinl strain at intervals from 2 months to 36 months remained healthy following challenge, and 83% (5/6) of the volunteers who were challenged at 48 to 66 months were protected(FOX et al., 1961). However, the E strain vaccine caused a late reaction in up to 14% of vaccinated persons 9-14 days after inoculation. The late reaction varied from simple malaise and mild headache to

modified typhus characterized by fever, headache, malaise and occasionally a rash in a small proportion of subjects. The reason for the late reaction was not known at the time. In 1970s a virulent revertant Evir strain was isolated from guinea pigs by passage of E strain in guinea pigs or mice.

Attenuation of Rickettsia by gene knockout. Because of the difficulty of transforming *Rickettsia*, scientists were unable to knock out rickettsial genes to determine their function and to create an attenuated rickettsial vaccine until recently. The phopholipase D (*pld*) gene was the first rickettsial gene that was genetically knocked out. The *pld*-inactivated Evir strain is avirulent for guinea pigs at doses for which the Evir strain is virulent and stimulates protective immunity to virulent *R. prowazekii* (Driskell et al., 2009). Genetic inactivation of *sca-2* in *R. rickettsii* results in loss of actin-based mobility in cell culture (Kleba et al., 2010). Sca-2 deficient *R. rickettsii* lacks the ability to cause disease in guinea pigs, but stimulates protection against challenge with virulent *R. rickettsii* (Kleba et al., 2010). Thus, Sca-2 and phospholipase D-deficient strains should be further evaluated as vaccines for Rocky Mountain spotted fever and epidemic typhus. Genetically attenuated *Rickettsia* are the strongest future prospect for developing an effective rickettsial vaccine.

Differentiation of strains of *R. prowazekii* and *R. rickettsii*. *R. prowazekii* has been isolated from humans, flying squirrels, and ticks for more than half century. Due to different sources and different passages, *R. prowazekii* strain can be grouped in to three virulence groups: high virulence group represented by Breinl strain, medium virulence group represented by flying squirrel isolates and low virulence or non-virulence group represented by Madrid E strain and its revertant strains. All strains of *R. prowazekii* can be differentiated by sequencing several loci of the genome of *R. prowazekii* (Zhu et al., 2008). *R. rickettsii* strains were not genetically typed except for virulent R strain and nonvirulent Iowa strains, whose whole genomes were completely sequenced (Ellison et al., 2008).

5. Conclusion

There has been progress in molecular biology, cellular biology and immunology and pathogenesis of *Rickettsia*. However, diagnosis of rickettsial diseases is still difficult and is usually retrospective. Rapid diagnostic methods are required for diagnosis of rickettsial diseases and in response to bioterrorism.

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This book consists of nine chapters, written by international authorities, discussing various aspects of bioterrorism preparedness and response. Five of the chapters are agent-specific and highlight the pathogenesis, prevention and treatment, and the potential of specific organisms (Rickettsia and Yersinia pestis) or toxins (ricin, botulinum neurotoxins, and staphylococcal enterotoxins) to be used for nefarious purposes. Four chapters discuss different aspects of detecting and responding to a bioterrorism attack. These include methods for spatio-temporal disease surveillance, international laboratory response strategies, detection of botulinum neurotoxins in food and other matrices, and the use of physical methods (ie Raman spectroscopy) to detect spores.

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