

# Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events

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**Abstract** | Rickettsiae cause some of the most severe human infections, including epidemic typhus and Rocky Mountain spotted fever. Substantial progress has been made in research into the genomics, vector relationships, pathogenesis and immunity of these obligate, intracellular, arthropod-transmitted bacteria. This Review summarizes our understanding of the early and late events in pathogenesis and immunity, modulation of the host response to rickettsial infection by the vector, host defence, virulence mechanisms and rickettsial manipulation of host cells.

## Housekeeping gene

A gene that is involved in the basic functions that are required for normal cell metabolism and is constitutively expressed.

## Hypovolaemia

Decreased blood volume — more specifically, a decrease in the volume of blood plasma.

## Hypotensive shock

Shock in which blood pressure is lower than normal and does not supply blood to the organs.

Pathogenic members of the *Rickettsia* genus are Gram-negative, obligate, intracellular bacteria that have a life cycle which involves both an arthropod vector and a vertebrate host<sup>1–3</sup> (FIG. 1). Rickettsiae are classified into four groups based on their biological, genetic and antigenic characteristics<sup>4</sup>: the spotted fever group (SFG), typhus group, transitional group and ancestral group. SFG rickettsiae include highly pathogenic organisms, such as tick-transmitted *Rickettsia rickettsii* (Rocky Mountain spotted fever (RMSF))<sup>5,6</sup>, *Rickettsia conorii* (Mediterranean spotted fever)<sup>1,2,7</sup>, *Rickettsia africae* (African tick-bite fever)<sup>8,9</sup>, *Rickettsia parkeri* (mild-to-moderate spotted fever rickettsiosis, found in North and South America)<sup>10,11</sup>, *Rickettsia slovaca* (tick-borne lymphadenopathy)<sup>12,13</sup>, *Rickettsia sibirica* (North Asian tick typhus and lymphangitis-associated rickettsiosis)<sup>14</sup>, *Rickettsia honei* (found in Australia and Southeast Asia)<sup>15</sup>, *Rickettsia japonica* (found in Japan and Korea)<sup>16,17</sup> and the apparently harmless *Rickettsia montanensis*, *Rickettsia peacockii* and *Rickettsia rhipicephali*<sup>2,18,19</sup>. The typhus group includes the highly pathogenic *Rickettsia prowazekii* (epidemic typhus) and *Rickettsia typhi* (murine typhus)<sup>20–23</sup>. The ancestral group includes *Rickettsia bellii*<sup>24</sup> and *Rickettsia canadensis*<sup>25</sup>; whether these species are pathogens is unknown. The transitional group comprises *Rickettsia akari* (rickettsialpox)<sup>26</sup>, *Rickettsia australis* (Queensland tick typhus)<sup>27</sup> and *Rickettsia felis* (flea-borne spotted fever)<sup>28</sup> (TABLE 1). *Rickettsia* phylogeny has been addressed by sequence analyses of different genes, varying from housekeeping genes, which are useful for distinguishing distinct strains, to genes that are under evolutionary pressure, such as

those that encode variable immunodominant outer-membrane proteins. This phylogenetic analysis has substantially affected the proposed taxonomy of rickettsiae. However, rickettsial taxonomy remains a controversial subject, owing to the absence of a universal consensus on those criteria that should be used for the designation of species (BOX 1).

Rickettsiosis can present with an array of clinical signs and symptoms<sup>6–9,11–16,29–31</sup>. Highly lethal RMSF<sup>29,30,32,33</sup> is characterized by headache, fever, myalgia, nausea and vomiting early in the illness; however, if untreated, severe injury can develop that sometimes progress to multi-organ failure. Systemic vascular infection in RMSF results in encephalitis, which leads to stupor, coma and seizures, interstitial pneumonia, non-cardiogenic pulmonary oedema and adult respiratory distress syndrome. In severe cases, hypovolaemia and hypotensive shock result in acute renal failure. Infection of a network of endothelial cells at the site of tick or mite inoculation of most SFG rickettsiae is followed by local dermal and epidermal necrosis that forms an eschar<sup>31</sup>. Disseminated infection, further injury to the vascular endothelium and infiltration of perivascular mononuclear cells leads to vasodilation, an increase in fluid leakage into the interstitial space and a characteristic rash. Epidemic typhus, which moulded world history for five centuries, is characterized by fever, headaches, mental confusion and a rash<sup>22,34</sup> (BOX 2). Similar to RMSF, epidemic typhus can develop into life-threatening conditions in previously healthy, immunocompetent individuals, unless they are treated early with an appropriate antibiotic. However, unlike RMSF, *R. prowazekii* causes latent infection in

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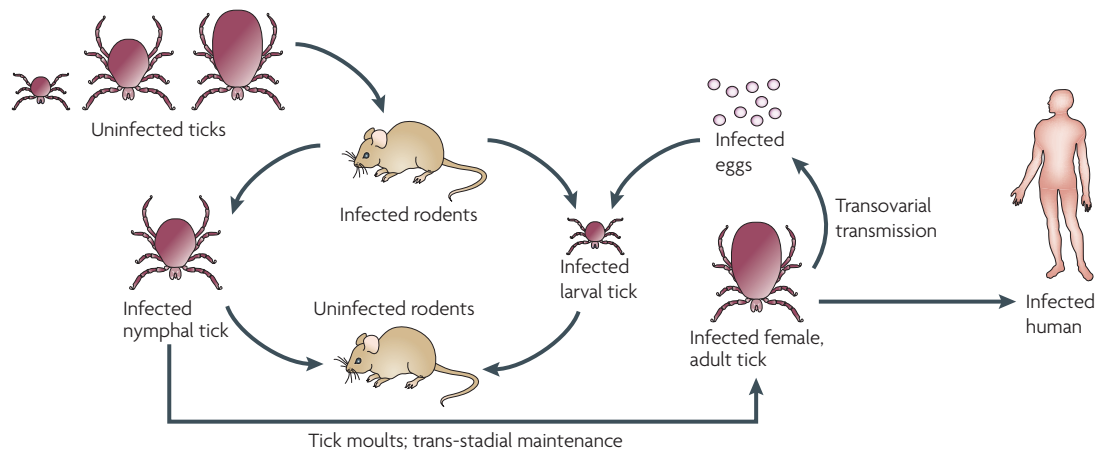


Figure 1 | **The life cycle of tick-borne rickettsiae.** Spotted-fever-group rickettsiae are maintained in nature by transovarial and trans-stadial transmission in ticks and horizontal transmission to uninfected ticks that feed on rickettsemic rodents and other animals.

convalescent individuals, and recrudescence of latent *R. prowazekii* infection results in Brill–Zinsser disease, which is characterized by fever, rash and less-severe illness that nevertheless can infect feeding lice and ignite an epidemic<sup>35,36</sup>.

Interest in the pathogenesis of *R. prowazekii* and *R. rickettsii* has increased following their classification as select agents and as category B and C agents of potential bioterrorism, respectively, by the United States Centers for Disease Control and Prevention<sup>37</sup> (see Further information). These pathogens are highly infectious agents that are easily disseminated and cause high morbidity and fatal disease, and thus require specific improvement in diagnostic tests and disease surveillance<sup>16</sup> — the use of *R. prowazekii* as a biological weapon was initiated by the Soviet Union in the 1930s and Japan during the Second World War<sup>21,38</sup>.

The body of knowledge of rickettsial pathogenesis and immunity is based on disseminated infection of endothelial cells, the principal target host cells for rickettsiae. Human infections have rarely been investigated until the middle or late, and often fatal, stages of the illness. The best animal models for SFG rickettsioses use *R. conorii* and *R. australis* or typhus group rickettsiosis (using *R. typhi*) in susceptible mice that are inoculated intravenously: these manifest systemic endothelial-cell infection and characteristic pulmonary and cerebral lesions that recapitulate the clinical and pathological manifestations of the disease in humans. Infections of guinea pigs with *R. rickettsii* and *R. prowazekii* provide models for RMSF and epidemic typhus, respectively.

This Review highlights how the arthropod host acquires, maintains and transmits rickettsiae, the initial steps in pathogenesis and the subsequent interaction of the bacteria with cells in the endothelium, the main target cells. These events include: rickettsial entry, phagosomal escape, actin-based motility, cell-to-cell spread and the induction of cell injury. Regarding the host immune response to rickettsial infection, we will address innate and acquired immunity, with emphasis

on recent data that illustrate the interaction of rickettsiae with dendritic cells (DCs). We also highlight some of the potential immunomodulatory effects of tick saliva on host defences and the immune response against *Rickettsia* spp.

**Acquisition, interference and immunomodulation**

**Acquisition.** Vertebrate hosts are infected with rickettsiae via direct inoculation by a feeding tick or mite or by scratching infected louse or flea faeces into their skin. Ticks with hard exoskeletal chitin are vectors and reservoirs for SFG rickettsiae. The principal vectors of RMSF in the United States are *Dermacentor variabilis* and *Dermacentor andersoni* (TABLE 1), which are most active during the late spring and summer, when RMSF peaks. Epidemic typhus (BOX 2) caused by *R. prowazekii* is associated with cold weather and lack of hygiene<sup>22</sup>, and has re-emerged in louse-infested populations. Humans in endemic regions, as well as the eastern flying squirrel *Glaucomys volans volans*<sup>20,39</sup>, and its flea and louse in the United States, and ticks in Mexico and Africa are the known reservoirs of *R. prowazekii*<sup>40,41</sup>.

**Interference.** Infection of a tick with one SFG rickettsial species seems to interfere with infection by a second SFG rickettsial species. It was suggested that rickettsial infection of tick ovaries might alter the molecular-expression profiles of the oocytes and cause interference or blocking of the second infection<sup>42,43</sup>. This process of rickettsial ‘interference’ might affect the frequency and distribution of different pathogenic rickettsiae, and could explain the limited distribution of virulent *R. rickettsii* in the eastern part of the Bitterroot Valley, Montana, USA, where they infect less than 1% of wood ticks<sup>42,44</sup>. The low infection rate of *R. rickettsii* is attributed to the high infection rate of female wood ticks (*D. andersoni*) in the eastern, but not western, Bitterroot Valley with non-virulent rickettsiae, particularly *R. peacockii* (70% in the eastern compared with 4% in the western side of Bitterroot Valley)<sup>19,44</sup>. In most

Table 1 | Rickettsial diseases in humans

Disease	Organism	Arthropod vector	Life cycle	Geographic area	Eschar	Rash	Regional lymphadenopathy	Symptoms or fever	Mortality rate*
<b>Tick-transmitted spotted fevers</b>									
Rocky Mountain spotted fever	<i>Rickettsia rickettsii</i>	<i>Dermacentor variabilis</i> , <i>Dermacentor andersoni</i> , <i>Rhipicephalus sanguineus</i> , <i>Amblyomma cajennense</i> and <i>Amblyomma aureolatum</i>	Transovarian in ticks and rodent ticks	Western hemisphere	Rare	Yes	No	Yes	High
Boutonneuse fever	<i>Rickettsia conorii</i>	<i>R. sanguineus</i> and <i>Rhipicephalus pumilio</i>	Transovarian in ticks	Southern Europe, Africa and southern Asia	Frequent	Maculopapular	No	Yes	Mild to moderate
African tick-bite fever	<i>Rickettsia africae</i>	<i>Amblyomma hebraeum</i> and <i>Amblyomma variegatum</i>	Transovarian in ticks	Africa and the West Indies	Frequent and often multiple	Papular or vesicular; often sparse or absent	Yes	Yes	None reported
Maculatum disease	<i>Rickettsia parkeri</i>	<i>Amblyomma maculatum</i> and <i>Amblyomma triste</i>	Ticks	Western hemisphere	Yes	Often	Yes	Yes	None reported
<b>Flea-transmitted diseases</b>									
Flea-borne spotted fever	<i>Rickettsia felis</i>	<i>Ctenocephalides felis felis</i>	Transovarian in the cat flea	Worldwide	Sometimes	Sometimes	No	Yes	None reported
Murine typhus	<i>Rickettsia typhi</i>	<i>Xenopsylla cheopis</i> and <i>Ctenocephalides felis</i>	Rat-flea for <i>X. cheopis</i> and Opossumflea for <i>C. felis</i>	Worldwide	No	Yes	No	Yes	Low
<b>Louse-transmitted disease</b>									
Epidemic typhus	<i>Rickettsia prowazekii</i>	<i>Pediculus humanus humanus</i>	Human louse	Worldwide	No	Yes	No	Yes	High
Epidemic typhus	<i>R. prowazekii</i>	Fleas and lice of flying squirrels and <i>Glaucomys volans volans</i>	Flying-squirrel flea and louse ectoparasite	United States	No	Yes	No	Yes	Low
<b>Mite-transmitted diseases</b>									
Rickettsialpox	<i>Rickettsia akari</i>	<i>Liponyssoides sanguinus</i>	Transovarian in mites	Worldwide	Yes	Yes	Yes	Yes	None reported

\*High mortality is >15%; moderate mortality is 7–15%; mild-to-moderate mortality is 2–7% and low mortality is ≤1%.

**Superinfection**

Infection by a microorganism of a cell that is already infected by another microorganism.

**Transovarial**

Passage of parasites or infective agents from the maternal body to eggs within the ovaries and subsequently to the larvae that hatch from the eggs.

geographic locations, fewer than 0.1% of *Dermacentor* spp. ticks carry *R. rickettsii*<sup>45</sup>. These data correspond to the focality of RMSF in the west side of the valley, where most human cases result from exposure to west-side ticks (*D. andersoni*). Unlike pathogenic *R. rickettsii*, which is lethal for ticks<sup>46</sup> and highly virulent in guinea pigs<sup>47</sup>, infection with *R. peacockii* does not cause a reduction in tick viability, and might even be beneficial for tick hosts by antagonizing superinfection of ovarian tissues by *R. rickettsii*.

Ticks acquire SFG rickettsial species through transovarial transmission (adult female to egg) and trans-stadial

passage (egg to larva to nymph to adult), and by horizontal acquisition during feeding on a rickettsemic host. Most SFG rickettsiae are probably maintained in nature by all these mechanisms (FIG. 1). Therefore, the adverse effect of virulent *R. rickettsii* on the viability of adult ticks and maintenance of *Rickettsia* spp. in nature are probably balanced by the feeding of susceptible ticks on a rickettsemic host, which functions as an amplifying reservoir for rickettsiae. In fact, it has been shown that despite the high mortality of experimentally infected ticks, many larvae that acquire rickettsiae during feeding survive and are capable of transmitting the infection as

Box 1 | **Rickettsial taxonomy**

Despite the major advances in serotyping and molecular genotyping of rickettsial isolates from defined geographic locations, *Rickettsia* taxonomy is still an evolving field. Novel *Rickettsia* isolates have been described in recent years, with the overenthusiastic designation of many new species, which vary much less from one another than the species of other bacterial genera<sup>107</sup>. The issue is not whether the isolates can be distinguished from one another, but rather whether the differences merit designation at the taxonomic level of species or even subspecies. Historically, different species of prokaryotic pathogens were defined based on the diseases that they caused, regardless of other ecological or evolutionary considerations. However, the clinical manifestations of most rickettsioses are neither specific to a particular agent nor to a geographic distribution. Thus, a consensus of taxonomic criteria has yet to be achieved for *Rickettsia*. A proposal to adopt the genetic-diversity limits of previously named *Rickettsia* species for several convenient, but not uniformly appropriate, genes is an approach that has been specifically rejected by experts in prokaryotic taxonomy<sup>108</sup>. In our opinion, if the classification of *Rickettsia* were congruent with other intracellular bacteria, many of the current species names would be designated as subspecies and scientists would recognize important new isolates as distinct strains without needing a new species name.

nymphs. This suggests that nymphs are a crucial link for *R. rickettsii* maintenance and transmission between vertebrates. Importantly, colonies of *R. rickettsii*-infected ticks have been observed to maintain the infection without overt deleterious effects for several generations. The pathological effect of rickettsiae on ticks would explain the occurrence of RMSF in endemic regions, despite the low prevalence of naturally infected adult ticks<sup>45,46</sup>.

**Immune modulation.** Studies of the virulence of rickettsiae within their tick vector revealed that feeding ticks or incubating them at 37°C for 24–48 hours before their inoculation onto non-immune guinea pigs results in severe disease, compared with asymptomatic infection following inoculation of guinea pigs with infected ticks that were maintained at 4°C or starved for a prolonged period<sup>48</sup>. This observation, described as the reactivation phenomenon by Parker and Spencer<sup>44</sup>, refers to changes in the virulence of rickettsiae that are linked to the physiological status of the ticks.

As an immune evasion or modulation mechanism that allows the ticks to feed for several days or weeks, ticks inoculate their saliva with anti-haemostatic components that are crucial for the enhancement of blood feeding and salivary immunomodulatory components that enhance pathogen transmission and prevent the host from rejecting the ticks<sup>49–53</sup>. For example, the saliva of ticks inhibits neutrophil function<sup>52</sup>, interferes with the complement system<sup>49–51</sup>, natural killer (NK) cell and macrophage activity<sup>54</sup>, decreases the production of cytokines, such as interleukin-12 (IL-12) and interferon- $\gamma$  (IFN- $\gamma$ ), and decreases T-cell proliferation<sup>55,56</sup>. Tick-infested mice do not develop resistance to further infestations with *Rhipicephalus sanguineus*, and the immune response in infested mice exhibits a T helper 2 (T<sub>H</sub>2)-type pattern<sup>56,57</sup>. Tick saliva might influence T-cell-effector functions through its initial interaction with professional antigen-presenting cells, namely DCs<sup>58</sup>. Such initial interactions can subsequently influence the differentiation towards either a T<sub>H</sub>2-cell phenotype (an ineffective acquired immune response against intracellular pathogens such as *Rickettsia* spp.) or an immunosuppressive phenotype<sup>58,59</sup>. Indeed, the addition of tick saliva to bone-marrow-derived DCs inhibits their maturation by decreasing the expression of co-stimulatory (CD40, CD80 and CD86)

and adhesion (CD54) molecules<sup>58,59</sup>. Furthermore, the maturation of DCs that is stimulated by lipopolysaccharide in the presence of tick saliva results in reduced expression of co-stimulatory molecules and reduced production of IL-12, but not immunosuppressive IL-10. More importantly, DCs cultured with tick saliva are inefficient in the induction and activation of antigen-specific, cytokine-producing T cells<sup>58,59</sup>. As discussed below, fully mature DCs are crucial for induction of an effective T<sub>H</sub>1 response against *Rickettsia* spp. Therefore, it is possible that suppression of DC maturation by tick saliva during the initial stages of rickettsial infection could interfere with their co-stimulatory and antigen-presentation functions. Such suppression of DC maturation would adversely influence the acquired immune response against tick-transmitted *Rickettsia* spp., thereby leading to increased host susceptibility to severe and fatal rickettsial disease. However, because the tick host is an important component in the life cycle of rickettsiae, further studies are required to address important questions related to vector biology and disease pathogenesis, such as whether tick saliva enhances *Rickettsia* spp. infectivity during natural transmission and whether pre-exposure to saliva from uninfected ticks that generates immunity to saliva protects the vertebrate host, particularly in endemic areas, from natural tick-transmitted rickettsial infection. If immunity to salivary components can

Box 2 | **Epidemic typhus**

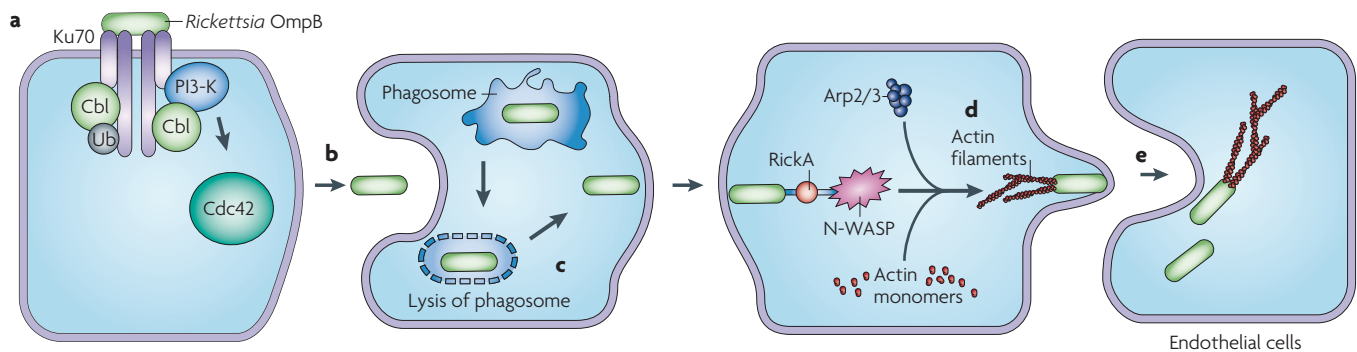
Epidemic typhus determined the outcome of European wars from the sixteenth century to the twentieth century. In Russia, during the First World War, the revolution and its aftermath, 30 million people suffered from typhus fever and 3 million of them died. The first description of typhus originated from the siege of Naples in 1528, but the role of the human body louse as a vector was not recognized until 1909, for which Charles Nicolle was awarded a Nobel Prize. Among the enigmas of typhus, two of the most intriguing questions are: in what cells and organs of the body does latent *Rickettsia prowazekii* reside during the period after recovery from the acute infection; and what factors and mechanisms are responsible for the reactivation of infection that leads to rickettsemia and potential louse-borne spread of another epidemic?

**Trans-stadial**

Passage of a microorganism from one developmental stage (stadium) of the host to a subsequent stage (or stages).

**Haemostatic**

Stops blood flow.



**Figure 2 | Host cell interactions of rickettsiae.** **a** | Spotted-fever-group rickettsiae attach to Ku70 on the surface of human target cells (the endothelium) via outer-membrane-protein B (OmpB) and to an unknown receptor via outer-membrane-protein A. **b** | Cbl ubiquitinates Ku70 (REF. 61), and signal-transduction events that involve Cdc42, protein tyrosine kinase, phosphatidylinositol 3'-kinase (PI3-K) and Src-family kinases activate the Arp 2/3 complex to induce cytoskeletal actin to phagocytose the rickettsia<sup>62</sup>. **c** | Membranolytic phospholipase D and haemolysin C mediate rickettsial phagosomal escape<sup>85</sup>. **d** | RickA-stimulated activation of Arp2/3-mediated polymerization of host actin propels the bacterium through the cytosol and into filopodia. **e** | Rickettsiae are then either released from filopodia extracellularly or spread into the adjacent cell<sup>84–88</sup>. Cbl, family of ubiquitin ligases; N-WASP, neural Wiskott–Aldrich syndrome protein; Ub, ubiquitin.

protect against natural rickettsial infection, an effective anti-rickettsial vaccine could be designed that contains tick salivary proteins that act as an adjuvant with specific rickettsial antigens.

#### Rickettsia–endothelial cell interactions

**Rickettsial entry.** *R. conorii* OmpB binds specifically to Ku70 (FIG. 2), a component of the DNA-dependent protein kinase<sup>60</sup>. The binding and recruitment of Ku70 to the plasma membrane are important events in the entry of *R. conorii* into non-phagocytic mammalian cells<sup>60</sup>. Although nuclear Ku70 is translocated to the cytoplasm and plasma membrane, where it inhibits apoptosis and mediates homologous and heterologous cell adhesion and fibronectin binding, it has been proposed that the presence of Ku70 within lipid rafts might have an important role in the signal transduction that leads to induced phagocytosis. The role of cholesterol as an essential component of the membrane receptor that binds to *R. prowazekii* was described previously<sup>61</sup>. Similar to other intracellular pathogens, such as *Listeria monocytogenes*, the entry of *R. conorii* into non-phagocytic cells is dependent on membrane cholesterol. Ku70 is present within lipid microdomains that are enriched in lipid-raft components. The association of Ku70 with lipid microdomains and its binding to *R. conorii* suggest that Ku70 has an important role in cholesterol-dependent bacterial entry<sup>60</sup>. Although the exact mechanism by which Ku70 supports the entry of *R. conorii* into non-phagocytic cells remains unclear, the binding of *R. conorii* OmpB to Ku70 might activate membrane Ku70, which is postulated to lead to the activation of a cascade of signalling events, including the small GTPase, Cdc42, phosphoinositidyl-3-kinase, src-family tyrosine kinases and the tyrosine phosphorylation of focal adhesion kinase<sup>62</sup>. These signalling events are known to be strongly associated with  $\beta$ 1-integrin activation and bacterial entry<sup>63</sup> (FIG. 2). Similar to the entry of *L. monocytogenes* into its host cells, *R. conorii* infection stimulates the ubiquitination

of Ku70. In addition, the ubiquitin ligase c-Cbl is recruited to *R. conorii*-entry foci, and downregulation of endogenous c-Cbl blocks bacterial invasion and Ku70 ubiquitination<sup>60,62</sup>. The binding of Ku70 to OmpB and the role of Ku70 in bacterial entry into host cells correlate with the decreased expression of OmpB that is associated with reduced virulence of *R. rickettsii* str. Iowa<sup>64</sup> and the observation that anti-OmpB antibodies protect animals from an otherwise lethal challenge of *R. conorii*<sup>65–67</sup>. However, it is possible that SFG rickettsial OmpA or other unidentified rickettsial outer-membrane proteins also mediate adhesion by binding to unknown receptors<sup>68</sup>.

**Rickettsial diseases and endothelial pathogenesis.** Most of the clinical characteristics of rickettsial diseases are attributed to disseminated infection of the endothelium, where they grow and stimulate oxidative stress, thereby causing injury to the endothelial cells. Severe morbidity and mortality of RMSF are due to effects such as cerebral oedema and non-cardiogenic pulmonary oedema. The most prominent pathophysiological effects of rickettsial infection of endothelial cells include: an increase in vascular permeability; generalized vascular inflammation; oedema; increased leukocyte–endothelium interactions; and release of powerful vasoactive mediators that promote coagulation and pro-inflammatory cytokines<sup>69,70</sup>. Evidence that supports a pro-coagulant and pro-inflammatory phenotype of the host response is provided by studies of cultured endothelial cells in which rickettsial infection causes increased expression of tissue factor, thrombomodulin plasminogen-activator inhibitor 1, IL-1, IL-6, IL-8 and E-selectin<sup>70–73</sup>. Increased plasma levels of von Willebrand factor that are associated with increased levels of inflammatory cytokines, such as IL-6, have also been detected in patients with African tick-bite fever and Mediterranean spotted fever<sup>69,74</sup>. Prostaglandins and leukotrienes are crucial vasoactive modulators of vascular tone and permeability that are potential mediators

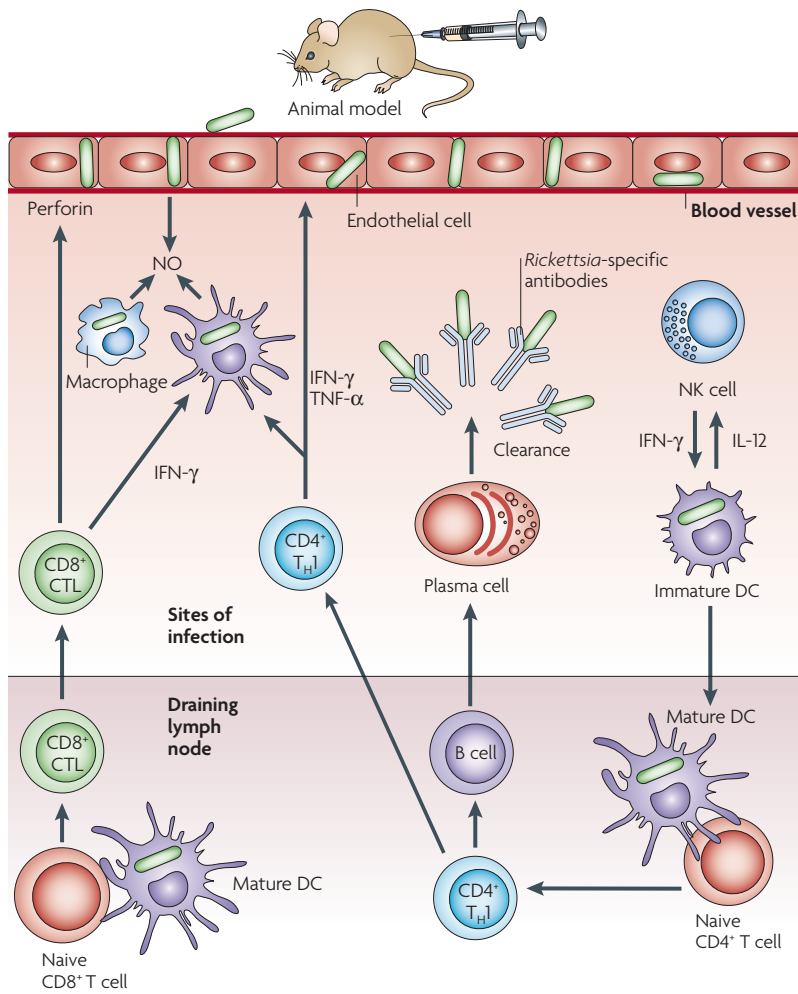
**Vasoactive**  
Causes constriction or dilation of blood vessels.

of microvascular injury and vasculitis in rickettsial infection<sup>75,76</sup>. These vasoactive substances are generally generated by an inducible isoenzyme cyclooxygenase (COX)<sup>75</sup>. Transcriptional activation of host endothelial cells in response to stimulation with *R. rickettsii* or *R. conorii* involves rapid regulation of COX2 expression and inhibition of COX2 activity during infection, which leads to decreased levels of secreted prostaglandins. As a regulatory mechanism that prevents the development of extensive vascular injury, endothelial cells that are infected with *R. rickettsii* produce haem oxygenase, an antioxidant, anti-inflammatory and vasoprotective enzyme that controls COX2 activity<sup>77</sup>. The production of this antioxidant mechanism by *R. rickettsii*-infected cells seems to be dependent on several factors, including: dose and kinetics of rickettsial infection; viability of the host cells; *de novo* protein synthesis by host cells; adhesion and entry of *Rickettsia* spp. to the host cell membrane; rickettsial replication; and viability<sup>77</sup>. Viability is probably influenced by the host immune status, as well as whether patients are treated with doxycycline at an early stage of infection. Nevertheless, the balance between the production of vasoprotective, anti-inflammatory and antioxidant haem oxygenase and the generation of vasoactive substances by COX2 in *Rickettsia*-infected endothelial cells might determine the outcome of rickettsial infection and thus the susceptibility or resistance to severe, and sometimes fatal, disease.

In an *in vitro* model of human microvascular endothelium, *R. rickettsii* causes early-dose-dependent increased vascular permeability. Furthermore, pro-inflammatory cytokines, such as IL-1 $\beta$  and tumour necrosis factor (TNF), which are probably produced *in vivo* by perivascular T lymphocytes and macrophages, cause a further enhancement of vascular permeability that is not dependent on nitric oxide production<sup>78</sup>. Increased permeability of endothelial monolayers that are infected with *R. rickettsii* in the presence of pro-inflammatory cytokines is associated with disruption of intercellular adherens junctions and redistribution of p120 and  $\beta$ -catenin proteins — these proteins attach the endothelial cells to the extracellular matrix and regulate the functional interaction of vascular endothelial (VE)-cadherin with the actin cytoskeleton — from the cell–cell junction by a nitric-oxide-independent mechanism. The cellular and molecular mechanism by which rickettsiae directly increase endothelial permeability is not yet clear. *R. rickettsii*-infected human-derived microvascular endothelial cells produce nitric oxide, which has been shown to play a part in increasing vascular permeability during rickettsial infection. However, blocking nitric oxide production does not influence endothelial-cell monolayer integrity despite an increase in the number of intracellular rickettsiae. These data suggest that other downstream or upstream intracellular molecules produced by endothelial cells at early stages after infection cause increased vascular permeability. Possible effectors could include reactive oxygen species or vascular endothelial growth factor, which have been associated with vascular dysfunction in endothelial-cell infections that are caused by other pathogens.

Rickettsiae inhibit endothelial-cell apoptosis by a mechanism that involves nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation<sup>79,80</sup>, an immune evasion strategy that enables rickettsiae to survive and replicate within the endothelium. NF- $\kappa$ B is a transcription factor that regulates many inflammatory genes that are involved in cytokine and chemokine production. The anti-apoptotic effect of rickettsiae on endothelial cells is linked to the pro-inflammatory phenotype of those cells. Lymphocyte adhesion to the endothelium is a crucial step for transmigration of lymphocytes to the areas of inflammation that are beneath the endothelium. The production of pro-inflammatory cytokines, such as IL-1 $\alpha$ , IL-6 and IL-8, by endothelial cells promotes the expression of endothelial-cell adhesion molecules, such as intercellular-adhesion molecule 1 and vascular-cell-adhesion molecule 1, which support the recruitment of T cells to the site of infection. Rickettsiae also enhance the expression of chemokine receptors, such as CXC-chemokine receptor 3, and the production of chemokines, such as CXC-chemokine ligand 9 (CXCL9; also known as MIG) and CXCL10 (also known as IP10), by infected endothelial cells<sup>81,82</sup> (FIG. 3). The peak of expression of these chemokines correlates with maximal T-cell infiltration (mainly CD8<sup>+</sup> T cells) at the site of infection<sup>82</sup>. However, it is not yet clear whether greater chemokine production and increased T-cell migration to the site of infection contribute more to the pathogenesis of severe disease or to protection against rickettsial infection.

**Actin-dependent movement of rickettsiae.** The entry of both SFG and typhus-group rickettsiae into mammalian cells is a dynamic process between metabolically active *Rickettsia* spp. and mammalian cells. Typhus-group rickettsiae enter the endothelial cells through adherence to undefined receptors and induced phagocytosis, followed by escape to the cytosol, which is mediated by phospholipase D and haemolysin C<sup>83</sup>. Replication of *R. prowazekii* inside host cells is followed by the rupture of infected cells and release of rickettsiae, which infect neighbouring cells. Spotted-fever rickettsiae can manipulate host cell signalling and endocytic pathways to their advantage, similar to *L. monocytogenes*<sup>84</sup>. SFG *Rickettsia* spp. harness the actin-polymerization machinery in the cytoplasm to facilitate intracellular and intercellular movement. After invasion of the host and entry into the cytosol, most SFG rickettsiae are propelled by polymerization of the host cell cytoskeletal protein actin at the surface of one pole of the rickettsiae. Unlike *Listeria* spp. and *Shigella* spp., which also escape into the cytosol, the actin tails of *Rickettsia* spp. are much less dense and comprise several distinct bundles of long, unbranched filaments that are similar to those present in filopodia<sup>85,86</sup>. The spread of *Rickettsia* spp. from cell to cell enables rickettsiae to avoid contact with immune-cells and antibodies, which could be a possible immune-evasion mechanism. A comparison of the complete genome sequence of *R. conorii* with that of typhus group *R. prowazekii*, which does not have actin tails and thus does not manifest an actin-based motility<sup>85</sup>, identified a 2 kb *R. conorii*-specific region that encodes a predicted protein of 517 amino acids, known as RickA<sup>86</sup>.



**Figure 3 | Model of protective immunity in rickettsial infection.** This hypothetical model is based on *in vitro* and *in vivo* studies in an animal model of mild disseminated spotted-fever rickettsiosis that was caused by sublethal rickettsial infection. Immature dendritic cells (DCs) that encounter rickettsiae in the peripheral tissues, such as skin and lung, undergo maturation and migrate to secondary lymphoid tissues (for example, draining lymph nodes), where they activate antigen-specific T lymphocytes. Rickettsial infection stimulates DC maturation (upregulation of the major histocompatibility complex and co-stimulatory molecules CD80, CD86 and CD40). Interferon- $\gamma$  (IFN- $\gamma$ ) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), which are produced by other cells of the innate immune system, such as natural killer (NK) cells or infected endothelial cells, could promote complete DC maturation, which is marked by the production of interleukin-12 (IL-12). IL-12 activates NK cells, which are crucial for the early clearance of rickettsiae. Mature *Rickettsia*-infected DCs enter lymph nodes through afferent lymphatic vessels, where they display antigens to naive antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells and provide co-stimulatory signals that activate antigen-specific T cells. Activated antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells proliferate and differentiate into antigen-specific effector-type-1 cells that produce IFN- $\gamma$  and TNF- $\alpha$ . Induction of T<sub>H</sub>1 cells is mediated, in part, by IL-12 production by DCs. In addition, antigen-specific B cells proliferate and differentiate into antibody-secreting cells. *Rickettsia*-specific antibodies have an important role in protection against re-infection. These differentiated B and T lymphocytes migrate back to the blood and then to the site of infection, where they mediate the clearance of rickettsiae. Production of chemokines, such as CXC-chemokine ligand 9 (CXCL9) and CXCL10, by infected endothelial cells enhances lymphocyte migration. Production of IFN- $\gamma$  and TNF- $\alpha$  by CD4<sup>+</sup> T<sub>H</sub>1 effectors and CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) activates infected target cells, such as DCs, macrophages and endothelial cells. Activated infected cells kill rickettsiae by producing intracellular rickettsicidal molecules; for example, nitric oxide. Further clearance of rickettsial infection requires cytotoxic antigen-specific CD8<sup>+</sup> T cells that eliminate *Rickettsia*-infected cells by a perforin-mediated mechanism.

RickA contains a central proline-rich domain and a carboxy-terminal WH2 (Wiskott–Aldrich syndrome protein (WASP) homology 2) domain<sup>87</sup>. WH2 binds to actin monomers, which is followed by binding to a region with homology to the central and acidic domain of WASP-family proteins, including an amphipathic helix that is predicted to bind the Arp2/3 complex<sup>87,88</sup> (FIG. 2). Activation of Arp2/3 then results in nucleation of actin polymerization and induction of the formation of a network of long, unbranched filaments in *Rickettsia* spp. actin tails<sup>87</sup>. RickA proteins from *R. conorii* and *R. rickettsii* have a single WH2 domain that is similar to WASP, whereas *R. montanensis* RickA has two WH2 domains that are similar to neural-WASP. The exact role of RickA in intracellular motility, cell-to-cell spread and virulence of SFG rickettsiae has not been completely determined owing to a lack of genetic tools. However, lack of motility and virulence in *R. peacockii*, an SFG rickettsia that has a naturally occurring transposon insertion in *ricketA*, indicates a possible role for RickA in the motility (and perhaps virulence) of SFG rickettsiae<sup>89</sup> (TABLE 2).

### Host response to infection

**Innate immunity.** Early resistance to infection is attributed to the production of IFN- $\gamma$  by NK cells and the resultant activation of infected target cells, principally endothelial cells, DCs and macrophages (FIG. 3). IFN- $\gamma$  and TNF are essential for primary defence against infection with *Rickettsia* spp., and mice that lack these cytokines develop an overwhelming infection and succumb to an ordinarily sublethal dose of rickettsiae<sup>90,91</sup>. Eschar lesions from patients with mild-to-moderate boutonneuse fever express high mRNA levels of TNF, IFN- $\gamma$ , IL-10, RANTES, indoleamine-2,3-dioxygenase (an enzyme that is involved in limiting rickettsial growth by tryptophan degradation) and inducible nitric oxide synthase (a source of microbicidal nitric oxide)<sup>92</sup>. Significantly high levels of intralésional IL-10 are inversely correlated with low levels of IFN- $\gamma$  and TNF. Skin lesions from patients with severe BF express higher levels of RANTES than those with mild or moderate disease<sup>92</sup>. However, it is not clear whether these cytokine and chemokine responses in patients with BF are simply a correlate of mild and severe disease or contribute to anti-rickettsial immunity and pathogenesis.

At the cellular level, cytokine-activated endothelial cells and macrophages are the principal mediators of the killing of rickettsiae, as they are also the major target cells. Although the mechanisms of bacterial killing by recruited lymphocytes and inflammatory cells *in vivo* are not completely defined, the induction of nitric oxide (FIG. 3), oxidative burst, production of hydrogen peroxide and/or tryptophan degradation by endothelial cells and macrophages contribute to pathogen clearance *in vitro*<sup>93</sup>.

**Interaction of rickettsiae with dendritic cells.** Although extensive studies have examined the acquired immune response against *Rickettsia* spp., there is still a large gap in our understanding of the initial interaction between tick-transmitted rickettsiae and host immune cells at the

Table 2 | Candidate rickettsial virulence genes

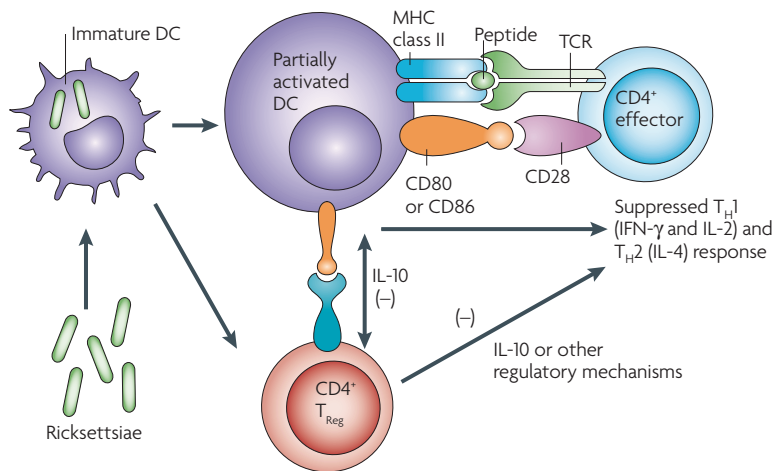
Rickettsial gene	Encoded product	Potential function
<i>pat1</i>	Patatin B1 precursor	Membranolytic phospholipase A host cell escape
<i>tlyA</i>	Haemolysin A	Membranolytic traversal of host cell membrane
<i>tlyC</i>	Haemolysin C	Membranolytic phagosomal escape
<i>pld</i> <sup>118</sup>	Phospholipase D	Membranolytic phagosomal escape
<i>invA</i> <sup>119,120</sup>	Dinucleoside polyphosphate hydrolase	Hydrolysis of toxic dinucleoside polyphosphates to ATP
<i>coxABC</i>	Cytochrome c oxidase	Aerobic respiration under optimal aerobic conditions
<i>cydAB</i>	Cytochrome d oxidase	Aerobic respiration under low-oxygen conditions
<i>sodB</i>	Superoxide dismutase	Neutralizes oxidative stress of reactive oxygen species
Lipopolysaccharide synthesis genes	Lipopolysaccharide	Endotoxin-mediated inflammation
<i>sca</i> genes <sup>121,122</sup>	Surface cell antigens, except for <i>sca4</i> , which is an intracellular protein	Autotransporter outer-membrane proteins
<i>ompA</i>	Outer-membrane protein A	Spotted-fever-group rickettsial attachment to host cell
<i>ompB</i>	Outer-membrane protein B	Rickettsial attachment to host cell
<i>virB4</i> , <i>virB6</i> , <i>virB7</i> , <i>virB8</i> , <i>virB9</i> , <i>virBro</i> , <i>virB11</i> and others <sup>123</sup>	Type IV secretion system	Transport of rickettsial proteins or DNA into host cytosol
<i>rickA</i> <sup>87,88</sup>	Actin-tail polymerization gene	Formation of actin tail and mediation of intracellular and intercellular rickettsial spread

site of inoculation (the dermis of the skin) and the effect of this interaction on the acquired immune response in secondary lymphoid organs. Transmission of rickettsiae through the dermis suggests that resident DCs might have an important role in innate and acquired immunity. *Rickettsia* spp. effectively infect bone-marrow-derived DCs (BMDCs). Compared with their immediate and exclusive cytoplasmic localization within endothelial cells, rickettsiae efficiently enter and localize in both phagosomes and the cytosol of BMDCs<sup>94</sup>. The dual intracellular localization of rickettsiae within BMDCs favours the access of rickettsial antigen to both major histocompatibility complex (MHC) class I and II pathways, thus promoting the activation of *Rickettsia*-specific CD8<sup>+</sup> and CD4<sup>+</sup> T cells, respectively. Rickettsiae have been shown to induce the maturation of BMDCs, which results in the upregulation of CD40, CD80, CD86 and MHC-II and production of IL-2, IL-12p40 and IL-23 — IL-12p40 and IL-23 are prototypic T<sub>H</sub>1-promoting cytokines<sup>94</sup>. DCs efficiently present rickettsial antigens to T cells, as shown by the ability of *R. conorii*-infected DCs to effectively activate immune CD4<sup>+</sup> and CD8<sup>+</sup> T cells that were derived from *R. conorii*-infected mice and stimulate the T<sub>H</sub>1 response owing to the production of substantial quantities of IFN- $\gamma$ <sup>94,95</sup> (FIG. 3). Interestingly, *Rickettsia*-infected DCs activate naive CD8<sup>+</sup> T lymphocytes *in vitro* in the absence of CD4<sup>+</sup> T-cell help<sup>95</sup> (FIG. 3) together with the induction of DC maturation by rickettsiae. Thus, *Rickettsia*-infected DCs provide signal 1 (TCR stimulation) and signal 2 (IL-2 and T<sub>H</sub>1 cytokines) to naive T cells, which accounts for their ability to induce activation and differentiation of T cells (FIG. 3). Interestingly, the transfer of rickettsiae-stimulated DCs protects mice from lethal

rickettsial challenge by limiting rickettsial proliferation *in vivo*, whereas partial protection is observed in mice that receive LPS-stimulated DCs<sup>95</sup>. Protection against *R. conorii* following the transfer of DCs is associated with the production of antigen-specific IFN- $\gamma$  by T cells and the expansion of NK cells.

The role of DCs in resistance and susceptibility to fatal SFG rickettsiosis has been investigated in susceptible C3H/HeN mice and highly resistant C57BL/6 mice, which remain healthy after a dose of *R. conorii* that kills 100% of C3H/HeN mice<sup>94</sup>. Early effective bacterial elimination in C57BL/6 mice suggests that the crucial immune effectors that determine the resistance or susceptibility to fatal disease are determined by innate immune cells. BMDCs of resistant mice internalize greater quantities of rickettsiae, kill rickettsiae more effectively, express higher levels of MHC-II, produce more IL-12p40 and are more potent in priming naive IFN- $\gamma$  to produce CD4<sup>+</sup> T<sub>H</sub>1 cells. By contrast, BMDCs of susceptible mice fail to induce CD4<sup>+</sup> T-cell activation and differentiation into the T<sub>H</sub>1 or T<sub>H</sub>2 phenotype in an *in vitro* co-culture system. The CD4<sup>+</sup> T-cell suppressive function of *Rickettsia*-infected BMDCs from susceptible mice is associated with the expansion of forkhead box P3 (Foxp3)<sup>+</sup>CD4<sup>+</sup> T-regulatory cells in an *in vitro* co-culture system<sup>94</sup> (FIG. 4). These data suggest that rickettsiae stimulate DCs to develop a protective T<sub>H</sub>1 response in resistant hosts but induce suppressive adaptive immunity in susceptible hosts, which is probably mediated by infection-induced T-regulatory cells<sup>94</sup>. Our preliminary data suggest that a unique subset of inducible T-regulatory cells play a crucial part in mediating fatal disease in an animal model of disseminated, lethal SFG rickettsiosis. We are currently undertaking





**Figure 4 | Early interactions between rickettsiae and dendritic cells (DCs) and acquired immunity.** Rickettsial infection stimulates partial DC maturation, as shown by the upregulation of major histocompatibility complex (MHC) and co-stimulatory molecules. Bone-marrow-derived DCs from susceptible murine hosts that are infected *in vitro* with *Rickettsia* fail to stimulate *Rickettsia*-specific CD4<sup>+</sup> T-cell differentiation into T<sub>H</sub>1 or T<sub>H</sub>2 cells that produce interferon- $\gamma$  (IFN- $\gamma$ ) or interleukin (IL)-4, respectively. Suppressed effects on CD4<sup>+</sup> T-cell responses are associated with IL-10 production by DCs as well as increased numbers of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T-regulatory (T<sub>Reg</sub>) cells that also secrete IL-10. This process suggests that immune suppression is a mechanism that contributes to the development of progressive, fatal spotted-fever rickettsiosis.

several approaches to identify the immune regulatory mechanism (or mechanisms) by which T-regulatory cells mediate immune suppression in fatal SFG rickettsiosis. Taken together, studies of the early interaction between SFG *Rickettsia* spp. and DCs not only enhance our understanding of the cellular immune mechanisms that account for mild and severe rickettsial disease, but can also be used to help develop effective and optimal immunotherapeutic approaches that both enhance protective immunity and abrogate the immunosuppressive mechanisms that are responsible for fatal spotted-fever rickettsial diseases.

NK cells provide an early source of IFN- $\gamma$  in immune responses to species of *Rickettsia* (FIG. 3), with a significant expansion of NK cells occurring 2 days after rickettsial infection. Depletion of NK cells enhances the susceptibility of mice to *R. conorii* infection<sup>96</sup>. Enhanced NK activity during rickettsial infection is associated with high serum levels of IL-12 and IFN- $\gamma$  (FIG. 3), which suggests that NK cells have a role in mediating the protective T<sub>H</sub>1 response in anti-rickettsial immunity<sup>96</sup>. Current studies in our laboratory are directed towards analysing early DC–NK crosstalk *in vitro* and *in vivo* following sublethal and lethal rickettsial disease in the presence or absence of tick saliva. These studies could provide essential information that is necessary for molecular identification of rickettsial ligands or vaccine candidates that stimulate innate immune cells and protective specific immunity against *Rickettsia* spp.

**Acquired immune response against *Rickettsia* spp.** Animal models of disseminated rickettsiosis caused by intravenous *R. conorii* or *R. australis* infection in C3H/HeN

or C57BL/6 mice, respectively, have revealed the crucial roles of T- and B-cell responses against rickettsiae (FIG. 3). Experiments with mice that were depleted of, or deficient in, particular subsets of cells or cytokines<sup>90,91,97</sup> demonstrated that a deficiency of CD8<sup>+</sup> T cells, IFN- $\gamma$  or TNF markedly increases the susceptibility of mice to infection with *R. conorii*. CD8<sup>+</sup> T cells mediate their effector function against *Rickettsia* spp. through both IFN- $\gamma$  production and cytotoxic killing of infected target cells. In humans, perivascular infiltrates of CD8<sup>+</sup> and CD4<sup>+</sup> T cells are present in skin lesions of patients with SFG rickettsiosis<sup>98</sup>. Depletion and adoptive-transfer experiments indicate that CD4<sup>+</sup> T<sub>H</sub>1 cells mediate protective immunity against *Rickettsia* spp. through several pathways: the production of IFN- $\gamma$  and TNF, which activate microbicidal activities of infected target cells; *Rickettsia*-specific CD4<sup>+</sup> T-cell help to antigen-specific B cells and therefore antibody production, an important host defence against re-infection; and CD4<sup>+</sup> T-cell help for the induction of cytotoxic CD8<sup>+</sup> T cells, an indispensable component of protective cellular immunity against species of *Rickettsia* (FIG. 3). Polyclonal antibodies to *R. conorii* and monoclonal antibodies to OmpA and OmpB provide effective passive immunity in infected severe combined immunodeficient mice<sup>66</sup>. This antibody-mediated protection is Fc-dependent, suggesting that antibodies have a role in opsonization and phagocytosis of extracellular rickettsiae. Interestingly, polyclonal antibodies and an anti-OmpB monoclonal antibody inhibit the escape of *R. conorii* from the phagosome of endothelial cells or macrophages, which results in phagolysosomal killing through nitric oxide, reactive oxygen intermediates and L-tryptophan starvation<sup>99</sup>. These observations suggest that anti-rickettsial antibodies, including anti-OmpB, neutralize an antigenic determinant on a rickettsial protein that plays a part in the escape of rickettsiae to the cytosol. Similar to SFG rickettsiae, the immune reactivity and immunogenicity of *R. typhi* and *R. prowazekii* OmpB have been characterized in an attempt to identify synthetic antigens that can be used in specific diagnostic immunoassays or candidate vaccines. These studies show that *R. typhi* and *R. prowazekii* OmpB are indeed immune reactive and immunogenic as measured by the ability of purified OmpB from *R. typhi* or *R. prowazekii* (Madrid E and Breinl strains, respectively) to induce strong proliferation and IFN- $\gamma$  production *in vitro* through human CD4<sup>+</sup> T-cell clones from individuals who are seropositive for *R. typhi*<sup>100</sup> and the ability of purified OmpB of *R. typhi* to stimulate humoral and cell-mediated immune responses in guinea pigs and mice<sup>101,102</sup>. The antibody-binding sites and linear peptide epitopes on these proteins were characterized<sup>102</sup>. However, the limited numbers and weak immune reactivity of these linear peptide epitopes against patient sera suggest that they are not useful for diagnosis. Yet these studies do not exclude the possibility that conformational epitopes of OmpB or other outer-membrane or cytoplasmic proteins of SFG or typhus-group rickettsiae play an important part in protection. In support of this possibility is the recent finding that immunization with avirulent *R. rickettsii* str. Iowa that is

## Box 3 | Genomics

Rickettsiae have undergone dramatic genome reduction (1.1–1.3 Mb) by relying on the host for the synthesis of many amino acids and nucleotides<sup>109</sup>. The rickettsiae have a close evolutionary relationship with the ancestor of mitochondria<sup>110,111</sup>. The genomes of seven Rickettsiaceae have been sequenced and annotated<sup>23,24,111–113</sup>. The *Rickettsia bellii* and *Rickettsia canadensis* genomes exhibit little synteny with spotted-fever-group or typhus-group rickettsiae, which suggests that these species may have retained ancestral features that were lost from other lineages in the course of evolution<sup>4,23,24</sup>. Plasmid sequences have recently been identified in *Rickettsia felis*, *R. bellii*, *Rickettsia monacensis*, *Rickettsia amblyomii*, *Rickettsia helvetica*, *Rickettsia peacockii* and *Rickettsia massiliae*, some of which are integrated into the chromosome<sup>4,113–115</sup>. *Rickettsia* species contain many pseudogenes, which are segments of DNA that do not produce a functional protein<sup>23,116</sup>. For example, both *Rickettsia prowazekii* and *Rickettsia conorii* contain intact genes that mediate the formation of succinate dehydrogenase, nicotinamide adenine dinucleotide (NADH), cytochrome reductase and cytochrome oxidase<sup>23</sup>. However, these genes are absent from *Rickettsia typhi*. Similarly, both *R. prowazekii* and *R. conorii* contain the genes *coxABC* and *cydAB*, which encode a putative cytochrome *c* oxidase and cytochrome *d* ubiquinol oxidase, respectively<sup>23,111,112,117</sup> — monomeric proteins that are used for aerobic respiration under differing oxygen concentrations. However, it has been shown that *R. typhi* has only one terminal oxidase for aerobic respiration of the cytochrome *d* type, which is usually expressed during stress conditions, such as low-oxygen concentration<sup>23,117</sup>. The presence of these pseudogenes might explain the different clinical manifestations and disease severities that are caused by human infection with *R. typhi* compared with those caused by *R. prowazekii* and *R. conorii*.

deficient in OmpA and defective in the processing of OmpB from the 168 kDa precursor form to the 135 and 32 kDa forms of the protein protects 90% of guinea pigs against disease that is caused by virulent *R. rickettsii* str. Sheila Smith<sup>103</sup>. Interestingly, lack of protection in one of the vaccinated animals was associated with defective antibody production, which is consistent with studies which indicate that antibodies of an as-yet-undefined antigen specificity or isotype have a role in protection against *Rickettsia* spp. Interesting questions include whether T-cell mediated immunity is responsible in large part for protection in these animals and which immunodominant or subdominant rickettsial antigens or peptides are recognized by B and T cells in protected animals that are vaccinated with avirulent *R. rickettsii* str. Iowa. That OmpA, OmpB and the unrelated T-cell-responsive antigens are crucial stimulators of immunity has been clearly established<sup>104–106</sup>.

### Conclusions and future directions

Analysis of rickettsial genome sequences (BOX 3) and investigation of rickettsiae–host cell interactions have identified rickettsial adhesins, a host cell receptor, components of signal transduction that affect rickettsial entry and apparent mediators of phagosomal escape and manipulation of host cell function, such as the activation of NF- $\kappa$ B to inhibit apoptosis, actin-based motility and cell-to-cell spread. However, most of these studies were exploratory rather than mechanistic, which is confounded by the problem of *in vitro* studies in which an interaction between *Rickettsia* spp. and one particular host cell has often been studied without considering the complex host–microbial interaction that occurs *in vivo*. In addition, the lack of an effective genetic system for rickettsiae hinders

the identification of virulence genes and elucidation of their effect on the host. Thus, although rickettsial research has generated a wealth of data, we still lack crucial information that is directly relevant to human disease and can be effectively applied towards vaccines and immunotherapy.

Examples of the gaps in our knowledge include: the crucial rickettsial virulence factor (or factors) that mediates immune evasion or modulation and intracellular survival; the immunodominant and subdominant T-cell epitopes that mediate protective immunity against species of *Rickettsia*; and the functions of rickettsial genes that have been annotated without experimental analysis. The exact pathophysiological and immune mechanism (or mechanisms) that accounts for host resistance or susceptibility to virulent *Rickettsia* species, such as *R. rickettsii* and *R. conorii*, or accounts for mild disease following infection with less-virulent rickettsial species; the microbial or host-related regulatory mechanisms that abrogate or enhance tissue injury and progression of the disease; and the mechanisms that control the induction of these regulatory mechanisms also remain unknown. Furthermore, the effect of variables such as infectious dose, route of infection and host genetic susceptibility on host defence against *Rickettsia* species; the immunomodulatory effects of tick saliva at the site of inoculation; the course and route of rickettsial spread from the skin; the mechanism and importance of rickettsial-infection-associated immunosuppression; and the mechanism of perivascular emigration of immune CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes and macrophages at the site of microvascular infection require further elucidation.

More importantly, what is the relevance of these *in vitro* studies and *in vivo* animal studies to humans, taking into consideration important factors such as age, gender, genetic polymorphisms, immune status, pre-existing morbidity and risk factors, such as diabetes and alcoholism. Future progress is required to identify the complete set of rickettsial adhesins and host cell receptors, the importance of *Rickettsia*-induced oxidative stress on endothelial cells *in vivo* and other mechanisms of cell and tissue injury in rickettsial diseases. A reliable genetic system is needed for inactivation of rickettsial genes to attribute functions to putative virulence factors, including phospholipase D, haemolysin C and RickA (TABLE 2). The contributions of cytokines, cytotoxic T lymphocytes and T-regulatory cells to the pathogenesis of tissue injury in rickettsial infection also need to be further determined. To understand the mechanisms of the most important pathophysiological mechanisms in rickettsioses, the relative roles of oxidative stress, pro-inflammatory cytokines, vascular endothelial growth factor and other factors should be analysed. For vaccine development, it is important to identify the immunodominant rickettsial antigens that stimulate CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes to secrete protective cytokines, stimulate protective antibodies, in addition to anti-OmpA and anti-OmpB, and stimulate cytotoxic CD8<sup>+</sup> T lymphocytes that eliminate rickettsiae-infected endothelial cells.

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**Acknowledgements**

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**DATABASES**

Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomemap>  
[Listeria monocytogenes](#) | [Rickettsia africae](#) | [Rickettsia akari](#) | [Rickettsia bellii](#) | [Rickettsia canadensis](#) | [Rickettsia conorii](#) | [Rickettsia felis](#) | [Rickettsia prowazekii](#) | [Rickettsia rickettsii str. Iowa](#) | [Rickettsia rickettsii str. Sheila Smith](#) | [Rickettsia sibirica](#) | [Rickettsia slovaca](#) | [Rickettsia typhi](#)

**FURTHER INFORMATION**

CDC emergency preparedness and response: bioterrorism agents and diseases (by category): <http://www.bt.cdc.gov/agent/agentlist-category>

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