MINIREVIEW

Rickettsia-host interaction: strategies of intracytosolic host colonization

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One sentence summary: Rickettsiae deploy their effector arsenal to manipulate membrane dynamics, actin cytoskeleton, phosphoinositide metabolism, intracellular trafficking and immune defense mechanisms; to gain access, and promote their intracytosolic lifespan to ultimately expedite transmission.

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ABSTRACT

Bacterial infection is a highly complex biological process involving a dynamic interaction between the invading microorganism and the host. Specifically, intracellular pathogens seize control over the host cellular processes including membrane dynamics, actin cytoskeleton, phosphoinositide metabolism, intracellular trafficking and immune defense mechanisms to promote their host colonization. To accomplish such challenging tasks, virulent bacteria deploy unique species-specific secreted effectors to evade and/or subvert cellular defense surveillance mechanisms to establish a replication niche. However, despite superficially similar infection strategies, diverse Rickettsia species utilize different effector repertoires to promote host colonization. This review will discuss our current understandings on how different Rickettsia species deploy their effector arsenal to manipulate host cellular processes to promote their intracytosolic life within the mammalian host.

Keywords: Rickettsia-host interaction; spotted fever group; transition group; typhus group; bacterial effector molecules; bacterial adherence and engulfment; phagosomal escape; phosphoinositide metabolism; intracellular trafficking; host defenses

INTRODUCTION

Rickettsiae host cell invasion is a dynamic process that involves a complex interplay between the invading pathogens and the host. During invasion, intracellular bacteria generate regulatory control over host cells by modulating membrane dynamics, actin cytoskeleton, phosphoinositide (PI) metabolism, intracellular trafficking and immune defense mechanisms; to gain access, and promote their survival and proliferation to ultimately expedite transmission (Ray et al. 2009; Pizarro-Cerdá, Kühlbacher and Cossart 2015; Personnic et al. 2016; Lamason and Welch 2017). After internalization, intracellular pathogens encounter innate defense surveillance initiated upon pathogen sensing or other danger signals in the host cytosol. Pathogenic bacteria have evolved multiple strategies with numerous effectors, to evade and/or subvert innate defense surveillance to successfully colonize the host (Huang and Brumell 2014; Mitchell and Isberg 2017). Akin to other intracytosolic bacteria, including Listeria, Shigella, Burkholderia and Francisella (Ray et al. 2009; Personnic et al. 2016), Rickettsia internalization by phagocytosis and subsequent escape of pathogens into host cytosol is required for the survival and ultimately colonization of the host cell (Hackstadt 1996; Hackstadt 1998; Gillespie et al. 2015; Gillespie et al. 2016; Sahni et al. 2018). Recent work in our laboratory...
and others have identified several rickettsial effectors that function during the early stage of host cell invasion. While several surface proteins characterized for adhesion and/or entry of host cell are conserved, others are only sporadically encoded across rickettsial lineages, suggesting that, despite superficially similar infection strategies, diverse Rickettsia species (spp) employ distinct biochemical mechanisms to enable host colonization (Sears et al. 2012; Rahman et al. 2013; Gillespie et al. 2015; Rennoll-Bankert et al. 2015; Gillespie et al. 2016; Lamason et al. 2016; Rennoll-Bankert et al. 2016; Sahni et al. 2018; Lehman et al. 2018; Engström et al. 2019; Voss et al. 2020; Aistleitner et al. 2020). In this review, we will discuss recent advances in our understanding of how diverse Rickettsia spp utilize their unique effector arsenal to alter host dynamics and spatiotemporal biochemical processes to establish an intracytosolic replication niche.

**RICKETTSIA SPECIES**

Members of the genus Rickettsia, belonging to the class of Alphaproteobacteria; in the family of Rickettsiaceae and order of Rickettsiales; are Gram-negative obligate intracellular bacteria, which can invade a wide range of eukaryotes, including blood- or sap-feeding arthropods. Based on molecular phylogeny estimation, Rickettsia spp are classified into four groups: spotted fever group (SFG), typhus group (TG), transitional group (TRG) and ancestral group (AG) (Gillespie et al. 2007; Gillespie et al. 2009; Gillespie et al. 2010). Among these four Rickettsia lineages, we have very little information about members of AG, while the remaining three lineages: SFG, TRG, and TG, that harbor many deadly human pathogens, are well studied (Gillespie et al. 2007; Walker and Ismail 2008; Weinert et al. 2009; Gillespie et al. 2010; Murray et al. 2016). Rickettsia from the SFG, like R. rickettsii and R. conorii, are maintained naturally within various species of ticks, while TRG members, such as R. akari, R. australis, and R. felis, are maintained and transmitted to humans respectively via mites, ticks and fleas (Fig. 1). In addition, species from the TG, such as R. prowazekii and R. typhi, can be found throughout the world in fleas and lice (Fig. 1). Intriguingly, regardless of the phyletic relationship only a selected few of Rickettsia spp are considered highly pathogenic by causing disease in humans, while others show little to no pathogenicity. In particular, rickettsial infections causing fatal disease in humans, include Rocky Mountain Spotted Fever (R. rickettsii, Sheila Smith, (SS)), Boutonneuse fever (R. conorii), rickettsial pox (R. akari), epidemic typhus (R. prowazekii), or murine typhus (R. typhi) (Fig. 1).

Rickettsiae are zoonotic pathogens, with a worldwide distribution, that are transmitted to humans by the bite of arthropods (e.g. ticks) or via the feces of infected arthropods, like lice and fleas (Fig. 2) (Hackstadt 1996; Hackstadt 1998; Walker and Ismail 2008; Gillespie et al. 2009; Gillespie et al. 2015). Apart from the historical record, the global impact of arthropod-borne rickettsial infections is illustrated by the resurgence of long-known pathogens, as well as the emergence of newly recognized spp (Sanchez-Vicente et al. 2019). Infections of humans with R. rickettsii continue to cause severe consequences in South and Central America (Bermúdez and Troyo 2018), and the resurgence of R. conorii in Europe, the Middle East, and Africa further highlights the current threats of rickettsial diseases (Levin et al. 2009). In the USA, tick- and flea-borne rickettsial diseases are also on the rise, as exemplified by recent outbreaks of R. rickettsii in Arizona (Drexler et al. 2014) and of R. typhi in California (Billetter and Metzger 2017) and Texas (Blanton et al. 2016). There are currently no vaccines to prevent rickettsiosis. Additionally, a poor understanding of rickettsial intracellular lifestyle present an immense challenge to research and hinders progress towards development of effective intervention against these increasingly recognized rickettsioses (Sahni et al. 2018).

Rickettsiae infect a wide range of host cells where the metabolite-enriched host cytosol sustains their survival and growth in the face of reduced genomes that lack genes for many metabolic pathways (Driscoll et al. 2017). Rickettsial infection into the host begins with the inoculation of Rickettsia spp via infected arthropod vectors, like ticks, mites, lice or fleas, at the host’s dermis (Fig. 2), where they encounter tissue-resident CD68+ macrophages (MΦ) and dendritic cells (Sahni et al. 2018). In fact, MΦ play a critical role in either terminating an infection at an early stage or succumbing to pathogen colonization and thus facilitating bacterial replication and host dissemination to distant organs, including lung, liver, heart and brain (Sahni et al. 2018). Given that intracytosolic survival depends on the escape from phagosomes and subversion of host cytosolic defense responses, in particular autophagy and inflammasomes, Rickettsia spp have develop sophisticated strategies to facilitate host invasion and to circumvent host immune defenses (discussed later in this review) (Ray et al. 2009; Personnic et al. 2016). Here, we will discuss the current advances of how virulent Rickettsia spp utilize their sec-dependent and -independent secretion pathways to translocate immunodominant outer membrane proteins [e.g. surface cell antigens (Scas)], actin modulating factor, RaIF, the Rickettsia ankyrin repeat proteins (RAARF-1/-2), Rickettsia intracellular secreted kinase-1 (Risk1), and other effectors [i.e. phospholipases (Pat1/Pat2) (Fig. 3) (Ammerman, Rahman and Azad 2008; Rahman et al. 2010; Sears et al. 2012; Kaur et al. 2012; Rahman et al. 2013; Gillespie et al. 2015; Rennoll-Bankert et al. 2015; Gillespie et al. 2016; Lehman et al. 2018; Voss et al. 2020), to target host PI metabolism and evade host defense surveillance to establish habitable intracytosolic replication niche (Figs 4 and 5).

**HOST INVASION: SURFACE CELL ANTIGEN PROTEINS**

Despite their reductive genomes, Rickettsia spp are highly complex organisms that choreograph the expression of multiple surface proteins for successful entry and to establish a replication niche within a nutrient rich host cytosol. Host invasion of Rickettsia spp requires ligand engagement of specific receptors, as well as hijacking of specific host signaling cascades, which ultimately results in the rearrangements of actin cytoskeleton and membrane dynamics. Key molecules contributing to Rickettsia invasion are surface cell antigens (Scas), a class of immunodominant outer membrane (OM) proteins, for which nearly each spp of Rickettsia encodes a different Sca arsenal (Chan, Riley and Martinez 2010; Gillespie et al. 2015). Initial computation analysis of rickettsial genomes designated 17 distinct Sca family member (Blanc, Renesto and Raoult 2005) of which only 6 members are encoded in most rickettsial genomes: Sca0 (rOmpA), Sca1, Sca2, Sca3, Sca4 and Sca5 (rOmpB) (Sears et al. 2012; Gillespie et al. 2015). The secretion of Sca proteins is mediated by a sec-dependent type V secretion system (T5SS) to the OM of rickettsia, except Sca4, a molecule which lacks the β-domain; and was extensively discussed in our earlier review (Gillespie et al. 2015) and is summarized in Fig. 3. Conserved in all rickettsiae spp, the ubiquitous Sca5 (rOmpB) was shown to mediate bacterial invasion through its association with the host cell-specific receptor Ku70 (subunit of a nuclear DNA-dependent PK) (Martinez et al. 2005; Chan et al. 2009). In turn, association of Ku70
Figure 1. Rickettsia species-specific pathogenicity and disease development in humans. Natural transmission of rickettsiae to humans is accomplished by various arthropod vectors (ticks, fleas, lice, or mites) resulting in rickettsiosis with various degrees ranging from highly severe e.g. Rocky Mountain Spotted Fever (RMSF), to moderate (like cat-flea typhus), or ultimately asymptomatic (Hackstadt1996; Hackstadt1998; Azad and Beard1998; Sahni et al. 2013; Clark et al. 2015; Curto et al. 2016). SFG: Spotted Fever Group; TRG: Transition Group; TG: Typhus Group; R. rickettsii [Sheila Smith, (SS)], R. rickettsii [Iowa, (IA)].

Figure 2. Life cycle of ticks and fleas and their natural transmission of rickettsiae. Black arrow lines emphasis key events of ticks natural cycle: (1) oviposition by engorged female ticks; (2) eggs hatched into larvae; (3) larvae feeding on rodents; (4) larvae molting to nymphs; (5) feeding of nymphs on larger animals; and (6) molting of nymphs into adult ticks that can feed on larger animals or infect humans (diagram was modified from (Eremeeva and Dasch2015)). Broken black lines highlights transovarial (7) and transstadial transmission (8) of rickettsiae, while broken red lines show transmission of rickettsiae to humans through a direct bite of nymphs (9, 10) or adult ticks (11). Blue arrow lines emphasis key events of fleas natural cycle: (12) female fleas shed eggs into the environment (13) eggs hatched into larvae; (14) larvae form pupae; (15) pupae hatch to adult fleas; while transmission of rickettsiae to animals (16) or humans (broken red line, 17) occurs through inoculation of bacteria-laden flea feces onto flea bite wounds or mucous membranes (Azad et al. 1997; Anstead2020).

with rOmpB results in the recruitment of ubiquitin ligase c-Cbl (causing ubiquitination of Ku70), clathrin and caveolin-2 to facilitate the engulfment of the bacteria (Martinez et al. 2005). The less conserved Sca0 (rOmpA), absent in most non-SFG spp (Fig. 3B), was also shown to be important for SFG rickettsiae internalization via its interaction with α2β1 integrin (Hillman, Baktash and Martinez2013). However, further studies revealed that rOmpA was dispensable for the bacterial virulence in vivo (Noriea, Clark and, Hackstadt2015). A very recent finding highlighted another host cell receptor FGFR1 (fibroblast growth factor receptor-1) as a potential target for rickettsial rOmpA to promote the internalization of SFG rickettsiae into host endothelium via a caveolin 1-dependent endocytosis (Sahni et al. 2017).

Genome analysis revealed the presence of full-length Sca1 in all Rickettsia spp, except being divided in R. prowazekii (Fig. 3B) and R. canadensis (Ngwamidiba et al. 2006). Autotransporter Sca1 is expressed in R. conorii and R. typhi and was shown to play a role in the adherence of the bacteria to the target cell, however, its function during rickettsial invasion and the identity of its mammalian receptor remains elusive (Ngwamidiba et al. 2006; Riley et al. 2010; Sears et al. 2012). The surface cell antigen Sca2 is ~150 kDa protein and conserved among most SFG and TRG Rickettsia spp, while fragmented in R. prowazekii (Ngwamidiba et al. 2006; Dreher-Lesnick et al. 2008; Cardwell and Martinez 2009) (Fig. 3B). Sears et al. 2012 Sca2 was shown to be involved in the adherence to and engulfment of host cells (Cardwell and
Figure 3. Effector section systems and distribution across divergent Rickettsia species. (A) Rickettsia spp utilize two distinct secretory pathways (adopted and updated from our previous reporting (Gillespie et al. 2015)). The T5SS and Sec-TolC pathway are two Sec-dependent pathways. The T5SS system is considered to be involved in the secretion of the surface cell antigen (Sca) family. Currently, Rickettsia ankyrin repeat protein 1 (RARP-1) is the only effector secreted by the Sec-TolC system and involves its N-Terminal secretion signal (Sec SS), C-Terminal ankyrin (ANK) domain, and the TolC protein. The highly conserved sec-independent secretory pathways included the twin-arginine translocation (Tat), T1SS and T4SS systems. The Tat system is composed three components (TatA, TatB and TatC) and involved in the translocation of folded substrates across the inner membrane (IM). The TolC protein combined with additional IM proteins (AprE and AprD) form the functional T1SS system. The Rickettsiales vir homolog (rvh) T4SS, is highly similar to the vir structure of Agrobacterium tumefaciens, however with some difference including the duplication of several scaffold molecules and the lack of a pilus. CP: cytoplasm; PP: periplasm; OM: outer membrane; LPS: lipopolysaccharide; ?: unknown effectors or mechanism remains to be determined. (B) Phylogeny and effector molecules distribution across transitional group (TRG), spotted fever group (SFG) and typhus group (TG) Rickettsia spp (Rahman et al. 2010; Sears et al. 2012; Kaur et al. 2012; Rahman et al. 2013; Gillespie et al. 2015; Rennoll-Bankert et al. 2015; Gillespie et al. 2016; Lehman et al. 2018; Voss et al. 2020). Full length protein; Truncated with active site; D denotes divergent passenger domain fused to Sca2 β-domain; n denotes ankyrin repeat number within full length protein; X not detected.

Martinez 2009), although its mammalian target receptor remains unknown. Autotransporter Sca3 is exclusively encoded within the genomes of TG rickettsiae and flea-associated R. felis of TRG rickettsiae (Fig. 3B) and is considered as the largest rickettsial surface protein among the Sca family. However, Sca3 functional importance in the adherence to and engulfment of target cells remains to be uncovered (Sears et al. 2012). Genome sequence analysis showed the presence of Sca4 in all rickettsial groups, including R. prowazekii, R. typhi (Fig. 3B) and R. bellii, while absent in R. canadensis (Blanc, Renesto and Raoult 2005; Sears et al. 2012). Intriguingly, Sca4, although lacking its autotransporter domain, is transported by a currently unknown mechanism to the rickettsial surface resulting in the activation of vinculin at the focal adhesion sites supporting a role for Sca4 in facilitating the invasion of target cells (Blanc, Renesto and Raoult 2005; Park et al. 2011; Gillespie et al. 2015).
HOST ACTIN CYTOSKELETON REARRANGEMENT AND CELL-TO-CELL SPREAD

As bacterial dissemination into neighboring cells is vital for a successful host colonization, rickettsial invasion involves host actin polymerization to form the typical F-actin ‘comet tail’. Notably, polar actin tail formation occurs exclusively in most SFG rickettsiae with the exception of *R. peacockii*, whereas minimal to no tail formation was observed among TG members (*R. typhi* and *R. prowazekii*) (Teysseire, Chiche-Portiche and Raoult 1992). As a result, infection of SFG rickettsiae initiates the activation of a signaling cascade involving Cdc42 (a GTPase), a phosphoinositide 3-kinases (PI3Ks), c-Src and likely additional protein tyrosine kinases, ultimately leading to the activation of Arp2/3 complex (Martinez and Cossart 2004). In fact, in vitro cell culture studies revealed that various Rickettsia spp from both SFG and TRG (e.g. *R. conorii*, *R. rickettsii*, *R. montanensis*, *R. australis*, *R. parkeri* and *R. felis*) utilize the effector RickA (absent in TG rickettsiae), a Wiskott–Aldrich syndrome protein (WASP), to promote actin-based motility and recruitment of the Arp2/3 complex (Heinzen et al. 1993; Gounin et al. 2004; Ogata et al. 2005; Balraj et al. 2008). Intriguingly, more recent findings support a model of biphasic rickettsial motility in which during early phase of infection (~30 min) motility is dependent on RickA and the Arp2/3 complex, while upon persistent infection (~24–48 hrs), motility is independent of Arp2/3 complex and RickA; and involves rickettsial Sca2 protein (Reed et al. 2014). Specifically, Sca2 nucleates the assembly of linear actin filaments, via its three WASP homology 2 (WH2) domains, resulting in the filament elongation by profilin and the inhibition capping protein activities (Haglund et al. 2010; Kleba et al. 2010; Lamason and Welch 2017).

Although, members of TG rickettsiae do not encode a functional orthologue of Sca2 (Fig. 3B), various spp of SFG and TRG rickettsiae; including *R. rickettsii*, *R. australis*, *R. conorii*, *R. ariacae*, and *R. akari*; encode Sca2, it is speculated that majority of SFG and TRG rickettsiae use the eukaryotic formin-like properties of Sca2 as a key mechanism for dissemination to neighboring host cells (Kleba et al. 2010; Gillespie et al. 2015). Recent report showed *R. parkeri* secretes an effector Sca4 that binds to the cell adhesion protein vinculin, inflicting the disruption of donor cell interaction between vinculin and α-catenin of recipient cell, which in turn promotes the cell to cell spread by enhancing protrusion engulfment efficiency (Lamason et al. 2016).

Another actin-binding molecule, RalF, expressed only in prokaryotes of some Rickettsia (Fig. 3B) and Legionella spp, is unique as it contains a N-terminal Sec7 domain (S7D) and C-terminal Sec7 capping domain (SCD) (Cox et al. 2004; Alix et al. 2012; Rennoll-Bankert et al. 2015). This domain is found among eukaryotic guanine nucleotide exchange factors (GEF) that activate ADP-ribosylation factors (Arfs), proteins involved in vesicle trafficking and actin remodeling (Casanova 2007). Both Legionella RalF (RalF₁) and Rickettsia RalF (RalF₄) function as ArfGEF that activates ArfGTPases by its catalytic S7D. However, RalF function differ significantly across these different pathogens and have divergent subcellular localization patterns mediated by the intrinsic determinants of SCD that confers distinct effector function in the host cells (Alix et al. 2012; Folly-Klan et al. 2013; Rennoll-Bankert et al. 2015; Rennoll-Bankert et al. 2016).
Legionella pneumophila RalF (RalF<sub>Lp</sub>) binds and activates host Arf1 to localize the Legionella-containing vacuole (LCV) to the endoplasmic reticulum (ER) (Nagai et al. 2002; Nagai et al. 2005). The SCD of RalF<sub>Lp</sub> likely contributes in the interception of host secretory vesicles, while that of Rickettsia prowazekii RalF (RalF<sub>Rp</sub>) targets the protein to the host PM to modulate actin dynamics (Alix et al. 2012; Folly-Klan et al. 2013). The role of SCD of RalF was investigated by chimera domain (SCD) swapping experiments between RalF<sub>Lp</sub> and RalF<sub>Rp</sub>. The study revealed that the chimeric protein having S7D of RalF<sub>Rp</sub> and SCD of RalF<sub>Lp</sub> is as efficient in recruiting Arf1 to the LCV as wild type RalF<sub>Lp</sub>. However, the reverse chimeric protein having S7D of RalF<sub>Lp</sub> and SCD of RalF<sub>Rp</sub> was inefficient in mediating Arf1 recruitment to LCV (Alix et al. 2012). Furthermore, the SCD of both RalF<sub>Lp</sub> and RalF<sub>Rp</sub> auto-inhibit the catalytic S7D in solution, and a favorable membrane environments derepress ArfGEF activity in both proteins (Folly-Klan et al. 2013). The membrane sensor within the SCD of RalF<sub>Lp</sub> and RalF<sub>Rp</sub> revealed the differential enrichment in aromatic/charged residues determining distinct membrane localization that regulates ArfGEF activity (Folly-Klan et al. 2013). The investigation of ArfGEF activity on membranes through chimeric proteins containing S7D of RalF<sub>Lp</sub> and SCD of RalF<sub>Rp</sub> (LpRpRalF) or S7D of RalF<sub>Rp</sub> and SCD of RalF<sub>Lp</sub> (RpLpRalF) revealed that RplpRalF activates Arf1. However, LpRpRalF was almost inactive, suggesting that a structural transition to the active form was blocked. The crystal structure analysis revealed the formation of serendipitous salt bridges between the residues of S7D of RalF<sub>Lp</sub> and SCD of RalF<sub>Rp</sub> in LpRpRalF chimera which was further highlighted as the cause of impaired LpRpRalF activity on the membranes, either by enforcing strong auto-inhibition or compromising the conformation of membrane bound LpRpRalF (Folly-Klan et al. 2015). Further follow up studies on rickettsial RalF (RalF<sub>R</sub>), allowed us to show that R. typhi RalF (RalF<sub>t</sub>) localized to the host PM (Fig. 5). Specifically, R. typhi RalF (RalF<sub>t</sub>) localization to the PM required recruitment of PI(4,5)P<sub>2</sub>, via the activation of Arf6, which in turn initiated actin cytoskeleton rearrangement that was critical for the invasion of R. typhi suggesting a role for this effector in modulating PI metabolism (discussed later in this review) (Rennoll-Bankert et al. 2015; Rennoll-Bankert et al. 2016).
ANKYRIN-REPEAT-CONTAINING PROTEINS

One of the most conserved protein–protein interaction motifs in nature are ankyrin domains (Mosavi, Minor and Peng 2002), with ankyrin repeat-containing proteins (ARPs) playing key roles in the pathogenicity of intracellular bacteria (Pan et al. 2008; Al-Khodor et al. 2010). However, each ARP identified from intracellular pathogens like Anaplasma, Ehrlichia, Legionella, and Orientia seem to perform strain specific tasks, such as directly modulating gene transcription, manipulating vesicular trafficking, interrupting signaling pathways, or disrupting organelles (Pan et al. 2008; Zhu et al. 2009; Price et al. 2010; Rikihisa and Lin 2010; Yang et al. 2015; VieBrock et al. 2015). Despite that, all rickettsial genomes encode variable numbers of predicted ARPs their functional importance during pathogenesis remains ill-defined (Gillespie et al. 2015). The most conserved ARPs are RARP-1 and RARP-2 (Gillespie et al. 2015) with RARP-1 being a Sec-TolC secreted effector (Kaur et al. 2012), while RARP-2 is secreted by a type IV secretion system (T4SS) (Fig. 3) (Lehman et al. 2018). As rickettsial RARP-1 precise role remains to be determined, RARP-2 from R. rickettsii (SGF) but not from R. typhi (TG) was shown to target the ER and trans-Golgi network upon infection of host cells (fuab016) suggesting a role for this effector in targeting cellular organization to facilitate host colonization likely in species-specific manner.

MEMBRANOLYTIC EFFECTORS

Adherence and engulfment of rickettsiae into host cells is a relatively fast dynamic process. However, successful intracytosolic survival requires rickettsial evasion from host defense surveil- lance by lysosomal destruction. Soon after internalization Rick- etsia spp avoid phago-lysosome fusion by escaping into host cytosol (Ray et al. 2009). Rickettsiae utilize their secretory mem- branolytic effector arsenal, including hemolysins and phospholipases, to disrupt the phagosomal membranes and gain access to host cytosol (Radulovic et al. 1999; Renesto et al. 2003; Whitworth et al. 2005; Rahman et al. 2010; Housley, Winkler and Audia 2011; Rahman et al. 2013). All Rickettsia genome analysis revealed the presence of membranolytic enzymes: TlyA, TlyC, Pld, Pat1 and Pat2 (Gillespie et al. 2015). Although the precise functional role of TlyA for rickettsial pathogenicity remains unknown, a membranolytic activity of the rickettsial TlyC protein from the R. typhi spp was demonstrated (Radulovic et al. 1999). Pld (phospholipase D), encoded by the pld gene, is highly conserved in all sequenced rickettsial genomes (Gillespie et al. 2015). The phospholipase activity of Pld, that functions as a dimer, was demonstrated in vitro (Renesto et al. 2003; Whitworth et al. 2005) and in a in vivo model (guinea pig) of rickettsiosis (Driskell et al. 2009). Intriguingly, domain structure predictions of R. typhi Pld suggest that it likely localizes to the rickettsial OM via a secretory pathway similar to that of RARP-1 (Fig. 3) (Amerman, Rahman and Azad 2008; Gillespie et al. 2015). The phospholipase A2 (PLA2)-like activities have long being proposed to facilitate rickettsial phagosomal escape as well as host cell entry and exit (Winkler and Miller 1982; Winkler and Daugherty 1989; Silverman et al. 1992; Ojcius et al. 1995; Walker, Fong and Popov 2001). The sequence analysis of available genomes of Rickettsia spp, identified a conserved patatin (Pat)- like PLA2 encoding molecule, Pat1 within all Rickettsia genomes (Blanc, Renesto and Raoult 2005). A more recent report further characterized a second PLA2-encoding protein, Pat2, within the TG rickettsiae genomes, which was only sporadically present in SFG or TRG rickettsial genomes (Rahman et al. 2010; Rahman et al. 2013). The sequence analysis of Pat1 and Pat2 enzymes suggest that both proteins are likely secreted by either the T1SS or T4SS (Gillespie et al. 2015). Furthermore, we demonstrated that R. typhi Pat2 possesses a PLA2 activity and is secreted into the host cell cytoplasm during infection (Rahman et al. 2010). A later report further confirmed a similar PLA2 activity for the R. prowazekii Pat2 enzyme (Housley, Winkler and Audia 2011). In line with these findings, Pat1 also possesses a PLA2 activity and is secreted into host cell cytoplasm during infection (Rahman et al. 2013). Importantly, antibody neutralization of either Pat1 or Pat2 significantly reduced the survival of R. typhi, highlighting the roles for both proteins during bacterial infection (Rahman et al. 2013). Collectively, these data suggest that R. typhi, and likely R. prowazekii, use similar phospholipases during host infection, a mechanism that perhaps distinguishes TG rickettsiae from other Rickettsia spp.

LIPID MODIFYING EFFECTORS

Many intracellular pathogens have developed diverse strategies to avoid recognition and subsequent destruction by host micro- bicidal defense mechanisms to establish a successful host colon- ization (Hibiske and Stephens 2008; Ray et al. 2009; Mitchell and Isberg 2017; Sahni et al. 2018). In fact, after internalization into host cells, some intracellular bacteria such as Shigella, Francisella, and Rickettsia spp, escape phagosomal maturation into cytosol to evade lysosomal destruction; while others like Mycobacterium, Salmonella, and Legionella modify the vacular compartment, to create an intracellular replication niche (Ray et al. 2009; Pizarro-Cerdá, Kühbacher and Cossart 2015; Person- nic et al. 2016). To accomplish such delicate tasks, all intra- cellular pathogens employ numerous effectors to commander the host PI metabolism by selectively manipulating different PI interconversion pathways (Fig. 4) (Pizarro-Cerdá, Kühbacher and Cossart 2015; Mitchell and Isberg 2017; Huang and Brumell 2014; Walpole, Grinstein and Westman 2018). Specifically, bacte- rial effectors can modulate PI interconversion by either directly acting as eukaryotic-like PI kinase and phosphatase or indirectly functioning as regulator of host PI kinases and phosphatases (Fig. 4). Legionella pneumophila secretes P4-Kinase LepB and the 3-phosphatase SidF, two dot/icm T4SS effectors that contribute to the synthesis of P(i)(4)P on the Legionella-containing vacuole to avoids endolysosomal destruction and to create a replication permissive vacular compartment (Fig. 4) (Hsu et al. 2012; Dong et al. 2016). In addition, L. pneumophila secretes another dot/icm effector, LegA5, a class III PI3-Kinase, consistent with numerous PI-interacting dot/icm effectors functioning to establish an intracellular vacuolar niche for Legionella (Fig. 4) (Ledvina et al. 2018; Steiner, Weber and Hili 2018). The intracytosolic bacte- ria, Francisella tularensis, secretes the T6SS effector OpIA, a PI3- Kinase, to enhance the production of P(i)(3)P on the Francisella- containing phagosome, which consequently prevents endolysoso- mal fusion and promotes the escape of the bacteria into host cytosol (Fig. 4) (Ledvina et al. 2018). As obligate intracytosolic bacteria, Rickettsia invasion into target cells also involves the manipulation of PI-metabolism and the evasion of lysosomal destruction (Ray et al. 2009, Pizarro-Cerdá, Kühbacher and Cossart 2015; Walpole, Grinstein and Westman 2018). In this effort, we reported that secretion of R. typhi T4SS effector, RalF, was critical for host cell invasion (Fig. 4) (Rennoll-Bankert et al. 2015). Particularly, we showed that RalF was expressed early during R. typhi infection, colocalized with the PM and activated Arf6, which in turn recruited the host PIP5-Kinase to facilitate the conversion of P(i)(4)P to P(i)(4,5)P2 (Fig. 5, Stage 1). These findings
likely support a mechanism by which R. typhi RalF indirectly modulates the PI metabolism to facilitate bacterial host invasion (Rennoll-Bankert et al. 2015; Rennoll-Bankert et al. 2016). In our recent report (Voss et al. 2020), we showed the presence of an additional R. typhi T4SS effector, Risk1, targeting host PI metabolism during invasion (Fig. 4). Our informatic, biochemical and enzymatic analysis further characterized Risk1 as a PI3K with a substrate specificity for both PI and PI(4,5)P2, making it the first bacterial PI3K with both class I and class III activities (Voss et al. 2020). In addition, our findings support that Risk1-dependent PI3K activity was involved in the synthesis of PI(3)P and PI(3,4,5)P3, lipids critical for the engulfment of R. typhi into host cells and the subsequent escape from endolysosomal destruction (Fig. 5, Stages 2 and 3) (Rennoll-Bankert et al. 2016; Voss et al. 2020). Our data further suggest that Risk1 likely modulates R. typhi-induced autophagy through its association with Beclin-1 and contributes to the escape from endolysosomal destruction via the conversion of PI to PI(3)P on the vacuole (Fig. 5, Stages 4 to 6) (Voss et al. 2020). As PI(3)P consumption on either endosomal or autophagosomal membranes is required for their fusion with lysosomes, it is tempting to propose a conceptual model of R. typhi intracytosolic infection by which Risk1-dependent generation of PI(3)P on both the phagosomal and autophagosomal membranes results in the delay of their maturation. As a result, the delay in maturation of these structures would allow additional effectors, such as Pat1 and Pat2 phospholipases, to perforate their membranes to facilitate the escape of the rickettsiae into the host cytosol (Fig. 5, Stage 7).

Aside R. typhi, strategies to induce autophagy and subsequent exploitation of autophagosomes to enhance host invasion are shared among some other rickettsial members (e.g. R. australis (Bechelli et al. 2018)) and their relatives, including Anaplasma phagocytophilum and Ehrlichia chaffeensis (Rikihisa 2017). Also, R. typhi ability to delay autophagic maturation via secretion of an effector protein, is another layer of sophistication that is shared among various intracellular pathogens, including Mycobacterium marinum (Romagnoli et al. 2012), Chlamydia trachomatis (Yasir et al. 2011; Al-Younes et al. 2011), Yersinia pestis (Pujol et al. 2009), and Francisella tularensis (Asare and Kwaik 2011). However, it is important to note that a previous postulated model of ‘nutritional virulence’, in which autophagosomal cargo is rerouted to the vacuoles harboring these bacteria, is harder to envision for Rickettsia spp, as all bacteria exclusively replicate in the intracytosolic space and not within modified phagosomes. Intriguingly, R. parkeri, a mildly-violent member of SFG, was very recently shown to avoid autophagy induction and to evade autophagic recognition through its surface protein rOmpB (Engström et al. 2019). On the contrary, the virulent TRG member, R. australis, benefited from Atg5-dependent autophagy induction and suppression of inflammasome-dependent IL-1β production to colonize the host (Bechelli et al. 2018). In fact, recent reports further suggest that autophagy can act on intracellular microbes upstream of the inflammasome and thereby functions as a negative regulator by degrading inflammasome components (Mitchell and Isberg 2017; Sun et al. 2017). Thus, it is possible that virulent Rickettsia spp, like R. australis and R. typhi, employ different effector-mediated mechanisms to induce autophagy that allows subversion of inflammasome-dependent recognition and to facilitate their host colonization, as compared to mildly- or non-virulent spp (e.g. R. parkeri and R. montanensis), however the precise mechanism remains to be determined. In sum, these data for diverse rickettsial spp accentuate the divergent strategies utilized across Rickettsiales for intracellular parasitism and additional research is required to elucidate the mechanisms on how these parasites modulate intracellular trafficking and manipulate host defense pathways to promote host invasion and intracytosolic replication.

CONCLUSION AND FUTURE DIRECTION

As highlighted in this review significant lack of conservation is seen not only at the level of protein secretion systems or pathways but also among members of effector proteins. In fact, many effectors are either absent, truncated, fragmentated or predicted as pseudogenes in one or more Rickettsia spp. Thus, aside surface molecules, secretory effector proteins are highly variable across Rickettsia spp. In fact, findings from our laboratory and others suggest that virulent Rickettsia spp, like R. australis and R. typhi, utilizes different effector-mediated mechanisms to hijack intracellular trafficking to subvert host defense pathways, like autophagy and inflammasomes, to facilitate host infection and dissemination, as compared to mildly- or non-virulent spp (e.g. R. parkeri and R. montanensis).

Future research is required to identify the precise mechanisms: (i) on how virulent Rickettsia spp utilize their effector repertoire to manipulate autophagic responses and (ii) by which effectors of virulent Rickettsia spp subvert inflammasome activation to establish intracytosolic replication niche and to promote host dissemination.

In sum, these studies will provide new insights on how pathogenic rickettsiae manipulate and evade host defenses, which ultimately will lead to the identification of a link that could be exploited for an anti-virulence strategy to develop better therapeutics to eradicate fatal rickettsial diseases.

AUTHOR CONTRIBUTIONS

OHV and MSR wrote and edited the review. All authors have read and agreed to the published version of the manuscript.

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