



Tropism and pathogenicity of rickettsiae

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Rickettsiae are obligate intracellular parasitic bacteria that cause febrile exanthematous illnesses such as Rocky Mountain spotted fever, Mediterranean spotted fever, epidemic, and murine typhus, etc. Although the vector ranges of each *Rickettsia* species are rather restricted; i.e., ticks belonging to *Arachnida* and lice and fleas belonging to *Insecta* usually act as vectors for spotted fever group (SFG) and typhus group (TG) rickettsiae, respectively, it would be interesting to elucidate the mechanisms controlling the vector tropism of rickettsiae. This review discusses the factors determining the vector tropism of rickettsiae. In brief, the vector tropism of rickettsiae species is basically consistent with their tropism toward cultured tick and insect cells. The mechanisms responsible for rickettsiae pathogenicity are also described. Recently, genomic analyses of rickettsiae have revealed that they possess several genes that are homologous to those affecting the pathogenicity of other bacteria. Analyses comparing the genomes of pathogenic and non-pathogenic strains of rickettsiae have detected many factors that are related to rickettsial pathogenicity. It is also known that a reduction in the rickettsial genome has occurred during the course of its evolution. Interestingly, *Rickettsia* species with small genomes, such as *Rickettsia prowazekii*, are more pathogenic to humans than those with larger genomes. This review also examines the growth kinetics of pathogenic and non-pathogenic species of SFG rickettsiae (SFGR) in mammalian cells. The growth of non-pathogenic species is restricted in these cells, which is mediated, at least in part, by autophagy. The superinfection of non-pathogenic rickettsiae-infected cells with pathogenic rickettsiae results in an elevated yield of the non-pathogenic rickettsiae and the growth of the pathogenic rickettsiae. Autophagy is restricted in these cells. These results are discussed in this review.

Keywords: *Rickettsia*, tropism, pathogenicity, spotted fever group, typhus group, vector, tick, insect

INTRODUCTION

Rickettsioses in the broad sense are caused by a variety of gram-negative bacteria from the *Rickettsia*, *Orientia*, *Ehrlichia*, *Anaplasma*, or *Neorickettsia* genera. *Rickettsia* are now further classified into the typhus group (TG) and spotted fever group (SFG; **Table 1**), although *Orientia* used to belong to the scrub TG of *Rickettsia* (Tamura et al., 1995; Fournier et al., 2005). *Coxiella*, the causative agent of Q fever, used to be classified into the *Rickettsiales* order but now belongs to the *Legionellales* order (Weisburg et al., 1989). As *Rickettsia* are obligate intracellular parasites, many of the mechanisms involved in their attachment and internalization into host cells are shared by viruses.

Rickettsiae cause febrile exanthematous illnesses, such as spotted fever, epidemic, and murine typhus, etc. (Dyer et al., 1931; Zinsser and Castaneda, 1933). Until nearly two decades ago, only five species of SFG rickettsiae (SFGR), *R. rickettsii*, *R. conorii*, *R. sibirica*, *R. australis*, and *R. akari*, which are responsible for Rocky Mountain spotted fever (Wolbach, 1919), Mediterranean spotted fever or Boutonneuse fever (Brumpt, 1932), North Asian tick typhus or Siberian tick typhus (Shmatikov and Velik, 1939), Queensland tick typhus (Plotz et al., 1946), and Rickettsialpox (Huebner et al., 1946), respectively, were known to be pathogenic. However, since the isolation and identification of a new

SFGR species, *R. japonica*, from Japanese patients with a form of SFG rickettsiosis known as Japanese spotted fever or Oriental spotted fever (Uchida et al., 1988, 1989, 1991, 1992; Uchiyama and Uchida, 1988; Uchiyama et al., 1991), many pathogenic SFGR species have been newly isolated and identified, and some of the previously identified SFGR species have now been recognized as pathogenic (**Table 1**; Beati and Raoult, 1993; Beati et al., 1993, 1997; Kelly et al., 1996; Raoult et al., 1997; Stenos et al., 1998; Fournier et al., 2003, 2005; Mediannikov et al., 2004; Paddock et al., 2004). More recently, *R. bellii* was isolated from amebas, and it was suggested that it should be classified into the ancestral group (AG), which contains *Rickettsia* species that have been demonstrated by phylogenetic analyses to have the oldest genomic features among the previously isolated and identified *Rickettsia* species (Philip et al., 1983; Stohard et al., 1994; Gillespie et al., 2012). *R. bellii* displays weaker pathogenicity in experimental animals than *R. conorii*, although its pathogenicity in humans remains unknown (Ogata et al., 2006). Although the mechanism by which rickettsiae cause disease has not been well established, it is thought that rickettsial pathogenicity involves the infiltration of cells into the vascular environment, hemorrhaging, and thrombosis due to the degeneration of endothelial vein cells caused by the growth of the rickettsiae. Lipopolysaccharides (LPS) on the outer membranes of rickettsiae are also thought to participate in

Table 1 | Classifications, vectors, and reservoirs of *Rickettsia* that are known to be pathogenic to humans.

Antigenic group	Species	Disease	Vector	Reservoir(s)
Spotted fever group	<i>R. aeschlimannii</i>	Rickettsiosis	Tick	Unknown
	<i>R. africae</i>	African tick-bite fever	Tick	Ruminants
	<i>R. akari</i>	Rickettsialpox	Mite	Mice, rodents
	<i>R. australis</i>	Queensland tick typhus	Tick	Rodents
	<i>R. conorii</i>	Mediterranean spotted fever or Boutonneuse fever	Tick	Dogs, rodents
	<i>R. felis</i>	Cat flea rickettsiosis	Flea	Cats, rodents, opossums
	<i>R. heilongjiangensis</i>	Far Eastern spotted fever	Tick	Rodents
	<i>R. helvetica</i>	Aneruptive fever	Tick	Rodents
	<i>R. honei</i>	Flinders Island spotted fever, Variant Flinders Island spotted fever, Thai tick typhus	Tick	Rodents, reptiles
	<i>R. japonica</i>	Japanese spotted fever or Oriental spotted fever	Tick	Rodents
	<i>R. massiliae</i>	Mediterranean spotted fever-like disease	Tick	Unknown
	<i>R. parkeri</i>	Maculatum infection	Tick	Rodents
	<i>R. rickettsii</i>	Rocky Mountain spotted fever, Febre maculosa, São Paulo exanthematic typhus, Minas Gerais exanthematic typhus, Brazilian spotted fever	Tick	Rodents
	<i>R. sibirica</i>	North Asian tick typhus, Siberian tick typhus	Tick	Rodents
	<i>R. sibirica mongolotimonae</i>	Lymphangitis-associated rickettsiosis	Tick	Rodents
	<i>R. slovacae</i>	Tick-borne lymphadenopathy (TIBOLA), <i>Dermacentor</i> -borne necrosis and lymphadenopathy (DEBONEL)	Tick	Lagomorphs, rodents
	Typhus group	<i>R. prowazekii</i>	Epidemic typhus, Brill-Zinsser disease	Louse
<i>R. typhi</i>		Murine typhus	Flea	Rodents

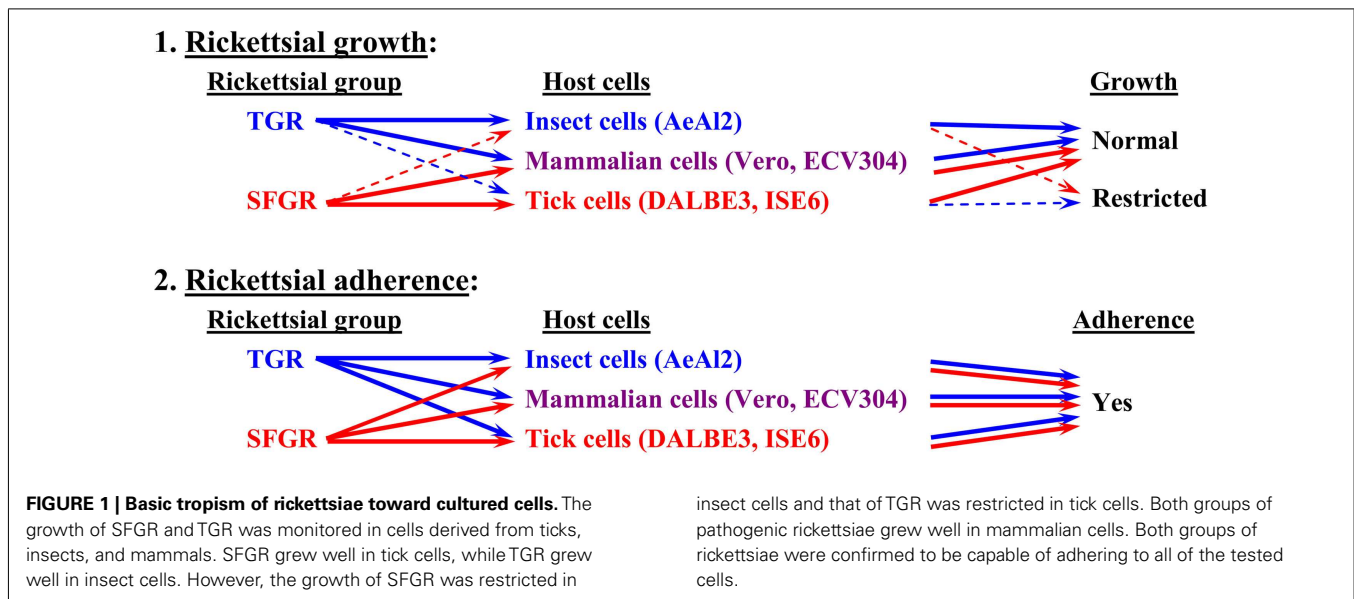
the eruption, pyrexia, and endotoxin shock observed during the course of rickettsial infection.

It is worth noting that the vector ranges of each rickettsial species are rather restricted; i.e., the vectors for SFGR species are usually ticks (except those for *R. akari* and *R. felis*, which are mites and fleas, respectively) belonging to *Arachnida*. On the other hand, those for TGR species are lice and fleas, which belong to *Insecta* (Table 1; Higgins et al., 1996). *R. felis* carries its pRF genes on a plasmid so it does not fully meet the criteria for the SFG or TG. Rather, this indicates that *R. felis* has participated in horizontal gene transfer involving the AG and might be better classified into a transitional group along with *R. akari*, which displays both SFG and TG characteristics (Gillespie et al., 2007, 2012). This plasmid might have been incorporated into the chromosomes of the other *Rickettsia* during the course of their evolution (Gillespie et al., 2007). The mechanisms responsible for the vector tropism of rickettsiae have not been studied in detail.

TROPISM OF RICKETTSIAE TOWARD ARTHROPOD VECTORS AND CULTURED CELLS

Although the relationships between rickettsiae and their vectors are relatively fixed, the mechanisms responsible for the tropism of rickettsiae toward arthropod vectors have not been elucidated. Studies using cell lines derived from arthropods are indispensable for clarifying these mechanisms. In studies using insect cells, Uchiyama reported that the growth of some SFGR species, *R. japonica*, and *R. montanensis*, was restricted in the NIAS-AeA1-2 (AeA12) insect cell line, which is derived from *Aedes albopictus*, even though SFGR species have been demonstrated to be capable

of adhering to and invading these cells (Figures 1–3; Mitsuhashi, 1981; Mizuki et al., 1999; Noda et al., 2002; Uchiyama, 2005). Scanning and transmission electron microscopy confirmed these results (Figure 3; Uchiyama, 2006). Rickettsiae seem to begin their invasion of AeA12 cells immediately after adhering to them. The superinfection of SFGR-infected AeA12 cells with a TGR species on day three of infection resulted in the growth of the TGR species but not the SFGR species. Furthermore, the SFGR-infected AeA12 cells suffered rapid cell death; however, as no DNA fragmentation, lobed nuclei, or peripheral chromosome condensation were observed, the growth inhibition of these cells was possibly due to their non-apoptotic necrotic cell death. Concerning this issue, induced cell death (subsequently renamed programmed necrosis), which is one of the candidates for the mechanism responsible for growth inhibition, has been found to act in opposition to anti-apoptotic factors (Laster et al., 1988; Holler et al., 2000; Chan et al., 2003; Cho et al., 2009; He et al., 2009). For example, cells infected with Cowpox virus cause tumor necrosis factor (TNF)-induced programmed necrosis, which is opposed by the anti-apoptosis factor CrmA (Chan et al., 2003). When T cells or fibroblastic cells are infected with the Vaccinia virus, apoptosis is inhibited by the anti-apoptotic factor B13R/Spi2; however, TNF-induced programmed necrosis can also occur (Cho et al., 2009). Thus, programmed necrosis might occur when AeA12 cells are infected with SFGR. Contrary to our results, a previous report found that some non-pathogenic SFGR species, *R. montanensis*, and *R. peacockii*, were able to grow in two mosquito cell lines (the *A. albopictus* cell line Aa23 and the *Anopheles gambiae* cell line Sua5B; Sakamoto and Azad, 2007). The reason for this discrepancy is poorly understood;



however, the abovementioned growth was only detected by FISH and PCR, rather than in an infective assay. Another report found that the transcription of *spoT* gene paralogs was suppressed during the maintenance of *R. conorii* in *A. albopictus* (C6/36) cells at 10°C for 38 days. Shifting the temperature to 37°C resulted in a rapid upregulation of *spoT1* gene expression (Roverly et al., 2005). Although *R. conorii* were confirmed to be able to survive in the cells at low temperature, their growth was not directly assayed after the temperature was increased.

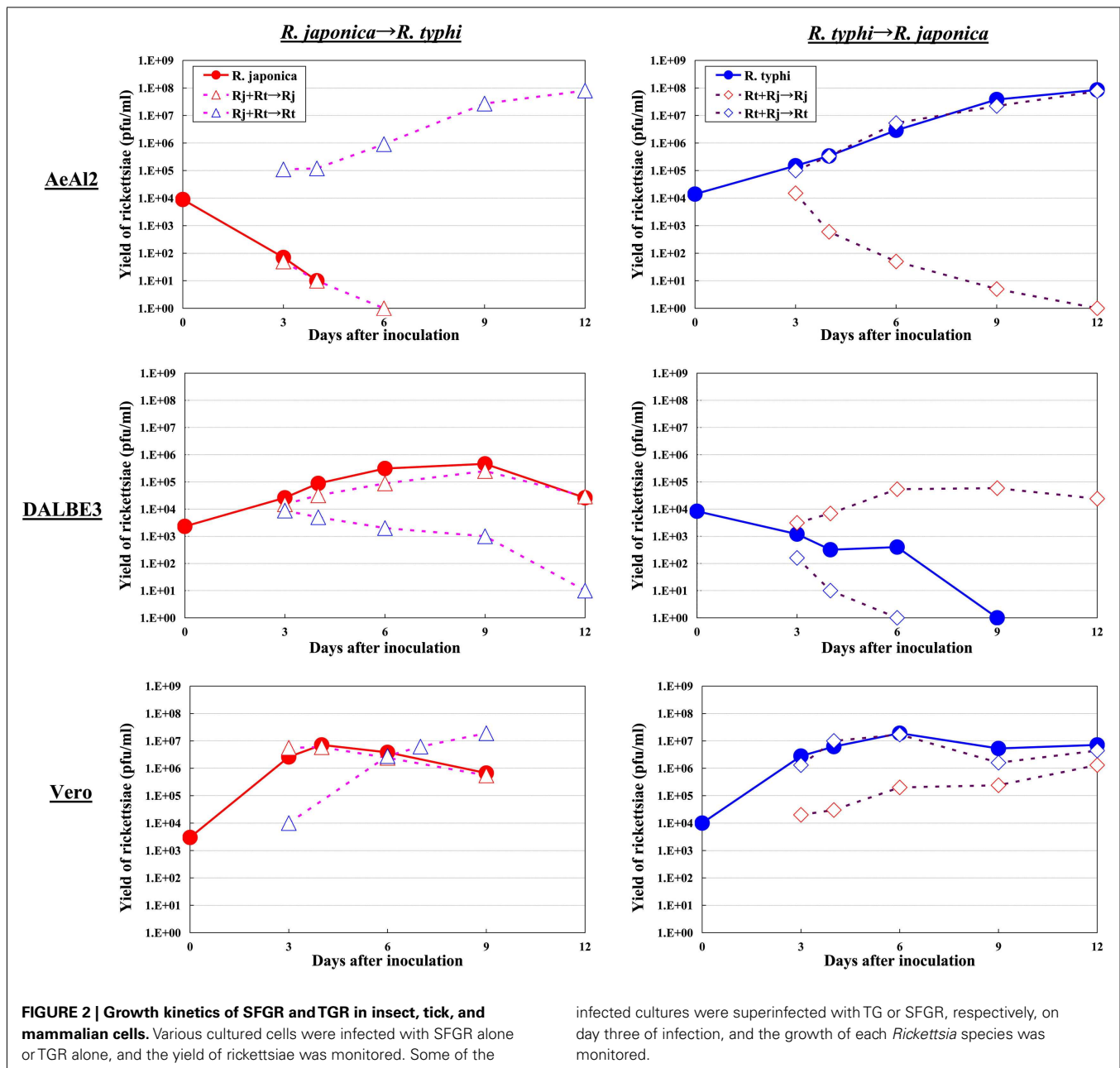
As for studies using tick cell lines, several reports have demonstrated the growth of SFGR species in cells derived from ticks (Policastro et al., 1997; Munderloh et al., 1998). In our study (Uchiyama et al., 2009), the DALBE3 cell line from *Dermacentor albipictus* and the ISE6 cell line from *Ixodes scapularis* were inoculated with *R. japonica* and *R. conorii* as SFGR species and *R. prowazekii* and *R. typhi* as TGR species. The SFGR grew well in these tick cells as well as in Vero, HeLa, and ECV304 mammalian cells (Figures 1 and 2). On the contrary, the growth of TGR was restricted in these tick cells, even though they successfully adhered to the cells, which was also true for other combinations of rickettsiae and host cells. These findings were confirmed by transmission electron microscopy. Rickettsiae were also found to be able to escape into the cytoplasm from phagosomes after being engulfed by the tick cells. Thus, the observed growth restriction occurred after these steps, although the precise mechanism responsible for it is yet to be elucidated. These results from studies using various combinations of SFGR or TGR and tick or insect cells suggest that the host vector tropism of rickettsiae is at least partially based on host cellular tropism (Figure 1).

PATHOGENICITY OF RICKETTSIAE

As shown in Table 1, many rickettsial species have displayed evidence of being pathogenic to humans. On the other hand, many other species have not displayed any evidence of being pathogenic to humans, some of which might be weakly pathogenic. To date, various putative factors that might be associated with the

pathogenicity of rickettsiae have been proposed; however, the molecular basis for the pathogenicity of rickettsiae is yet to be precisely established.

It is reasonable to think that the degree of growth of *Rickettsia* in human blood vessels; i.e., endothelial cells (EC), primarily determines the severity of their effects on the host, with the exception of *R. akari*, the causative agent of Rickettsialpox, which principally targets macrophages (Walker et al., 2007). Thus, every step of the growth of rickettsiae in host cells could affect their pathogenicity. The events involved in host cell infection by rickettsiae are summarized in Figure 4. The first steps involve the adherence of the rickettsiae to host cells and their subsequent invasion of these cells, since *Rickettsia* are obligate intracellular parasitic bacteria. Internalization occurs within minutes, and rickettsiae escape from phagosomes into the cytoplasm via the phospholipase activities of hemolysin C (TlyC) and phospholipase D (Pld; Teyssie et al., 1995; Whitworth et al., 2005). It has been clarified that among the 17 subfamilies of Sca auto-transporter proteins, rOmpA (=Sca0), and rOmpB (=Sca5) are involved in host cell adherence and invasion by rickettsiae. rOmpA is one of the major surface antigen proteins of SFGR, and treatment with the antibody against rOmpA or immunization with recombinant rOmpA protected animal models against infection by rickettsiae (Anacker et al., 1985, 1987; McDonald et al., 1987; Li et al., 1988; McDonald et al., 1988; Vishwanath et al., 1990; Sumner et al., 1995; Crocquet-Valdes et al., 2001). The role of rOmpA in the adherence of rickettsiae to host cells has also been examined using cultured cells (Li and Walker, 1998). However, TG rickettsiae do not possess rOmpA, although a remnant (369 bp) of its ORF (6,063 bp) still exists in the equivalent region in *R. prowazekii*. rOmpB, which is the only major surface antigen protein common to the genus *Rickettsia*, was also confirmed to play roles in host cell adherence and invasion by rickettsiae in studies using *Escherichia coli* expressing recombinant *R. japonica* rOmpB on their surface (Uchiyama, 1999; Uchiyama et al., 2006; Chan et al., 2009). rOmpB is well conserved among the *Rickettsia* genus



including SFGR and TGR, e.g., the rOmpB amino acid sequences of *R. prowazekii* and *R. conorii* share 70% homology, which might reflect the importance of the molecule for rickettsial growth (Carl et al., 1990; Gilmore et al., 1991; Hahn et al., 1993). rOmpB might also play other roles, e.g., in the maintenance of the structure of the bacteria or as a molecular sieve, etc. rOmpB associates with Ku70 on the plasma membrane (Martinez et al., 2005), and this interaction is sufficient to mediate the rickettsial invasion of non-phagocytic host cells (Chan et al., 2009). Clathrin and caveolin-2-dependent endocytosis are responsible for the internalization of rickettsiae. The recruitment of c-Cbl, a ubiquitin ligase, to the entry site is also required for the ubiquitination of Ku70 (Martinez et al., 2005). *R. conorii* enters non-phagocytic cells via an Arp2/3

complex-dependent pathway (Martinez and Cossart, 2004). Pathways involving Cdc42, phosphoinositide 3-kinase, c-Src, cortactin, and tyrosine-phosphorylated proteins activate Arp2/3, resulting in localized actin rearrangement during rickettsial entry. Furthermore, activation of the p38 mitogen-activated protein kinase module facilitates host cell invasion by *R. rickettsii* *in vitro* (Rydikina et al., 2005, 2008). Recently, it has been clarified that some of the other outer membrane proteins belonging to the Sca family, Sca1, and Sca2, also play roles in host cell adherence and invasion by rickettsiae (Cardwell and Martinez, 2009; Riley et al., 2010). Overlay assays involving biotinylated EC, 2D-PAGE, and mass spectrometry have demonstrated that the β -peptide, Adr1, and Adr2 are also putative rickettsial adhesins (Renesto et al., 2006).

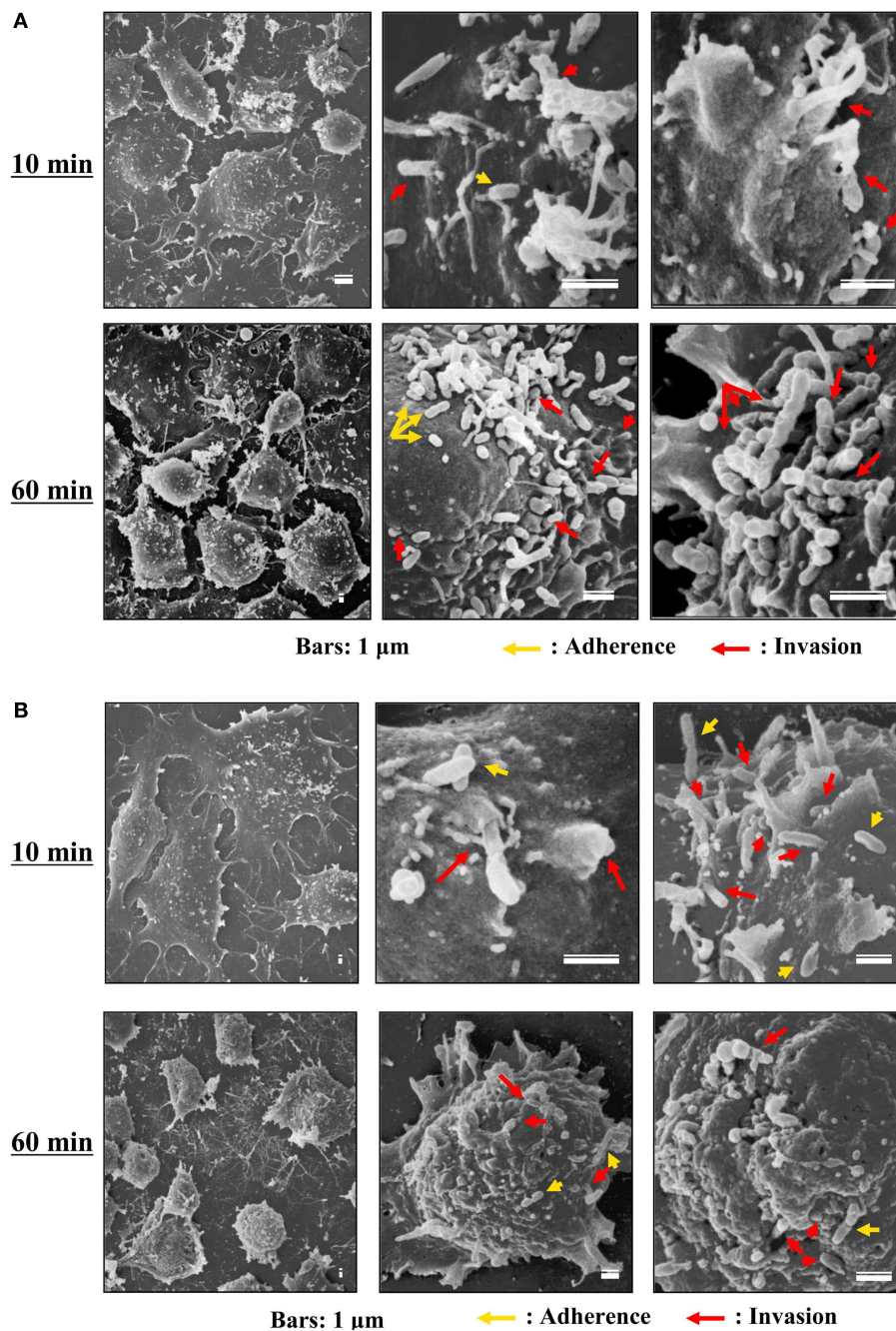
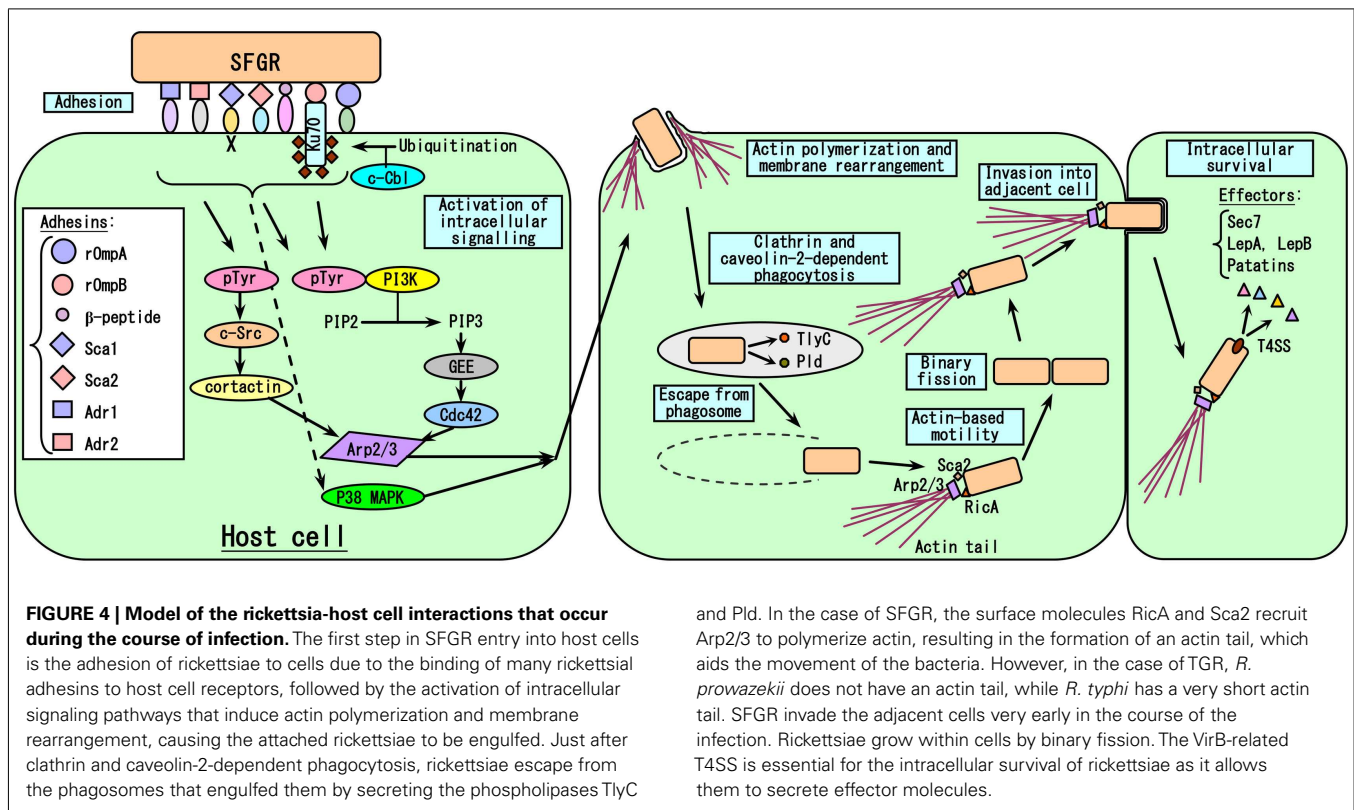


FIGURE 3 | Scanning electron microscopy of cells infected with TGR and SFGR. (A) AeA12 cells infected with *R. typhi* at 10 and 60 min after infection. **(B)** AeA12 cells infected with *R. japonica* at 10 and 60 min after infection.

Successful adherence to and invasion of AeA12 insect cells was achieved by both TGR and SFGR soon after their inoculation. The yellow and red arrows indicate adherent and invading rickettsiae, respectively.

In addition to studies of these early events during the course of rickettsial infection, there have been many genomic analyses of the pathogenicity of rickettsiae (Andersson et al., 1998; Li and Walker, 1998; Ogata et al., 2001; Uchiyama, 2003; Joshi et al., 2004; Sahni et al., 2005; Whitworth et al., 2005; Uchiyama et al., 2006; Chan et al., 2009; Fournier et al., 2009; Clark et al., 2011). Rickettsial genomes possess homologs of the *virB* operon, which is

known to be related to the type IV secretion system (T4SS) and might be associated with rickettsial pathogenicity. It was reported that Vero cells that had been infected with *R. conorii* displayed upregulated *virB* operon expression when they were exposed to nutrient stress (La et al., 2007). The factors secreted by the T4SS, such as Sec7, LepA, LepB, and patatins, might upregulate the synthesis of nutrients that allow rickettsiae to survive in stressful



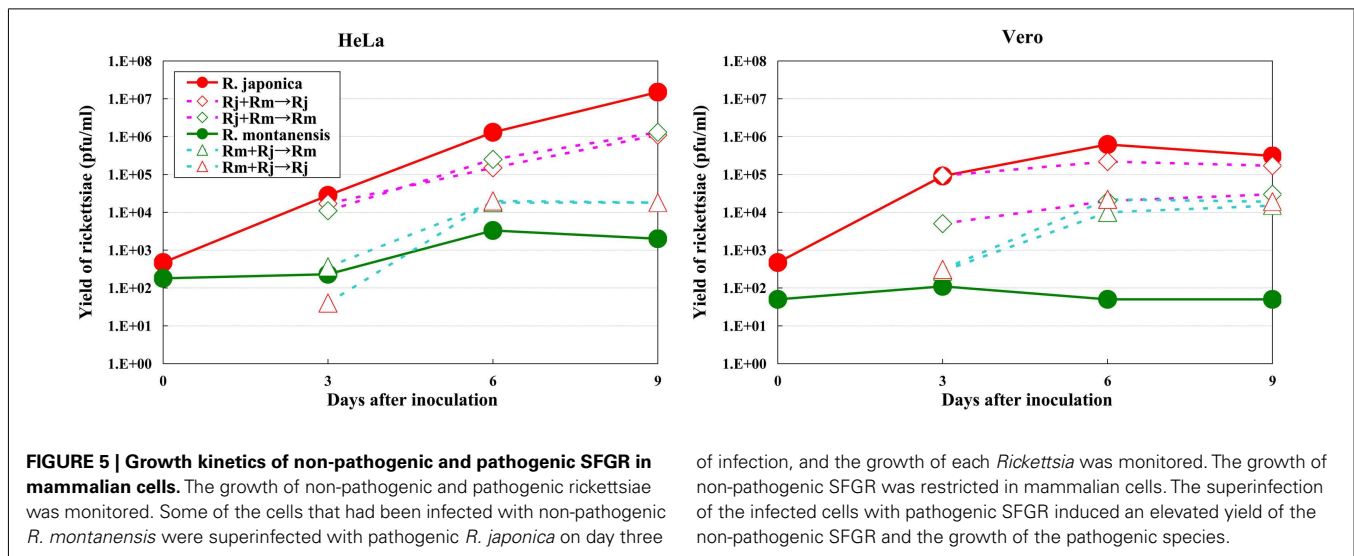
and Pld. In the case of SFGR, the surface molecules RicA and Sca2 recruit Arp2/3 to polymerize actin, resulting in the formation of an actin tail, which aids the movement of the bacteria. However, in the case of TGR, *R. prowazekii* does not have an actin tail, while *R. typhi* has a very short actin tail. SFGR invade the adjacent cells very early in the course of the infection. Rickettsiae grow within cells by binary fission. The VirB-related T4SS is essential for the intracellular survival of rickettsiae as it allows them to secrete effector molecules.

environments. Moreover, of the ten *Staphylococcus aureus* genes (*capA–M*) involved in the biosynthesis of capsular polysaccharides, which are related to pathogenicity, three have homologs in the *Rickettsia* genome (Lin et al., 1994).

The infection of cultured human EC with *R. rickettsii* induced the early cell-to-cell spread of the bacteria, resulting in widespread membrane damage and finally cell death (Silverman, 1984). However, EC are not only injured by infection, but also initiate cellular responses such as endothelial activation. Specifically, the infection of EC with *R. rickettsii* or *R. conorii* induces surface platelet adhesion (Silverman, 1986); the release of von Willebrand factor from Weibel–Palade bodies (Sporn et al., 1991; Teyssie et al., 1992); and increased expression of tissue factor (Teyssie et al., 1992; Sporn et al., 1994), E-selectin (Sporn et al., 1993), IL-1 α (Kaplan-ski et al., 1995; Sporn and Marder, 1996), cell-adhesion molecules (Dignat-George et al., 1997), and plasminogen activator inhibitor-1 (Drancourt et al., 1990; Shi et al., 2000). The infection of EC with the TGR *R. prowazekii* resulted in enhanced prostaglandin secretion (Walker et al., 1990). Thus, EC infected with rickettsiae demonstrate procoagulant and proinflammatory features, which might contribute to the severity of rickettsioses. Rickettsial infection of EC also activates nuclear factor (NF)- κ B, which inhibits apoptosis and mediates the production of the proinflammatory cytokines IL-6, IL-8, and monocyte chemoattractant protein 1 (Joshi et al., 2004; Bechelli et al., 2009). Conversely, EC activated by IFN- γ , TNF- α , IL-1 β , or RANTES degenerate intracellular rickettsiae through nitric oxide production and hydrogen peroxide production (Valbuena et al., 2002; Rydkina et al., 2004).

A comparative study of rickettsial genomes suggested that the inactivation of some genes by genome reduction during the course of their evolution abrogated host-induced rickettsial growth restriction (Blanc et al., 2007). In fact, a conflicting relationship was detected between a smaller genome size and increased pathogenicity in rickettsiae, e.g., *R. prowazekii*, which possesses a smaller genome, causes more severe symptoms than *Rickettsia* species with larger genomes such as *R. conorii* (Fournier et al., 2009; Botelho-Nevers and Raoult, 2011). A comparison of the growth of the virulent and avirulent strains of *R. rickettsii* revealed that the *relA/spoT* gene is essential for growth restriction (Clark et al., 2011).

The growth kinetics of pathogenic rickettsiae in mammalian cells were compared with those of non-pathogenic rickettsiae. Vero and HeLa cells derived from mammals were inoculated with a non-pathogenic species of SFGR, *R. montanensis* (Bell et al., 1963; Uchiyama et al., 2012). The growth of *R. montanensis* in the mammalian cells was restricted; however, the infection was persistent, and low levels of rickettsiae were produced throughout its course (Figure 5). On the other hand, superinfection of the *R. montanensis*-infected cells with the pathogenic *R. japonica* resulted in increased yields of the non-pathogenic *R. montanensis* and *R. japonica* growth. Western blotting confirmed that autophagy had been induced in the cells infected with *R. montanensis* alone. On the contrary, autophagy was restricted in the *R. montanensis*-infected cells that had been superinfected with pathogenic *R. japonica*. These results were consistent with the findings of ultrastructural observations (Figure 6). Thus, it is suggested



of infection, and the growth of each *Rickettsia* was monitored. The growth of non-pathogenic SFGR was restricted in mammalian cells. The superinfection of the infected cells with pathogenic SFGR induced an elevated yield of the non-pathogenic SFGR and the growth of the pathogenic species.

that the growth restriction of the non-pathogenic species, *R. montanensis*, was at least partly due to the occurrence of autophagy in the infected cells and that the pathogenic species, *R. japonica*, might secrete an unidentified autophagy restriction factor(s). Although autophagy is one of the innate defense systems against invading microbes, other pathogenic bacteria that display intracellular growth, such as *Shigella*, *Listeria*, and *Burkholderia*, also possess mechanisms for escaping from autophagic degeneration (Sasakawa, 2010). *Shigella* escapes from autophagic recognition by secreting IcsB via the type III secretion system (TTSS; Ogawa et al., 2005). *Listeria* recruits the Arp2/3 complex and Ena/VASP to its surface via the bacterial ActA protein and disguises them from autophagic recognition (Yoshikawa et al., 2009), and *Burkholderia* secretes the BopA protein via the TTSS to evade autophagy (Cullinane et al., 2008). It is also known that the BopA protein shares 23% homology with IcsB of *Shigella*. Another of the putatively non-pathogenic SFGR, *Rickettsia* sp. LON, which was isolated from *Haemaphysalis longicornis* (a tick), but has never been isolated from human spotted fever patients in Japan (Fujita, 2008; Hanaoka et al., 2009), is genetically closely related to *R. japonica* and in fact is classified within the *R. japonica* group. Its growth in mammalian cells was examined in a recent study (Uchiyama and Fujita, 2012). The growth of *Rickettsia* sp. LON is restricted in mammalian cells, as was found for *R. montanensis*. However, its growth can be recovered by superinfection of the pathogenic *R. japonica*. These results further strengthen the hypothesis that the degree of rickettsial growth in mammalian cells basically determines the pathogenicity of *Rickettsia*. Another non-pathogenic *Rickettsia*, *R. peacockii*, which is also known as the East Side agent, was isolated from Rocky Mountain Wood ticks (*Dermacentor andersoni*) from Montana, USA (Bell et al., 1963; Burgdorfer et al., 1981). *R. rickettsii*-carrying *D. andersoni* display a markedly reduced prevalence on the east side of the Bitterroot Valley, while Rocky Mountain spotted fever predominantly occurs on the west side of the valley (Philip and Casper, 1981). Thus, the presence of *R. peacockii* in *D. andersoni* ticks might prevent the transovarial transmission of *R. rickettsii* and limit its spread in the tick population, although it is uncertain whether *R.*

peacockii actively interferes with *R. rickettsii* in ticks or whether ticks carrying *R. peacockii* have a reproductive advantage over those carrying *R. rickettsii*. *R. rickettsii* has been demonstrated to have a lethal effect on its tick vector *D. andersoni* (Niebylski et al., 1999). A comparative study has also been performed of the genome sequences of the pathogenic *R. rickettsii* and the non-pathogenic *R. peacockii* (Niebylski et al., 1997; Felsheim et al., 2009). In *R. peacockii*, the genes encoding an ankyrin repeat containing protein, DsbA, RickA, protease II, rOmpA, Sca1, and a putative phosphoethanolamine transferase, which are related to its pathogenicity, were deleted or mutated. The gene coding for the ankyrin repeat containing protein is especially noteworthy as it is also mutated in the attenuated Iowa strain of *R. rickettsii*. The precise mechanisms by which these factors contribute to the pathogenicity of SFGR are yet to be clarified.

PERSPECTIVES

The vector tropism of rickettsiae seems to correspond with their growth in cultured mammalian cells. It has been clarified that the growth restriction of SFGR in AeA12 cells depends on the non-apoptotic cell death induced after host cell adherence and invasion by rickettsiae. It is important to analyze the mechanisms responsible for this cell death and the cell death inhibition observed in AeA12 cells infected with TGR. Moreover, the mechanisms responsible for the restriction of TGR growth in tick cells and the abrogation of the growth restriction in tick cells infected with SFGR also need to be elucidated.

A relationship was detected between the ability of *Rickettsia* species to grow in cultured mammalian cells and their pathogenicity; however, the growth abilities of *Rickettsia* species are affected by many host and rickettsial factors during the various stages of rickettsial infection. In order to elucidate the mechanisms governing rickettsiae pathogenicity, it is necessary to compare these factors between pathogenic and non-pathogenic strains.

Although I have attempted to elucidate the relationships between various rickettsiae species and cell types in this review, it is also necessary to clarify the roles of innate and acquired immunity against rickettsiae infection.

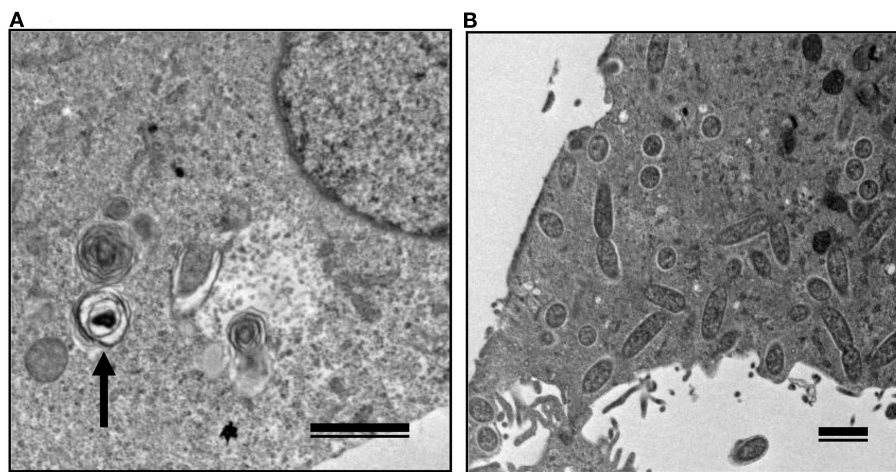


FIGURE 6 | Transmission electron microscopy of Vero cells infected with non-pathogenic and pathogenic SFGR. (A) Vero cells infected with *R. montanensis* alone was observed at 7 days after infection. An arrow marks a degenerating rickettsia in an autophagosome-like vacuole. **(B)** *R.*

montanensis-infected cells were superinfected with *R. japonica* on day three of infection and observed at 7 days after the first infection. Many free rickettsiae around 1 μm in length surrounded by halos and those in the course of binary fission were seen in the cytoplasm. Bars, 1 μm .

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