Pathogenic Mechanisms of Diseases Caused by *Rickettsia*

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ABSTRACT: The specter of bioterrorism employing genetically engineered *Rickettsia* resistant to all antibiotics should reawaken the world's desire to elucidate the pathogenesis of typhus and spotted fever rickettsioses in a search for mechanisms vulnerable to interdiction. The pathogenetic sequence includes rickettsial entry into the dermis, hematogenous dissemination to vascular endothelial cells (most critically in brain and lungs), increased vascular permeability, edema, and immunity mediated by NK cells, IFN-γ, TNF-α, RANTES, antibodies, and cytotoxic T lymphocytes. Silverman has demonstrated the role of reactive oxygen species (ROS) produced by *R. rickettsii*–infected endothelial cells in peroxidative damage to cell membranes *in vitro*, and Heinzen has described actin-based rickettsial intracellular mobility and intercellular spread. At this point the availability of sequences of rickettsial genomes and excellent animal models of rickettsioses have yielded insufficient progress towards the identification of rickettsial virulence factors and knowledge of the importance of injury mediated by ROS, phospholipase A_2, protease(s) or other mechanisms *in vivo*. Attention to the rickettsiosis-associated procoagulant state led to determination that hemostatic mechanisms largely prevent major hemorrhage without disseminated intravascular coagulation or thrombosis-mediated ischemia. Particularly lacking is knowledge of early events *in vivo* at the portal of entry in skin (or lung), of the effects of the inoculum medium (arthropod saliva or feces), mediators produced by infected endothelium under conditions of flow and of the contributions *in vivo* of immune effectors to pathology, of the role of apoptosis in rickettsial infection, and of the endothelial cell alterations that account for increased vascular permeability. The host cell receptor for the *Rickettsia* ligand and the mechanism of rickettsial escape from the phagosome need to be elucidated.

KEYWORDS: *Rickettsia*; pathogenesis; pathophysiology; host defenses

INTRODUCTION

Rickettsiology claims few discoveries of novel scientific concepts and principles. Most rickettsiologists justify the research described on their grant applications as providing knowledge that will lead to a vaccine or will elucidate rickettsial pathogenesis rather than as a pursuit of pure unapplied science. Because rickettsiae include some of the most pathogenic bacteria known, they are a prime subject for the investigation of pathogenesis.

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Before critical analysis of the knowledge of rickettsial pathogenesis, the concept of pathogenesis of an infectious disease should be defined. It has two essential components:

1. the sequence of events (transmission, entry at a particular anatomic site, for many diseases spread via a particular anatomic route, evasion of host defenses, and growth within its target location in the body) and
2. the mechanism(s) of injury to cells, tissues, organs, and the host as a whole.

For the obligately intracellular organisms of the genus *Rickettsia*, there are also the sequence of steps of interaction with the host cell and their underlying mechanisms (adhesion to a host cell membrane receptor, induction of phagocytosis, rapid escape from the phagosome, avoidance of intracytoplasmic rickettsicidal mechanisms, acquisition of nutrients, relatively slow growth, and spread to other host cells).

It is important to emphasize the rationale for seeking understanding of rickettsial pathogenesis. Knowledge of the pathways by which rickettsiae cause disease would enable the design of pharmacologic, immune, and other interventions to prevent or mitigate the damage caused by rickettsiae. Rickettsiology has suffered lack of support because of the misconception that tetracycline and chloramphenicol had solved all of the problems. The specter of bioterrorism employing genetically engineered *Rickettsia* resistant to all antibiotics should reawaken the world's desire to elucidate the pathogenesis of typhus and spotted fever rickettsioses in search for mechanisms vulnerable to interdiction. In the 96 years since the discovery of *Rickettsia rickettsii,* a substantial catalogue of knowledge of the pathogenetic sequence, rickettsia-host cell interaction, mechanisms of injury to infected cells, pathophysiology, mechanisms of tissue and organ injury *in vivo*, and host defenses of various diseases caused by *Rickettsia* has been established and will be the subject of this review.[1–8]

PATHOGENETIC SEQUENCE OF RICKETTSIOSES

Transmission to humans is not essential to the maintenance of any *Rickettsia* in its natural cycle, including *R. prowazekii,* which is maintained effectively in a zoonotic cycle involving North American flying squirrels and their ectoparasites.[9] However, investigation of the steps occurring in rickettsial diseases has a valid anthropocentric medical justification. Many of the pathogenetic steps in rickettsial pathogenesis are firmly established (TABLE 1).

RICKETTSIA-HOST CELL INTERACTION

Evidence that rickettsial outer membrane protein A (OmpA) is an adhesin of *R. rickettsii* and that OmpA and OmpB are adhesins of *R. japonica* has been documented.[10–11] Rickettsiae attach to a protein-dependent receptor on the host cell membrane and induce focal host cell cytoskeletal rearrangements at the site of attachment, resulting in their entry into the host cell even in nonprofessional phagocytes by a mechanism requiring rickettsial metabolic activity.[12,13] Rickettsiae rapidly lyse the phagosomal membrane and escape into the cytosol prior to phagolysosomal fusion avoiding exposure to the lysosomal enzymes.[14,15] In the

TABLE 1. Pathogenetic sequence in rickettsial diseases

Pathogenetic Step	Observations
Transmission	Salivary inoculation by feeding tick (e.g., RMSF, MSF), mite (rickettsialpox), or flea (murine typhus)
	Deposition in feces of louse (epidemic typhus) or flea (murine typhus)
	Aerosol (especially epidemic typhus, murine typhus, RMSF, MSF)
Entry	Skin (all rickettsioses)
	Mucous membranes (potentially all rickettsiae, e.g., conjunctiva)
	Lungs (potentially all rickettsiae as in a laboratory accident or bioterrorist event)
Spread	Lymphatic vessels from portal of entry to regional lymph nodes (likely, but unproven)
	Bloodstream to all organs
Target Cells/Organs	Endothelium > macrophages > ?hepatocytes
	Disseminated endothelial infection of all organs with brain and lungs as critically affected vital organs
Evasion of Host Defenses	Escape from phagosome Selection for IFN-γ resistance Latency (*R. prowazekii*) Cell-to-cell spread (SFG rickettsiae)

cytosol they acquire their nutrients (e.g., glutamate), a part of their energy requirements (ADP/ATP transporter), and many components required for growth (e.g., amino acids).[16] The evolutionary factors leading to the relatively long generation time (8–10 h) are unclear in the context of infection of vertebrate hosts and the production of rickettsemia of sufficient titer and duration to infect other feeding arthropod vectors. Slow growth might be a good survival strategy to avoid harming the arthropod vector or in a latent *R. prowazekii* infection in humans, but would seem a poor approach to horizontal transmission of *R. rickettsii, R. typhi, R. sibirica,* or *R. prowazekii* from their rodent host to the uninfected feeding arthropod hosts. Heinzen's studies on actin-based mobility of spotted fever group (SFG) rickettsiae have opened the door to elucidating their cell-to-cell spread in contrast with the release of typhus group (TG) rickettsiae by bursting of the massively infected host cell.[17–19]

MECHANISMS OF CELL INJURY BY RICKETTSIAE

The steady efforts of Silverman and Ermeeva have demonstrated the importance of reactive oxygen species (ROS) produced by endothelial cells infected by *R. rickettsii* in damaging the infected cells via lipid peroxidation of host cell

membranes.[20–24] The oxidative stress-mediated injury of cultured endothelial cells is associated with depletion of host components such as glutathione and increased levels of catalase, which increases the concentration of hydrogen peroxide, and striking reduction in enzymes such as glucose-6-phosphate dehydrogenase, glutathione peroxidase, and catalase that are host defenses against ROS-induced damage.[20,25] Further proof of the significance of rickettsia-induced oxidative stress as a pathogenic mechanism is the ameliorating effect of the antioxidant molecules, α-lipoic acid and desferroxamine, in the infected endothelial cell culture system.[26] Less evidence has been generated that a rickettsial phospholipase A_2 or protease plays a pathogenic role.[15,27–30] It is possible that phospholipase A_2 and ROS may act synergistically in damaging the host cell.

Rickettsiae can kill infected cells in the absence of immune effectors, and the immune system acts overall to the benefit of the rickettsia-infected host.[31–34] However, some immune effector mechanisms (e.g., CD8 cytotoxic T-lymphocytes) appear to eliminate infected cells by inducing apoptosis.[35] If the infected endothelial cell target of these cytotoxic T-lymphocytes is very extensive at the time of their clonal expansion and activation, an immunopathologic effect can occur, as has been observed in our laboratory under certain artificial conditions. The mode of death of rickettsia-infected cells usually appears to be necrosis, although cytotoxic T-lymphocytes remove infected cells by inducing their apoptosis. An effect of activation of NF-κB by a direct *R. rickettsii*-mediated, proteosome-independent mechanism is inhibition of apoptosis.[36–38] This result would allow prolonged intracellular rickettsial survival and growth but does not absolutely prevent apoptosis and can be overridden.[35]

PATHOPHYSIOLOGY OF RICKETTSIAL DISEASES

The functional effect of rickettsial infection of numerous foci of contiguous networks of endothelial cells of the microcirculation of every organ is increased vascular permeability.[39,40] The results are the leakage of fluid from the bloodstream, its accumulation in the surrounding tissues (edema), and a lower volume of fluid remaining in the circulation (hypovolemia). The presence of edema can pose a life threatening situation in the lungs and the brain, which lacks lymphatic vessels to remove interstitial fluid.[41] Swelling of the brain, in the rigid cranial vault eventually prevents the blood from entering and squeezes out the brain itself. Filling of the airspaces of the lung with fluid limits gas exchange leading to hypoxemia. The reduced blood volume leads to poor perfusion of organs such as the kidney, where renal function is impaired.[42,43] The general and localized effects of multifocal lesions in the central nervous system may cause loss of neurologic function related to the area of the brain involved. Focal lesions in many organs are not sufficiently extensive to result in organ failure.[44] For example, hepatic infection may result in focal death of a very small portion of the hepatocytes, enough to cause elevated serum transaminase concentrations, but not hepatic failure.[45]

MECHANISMS OF TISSUE AND ORGAN INJURY

It has been hypothesized that, in addition to the reduced delivery of nutrients and oxygen to tissues owing to decreased blood volume-associated hypoperfusion and pulmonary edema-associated hypoxemia, thrombus-mediated vascular occlusion, including disseminated intravascular coagulation (DIC), might be an important pathogenic mechanism in severe rickettsioses.[46–49] Indeed, Rocky Mountain spotted fever and boutonneuse fever induce a procoagulant state.[50] However, DIC rarely occurs, and postmortem studies of fatal cases reveal that thrombi are few and do not constitute an important general pathogenic mechanism but rather a physiological response to endothelial denudation. Recent detailed studies of the coagulation system throughout the course of SFG rickettsial infection in an animal model with a lethal or sublethal dose of rickettsiae demonstrated that DIC does not occur and that fatal rickettsiosis is not a thrombotic disease.[51] It is remarkable that the homeostatic mechanisms of the coagulation system function in a disease with severe systemic endothelial infection and injury are usually effective in preventing life threatening hemorrhage and in avoiding thrombosis-mediated pathogenesis. The pathogenic mechanisms that mediate the vascular permeability changes associated with rickettsial infections have not been elucidated. Multiple mechanisms, which likely operate simultaneously, may explain the increased vascular permeability observed in patients with rickettsioses. Endothelial denudation may explain some of the changes in the most heavily parasitized segments of the vasculature; however, this by itself could not explain the systemic manifestations of moderately to severely ill patients.

A second mechanism implies an active role of the endothelium. These cells should not be viewed as passive targets of the infection. Their capacity to express an activated phenotype upon rickettsial infection and to kill rickettsiae intracellularly suggests an active role in the pathogenesis of these diseases. Activated rickettsia-infected endothelial cells can interact with leukocytes, which can transmigrate and/or secrete cytokines as a consequence of the encounter. This situation is analogous to any other type of inflammation with its associated localized edema; however, in the case of rickettsial infections, the multifocal and segmental nature of the infection is likely to greatly multiply the effects. The total profile and kinetics of cytokines and chemokines produced by endothelial cells from different organs in response to rickettsial infection are still unknown. It is possible that rickettsiae stimulate the production of a unique signature profile of cytokines that may explain the massive changes in permeability.

A third and more intriguing mechanism, although still unexplored, is direct triggering of increased vascular permeability by intra-endothelial rickettsiae via changes in the cytoskeleton and the proteins of the interendothelial junctional complexes (adherens and tight junctions). The documented detection of circulating endothelial cells during human rickettsioses suggests that changes in the adhesiveness of these cells may indeed play a role.[46] Notably, these viable, infected, circulating endothelial cells may provide a protective vehicle for rickettsiae so that they can establish new foci of infection in the capillaries where they lodge.

Understanding the mechanisms of increased vascular permeability and particularly the early changes and mediators during rickettsial infections would allow the design of strategies to block this effect. This issue is of special importance in severe

and advanced cases, which can be fatal or have neurologic sequelae despite antibiotic treatment.

The paradox of ROS appearing to act as both a host defense and a pathogenic mechanism is unresolved, but presumably involves different subcellular compartments.

HOST DEFENSES AGAINST RICKETTSIAE

The topic of immunity to rickettsiae has been reviewed recently and was presented a year ago at the rickettsiology meeting.[52] The essential principles are the paramount importance of cellular immunity expressed as cytokine activation of intraendothelial killing of rickettsiae and the ultimate clearance of rickettsial infection by CD8 cytotoxic T lymphocyte activity.[35,53,54] In mice IFN-γ and TNF-α act synergistically to activate nitric oxide synthase 2-dependent intraendothelial killing.[33,53] In humans, IFN-γ, TNF-α, IL-1β, and RANTES activate intracellular rickettsicidal activity in various combinations, by three different mechanisms, in different host cell types when investigated *in vitro*.[54] The antirickettsial mechanisms include nitric oxide, ROS, and limitation of availability tryptophan via its degradation by indoleamine -2, 3-dioxygenase.

Natural killer cells are effectors of antirickettsial innate immunity by secretion of IFN-γ early in the course of infection.[55] Antibodies to epitopes of OmpA and OmpB, but not lipopolysaccharides, also contribute to immune protection against SFG rickettsiae. The ultimate conclusion is that the components of the immune system act in concert to dampen and eventually clear rickettsial infections with subsequent strong group-crossprotective immune protection.

RICKETTSIA AS THE CAUSE OF CHRONIC DISEASE?

Nilsson and colleagues have challenged the classic paradigm that rickettsioses are all acute infectious diseases.[56,57] Asymptomatic, latent infection with *R. prowazekii* follows typhus fever, and a unique case report described asymptomatic persistence of *R. rickettsii* in the lymph node of a patient after Rocky Mountain spotted fever.[58,59] However, the generally accepted principle is that *Rickettsia* cause acute illnesses, and the patients who have not lost critical neurons or undergone amputation of gangrenous extremities will return to their usual state of health after a convalescent period.

In contrast, the two Swedish studies reported the association of *R. helvetica* with (1) chronic perimyocarditis and sudden death or (2) sarcoidosis.[56,57] The identification of *R. helvetica* was based upon the DNA sequence of the product of hemi-nested polymerase chain reactions. One of the sudden death cases, but none of the sarcoidosis cases, had supporting serologic evidence of a SFG rickettsiosis. Histochemical stains (May-Grünwald Giemsa, Grocott silver methenamine, and acridine orange) were interpreted as showing structures consistent with small bacteria, but these nonspecific methods are notorious for staining small unidentified particles in any damaged tissue. Immunohistochemistry using a previously undescribed method with an antibody to *Proteus* OX-19 and fluorescence yielded results shown in the published

figures as bright dots that could not be determined as intracellular or extracellular, much less as to the associated cell type, if any. It is possible that the vigorous immune response that resulted in the granulomas reduced the rickettsial burden to single isolated organisms rather than the usual pairs of small bacilli. The negative PCR controls and the results of immunohistochemistry using SFG-specific antibodies in three of the sarcoidosis cases strengthen the diagnosis, but the notorious pitfalls of nested PCR beg for further specific supporting data. Unfortunately the ultrastructural illustrations do not provide it. None of the transmission electron photomicrographs from the heart or the sarcoidosis tissues contains rickettsiae. The structures do not have the cell wall structure of *Rickettsia*, and of course their quantities are inconsistent with the few structures shown by immunohistochemistry. The hypothesis that *R. helvetica* causes a chronic granulomatous infection may eventually be proven to be true. Isolation of the organism from the affected tissue would be the most convincing evidence, and production of chronic granulomatous disease by *R. helvetica* in experimentally infected animals would add further support.

GAPS IN OUR KNOWLEDGE OF RICKETTSIAL PATHOGENESIS

With the exception of limited evidence identifying the adhesins of rickettsiae, no rickettsial virulence factors have been determined despite the availability of annotated rickettsial genomes.[60,61] All the adhesins of all rickettsiae need to be identified. The gene encoding the rickettsial phospholipase A_2 has yet to be identified, much less experimentally proven. Until genetic systems are available that enable the reliable directed inactivation of rickettsial genes and transfer of active genes into rickettsiae, progress in determining the virulence factors of rickettsiae will continue to be slow. Important unidentified elements of the rickettsia-host cell interaction include the host cell membrane receptor for the rickettsial adhesin(s) and the rickettsial mechanism of escape from the phagosome. Although phospholipase A_2 activity has been hypothesized to effect phagosomal escape, there are no supportive experimental data. Significant gaps in our knowledge of rickettsial pathogenesis relate to lack of evidence that phenomena identified *in vitro* are important *in vivo.* The role and importance of ROS-mediated endothelial injury have not been established in animal models. Similarly the importance of hypothetical host-mediated pathogenic mechanisms, such as the effects of cytokines or cytotoxic T lymphocyte activity, has not been determined *in vivo*.

Indeed the actual mechanism of increased vascular permeability in rickettsial diseases has not been determined. The striking presence of rickettsial infection in endothelium suggests direct rickettsial-mediated endothelial injury, but the roles of host-derived inflammatory and immune mediators have not been examined carefully.

Some knowledge is lacking because the experimental models have yet to be established. Elucidation of the importance of tick saliva in modulating the infection and of the early infection events in the skin and possibly lymphatic vessels and regional lymph nodes require the development of a SFG rickettsial disease model incorporating tick bite transmission. Similarly a mouse model of aerosol transmission would be important for studies related to the use of typhus or spotted fever rickettsiae as agents of bioterrorism. The lack of needed data relates not only to too few

studies of rickettsiae in animal models. There are also important principles that have been documented in animal models but have not been examined in the actual human disease. For example, intracellular rickettsial killing has been investigated in human endothelial cells in cell culture, but what occurs in the human body remains undetermined. The answers will require well-designed studies that are careful to protect human subjects, or alternatively, employ humanized animal models. Animal models are available that are appropriate to address many of the current issues, in particular the disseminated endothelial-target C3H/HeN mouse models of SFG rickettsiosis (*R. conorii*) and TG rickettsiae (*R. typhi*).[62,63]

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