Rickettsiae and Rickettsial Infections: The Current State of Knowledge

David H. Walker
Department of Pathology, Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston

New human rickettsial pathogens have been discovered, and long-known rickettsiae of undetermined pathogenicity have been demonstrated to cause illness. Disease associated with Rickettsia slovaca has unique clinical manifestations, including prominent lymphadenopathy without fever and rash. Rickettsial genomes are highly conserved, with reductive evolution leading to a small genome that relies on the host cell for many biosynthetic functions. Advances in the evaluation of the pathogenesis of rickettsial disease include identification of rickettsial adhesins, a host cell receptor, signaling elements associated with entry of rickettsiae by induced phagocytosis, rickettsial enzymes mediating phagosomal escape, and host actin-based rickettsial cell-to-cell spread. Disruption of adherens junctions of infected endothelial cells likely plays a role in the critical pathophysiologic mechanism: increased microvascular permeability. Production of reactive oxygen species by infected endothelium injures these cells. However, disseminated intravascular coagulation rarely occurs. Immunity is mediated by reactive cytokine-activated rickettsicidal nitrogen and oxygen species and by clearance of rickettsiae by cytotoxic CD8 T cells.

TAXONOMIC CONUNDRUM

During the past 2 decades, many novel Rickettsia isolates have been characterized by recently developed methods for genetic analysis. A large number of these Rickettsia species are agents of human diseases in areas of the world where rickettsioses had not previously been investigated in depth (e.g., R. japonica in Japan and Korea; R. honei in Australia and Southeast Asia; R. africae throughout sub-Saharan Africa and in the French West Indies; R. felis globally; the R. sibirica mongolotimonae strain in Asia, Europe, and Africa; R. parkeri in North and South America; “R. helongiangensis” in northeastern Asia; and R. aeschlimanni in Africa) [1–7]. A novel isolate (Astrakhan strain) of Rickettsia conorii has been recovered from patients in Astrakhan, Russia, and in Chad and from ticks in Kosovo. R. conorii strain Israeli has been isolated not only from patients in Israel but also from those in Portugal.

Interestingly, this proliferation of named species has generated controversy among rickettsiologists regarding the appropriate taxonomy of Rickettsia species. One proposed set of criteria for establishing rickettsial species is based on the premise that all previously named species are substantially different from one another [8]. However, in a recent publication in Nature Reviews Microbiology discussing reevaluation of prokaryotic species, a group of taxonomic experts rejected this approach, stating that “Defining species limits by using levels of sequence similarity typically found within existing named species is clearly inappropriate” [9, p. 735]. For rickettsiae, a proposed criterion for the establishment of a new species (i.e., a divergence of the rrs gene of 0.2%) differs markedly from the threshold of genetic divergence (i.e., a divergence of the rrs gene of 0.5%) of variants of other bacteria with similar evolutionary selective pressure in an obligately intracellular lifestyle involving an arthropod host (i.e., Orientia tsutsugamushi, Coxiella burnetii, and Ehrlichia chaffeensis). Of note, such organisms as R. conorii, R. sibirica, R. africae, and R. parkeri could be considered to be strains
Dermacentor and closely related agent it produces painful regional lymphadenopathy, multiple apparently, non–life-threatening rickettsiosis [2, 7]. It differs from gene decay, with there being many pseudogenes and a high proportion of noncoding DNA. Their cytosolic niche, which is rich in nutrients, amino acids, and nucleotides, has allowed rickettsiae to jettison the genes encoding enzymes for sugar metabolism and for lipid, nucleotide, and amino acid synthesis, a characteristic most likely responsible for our inability to cultivate them in cell-free medium. Rickettsia species also contain as many as 5 autotransporters, proteins with 3 domains, a leader sequence that mediates transport across the cell membrane, a passenger sequence, and a transporter sequence that is inserted as a β-barrel into the outer envelope to transport the passenger sequence to the outer surface of the cell wall. Among the autotransporters, outer membrane protein (Omp) A is present only in rickettsiae in the spotted fever group, and OmpB is present in all Rickettsia species. Sca 1, Sca 2, and Sca 3 exist as split genes (interrupted into 2–4 open reading frames) in at least 1 Rickettsia species. Sca 4, which shares sequence similarity, is not an autotransporter, because it lacks the transporter domain.

CONTRIBUTIONS OF GENOME SEQUENCING TO UNDERSTANDING RICKETTSIAE

Rickettsial genomes are highly conserved, with similar gene synteny and content [15]. Their small genomes have resulted from gene decay, with there being many pseudogenes and a high proportion of noncoding DNA. Their cytosolic niche, which is rich in nutrients, amino acids, and nucleotides, has allowed rickettsiae to jettison the genes encoding enzymes for sugar metabolism and for lipid, nucleotide, and amino acid synthesis, a characteristic most likely responsible for our inability to cultivate them in cell-free medium. Rickettsia species also contain as many as 5 autotransporters, proteins with 3 domains, a leader sequence that mediates transport across the cell membrane, a passenger sequence, and a transporter sequence that is inserted as a β-barrel into the outer envelope to transport the passenger sequence to the outer surface of the cell wall. Among the autotransporters, outer membrane protein (Omp) A is present only in rickettsiae in the spotted fever group, and OmpB is present in all Rickettsia species. Sca 1, Sca 2, and Sca 3 exist as split genes (interrupted into 2–4 open reading frames) in at least 1 Rickettsia species. Sca 4, which shares sequence similarity, is not an autotransporter, because it lacks the transporter domain.
Figure 1. The most important neglected emerging infectious diseases and bioterror threats around the world. On the basis of criteria of low-dose aerosol infectivity, a high case-fatality rate, a high ratio of disease to asymptomatic infection, availability in nature, and the potential for genetic engineering of complete antibiotic resistance, *Rickettsia rickettsii* and *Rickettsia prowazekii* should be category A agents, and *Rickettsia typhi* and *Rickettsia conorii* category B agents. RMSF, Rocky Mountain spotted fever.

**Rickettsia–Host Cell Interactions**

Obligately intracellular rickettsiae attach to the host cell receptor Ku70 by means of the most abundant surface protein, OmpB (figure 2) [16]. Spotted fever group rickettsiae also use OmpA as an adhesin. Adhesion of OmpB to the membrane-spanning protein Ku70 results in recruitment of additional Ku70 molecules to the cell membrane, where further OmpB binding occurs. Ubiquitin ligase is also recruited to the future rickettsial entry site where Ku70 is ubiquitinated, and signal transduction events lead to recruitment of Arp2/3 complex. Cdc42 (a small guanidine triphosphatase), protein tyrosine kinase, phosphoinositide 3-kinase, and Src-family kinases activate Arp2/3, resulting in phagocytosis of the attached rickettsia occurring as a result of a zipper mechanism involving alteration of cytoskeletal actin at the entry site [17]. Another rickettsial protein—RickA, expressed on the rickettsial surface—activates Arp2/3, which initiates polymerization of host cell actin [18, 19]. The filaments of actin push the rickettsia to the surface of the host cell, where the host cell membrane is deformed outward and invaginates into the adjacent cell. Disruption of both cell membranes enables the rickettsia to enter the adjoining cell without being exposed to the extracellular environment. Some rickettsiae exit via the luminal surface of blood vessels into the bloodstream. Typhus rickettsiae do not stimulate actin-based mobility, and they accumulate to massive quantities intracellularly until the endothelial cell bursts, releasing rickettsiae into the blood.

To enter the cytosol of the host cell where nutrients, adenine triphosphate, amino acids, and nucleotides are available for growth and to avoid phagolysosomal fusion and death, rickettsiae must escape from the phagosome [20]. Rickettsiae secrete phospholipase D and hemolysin C, which disrupt the phagosomal membrane and permit the rapid escape of the rickettsiae.

**Rickettsial Pathogenic Mechanisms**

The major pathophysiologic effect of rickettsial infections is increased microvascular permeability due to the disruption of adherens junctions between infected endothelial cells, development of interendothelial gaps, formation of stress fibers, and conversion of the shape of endothelial cells from polygons to large spindles [21]. The current hypothesis regarding the mechanism of injury of rickettsia-infected endothelial cells concerns oxidative stress. Endothelial cells infected with spotted fever group rickettsiae in vitro produce reactive oxygen species that cause lipid peroxidative damage to the host cell membranes. There is evidence that rickettsial infection causes oxidative stress in experimentally infected animals [22], but the extent to which...
this mechanism explains the pathologic findings of spotted fever group rickettsioses and the possibility of other pathogenic mechanisms, such as the effects of cytokines, other mediators, or cytotoxic T cells, remains undetermined.

Rickettsial infection of endothelial cells activates nuclear factor κB, which inhibits apoptosis and mediates the production of proinflammatory cytokines [23]. Rickettsia-infected endothelium produces IL-6, IL-8, and monocyte chemoattractant protein 1. Delay of endothelial cell death allows further intracellular rickettsial growth. A promising avenue for discovering novel general principles of pathobiology and immunobiology is in vitro investigation of the effects of rickettsial infection on primary endothelial cells from relevant organs in the presence of cellular components of blood and physiologic shear forces of flow and with appropriate in vivo models.

Reports of cases of rickettsioses often claim, without supporting evidence other than thrombocytopenia, that the patient had disseminated intravascular coagulation. Case series based on such reports submitted to public health agencies and other reports based on retrospective reviews of medical records lacking sufficient coagulation studies have perpetuated the myth that disseminated intravascular coagulation occurs in a substantial portion of patients with severe rickettsiosis. Careful analysis of such data reveals that disseminated intravascular coagulation occurs only rarely in persons with rickettsial infections [24]. Patients with Rocky Mountain spotted fever and Mediterranean spotted fever develop a procoagulant state resulting from rickettsia-induced disseminated endothelial injury, release of procoagulant factors, and activation of the coagulation cascade with generation of thrombin, platelet activation, increased fibrinolytic factors, and consumption of natural anticoagulants. The result is a remarkably homeostatic state, considering the extent of the endothelial injury: life-threatening hemorrhages and vaso-occlusive thrombi leading to infarcts do not occur often, even in fatal rickettsioses. Even though hemorrhagic and thrombotic complications are not common, the presence of thrombin might play a role in increased vascular permeability, as has been demonstrated by in vitro experiments using endothelial cell monolayers.

Serial investigations of the coagulation system in a mouse model of lethal spotted fever rickettsiosis revealed severe multifocal endothelial injury, increased generation of thrombin, decreased levels of factor VII, increased factor V procoagulant activity, decreased prekallikrein and tissue plasminogen activator activity, and increased activity of plasminogen activator inhibitor without disseminated intravascular coagulation [25]. Mice with sublethal infection manifested an acute-phase reaction (e.g., increased plasma fibrinogen) and release of endothelial cell contents (e.g., von Willebrand factor), but without apparent activation of the coagulation system in the face of marked inhibition of fibrinolysis driven by endothelial cells. Neither lethal nor sublethal infection resulted in uncontrolled pathologic thrombosis or consumption of clotting factors.

MECHANISMS OF IMMUNITY TO RICKETTSIAE

Among the most interesting aspects of the pathogenesis of rickettsial infections are the host defenses. Studies of excellent murine models of spotted fever and typhus group rickettsioses have identified novel mechanisms of immunity, including cytokine-mediated activation of endothelial cell bactericidal control of intracellular infection and the role of autophagy in rickettsial killing. Murine endothelial cells activated by IFN-γ and TNF-α produce rickettsicidal nitric oxide via inducible nitric oxide synthetase [26]. Early in rickettsial infections, natural killer cells are activated and inhibit growth of rickettsiae in association with production of IFN-γ. Clearance of rickettsiae requires cytotoxic CD8 T cells, which eliminate infected endothelial cells by inducing apoptosis that is dependent, at least in part, on a perforin-mediated mechanism. Antibodies against rickettsial OmpA and OmpB, but not rickettsial lipopolysaccharide, are protective against reinfection [27, 28]. However,
antibodies to these proteins do not appear until after control of the rickettsial infection and recovery from the disease have occurred. Thus, antibodies do not play an important role in immunity during the first exposure to a pathogenic rickettsia.

Both immune CD4 and CD8 T cells contribute to protective immunity, and homing of lymphocytes and macrophages to foci of infection in the microcirculation is associated with clearance of rickettsiae [26]. Expression of the chemokines CXCL9 (Mig), CXCL19 (IP-10), and CXCCL1 (fractalkine) in rickettsia-infected murine endothelial cells and of CXCL9 and CXCL10 in cerebral endothelial cells from patients with Rocky Mountain spotted fever has not been mechanistically linked to immune cell chemotaxis to rickettsia-infected endothelium [29]. Expression of intercellular adhesion molecule–1 and vascular endothelial cell adhesion molecule–1 and of rickettsial antigens on infected endothelial cells could contribute to perivascular migration of T cells. Perivascular CD4 and CD8 T cells, macrophages, and dendritic cells are presumed to be the sources of the cytokines that activate endothelial rickettsicidal activities. Human endothelial cells activated by IFN-γ, TNF-α, IL-1β, and RANTES (regulated on activation, normally T cell expressed and secreted) kill intracellular rickettsiae through 2 bactericidal mechanisms, nitric oxide production and hydrogen peroxide production [26]. Human macrophages, a minor target of rickettsial infections, kill intracellular rickettsiae after activation by IFN-γ, TNF-α, and IL-1β via production of hydrogen peroxide and tryptophan starvation of rickettsiae associated with degradation of tryptophan by indoleamine-2,3-dioxygenase. The relevance of these bactericidal mechanisms to human rickettsioses is supported by the expression of inducible nitric oxide synthetase, IFN-γ, and indoleamine-2,3-dioxygenase in the skin lesions of patients with Mediterranean spotted fever.

The balance between the susceptibility of the host to pathogenic mechanisms and resistance to rickettsial growth is expressed as host risk factors for severity of illness. For humans, these factors include older age, glucose-6-phosphate dehydrogenase deficiency, treatment with a sulfonamide, diabetes mellitus, and male sex. Experimental infections have confirmed that older male animals are killed by lower median doses of rickettsiae. The underdetermined importance of immune responses and of oxidative stress in human rickettsioses emphasizes the unfortunate neglect of these life-threatening infections by infectious diseases physician-scientists.

The statements of Ted Woodward in his Maxwell Finland Lecture of 1972 regarding the need for scientists and physicians to enter the field of rickettsiology played a role in my choice of rickettsial diseases as a career focus [30]. Dr. Woodward’s gift to me of the 1922 classic The Etiology and Pathology of Typhus, written by Wolbach, Todd, and Palfrey and containing the signature of S. Burt Wolbach, is the most cherished volume in my library [31]. The studies of rickettsioses advanced by both Wolbach and Woodward remain unfinished.

Acknowledgments

I thank Doris Baker and Sherrill Hebert for expert secretarial assistance and Aaron Medina-Sanchez and Steve Schuenke for their expert preparation of the figures.

Financial support. National Institutes of Health (grant AI21242).

Supplement sponsorship. This article was published as part of a supplement entitled “Tribute to Ted Woodward,” sponsored by an unrestricted grant from Cubist Pharmaceuticals and a donation from John G. McCormick of McCormick & Company, Hunt Valley, Maryland.


References