Bacterial Pathogenesis of *Rickettsia rickettsii*

Where does it reside?

- *R. rickettsii* present on all continents except Antarctica, disease restricted to Americas
- Common in souteast and southcentral states in North America
- Prefer warm damp places
- Small animals can act as reservoirs to amplify bacteria in the blood and then pass it along to another vector
- Resides in cytoplasm or nucleus of host cells that line small to medium blood vessels

Characteristics of *R. rickettsii*

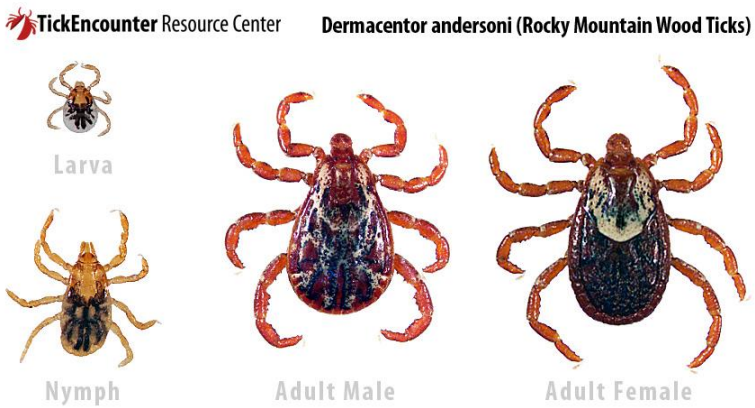
- Gram-negative, coccobacilli
- Has LPS, peptidoglycan, and slime layer - slime layer helps protect against dry environments, antibiotics, and allows for adherence to smoot surfaces
- Divide via binary fission in host
- Small (0.2 x 0.5 micrometers to 0.3 x 2.0 micrometers), allows them to live in cytoplasm and nucleus
- Able to metabolize host-derived glutamate via aerobic respiration and TCA cycle

The vector itself

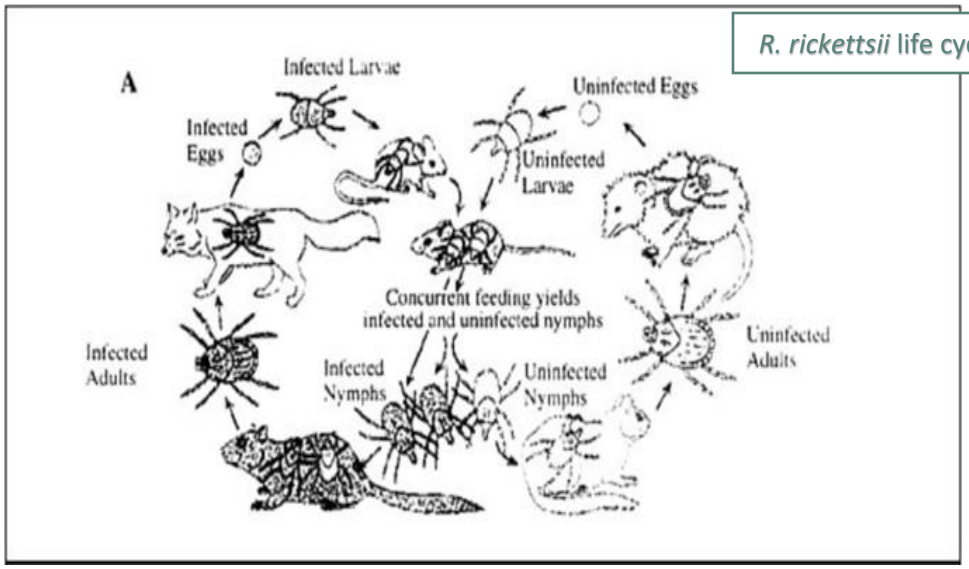
- Family of Ixodidae ticks, known as "hard-bodied" ticks, serve as main vector
- 3 most common species are: Dermacentor variabilis (American dog tick), Dermacentor andersoni (Rocky Mountain Wood Tick), Rhipicephalus sanguine (Brown dog tick)
- Several factors known to influence extent of vector-pathogen interaction and vector's competence of carriage and transmittance

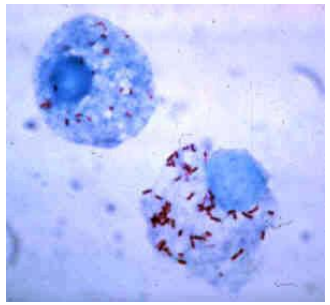
The infected tick

- Bacteria colonizes midgut for survival, then travels to salivary glands for transmission
- Bacteria must overcome the vector's defense mechanisms (e.g. phagocytosis by hemocytes, antimicrobial peptides, RNA interference)
- Once infected with R.rickettsii, the tick remains infective for life, and transmission to a human host is not necessary for continued survival
- Bacteria maintained in vector through transovarial transmission - infected female lays infected ova, hatch into infected larvae



R. rickettsii life cycle





Adherence to host cell

Entry to host

- Main route - through skin at point of tick bite
- May enter through mucous membranes or lungs

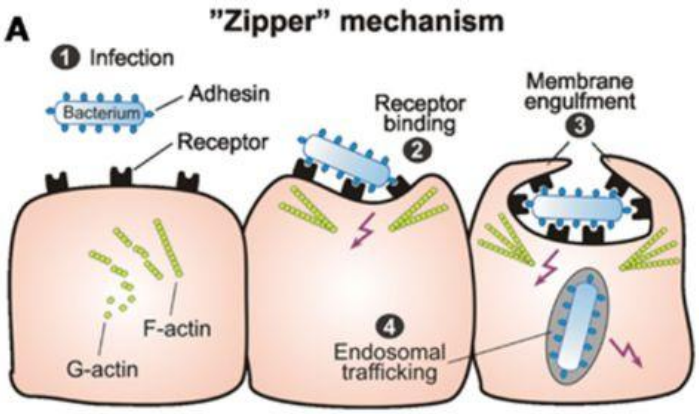
Prefered host cells

- First inoculates dermis of skin
- Adheres to and invades endothelial cells lining small and medium-sized blood vessels with high affinity

Adherence to host cells

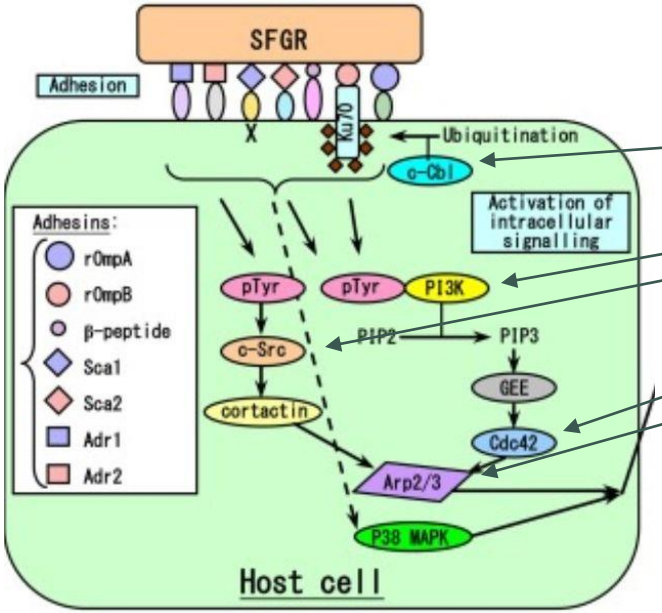
- Surface-exposed outer membranes of *R. rickettsii*, rOmpA and rOmpB, act as adhesins to attach to host's cell surface receptors
- rOmpA mediates bacterial adherence to endothelial cells
- rOmpB acts as bacterial ligand for Ku-70, a nuclear DNA-dependent protein kinase on host cell membrane - interaction triggers downstream signalling cascade for internalization

Mechanism of entrance into host cell

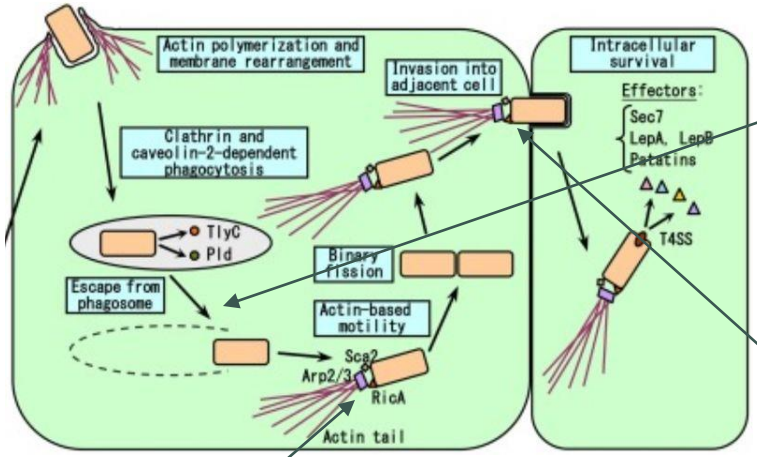


Zipper mechanism of invasion:

- *R. rickettsia* uses 2 adhesin molecules: rOmpA and rOmpB
- bacterial protein binds to host receptor on cell surface
- rOmpA – mediates adherence to endothelial cell
- rOmpB – binding to Ku-70 on host cell triggers cascade for phagocytic internalization



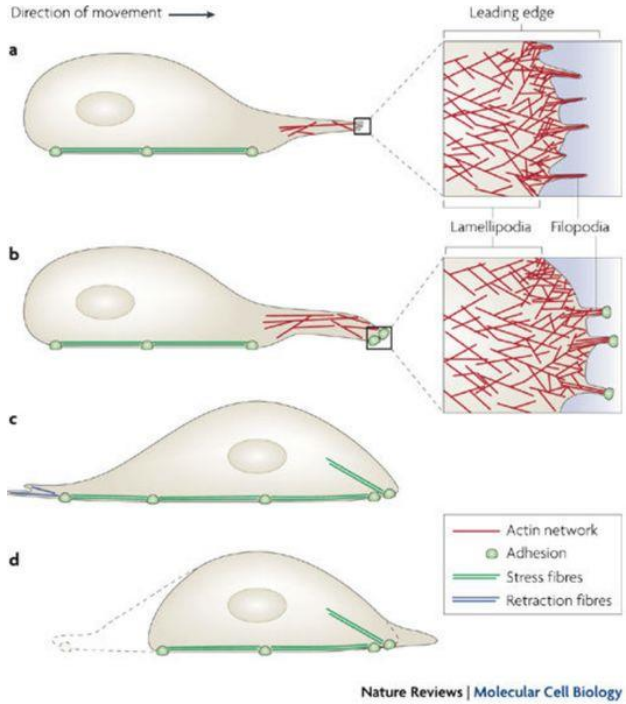
- rOmpB and Ku-70 binding signals recruitment of an ubiquitin ligase, c-Cbl - ubiquitination of Ku-70
- Activation of signalling cascades occur – Cdc42, phosphoinoside 3 kinase, C-src, Arp2/3 complex activated
- Cdc42 only GTPase involved in *R. rickettsii* invasion
- Arp2/3, an actin nucleating complex, instigates localized actin rearrangement to induce engulfment



- When internalized, *R. rickettsii* in phagosome, must escape before it fuses with lysosome
- Exact mechanism still unknown
- Phospholipase D and hemolysin C have membranolytic activities, these may be used for escape
- Phospholipase A2 may also be used
- *R. rickettsii* able to survive, grow, and multiply by using nutrients found in host cytosol

- *R. rickettsii* unique in that it requires very few divisions before exiting and infecting other cells and doesn't undergo lysis event
- To infect other cells, Arp2/3 required to activate bacterial RicA, a protein that directs actin mobilization
- RicA directs actin nucleation at one end of bacterium to form little tails
- Tails mediate movement around cytosol, when bacterium gains sufficient velocity it results in formation of filopodia on cell surface
- If filopodia form on basal side, *R. rickettsii* can spread to and infect vascular smooth muscle cells
- If filopodia form on luminal side, *R. rickettsii* can enter bloodstream and spread to organ cells and become a systemic infection

- Filopodia are long thin cell projections which pierce into adjacent cell
- Allows *R. rickettsii* to infect neighbouring cells and infect many cells in a small area quickly



R. rickettsii remain intracellular and induce various damages to the host. However, for bacterial survival, various mechanisms are used to maintain host cell integrity and the intracellular environment, which includes activation of host cell's intracellular signalling mechanisms and regulation of programmed cell death. Mechanisms involving host damage include oxidative stress, increase in inflammatory mediators, and alterations in vascular permeability.

Maintaining host cell integrity: intracellular signalling and regulation of apoptosis

R.rickettsii induces signalling mechanisms that lead to functional changes to maintain cell integrity, known as "endothelial activation"

Signalling cascade involves 2 main host factors - nuclear factor (NF)-κB and MAPK

MAPK signalling events have anti-apoptotic effects on endothelial cell, facilitating host-cell invasion

Activation of NF-κB leads to downstream signalling cascades

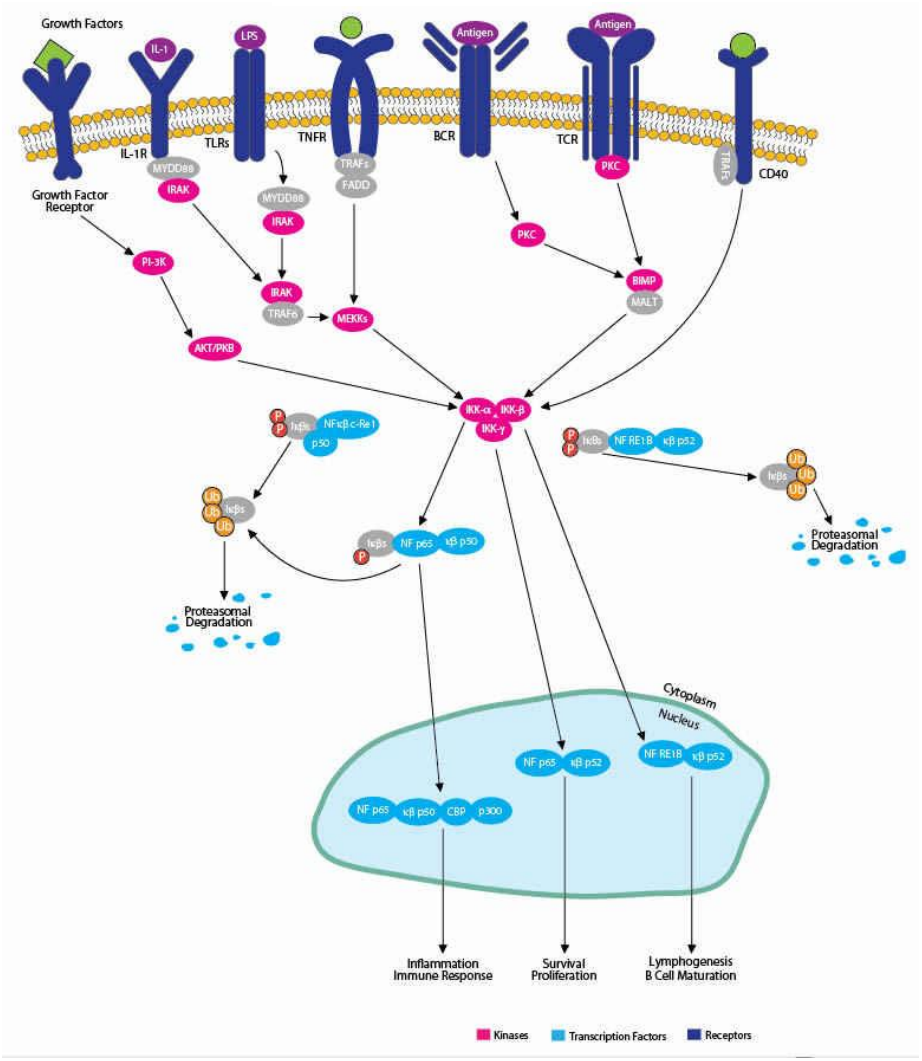
NF-κB can also inhibit caspase-8 and -9 mediated pathways of apoptosis

Alters expression of B-cell lymphoma-2 (bcl-2) family of proteins - includes mitochondrial proteins of both anti- and pro-apoptotic properties

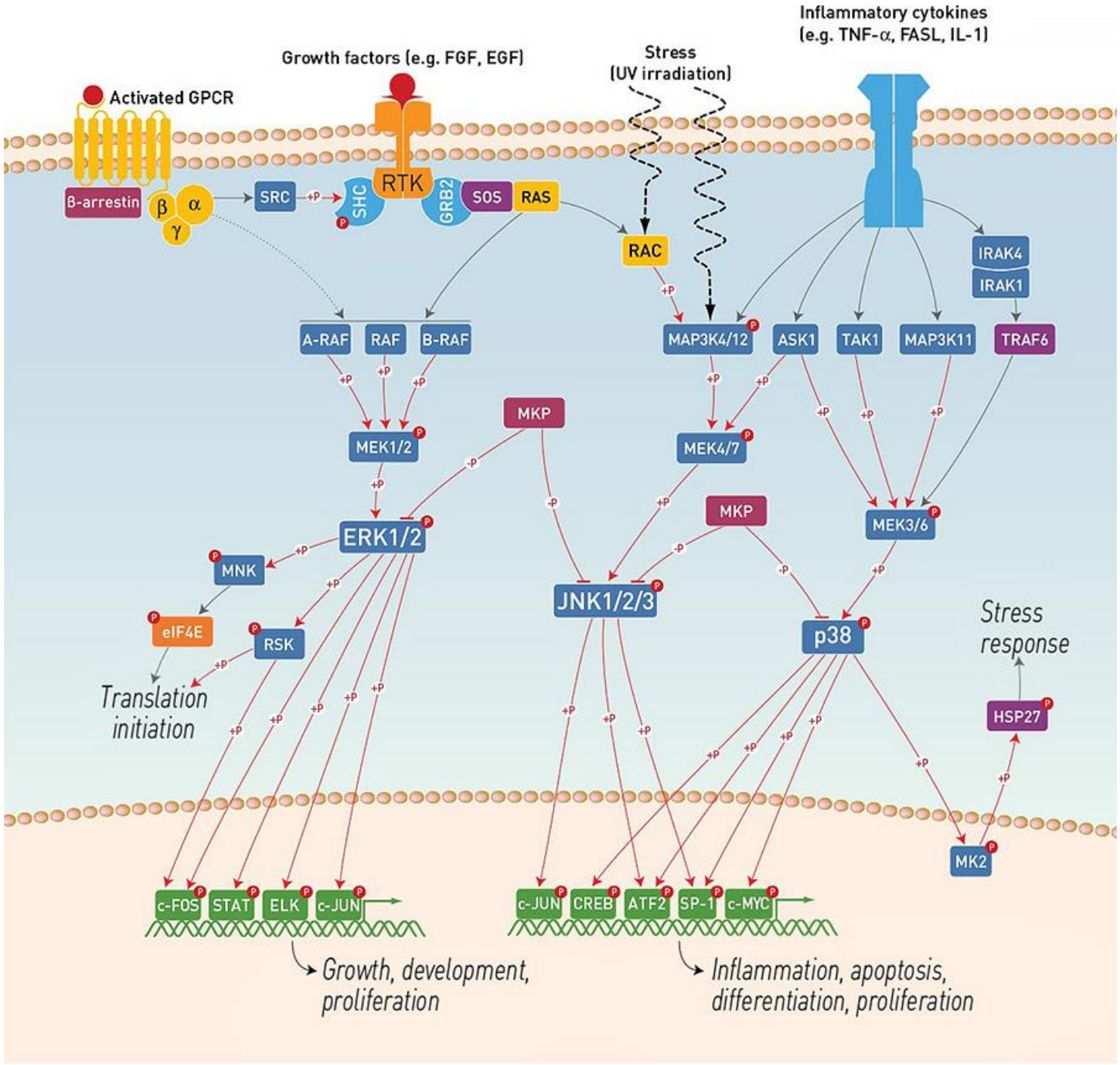
NF-κB activation yields maintenance of intracellular environment necessary for bacterial replication and spread

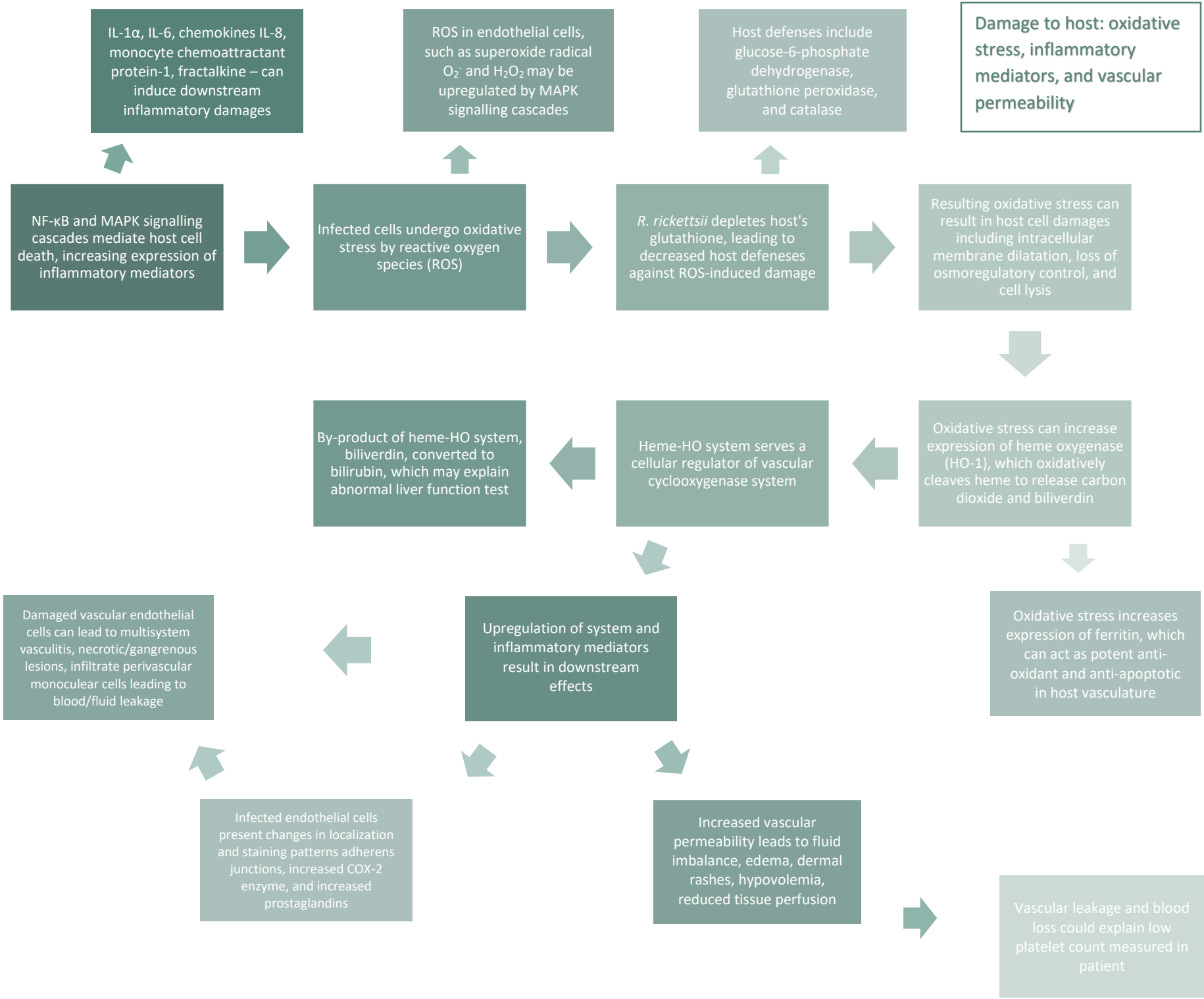
This signalling cascade yields net anti-apoptotic effect by allowing proteins to maintain integrity of host mitochondria

NF-κB signalling pathway



MAPK signalling pathway




Damage to host: oxidative stress, inflammatory mediators, and vascular permeability
NF-κB and MAPK signalling cascades mediate host cell death, increasing expression of inflammatory mediators
IL-1α, IL-6, chemokines IL-8, monocyte chemoattractant protein-1, fractalkine – can induce downstream inflammatory damages
Infected cells undergo oxidative stress by reactive oxygen species (ROS)
ROS in endothelial cells, such as superoxide radical O_2^- and H_2O_2 may be upregulated by MAPK signalling cascades
R. rickettsii depletes host's glutathione, leading to decreased host defeneses against ROS-induced damage
Host defenses include glucose-6-phosphate dehydrogenase, glutathione peroxidase, and catalase
Resulting oxidative stress can result in host cell damages including intracellular membrane dilatation, loss of osmoregulatory control, and cell lysis
Oxidative stress can increase expression of heme oxygenase (HO-1), which oxidatively cleaves heme to release carbon dioxide and biliverdin
Oxidative stress increases expression of ferritin, which can act as potent anti-oxidant and anti-apoptotic in host vasculature
Heme-HO system serves a cellular regulator of vascular cyclooxygenase system
By-product of heme-HO system, biliverdin, converted to bilirubin, which may explain abnormal liver function test
Upregulation of system and inflammatory mediators result in downstream effects
Damaged vascular endothelial cells can lead to multisystem vasculitis, necrotic/gangrenous lesions, infiltrate perivascular monocular cells leading to blood/fluid leakage
Infected endothelial cells present changes in localization and staining patterns adherens junctions, increased COX-2 enzyme, and increased prostaglandins
Increased vascular permeability leads to fluid imbalance, edema, dermal rashes, hypovolemia, reduced tissue perfusion
Vascular leakage and blood loss could explain low platelet count measured in patient