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Dysregulated Th1 Immune and Vascular Responses in Scrub Typhus Pathogenesis

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Abstract

Scrub typhus is an emerging, insect-transmitted disease caused by *Orientia tsutsugamushi,* a Gram- and LPS-negative bacterium that replicates freely within professional phagocytes and endothelial cells (EC). Scrub typhus is prevalent with high mortality rates, but information regarding its molecular pathogenesis, microbial virulence determinants, and key immune responses is limited. Improved animal models have recently developed that respectively resemble the pathological features of self-limiting or severe scrub typhus in humans. Strong activation of Th1 and CD8, but not Th2 and Treg, immune responses, accompanied by altered angiopoietin/Tie2-related regulation, are hallmarks of lethal infection in murine models. This review, based primarily on recent advances from clinical and experimental studies, highlights tissue- and EC-specific biomarkers that are indicative of immune dysregulation. The potential roles of neutrophils and damage-associated molecular pattern (DAMP) molecules at late stages of disease are discussed in the context of vascular leakage, pulmonary and renal injury, and scrub typhus pathogenesis.

Keywords

Orientia; Vascular responses; Scrub typhus; Pathogenesis

Scrub typhus is a zoonotic and life-threatening disease. More than one million new cases are diagnosed every year, mostly in a broad geographic region or “tsutsugamushi triangle” in Southeast Asia, with one-third of the world’s population at risk of infection [reviewed in (1, 2)]. Under-reporting and misdiagnosis in endemic regions and potential emerging of this infection in Africa and South America are major concerns for this neglected tropical disease (1–3). The disease is initiated by the bite of a larval *Leptotrombidium* mite or chigger, which transmits *Orientia tsutsugamushi* (formerly named *Rickettsia tsutsugamushi*), an obligate intracellular bacterium.

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Orientia comprises numerous strains of differing virulence, and approximately 50% and 25% of human infections are associated with infection with Karp- and Gilliam-related strains, respectively (4). After an incubation period of 7–14 days, some patients develop eschar, a unique and localized pathological skin lesion (5), followed by fever, rash, myalgia, headache, and non-specific flu-like symptoms. Pathological characteristics of fatal scrub typhus include diffuse interstitial pneumonia, hepatic lesions, meningoencephalitis, and coagulation disorders (6, 7). In some endemic areas, scrub typhus is a leading cause of non-malaria febrile illness (8, 9), but diagnosis of this infection is relatively difficult, due to initial non-specific clinical presentation and other challenges (10). The endothelial tropism of *Orientia* can lead to vasculitis that affects all organs, especially in severe cases (11–13). Patients can have fast and unpredictable progression and multi-organ failure, with up to 70% mortality, depending on bacterial strains involved and receipt of an accurate diagnosis (8, 14). Scrub typhus is treatable, as antibiotics like azithromycin and doxycycline are effective, if given at the onset of disease (6). The bacteriostatic nature of antibiotics in use, delayed diagnosis, persistent infection, and the lack of efficient vaccines are major issues (2, 15, 16). Adaptive immunity or cross-species protection in humans is short-lived and bacterial strain-related (17, 18), but the mechanism of waning immunity is unclear, which increases the challenge for developing effective vaccines for the control of scrub typhus.

This review focuses primarily on advances from recent *in vitro* studies in *Orientia*-infected target cells, clinical findings, and animal models of scrub typhus, highlighting tissue- and cell-specific biomarkers that are indicative of immune dysregulation. The potential mechanisms underlying alterations in immune responses and the possible roles of neutrophils and DAMPs during infection in murine models are discussed in the context of vascular leakage, pulmonary and renal injury, and severe scrub pathogenesis. Key players in scrub typhus pathogenesis and potential therapeutics are also discussed.

Bacterial Replication and Cellular Activation

Orientia has a small genome of ~1,500 genes with no pathogenicity islands or plasmids, but its biology remains poorly understood [reviewed in Refs.(19, 20)], because the organisms are osmotically sensitive and hard to propagate in cell cultures (21). For this genetically un-tractable bacterium, electron microscopic analyses or immunostaining have been the most important approaches, although labeling of bacteria with fluorescent probes has some success (22). *Orientia* entry into non-phagocytic cells can be divided into the adhesion and invasion stages, which involve bacterial 56-kDa type-specific antigens and surface cell antigens, as well as host fibronectin- and clathrin-dependent endocytic pathways (23–25). The bacterium can activate, but quickly escape from, cellular autophagy (26) and replicate freely but slowly in the cytosol (9 h for the dividing time). Bacteria can form perinuclear micro-colonies or exit from host cells via a budding-like process (20). The cytopathic effects of *Orientia* in human endothelial cells (EC) are milder than other endothelial-target pathogens such as spotted fever and typhus group of *Rickettsiae* (27) and hantaviruses [reviewed in (28)]. *Orientia* can destabilize the Golgi and ER and alter host secretory and apoptosis pathways via ankyrin repeat-containing protein-mediated mechanisms and other undefined processes (29–31).

Innate recognition of *Orientia* remains unclear (19, 32, 33). In contrast to other Gram-negative bacteria or *Rickettsia* spp. (LPS positive), *Orientia* lacks the classical lipopolysaccharide and peptidoglycan in its cell wall, although a peptidoglycan-like structure has been identified recently (34). TLR2 and nucleotide-binding oligomerization domain-containing protein 1 (NOD1)/IL-32-related pathway partially mediate *Orientia* recognition and inflammasome activation (32). In mouse macrophages, live *Orientia* (but not heat- or UV-inactivated bacteria) can trigger ASC inflammasome activation leading to IL-1β production, which is a critical innate immune response for effective host defense (35). Live *Orientia* are also highly competent in stimulating a M1-polarized phenotype in human monocytes/macrophages (36, 37). High levels of cytokines (TNF-α, IL-1β, IL-6, IL-12p40, IL-23p19), CXCR3-related chemokines (especially CXCL11), apoptosis-related genes, as well as type I IFN and its related genes, resemble host responses commonly elicited against viruses (36, 37). Unexpectedly, *Orientia* can propagate well in LPS-activated macrophages via NO-mediated pathways (38); however, the implication of these *in vitro* findings needs to be carefully evaluated in the context of in disease progress versus control (39).

The eschar biopsies have revealed that *Orientia* are mainly associated with dendritic cells (DCs, positive for CD1a/DCSIGN/S100/FXIIa and CD163), monocytes (positive for CD14/LSP-1/CD68), and vascular endothelium; these cells may contribute to local immunity and bacterial dissemination (12, 31). In cultured DCs or neutrophils, *Orientia* can rapidly escape from autophagy and replicate in DC cytosol (40), but neutrophils are not effective host cells (41). Infection stimulates DC activation, with increased expression of MHC II and costimulatory molecules (CD80, CD83, CD86, CD40), CCR7 (the receptor for CCL19 and CCL21), and inflammatory cytokines (IL-6, IL-12, TNF-α), as well as the potential of priming of IFN-γ-producing Th1 cells *in vitro* (40, 42). Compared with LPS-stimulated DCs, *Orientia*-infected DCs have much weaker levels of maturation, migration, and T cell-priming potential (40). But, *Orientia* is more potent to activate DCs than bacteria that reside in vacuolar compartments, including *Coxiella burnetii* (the agent of Q fever) and *Brucella abortus* (the agent of brucellosis) (43). Overall, *Orientia* infection stimulates sub-optimally activated, DC1-like phenotypes that preferentially induce Th1 cell activation *in vitro* (42).

Given the above findings, it may not be surprising that scrub typhus patients develop strong inflammatory immune responses, with unremarkable activation of Th2 cytokines (IL-4 and IL-13) (44) and peripheral Treg cells (45). IFN-γ-mediated deprivation of tryptophan partially contributes to *Orientia* growth restriction (46). However, massive T cell apoptosis, $CD4^+$ T lymphopenia, and neutrophilia in the acute phase of infection, followed by preferential increase of NK and $CD8^+$ T cells, may contribute to severe outcomes, as well as impaired immunological memory (45, 47). Disease severity is positively linked to the presence of eschar, the duration of skin rash, and the levels of serum cytokines (TNF-α, IL-8, IL-10) and circulating IFN-γ-producing NK cells (44, 48, 49). Disease severity is also positively linked to endothelial-related markers, including soluble L-selectin (50) and sCD163, VCAM-1 (51), and von Willebrand factor(52). Patients with low IL-10 and miR-155 levels are more vulnerable to a cytokine storm and severe pathology (53). Thrombocytopenia observed in severe scrub typhus patients (52, 54–56) and the failure of platelet count normalization, even after a general improvement of other markers of multi-organ dysfunction, imply for immune-mediated thrombocytopenia mechanism (57). These

clinical studies collectively indicate immune system dysregulation in scrub typhus patients, but the nature and the mechanisms of immune dysfunction have not been investigated. Since the lung is the major organ for *Orientia* infection in humans and in animal infection models (see below), as well as the site of hematopoietic progenitors and platelet formation (58), a better understanding of tissue- and cell-specific alterations is crucially important for scrub typhus biology and vaccine development.

Animal Models of Self-Limited Scrub Typhus

Host-*Orientia* interactions in animal models are understudied and require biosafety level 3 facilities. Non-human primates, especially *Macaca fascicularis* (cynomolgus macaques) and *Presbytis cristatus* (silvered leaf monkeys), have been used to study pathological changes and immunological responses to *Orientia* infections (5, 59). The Rhesus macaques intradermally-inoculated with *O. tsutsugamushi* Karp strain are excellent models for detailed analyses of initial target cells in the eschars, local immune responses and adaptive immunity, as well as for vaccine-based studies (60). The development of an *Orientia*-specific ELISpot assay for measuring IFN-γ-mediated cellular immunity is of relevance to monitor the control of scrub typhus in Rhesus macaques and humans (61) and in vaccine-based studies for protective immunity (60, 62). While non-human primates closely mimic human scrub typhus, they are not widely used in laboratories due to the high expense and other logistical issues.

Since 2012, several groups have established improved murine models for scrub typhus, which better mimic infection and pathology than the previously used, intraperitoneal-inoculation models that have several inherent drawbacks [reviewed in (28)]. The use of outbred mice fed with different mite lines or inoculated intradermally with different *Orientia* strains has the advantage of modeling natural infection, bacterial dissemination, and clinical features (63, 64); however, these models are technically challenging with a high possibility of variations in infection outcomes. The use of BALB/c and C57BL/6 inbred mice inoculated subcutaneously or intradermally with *Orientia* Karp strain can model self-limited scrub typhus in humans (39, 65). However, these skin-inoculated outbred or inbred mice fail to form eschar lesions. Nevertheless, inbred mouse models permit detailed immunological analyses, because of their defined kinetics of bacterial dissemination from injection sites to visceral organs, as well as the highest bacterial loads that are detected in the lungs, as in the case of infection in humans and non-human primates.

Following intradermal inoculation, C57BL/6 mice develop fever, marked hypothermia, moderate weight loss, and then gradually recover in three weeks. However, infectious bacteria can be detected in the kidneys and other organs for months, even though mice can maintain high titers of serum Ag-specific antibodies (>1:65,000) at 12 weeks post-infection (65). Analyses of serum and lung samples have revealed a Th1/Th2-mixed response profile, with marked elevations of inflammatory markers (IL-6, IL-12, IFN-γ, G-CSF, CCL5, CCL11, IL-1α/β, IL-2, TNF-α, GM-CSF), as well as modulatory cytokines (IL-9 and IL-13) (65). IL-9 is a pleiotropic cytokine mostly produced by a special subset of $CD4^+$ T (Th9) cells following TGF-β and IL-4 stimulation, and can modulate host immune responses and promote the resolution of inflammation (66, 67). Although IL-9 is involved in Th2-type

inflammatory responses (68, 69), its role in bacterial infections remains unclear. Regardless of the source or the role of IL-9, it seems that skin-inoculated mice can mount Th1/Th2-balanced immune responses at acute stages of infection (65). Since these infected mice also have transient thrombocytopenia, with signs of platelet alterations and anemia, this model can be used to examine subclinical and persistent infections that are often observed in humans (16).

Functional $CD8^+$ T cells are required for the restriction of *Orientia* growth at the acute and chronic stages of infection, as MHC $I^{-/-}$ and $CD8^{-/-}$ mice are highly susceptible to ordinarily sublethal doses of Karp stain and have lethal outcomes, regardless of the inoculation routes (70, 71). Depletion of $CD8^+$ T cells via neutralizing antibodies at the chronic stages can reactivate bacterial growth (70); however, antigen-primed $CD8^+$ cells can also mediate acute tissue injury (70, 71), implying a complex role of $CD8^+$ T cells at different stages of disease. Also, the role of NO-mediated responses in *Orientia* infection may be more complex than we previously appreciated, because 1) MHC $I^{-/-}$ and $CD8^{-/-}$ mice have significantly higher IFN-γ and iNOS levels, as well as bacterial loads, than their wild-type controls (70, 71); 2) NO-inducing conditions can enhance *Orientia* replication in macrophages in a bacterium strain-dependent manner (38, 72); and 3) high levels of iNOS in the lungs have been observed in autopsies of severely infected cases (73). At present, information on the roles of NK, NKT, and $CD4^+$ T cell subsets during infection in naïve or antigen-immunized mice is very limited (70, 74). There are no reports for the kinetics of expansion or retraction of *Orientia*-specific $CD8^+$ and $CD4^+$ T cell subsets in any murine models of scrub typhus. The use of cell-specific knockout and trackable transgenic mice will help define immune mechanisms for infection control.

Th1-Skewed, but Th2-suppressed, Responses during Severe Scrub Typhus

Following intravenous inoculation of *O. tsutsugamushi* Karp strain, C57BL/6 mice can have lethal or sublethal outcomes, depending on infection doses and host immune status (71, 75, 76). These mice develop disseminated endothelial infection with a spectrum of vasculitis, hemorrhage, interstitial pneumonia, or meningioencephalitis (75, 77), which resemble severe scrub typhus in humans. For lethal infection, mouse tissue bacteria reach peaks within a week and then come under control; however, body weight loss progresses, with 100% mortality in two weeks. PCR- and protein-based studies have revealed tissue-specific immunological features and argue for immunopathogenesis, rather than bacterial over-growth, in late-stage pathology and mortality (57). Firstly, Th1-skewed, but Th2-suppressed, immune profiles are consistently detected in the lung, liver, spleen, kidney, heart, and brain tissues during the course of infection (65, 78). As illustrated in Figure 1, the expression levels of inflammatory cytokines (IL-1β, IL-6, TNF-α, IFN-γ, CXCL9-11, etc.) and IL-10 are significantly elevated, but there are no signs for activation of, or even reduced baseline levels for, Th2 markers (IL-4, IL-13, and CXCL12) or regulatory molecules (IL-7, GATA3, and ROR-γt). At this stage, it remains unclear as to whether this polarized gene expression profile is the result of selective cellular recruitment or just an up-regulation of specific mediators by resident cells. Nevertheless, these trends are consistent with *in vitro* findings of M1- and DC1-type immune phenotypes (36, 37, 40, 42), implying a strong skew to Th1 immune responses during the course of disease progression (65, 76, 78).

Secondly, there are no overt signs of neutrophil recruitment/activation at early hours or days of infection. However, myeloperoxidase (MPO)-positive and $CD63^+Ly6G^+$ neutrophils become steadily evident around day 6 and are most extensive around day 10 in the lungs, liver, spleen, and heart (77, 78), which is consistent with the low recovery of $CD31^+$ and VE-cadherin$^+$ endothelial cells (EC) from infected lungs (manuscript in preparation). The loss of positive staining for EC-specific markers, accompanied with increased accumulation and activation of neutrophils, is one of the most important features for this lethal infection mode (Figure 1), and is highly relevant for the understanding of the pathogenesis of vasculitis in severe infections in humans (11–13).

These immunological changes are positively correlated with cellular apoptosis, vascular leakage, and multi-organ failure, as mice receiving lethal or sublethal doses succumb to infection in 2–3 weeks (77, 78). Although the intravenous inoculation models have intrinsic shortcomings as they bypass skin- and draining lymph node-mediated immune regulation, they are valuable tools for examining severe scrub typhus. Neutrophil-based studies are important for understanding *Orientia* biology (79), as it is still debatable as to whether neutrophils are target cells for *Orientia* infection in vivo. Neutrophil-based studies are clinically relevant, as neutrophilia and thrombocytopenia are positively associated with severe scrub typhus in humans (52, 54–56) and in experimental mice (65, 75). Also, neutrophil gelatinase-associated lipocalin can contribute to kidney injury in scrub typhus patients (80). Although neutrophils are short-lived, they can regulate EC and platelet functions and local immune responses via their released cytotoxic effector molecules, cytokines, and endogenous DAMP molecules.

DAMP Molecules in Scrub Typhus Pathogenesis

DAMP molecules can initiate and perpetuate an immune response under both noninfectious and infectious inflammatory conditions. Many molecules in the IL-1 super family, including IL-1α, IL-1β, IL-33, IL-36, and high mobility group box 1 (HMGB1), are considered as alarmins [as reviewed by (81, 82)]. IL-1 family alarmins share structural similarities and have dual functionality, depending on their secretion and activation process and intracellular location. For example, pro-IL-33 can be released from stressed and necrotic cells and processed by neutrophil elastase and cathepsin G. IL-33 binding to its IL-33R/ST2L receptor can regulate innate immunity and $CD8^+$ T-cell responses in a tissue-specific manner (83–85), or play deleterious roles in infectious diseases and models (86).

In *Orientia*-infected mice, IL-33 contributes to scrub typhus pathogenesis via exacerbating infection-triggered vascular activation and tissue damage (76). Organ-specific differences in IL-33 and IL-33R/ST2L expression profiles (Figure 1) suggest selective regulation of the IL-33/ST2L axis. Indeed, IL-$33^{-/-}$ mice show attenuated renal pathology after lethal infection, while rIL-33 treatment augments renal injury following a sublethal infection, via increasing CXCL1 and CXCL2, but altering anti-apoptotic gene BCL2 in the kidneys (76). At present, the tissue/cellular sources of IL-33 and the maturation process of released IL-33 are unclear. Given that infectious *Orientia* can persist in humans and mice for months or years (16, 65, 71), and that serum IL-33/ST2 levels serve as a surrogate of endothelial

dysfunction in human diseases (87), monitoring IL-33/ST2 levels during acute and persistent infection is desirable.

HMGB1 is another alarmin of potential interest, as serum HMGB1 levels are positively linked to the severity of human scrub typhus (52, 88). HMGB1 release in infection, inflammation, and cell death is well documented; its mature proteins can stimulate TLR expression and bind to the TLR4/RAGE receptor on the surface of activated EC and other cells [see reviews in (82, 89)]. In dengue virus infection, viral proteins translocated in the nucleus can trigger monocytes to release HMGB1; the binding of HMGB1 to TLR4/RAGE on the surface of EC contributes to the loss of vascular integrity and increased inflammatory responses (90). The role of HMGB1 in scrub typhus remains unknown (52, 88); however, *Orientia* infection selectively activates TLR2/4/9 expression in mouse brains during lethal and sublethal infection, especially at late stages of disease, which correlates with neuroinflammation and vascular stress (77). The breakdown of the blood-brain barrier (BBB) is featured by a marked loss of occludin^{+} tight junction staining, as well as an increased trafficking of T cells, in the cortex and cerebellum of infected mice (77). Since *Orientia* are sparse in the brains, it is speculated that inflammatory cytokines like IFN-γ and TNF-α can synergize with DAMP molecules to promote BBB opening (77). It will be important to examine the involvement of IL-1 family DAMP molecules, their receptors, and TLR2/4/9 at acute and convalescence stages via population-based genetic studies of scrub typhus patients (91) or pathway-focused studies in mouse models (33).

Vascular Responses and Dysfunction during severe Orientia Infection

The endothelium provides a crucial interface between tissues and circulating inflammatory cells. Sepsis and severe infections caused by viruses (dengue, influenza, West Nile viruses) and parasites (*Toxoplasma gondii*, *Plasmodium falciparum*) can lead to vascular dysfunction (92, 93). *Orientia*, like *Rickettsia,* is an endothelium-targeting pathogen, readily detectable within lectin^{+} ECs in the mouse brains (77) and other organs (75, 77). While molecular details of *Rickettsia*-EC interaction are becoming clear [see reviews in (94, 95)], there is a paucity of studies for *Orientia*-triggered vascular dysfunction. Recent studies of *Orientia* infection in primary HUVEC cultures and in C57BL/6 mice have revealed marked alterations in the levels of angiopoietins (the vascular growth factors important for embryonic and postnatal angiogenesis) and Tie2 (an endothelial tyrosine kinase receptor) (76, 77). Angiopoietin-1 (Ang1) is constitutively produced by vascular support cells, specialized pericytes in the kidney, and hepatic stellate cells in the liver; Ang1/Tie2 binding and Tie2 phosphorylation (pTie2) are critical for vessel maturation, adhesion, migration, and survival. Ang2, on the other hand, promotes cell death and disrupts vascularization, via competing for Tie2; increased Ang2 production from activated/damaged EC also promotes inflammation (93, 96). *Orientia* infection suppresses Ang1 expression, but greatly increases Ang2 expression and Ang1/Ang2 ratios, in HUVEC cultures and in mouse tissues (76–78). Impaired levels of Tie2 mRNA, as well as total Tie2 and pTie2 proteins, are found in *Orientia*-infected mouse brain and lungs at late stages of lethal infection (77). Dysregulated Ang/Tie2 axis is positively correlated with reduced tight junctions (occludin) and adherence junctions (VE-cadherin, Figure 1), contributing to scrub typhus pathogenesis.

Orientia-infected endothelium also contributes to immune regulation via other mechanisms, including cytokine production (IL-1β, IL-6, IL-8), leukocyte recruitment (ICAM-1, MCP-1/CCL2), and immune regulation (cellular apoptosis, receptor expression) (32, 97, 98). Infection dose/time-dependent expression of IL-33, soluble ST2, and membrane-bound ST2L in primary HUVEC cultures supports findings from *Orientia*-infected mice (76). Given the secretion of IL-33 by damaged and living cells and its important role in endothelial activation (99, 100), one can speculate that *Orientia*-primed endothelium can be a source of DAMPs, and the latter can further amplify vascular damage and inflammation (76).

Conclusions and Future Studies

Our understanding of the pathophysiology of scrub typhus is still limited, and there are sizable knowledge gaps as to how the host immune system responds to *O. tsutsugamushi* at the cellular or molecular level. It has become clear that strong Th1- and CD8-mediated immune responses are essential for restricting intracellular replication of bacteria. Relatively high levels of inflammatory cytokines and IFN-γ-inducible chemokines are common immunological features observed in *Orientia*-infected humans and in animal models. However, dysregulated immune responses, especially sustained activation of $CD8^+$ T cells and neutrophils after the control of bacterial growth, may contribute to acute tissue damage and host mortality. New evidence has emerged from clinical and experiential studies that suggests pathogenic roles of host cytokines (TNF-α, IL-8) and endogenous DAMPs (HMGB1, IL-33), neutrophil/platelet alterations, and their associations with endothelial dysregulation in severe scrub typhus. The identification of tissue/cell-specific gene regulation profiles and pathogenic biomarkers (Figure 1) will expedite mechanistic and translational research in the future.

Recent progress also raises several fundamental questions. What are the molecular mechanisms underlying Th1/CD8-skewed, but Th2/Treg-suppressed, immune responses during severe infection? What are the roles of neutrophils and platelets during *Orientia* infection? What is the critical window of time to restore immune balance? What is the therapeutic option to minimize vascular damage? Since waning adaptive immunity has been noticed in scrub typhus patients, a better understanding of effect and memory $CD4^+$ versus $CD8^+$ T cells is highly relevant for vaccine development. The establishment of biomarkers will help examine how a given DAMP and/or cytokine promotes bacterial clearance versus cellular injury. Monitoring serum levels of and genetic variations in pathogenic biomarkers will help assess disease progression and risk factors, as reported for other diseases (101–103). Such knowledge is essential for the development of effective vaccines or therapeutics for controlling scrub typhus.

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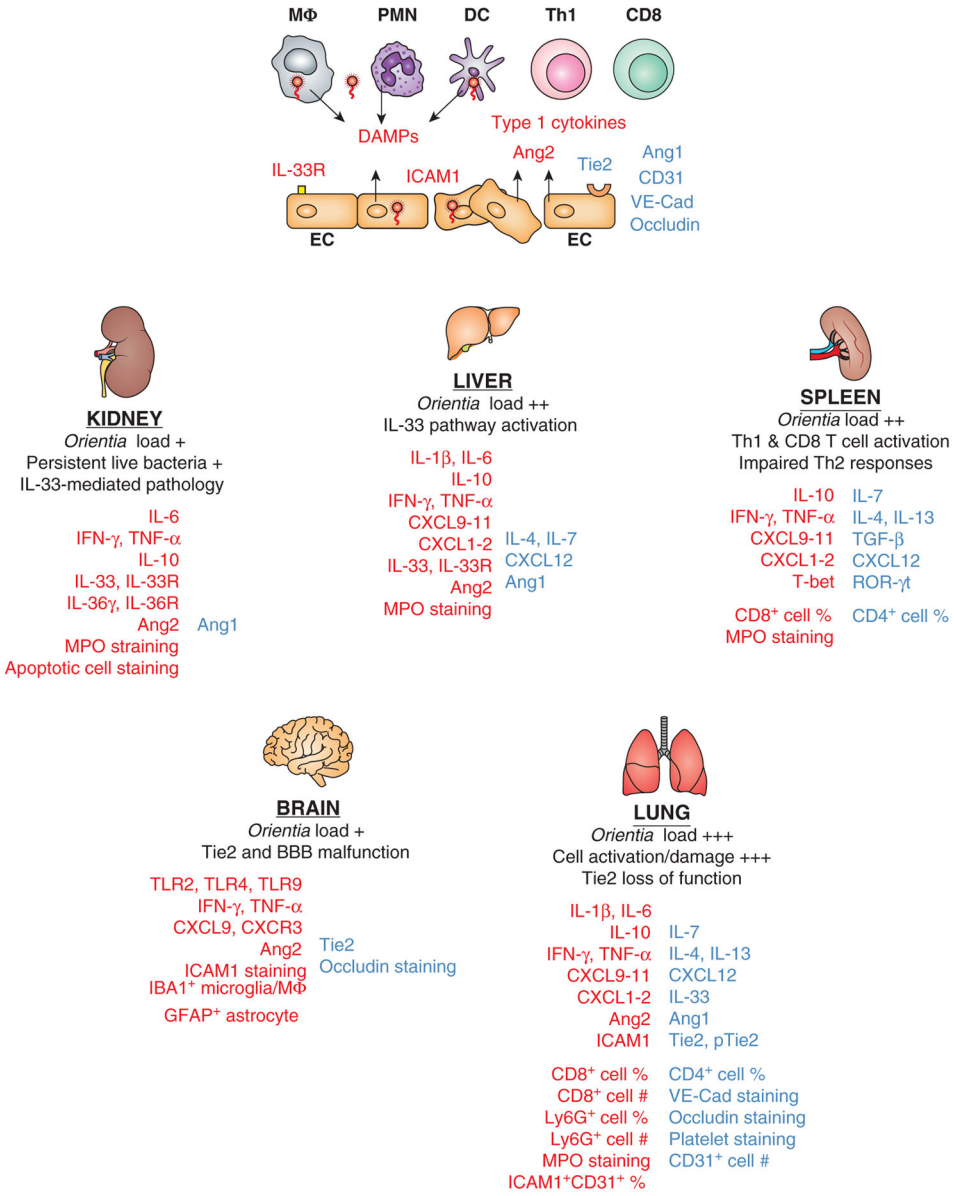

Figure 1. Dysregulated Th1 immune responses, accompanied by impaired Th2 responses, contribute to *Orientia tsutsugamushi*-induced acute tissue injury and vascular dysfunction

Biomarkers or cell subsets that are up-regulated (in red) or down-regulated (in blue) during *in vitro* infection of human cell cultures and lethal or sublethal infection in C57BL/6 mice are illustrated. At the cellular level (top panel of the figure), *Orientia* coccobacilli within target cells such as monocytes/macrophages (MΦ), dendritic cells (DC), neutrophils (PMN), and endothelial cells (EC) preferentially stimulate the activation of Th1 and $CD8^+$ T cells, but not Th2 cells, with high levels of type 1/inflammatory cytokines. Infected target cells can also release or activate damage-associated molecular pattern (DAMP) molecules such as IL-33 and other host factors. ECs can contribute to immune activation via surface expression of IL-33 receptors and ICAM1, or the release of vascular destabilizing factors such as angiopoietin protein-2 (Ang2). Sustained infection can trigger EC apoptosis and down-

regulate the expression of Ang1, CD31 (also known as platelet endothelial cell adhesion molecule-1), occludin (a tight junction-associated protein), and VE-cadherin (endothelial adhesion junctions) via yet-undefined mechanisms. At the tissue level (lower panels of the figure), bacterial loads at the acute or chronic stages of disease are illustrated comparatively, together with hallmarks of pathological changes in the examined organs. The common immunological changes revealed from recent publications (65, 71, 75–78) include the sustained production of Th1 cytokines, selective activation of $CD8^+$ T cells and Ly6G/MPO-positive PMNs, as well as organ-specific differences in the IL-33-like DAMPs. Vascular dysfunction is presented as reduced expression of Ang1, Tie2 (a tyrosine-protein kinase receptor for Ang1 and Ang2), CD31, occludin, and VE-cadherin at the severe stages of infection in mice. Data for lung flow cytometry and vascular staining, as well as kidney IL-36γ/IL-36R analysis, are unpublished. Collectively, these cell- and tissue-specific alterations contribute to the loss of vascular integrity, excessive tissue damage, and host mortality.