38
Molecular Pathology of Rickettsial Lung Infections

J. Stephen Dumler

Introduction

Rickettsial infections of humans comprise a diverse group of infections caused by pathogens that are obligate intracellular bacteria with a genetic relationship, including the genera *Rickettsia*, *Orientia*, *Ehrlichia*, and *Anaplasma*. The host cells of these pathogens largely belie the systemic clinical manifestations, because *Rickettsia* and *Orientia* infect endothelial cells, and *Ehrlichia* and *Anaplasma* infect circulating leukocytes (monocytes and neutrophils, respectively). Thus, the predominant manifestations (fever, headache, myalgia, with or without rash) do not usually focus attention on the respiratory system; however, the underlying pathogenesis of these infections involves degrees of vascular compromise either by direct injury and inflammation or by the action of vasoactive proinflammatory molecules such as cytokines, chemokines, and prostaglandins. Given that the lung possesses the largest vascular bed in the human body, it is not surprising that pulmonary involvement is periodically identified and, when severely affected, is considered a potentially life-threatening complication.[1,2]

The precise microbial molecular pathogenetic mechanisms and the relative contributions of molecular proinflammatory responses toward pulmonary infection with these pathogens are in general poorly understood. However, recent years have seen significant advances in understanding the principles of how these obligate intracellular pathogens interact with host cells to exert direct influence over cellular function and integrity and how the host immune system responds. The main purposes of this chapter are to briefly describe the histopathologic and pathophysiologic alterations observed with two major rickettsial infections that impact the lung, Rocky Mountain spotted fever (*Rickettsia rickettsii*) and human monocytic ehrlichiosis (*Ehrlichia chaffeensis*) and to describe the molecular basis of cellular alterations in the host and pathogen virulence mechanisms that belie the pulmonary pathology with these diseases.

Rickettsial Infections That Impact Lung Structure and Function

The order Rickettsiales is divided into two major families, Rickettsiaceae and Anaplasmataceae. The Rickettsiaceae family includes the genera *Rickettsia* and *Orientia*, obligate intracellular vasculotropic bacteria that live and propagate within the cytoplasmic compartments of endothelial cells in mammalian hosts. In contrast, genera within the family Anaplasmataceae include *Ehrlichia* and *Anaplasma*, which infect leukocytes, including those circulating in the bloodstream. Among these genera, important human infections that impact lung function and structure include the vasculotropic rickettsioses such as Rocky Mountain spotted fever (RMSF), epidemic typhus, and murine typhus, among other entities bearing geographic names. The major underlying theme of these is endothelial cell infection followed by vasculitis and increased vascular permeability.[3,4] Systemically, this leads to hypotension, organ ischemia and failure, and sometimes death. In the lung, this translates into potentially significant noncardiogenic pulmonary edema that can be life threatening.[3] Fundamental differences exist between spotted fever group rickettsiae, such as *R. rickettsii* that causes RMSF, and the typhus group rickettsiae, such as *Rickettsia prowazekii* and *Rickettsia typhi* that cause epidemic and murine typhus, respectively.[5] The molecular pathogenesis of typhus group infections is less well understood; thus, this chapter focuses in part on RMSF as an example of rickettsial pneumonitis.

In contrast, the Anaplasmataceae are known to infect predominantly circulating leukocytes in mammals, and, by virtue of this host cell niche, histopathologic vasculitis is not a significant component of *Ehrlichia* or *Anaplasma* infections in humans.[6] However, infections by *E. chaffeensis*, the cause of human monocytic ehrlichiosis, can present with a clinical picture similar to vasculitis, and this is now

believed to be due to the local or systemic release of proinflammatory vasoactive cytokines that impair endothelial integrity and lead to increased vascular permeability as observed in vasculitis.[7,8] Of those Anaplasmataceae that are significant causes of human disease, *E. chaffeensis* is the most frequent cause of infection in which lung structure and function are impaired and thus is the other main topic of this chapter.

Rocky Mountain Spotted Fever

Clinical Disease and Pathophysiology

Rocky Mountain spotted fever is an acute febrile illness that results after transmission of *R. rickettsii* into a human or animal host after the bite of competent vector ticks. The infection is limited to the Western hemisphere, but infections by related spotted fever group rickettsiae are documented on every continent except Antarctica.[9] After tick bite, the rickettsiae usually disseminate within hours to days via the blood or lymphatics.[10] These obligate intracellular bacteria attach to host endothelial cells in which they become internalized, escape the endocytic vacuole, and propagate within the cytosol.[1] During this process, endothelial cell functions are altered, leading to proinflammatory and procoagulant conditions that favor leukocyte infiltration, focal thrombus formation and increase in vascular permeability.[11–13] Endothelial cells either release the rickettsiae into the bloodstream or the infected cell is lysed, also releasing bacteria for hematogenous spread into all organs and tissues, including the lung. In the extensive microvasculature of the lung, *R. rickettsii* infection may be widely spread.[3] Host inflammatory response, usually manifest as some combination of interstitial mononuclear cell pneumonitis and edema, is observed in histopathologic preparations, leading to the occasional interstitial infiltrative pattern observed with chest x-rays in RMSF.[14] The fact that pulmonary vasculature permeability increases at a time when multiorgan failure and hypotension are also observed sometimes leads to overly aggressive fluid therapy that can precipitate or aggravate pulmonary edema. Prompt treatment with doxycycline can arrest and reverse many clinical manifestations, indicating that much of the inflammatory process is initiated and maintained by the bacterium.

Early Events in the *Rickettsia*–Endothelial Cell Interaction

The molecular mechanisms by which infection of endothelial cells results in vasculitis and altered vascular permeability in the lung is an area of active study. Initially, bacteria that are inoculated into the host replicate locally and then disseminate into the lymphatics.[10] Thereafter, the rickettsiae enter the bloodstream and interact with endothelial cells in the microvasculature of many organs including the lung. The initial rickettsia–endothelial cell interface is a receptor–ligand mediated event, dependent on rickettsial expression of outer membrane proteins A and B (OmpA and OmpB).[15–17] The host cell ligand for OmpA, only found in the spotted fever group rickettsiae, is not known. However, for both the spotted fever group rickettsiae *Rickettsia japonica* and *Rickettsia conorii*, OmpB ligation occurs via binding to host cell Ku70 that is recruited to host membrane lipid microdomains.[15] After OmpB binding, Ku70 is ubiquitinated by the protein tyrosine kinase adaptor protein Cbl, an event linked to internalization of the rickettsia-containing endosome because its inhibition blocks *R. conorii* entry.

Similarly critical for internalization of rickettsiae is the recruitment of Arp2/3, c-Src, and p80/85 cortactin to binding sites that leads to localized actin cytoskeletal rearrangements in part mediated by Cdc42, phosphatidylinositol-3 kinase, and the Src family of kinases.[18] Spotted fever group rickettsiae also express RickA on one pole of the bacterium, a protein that mediates actin polymerization via Arp2/3 complex assembly, effectively creating an intracellular scaffold that allows propulsion through the host cell and occasionally through host membranes.[19]

On entry, the endosomal membrane that contains the rickettsiae is rapidly degraded, presumably by the action of rickettsial phospholipase D or membrane hemolysin TlyC that are actively expressed during this interval.[20,21] Rickettsial genomes encode an intact tricarboxylic acid cycle, but otherwise have only a limited capacity for energy generation, lacking genes for enzymes for carbohydrate, lipid, nucleotide, and amino acid metabolism.[5] These observations and the demonstration of active adenosine triphosphate/adenosine diphosphate translocases establish the concept of rickettsiae as energy parasites. The presence of an intact pathway for a type IV secretion mechanism underscores the potential importance of transporting rickettsial proteins into the host cytosol.[5]

Cellular and Tissue Injury

Rickettsia rickettsii infection of endothelial cells leads to membrane injury that can be antagonized by antioxidants, and this membrane injury leads to loss of cellular osmoregulation and eventually cell lysis, even in the absence of large organism loads.[22,23] The degree of membrane injury in typhus group infections is much less substantial, and cytolysis is generally believed to be mechanical owing to the accumulation of large bacterial quantities within infected cells.[1]

Endothelial cells infected by *R. rickettsii* undergo a number of transcriptional changes, including upregulation

of proinflammatory cytokines and chemokines (interleukin [IL]-1α, IL-6, IL-8, monocyte chemoattractant protein 1), surface procoagulant activity and tissue factor expression, E-selectin upregulation, and release of von Willebrand factor multimers.[7,11,12,24,25] The net result of the intracellular infection is an increased proinflammatory and procoagulant endothelial cell phenotype. These changes are mediated in part by direct rickettsial activation and nuclear mobilization of nuclear factor (NF)-κB via a mechanism involving activation of inhibitory-κB kinase α and β and phosphorylation-proteolysis of the inhibitor protein IκBα.[26,27] This phenomenon is abrogated by the bacterial protein synthesis inhibitor doxycycline, implying a contribution of bacterial proteins toward NF-κB proinflammatory gene activation. The triggers for the transcriptional alterations are not completely defined but appear to involve interactions with protein kinase C isoforms, and are modulated in part by p38 mitogen-activated protein (MAP) kinase.[28,29] Interestingly, this process also inhibits apoptosis, prolonging survival of infected cells, an obvious advantage for the bacterium. Intracellular rickettsial infection also yields upregulated expression of heme oxygenase 1, a host defense against oxidative injury and a critical regulator of the cyclooxygenases, including cyclooxygenase-2, that are upregulated with rickettsial infection and is a key enzyme that governs prostaglandin production, increased release of prostaglandins I_2 and E_2, and, indirectly, vascular tone and integrity.[13]

The upregulated presence of E-selectin and procoagulant molecules on infected endothelial cells promotes inflammation and focal thrombosis, features considered typical of rickettsial vasculitis.[11,12] Although large-vessel thrombosis is atypical for RMSF, fibrin clots are not infrequently detected as eccentrically localized lesions among vessels in which only focal infection is demonstrated (Figure 38.1). When rickettsial infection occurs within the confines of the pulmonary parenchyma, capillaries are the dominant vascular structure and support the heaviest burden of rickettsiae.[3] As a consequence of infection in these small-caliber vessels, interstitial inflammatory cell infiltration is the dominant feature and appears as widened, hypercellular alveolar septae, some of which may be edematous; capillaries that are dispersed within the alveolar septae are often surrounded by mononuclear cells, chiefly lymphocytes and macrophages (Figure 38.2).

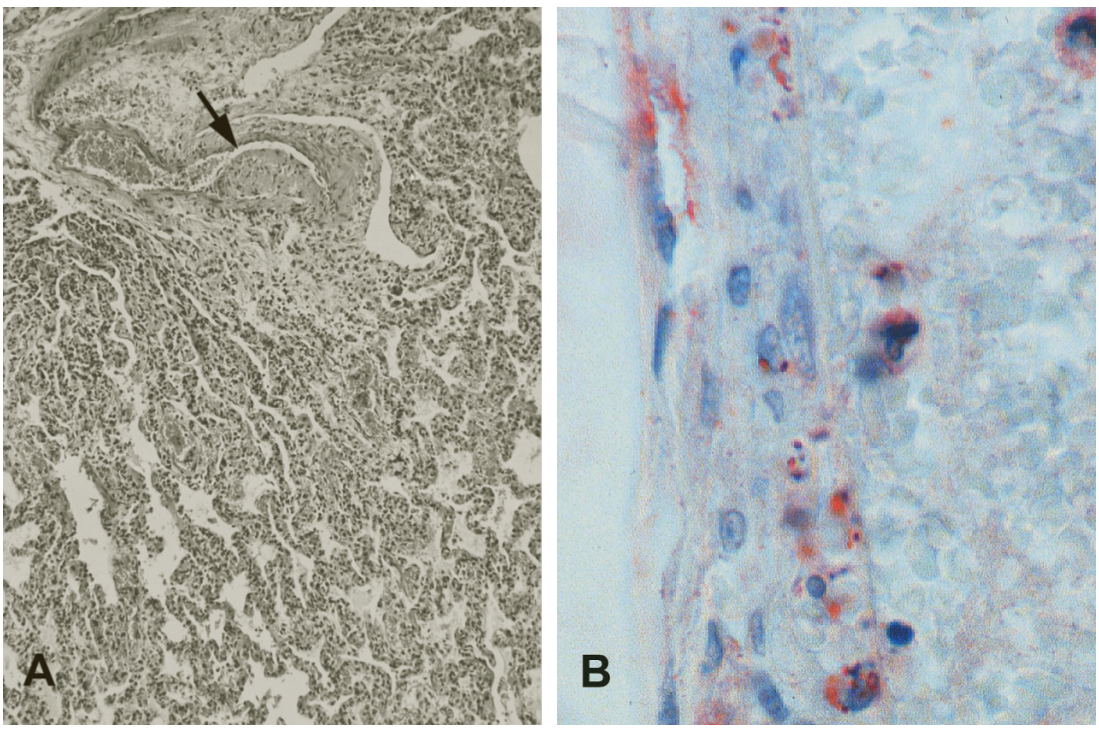

FIGURE 38.1. **(A)** *Rickettsia rickettsii* vasculitis with eccentric microthrombus (arrow) in pulmonary venule and diffuse interstitial pneumonitis. (Hematoxylin and eosin; original magnification, ×16.) **(B)** Note the intracellular distribution of infected endothelial cells along a venule as demonstrated by immunohistochemistry. (Anti-*Rickettsia rickettsii* with hematoxylin counterstain; original magnification, ×260.)

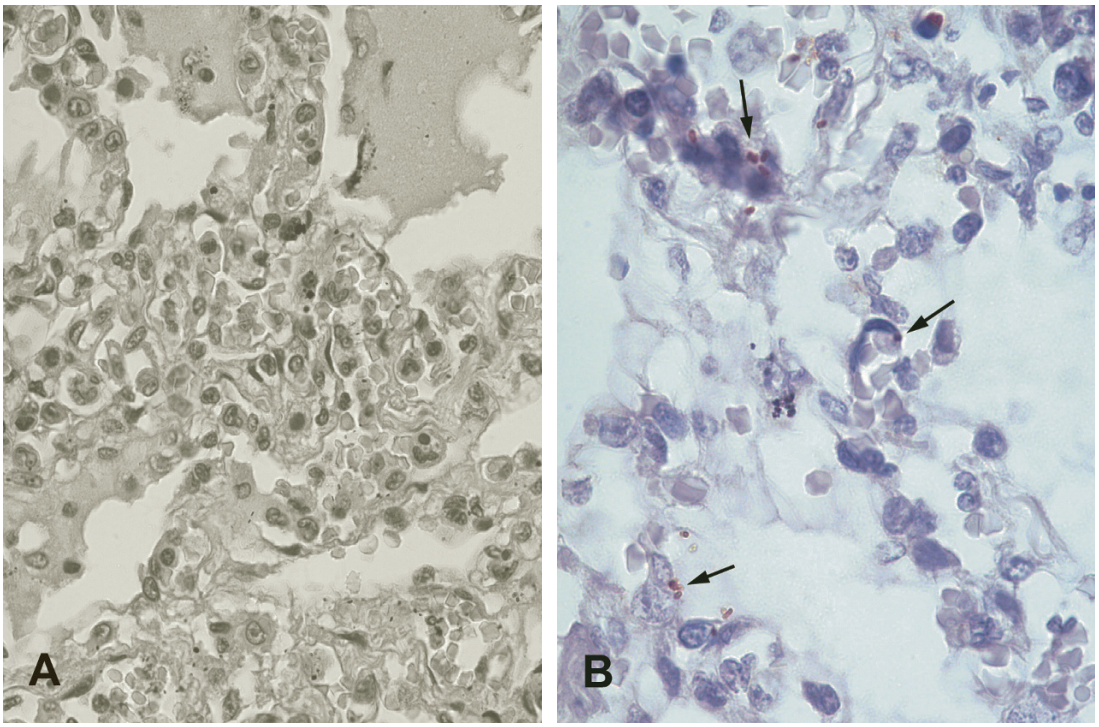

FIGURE 38.2. **(A)** Interstitial pneumonitis and capillaritis in Rocky Mountain spotted fever. (Hematoxylin and eosin; original magnification, ×160.) **(B)** Immunohistochemistry for *Rickettsia rickettsii* demonstrates the presence of small bacilli (arrows) within endothelial cells of capillaries in alveolar interstitial spaces. (Hematoxylin counterstain; original magnification, ×260.)

Host Innate and Adaptive Immune Responses to Infection

Host response to rickettsial infection is dominated by the infiltration of tissues and vessel walls by lymphocytes and macrophages. Immunophenotyping methods of rickettsial inflammatory lesions have identified a polymorphous mixture of CD4 and CD8 T lymphocytes, admixed with scattered B lymphocytes, macrophages, and occasional neutrophils.[1,30] Infection by rickettsiae leads to substantial chemokine production by endothelial cells, including IL-8, monocyte chemoattractant protein 1, and fractalkine (CX3CL1), and it is likely that these signals in part recruit and retain inflammatory cells and primed immune cells that participate in the localized tissue vasculitis.[30] As anticipated, effective antirickettsial immunity is predominantly dependent on cellular immunity, especially on expansion of adaptive immunity via CD4 and CD8 cells that produce interferon (IFN)-γ and mediate cytotoxic responses. However, recent investigations provide evidence that antibody plays a more important role than previously ascribed.[30]

The inflammatory and immune response to the presence of rickettsiae in cells is at first heralded by expansion of natural killer cell populations, and depletion of these cells allows greater rickettsial propagation and reduced production of suppressive IFN-γ. Depletion of both CD4 and CD8 T lymphocytes also adversely impacts survival in murine models, and the most dramatic effect implicates a role for major histocompatibility complex I–mediated, perforin-dependent adaptive immune responses.[1,30] The molecular mechanism of rickettsial restriction by immune cells appears to be dependent on the synergistic effects of IFN-γ, tumor necrosis factor (TNF)-α, IL-1β, and CCL5 produced from natural killer cells, CD8 T cells, and macrophages; critical effectors include both nitric oxide and hydrogen peroxide, accentuated by tryptophan starvation after enhanced host cell degradation of this amino acid essential for bacterial propagation. Interestingly, there appears to be a critical balance between beneficial and deleterious immune responses, because adoptive transfer of immune CD8 T cells into rickettsia-infected naïve animals accelerates death if introduced during early phases of infection.[1]

In the context of RMSF lung involvement, it is likely that many of the events are simultaneously occurring, with outcome dependent on degree of pulmonary microvascular infection and compromise and the degree to which a rapid and protective immune response is induced.[1,30] Fundamental studies have identified several novel targets for intervention at the level of the bacterium (inhibition of OmpB–Ku70 interaction,

inhibition of type IV secretion system activity, inhibition of phospholipase D or hemolysin activity) and at the level of the host (inhibition of ubiquitination of Ku70, inhibition of induced actin cytoskeletal rearrangements or signaling via protein kinase C, p38 MAP kinase, cyclooxygenase-2, and NF-κB nuclear translocation).

Diagnosis

When involvement of the lung by RMSF becomes evident, it is usually during the course of increasing decompensation, hypotension, and multiorgan failure. Diagnosis of the infection at this interval is often too late to prevent significant morbidity, long-term sequelae, or death, prompting physicians to have a low threshold for empirical doxycycline therapy at earlier times. Rocky Mountain spotted fever is best diagnosed during the active phase of infection by skin biopsy of petechial lesions followed by immunohistochemical or in situ hybridization demonstration of *R. rickettsii* in the tissue.[31] Antibodies are infrequently present during active infection, but demonstration of seroconversion or a fourfold titer change in convalescence can retrospectively confirm a clinical diagnosis. Molecular diagnostic methods are less often used and generally focus on polymerase chain reaction (PCR) amplification of *R. rickettsii* nucleic acids from whole blood samples obtained during the active phase of infection, although this is not currently considered highly sensitive.[32] Additional data suggest that PCR on freshly obtained skin biopsies or other tissues may also work well because the rickettsiae live predominantly within tissue-bound endothelial cells.[33] Immunohistochemistry is often used to establish a postmortem diagnosis, and PCR methods should be excellent adjuncts to this approach.[34]

Human Monocytic Ehrlichiosis

Clinical Disease and Pathophysiology

Human monocytic ehrlichiosis (HME) is a febrile illness with many similarities to rickettsial infections such as RMSF.[35] Some authors have used the terminology "spotless" spotted fever to indicate the clinical and historical similarity of HME to RMSF. After tick bite, *E. chaffeensis* gains access to the blood and may be visualized in peripheral blood smears from some patients after an incubation period of 7–10 days. The infection has been well-characterized to occur only in North America, although increasing evidence suggests that the pathogen and infection may be worldwide in distribution.[35]

Unlike the situation for *R. rickettsii*, *E. chaffeensis* infects almost exclusively mononuclear phagocytes in both tissues and blood. It has been detected in blood, bone marrow, lymph node, liver, spleen, and many tissues and organs that possess mononuclear phagocyte populations or acquire these cells via inflammatory cell infiltration.[2,6] Presumably, *E. chaffeensis* infects mononuclear phagocytes at the site of tick bite or passes via lymphatics to draining lymph nodes where initial infection occurs. Once *E. chaffeensis* attaches to and enters the mononuclear phagocyte, it accumulates in an endosomal vacuole that is arrested in maturation at the early endosome stage.[36] The bacteria replicate within this vacuole to form an intracytoplasmic inclusion called a morula that is occasionally visualized in peripheral blood monocytes on Romanowsky-stained blood smears. The infected cells undergo substantial alterations in function that presumably diminish innate and adaptive immune recognition and response; however, cells that manage to ingest *E. chaffeensis* via opsonophagocytosis generate considerable proinflammatory cytokine responses that could drive the underlying inflammatory cell infiltration and tissue necrosis observed in some cases.[2,37] This response does not occur via typical lipopolysaccharide or peptidoglycan-mediated Toll-like receptor signaling because *E. chaffeensis*, like other Anaplasmataceae, lacks biosynthetic pathways for both of these bacterial components.[38] The majority of clinical infections present with fever and myalgias accompanied by thrombocytopenia, leukopenia, anemia, and evidence of mild to moderate hepatic injury supported by elevated liver transaminase activities in serum.[2,35]

Although *E. chaffeensis* has no known predilection for the respiratory system, when circulating mononuclear phagocytes become activated for proinflammatory function during passage through the pulmonary microvasculature, the result would be increased vascular permeability and inflammatory cell infiltration of the interstitial spaces—the typical interstitial pneumonitis observed with HME in severe cases.[6,39,40] Infection can precede diffuse alveolar damage (Figure 38.3), even in the absence of large numbers of bacteria and a vigorous inflammatory cell infiltrate. In advanced stages, lung involvement can appear as macrophage-rich intraalveolar infiltrates, again absent substantial quantities of bacteria (Figure 38.4).[6] The clinical and histopathologic sequelae of lung involvement can take days or weeks to resolve. This process often occurs in the context of systemic inflammatory response with fulminant *E. chaffeensis* infections in patients with preexisting immune compromise such as with human immunodeficiency virus and immune suppression for organ transplantation or for autoimmune diseases.[2,41,42] Very often the presentation is septic-like or toxic shock–like and includes a component of acute respiratory distress syndrome.[39,43,44] Despite clinical similarities to RMSF and vasculitis, histopathologic investigations do not provide any support for vasculitis as a component

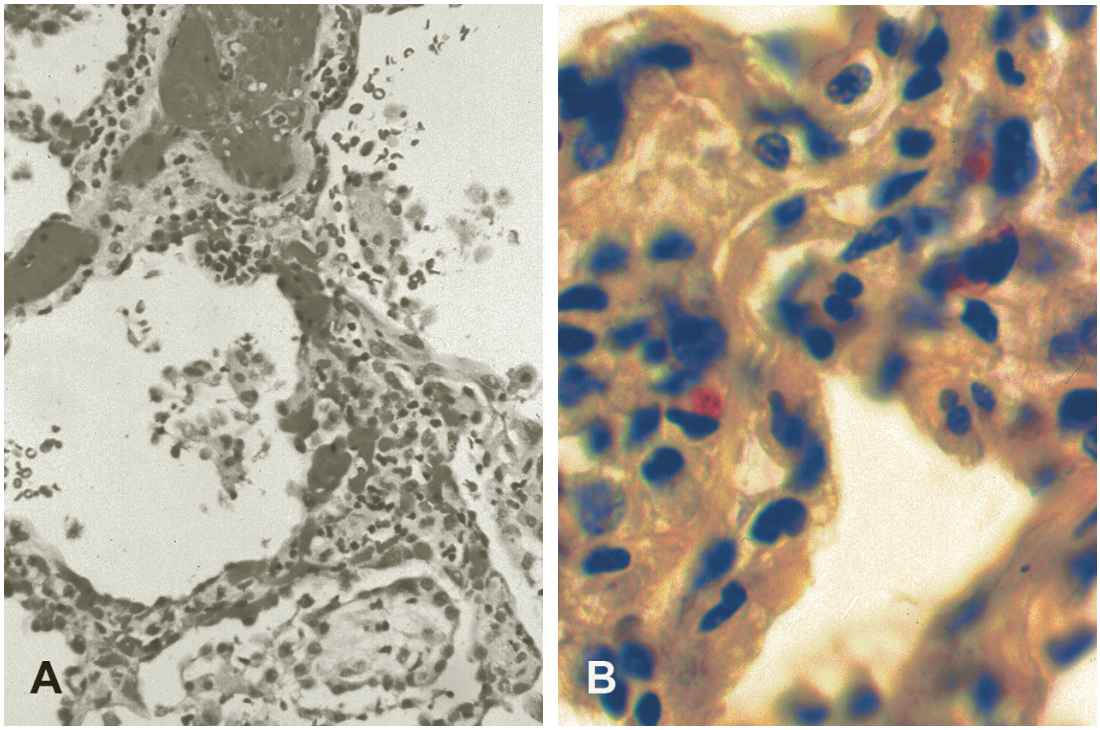

FIGURE 38.3. **(A)** *Ehrlichia chaffeensis*–induced interstitial pneumonitis accompanied by diffuse alveolar damage. (Hematoxylin and eosin; original magnification, ×80.) **(B)** Ordinarily, *E. chaffeensis* is infrequently found, except in patients with underlying immunocompromise. (*Ehrlichia chaffeensis* immunohistochemistry with hematoxylin counterstain; original magnification, ×400.)

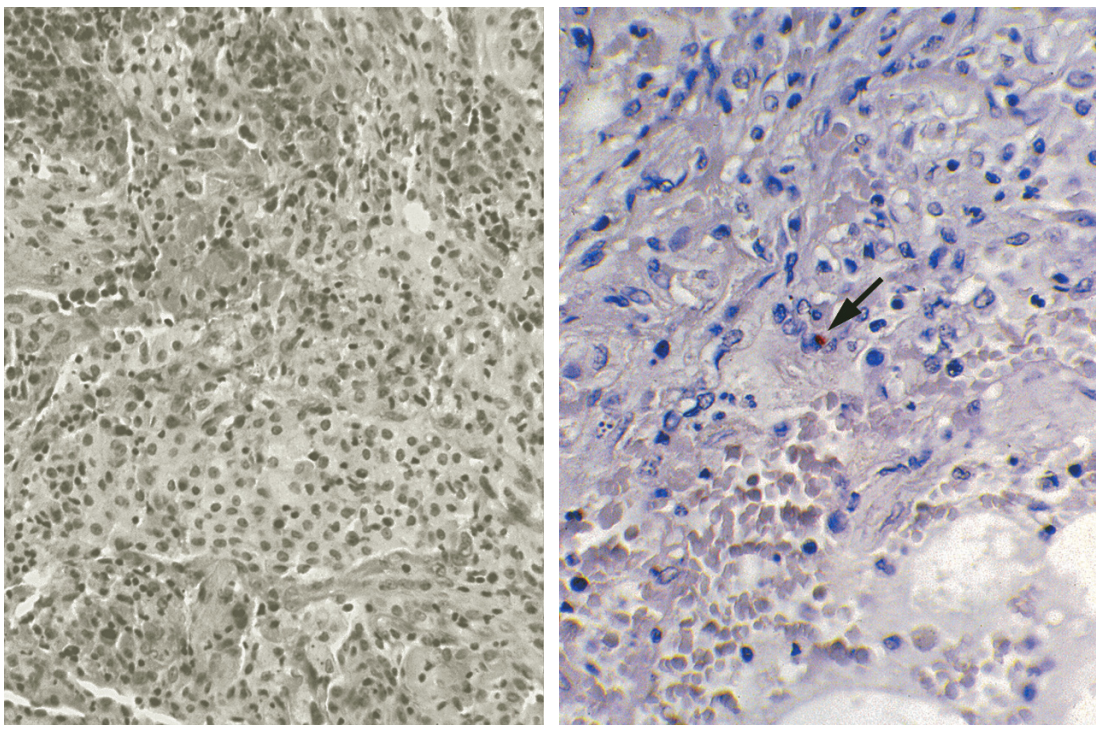

FIGURE 38.4. **(A)** *Ehrlichia chaffeensis*–induced macrophage-rich alveolar infiltrates and resolving diffuse alveolar damage in human monocytic ehrlichiosis. (Hematoxylin and eosin; original magnification, ×40.) **(B)** The tissue injury is usually greatly out of proportion to the bacterial load. (*Ehrlichia chaffeensis* immunohistochemistry with hematoxylin counterstain; original magnification, ×80.)

of HME.[6,41] Although a rapid clinical response is often demonstrated even after 1 to 2 days of doxycycline treatment, given the mononuclear phagocyte niche, it is difficult to explain pancytopenia and hepatic or pulmonary injury based on direct bacterial injury.[2] Most data now support a role for *E. chaffeensis* triggering of host proinflammatory response as a major pathogenetic feature in HME.[8,45]

Early Events in the *Ehrlichia*–Mononuclear Phagocyte Interaction

During the initial encounter with a mononuclear phagocyte, *E. chaffeensis* binds to E- and L-selectins probably via the bacterial membrane glycoprotein gp120.[46,47] Interestingly, there are two morphologic forms of *E. chaffeensis*, analogous to the situation with the Chlamydiae—a lower metabolic activity dense core form that expresses the gp120 adhesin and a more substantially metabolic reticulate form that expresses lower gp120 quantities and undergoes active binary fission.[47] The initial internalization leads to interactions via glycosylphosphatidylinositol-anchored proteins and caveolin in host cell membrane lipid rafts that is followed by intracellular calcium fluxes and changes in tyrosine phosphorylation, activation of phospholipase Cγ2, and inositol 1,4,5-triphophate production.[48] The emerging parasitophorous vacuoles accumulate only early endosomal markers, including an increasing amount of transferrin receptor, a characteristic of recycling endosomes diverted from the phagosome–lysosome fusion pathway.[36] Likewise, *E. chaffeensis*–infected THP-1 cells show downregulated transcription of *RAB5A*, *SNAP23*, and *STX16*, critical components of vesicular transport and fusogenic events.[49]

Intracellular entry also occurs in the absence of significant proinflammatory cell activation and triggering.[37] Although pretreatment of macrophages with IFN-γ leads to restriction of ehrlichial growth, infection is associated with inhibition of IFN-γ–inducing pathways such as JAK/STAT, and induction of important cytokines for maturation of Th1 and immune responses, such as IL-12, IL-15, IL-18, Toll-like receptors 2 and 3, and CD14.[48,49] The IFN-γ–mediated restriction occurs via its action in reducing expression of transferrin receptors, thereby reducing accessible free iron for bacterial growth, because *E. chaffeensis* lacks effective siderophores.[48] Similar to *R. rickettsii*, *E. chaffeensis* infection inhibits apoptosis of infected cells, presumably via its action on transcription of cell cycle proteins and inhibition of NF-κB activation.[48,49]

Cellular and Tissue Injury

Cytolysis of cells infected by *E. chaffeensis* in vitro is the usual outcome. However, most in vivo examinations demonstrate only meager quantities of bacteria, out of proportion to the degree of histopathologic injury, cytopenias, and hepatic injury in nonfulminant cases.[2] Because no good animal model of *E. chaffeensis* infection exists, data extrapolated from murine models of infection by related *Ehrlichia* species provides additional evidence that most tissue injury results from the induction of aberrant and dysfunctional immune responses.[8] Humans infected with *E. chaffeensis* have a marked expansion of CD8 T lymphocytes in lymph nodes and presumably other tissues, and this feature is associated with a high frequency of hemophagocytic macrophages, suggesting activation of macrophages as a component of the pathogenesis.[50] Similarly, a dose- and route-dependent induction of CD8 T lymphocyte overproduction of TNF-α has been implicated as a mechanism of severe tissue injury in murine models.[8,45] Infection of TNF receptor–deficient mice substantially abrogates manifestations of shock in murine models, yet depletion of TNF-α from mice does not alter shock manifestations.[51] In contrast, at least one severe infection in a human occurred while receiving the TNF-α inhibitor etanercept, seeming to contradict the murine model data.[52] Despite these advances, little investigation of the specific effectors of tissue injury, whether immunologic or not, has been conducted.

Host Innate and Adaptive Immune Responses to Infection

Ehrlichia chaffeensis subverts many innate immune responses via interactions with nonopsonophagocytic macrophage receptors (L-selectin or E-Selectin) and by its early downregulation of inflammation- and immune-inducing signals, receptors, and signaling pathways.[48,49] Classic cellular immune pathways appear important for restriction of *E. chaffeensis* infection.[53] In mice that ordinarily are not susceptible, infection persists when devoid of Toll-like receptor 4 and major histocompatibility complex II.[54,55] Murine models of monocytic ehrlichiosis generally employ the related species *Ehrlichia muris* or an *Ehrlichia* species isolated from *Ixodes ovatus* ticks.[8,56,57] In this model, infection and severity are decreased with low infectious doses and intradermal inoculation, and resistance to challenge depends on CD4 but not CD8 T lymphocytes and requires IL-12, IFN-γ, and TNF-α but not IL-4.[8,45,51,58] Likewise, intact adaptive immunity is critical because severe combined immunodeficiency mice cannot resist infection.[53,59] Despite the evidence that cellular immunity, chiefly Th1-polarized responses, are critical for control of *Ehrlichia* infections, a role for antibody has also been demonstrated with passive immunization using anti-*E. chaffeensis* or *Ehrlichia* monoclonal antibodies or with infection of animals devoid of Fc receptors or effector pathways such as phagocyte oxidase (gp91^{phox} knockout mice).[53,57,60–62]

In the context of HME lung involvement, it is most likely that host immune response is a critical determinant of pathologic injury and outcome. The tissue injury that is disproportionate to bacterial load and the lack of any direct evidence of *Ehrlichia*-mediated cellular injury argue that the predominant pathologic force is an overly aggressive or misdirected host inflammatory or immune response triggered by active infection.[2,41,45,50] Why some infections are asymptomatic yet others are fatal is not understood, although studies with mouse models are yielding important clues regarding infectious load and genetic background.[8,53,58] If pathologic injury is driven primarily by host response, the most prudent approach as a supplement to antimicrobial treatment involves strategies to dampen overly aggressive production of proinflammatory cytokines or strategies that dampen vigorous Th1 responses culminating in excessive macrophage activation. That fulminant infection occurs with defects in T-cell immunity dictates that this approach must be carefully evaluated and implemented with great caution.[6,41,42,45,52] Molecular tools that could interfere with *Ehrlichia*-mediated host transcriptional changes or that interfere with *Ehrlichia*-initiated signal transduction events and apoptosis delay might provide adjunctive treatments, especially among immunocompromised patients with fulminant infections.[2,49]

Diagnosis

Approximately 20% of patients with HME demonstrate cough or other respiratory manifestations; however, significant respiratory disease is relatively infrequent, presenting as acute respiratory distress syndrome, and usually accompanied by other severe systemic manifestations such as multiorgan failure, a shock syndrome, and meningoencephalitis.[2,41] Even at this late stage, a diagnosis may prevent and reverse adverse outcomes by prompting doxycycline treatment. Although examination of peripheral blood smears will identify bacterial inclusion vacuoles in circulating monocytes in less than 10% of cases, and antibodies will be present in a small minority of infected persons, approximately 60% or more will have *E. chaffeensis* DNA demonstrable in peripheral blood by PCR.[2,52] Tissue examination by immunohistochemistry and in situ hybridization is useful in some cases, although these methods may lack sensitivity.[63,64] Polymerase chain reaction has also been applied as a diagnostic tool for *E. chaffeensis* on tissues including lung, although no careful evaluation of this approach has been conducted.

Conclusion

Many current advances in understanding the molecular pathogenesis of RMSF and HME have been facilitated by the availability and improved annotations of rickettsial genomes; however, these efforts continue to be undermined by the lack of effective gene ablation methods for these pathogens.[5,65–67] In time even this research bottleneck will be circumvented, and rickettsial organisms will release their unique secrets for the molecular and cellular perturbations that allow their intracellular survival and pathogenicity.

References

1. Walker DH, Valbuena GA, Olano JP. Pathogenic mechanisms of diseases caused by *Rickettsia*. Ann NY Acad Sci 2003;990:1–11.
2. Dumler JS. Anaplasma and ehrlichia infection. Ann NY Acad Sci 2005;1063:361–373.
3. Walker DH, Crawford CG, Cain BG. Rickettsial infection of the pulmonary microcirculation: the basis for interstitial pneumonitis in Rocky Mountain spotted fever. Hum Pathol 1980;11:263–272.
4. Walker DH, Mattern WD. Rickettsial vasculitis. Am Heart J 1980;100:896–906.
5. Walker DH, Yu XJ. Progress in rickettsial genome analysis from pioneering of *Rickettsia prowazekii* to the recent *Rickettsia typhi*. Ann NY Acad Sci 2005;1063:13–25.
6. Walker DH, Dumler JS. Human monocytic and granulocytic ehrlichioses. Discovery and diagnosis of emerging tick-borne infections and the critical role of the pathologist. Arch Pathol Lab Med 1997;121:785–791.
7. Sporn LA, Marder VJ. Interleukin-1 alpha production during *Rickettsia rickettsii* infection of cultured endothelial cells: potential role in autocrine cell stimulation. Infect Immun 1996;64:1609–1613.
8. Ismail N, Soong L, McBride JW, et al. Overproduction of TNF-alpha by $CD8^+$ type 1 cells and down-regulation of IFN-gamma production by $CD4^+$ Th1 cells contribute to toxic shock-like syndrome in an animal model of fatal monocytotropic ehrlichiosis. J Immunol 2004;172:1786–1800.
9. Walker DH. Rickettsioses of the spotted fever group around the world. J Dermatol 1989;16:169–177.
10. Murphy JR, Wisseman CL Jr, Fiset P. Mechanisms of immunity in typhus infection: some characteristics of *Rickettsia mooseri* infection of guinea pigs. Infect Immun 1978;21:417–424.
11. Shi RJ, Simpson-Haidaris PJ, Lerner NB, et al. Transcriptional regulation of endothelial cell tissue factor expression during *Rickettsia rickettsii* infection: involvement of the transcription factor NF-kappaB. Infect Immun 1998;66:1070–1075.
12. Sporn LA, Lawrence SO, Silverman DJ, Marder VJ. E-selectin-dependent neutrophil adhesion to *Rickettsia rickettsii*–infected endothelial cells. Blood 1993;81:2406–2412.
13. Sahni SK, Rydkina E, Sahni A, et al. Potential roles for regulatory oxygenases in rickettsial pathogenesis. Ann NY Acad Sci 2005;1063:207–214.
14. McCook TA, Briley C, Ravin CE. Roentgenographic abnormalities in Rocky Mountain spotted fever. South Med J 1982;75:156–157.
15. Martinez JJ, Seveau S, Veiga E, et al. Ku70, a component of DNA-dependent protein kinase, is a mammalian

receptor for *Rickettsia conorii*. Cell 2005;123:1013–1023.
16. Uchiyama T, Kawano H, Kusuhara Y. The major outer membrane protein rOmpB of spotted fever group rickettsiae functions in the rickettsial adherence to and invasion of Vero cells. Microbes Infect 2006;8:801–809.
17. Li H, Walker DH. rOmpA is a critical protein for the adhesion of *Rickettsia rickettsii* to host cells. Microb Pathog 1998;24:289–298.
18. Martinez JJ, Cossart P. Early signaling events involved in the entry of *Rickettsia conorii* into mammalian cells. J Cell Sci 2004;117:5097–5106.
19. Gouin E, Egile C, Dehoux P, et al. The RickA protein of *Rickettsia conorii* activates the Arp2/3 complex. Nature 2004;427:457–461.
20. Renesto P, Dehoux P, Gouin E, et al. Identification and characterization of a phospholipase D–superfamily gene in rickettsiae. J Infect Dis 2003;188:1276–1283.
21. Whitworth T, Popov VL, Yu XJ, et al. Expression of the *Rickettsia prowazekii pld* or *tlyC* gene in *Salmonella enterica* serovar *Typhimurium* mediates phagosomal escape. Infect Immun 2005;73:6668–6673.
22. Eremeeva ME, Silverman DJ. Effects of the antioxidant alpha-lipoic acid on human umbilical vein endothelial cells infected with *Rickettsia rickettsii*. Infect Immun 1998;66:2290–2299.
23. Silverman DJ, Santucci LA. Potential for free radical-induced lipid peroxidation as a cause of endothelial cell injury in Rocky Mountain spotted fever. Infect Immun 1988;56:3110–3115.
24. Clifton DR, Rydkina E, Huyck H, et al. Expression and secretion of chemotactic cytokines IL-8 and MCP-1 by human endothelial cells after *Rickettsia rickettsii* infection: regulation by nuclear transcription factor NF-kappaB. Int J Med Microbiol 2005;295:267–278.
25. Sporn LA, Shi RJ, Lawrence SO, et al. *Rickettsia rickettsii* infection of cultured endothelial cells induces release of large von Willebrand factor multimers from Weibel-Palade bodies. Blood 1991;78:2595–2602.
26. Clifton DR, Rydkina E, Freeman RS, Sahni SK. NF-kappaB activation during *Rickettsia rickettsii* infection of endothelial cells involves the activation of catalytic IkappaB kinases IKKalpha and IKKbeta and phosphorylation-proteolysis of the inhibitor protein IkappaBalpha. Infect Immun 2005;73:155–165.
27. Sporn LA, Sahni SK, Lerner NB, et al. *Rickettsia rickettsii* infection of cultured human endothelial cells induces NF-kappaB activation. Infect Immun 1997;65:2786–2791.
28. Rydkina E, Silverman DJ, Sahni SK. Activation of p38 stress-activated protein kinase during *Rickettsia rickettsii* infection of human endothelial cells: role in the induction of chemokine response. Cell Microbiol 2005;7:1519–1530.
29. Sahni SK, Turpin LC, Brown TL, Sporn LA. Involvement of protein kinase C in *Rickettsia rickettsii*–induced transcriptional activation of the host endothelial cell. Infect Immun 1999;67:6418–6423.
30. Valbuena G, Feng HM, Walker DH. Mechanisms of immunity against rickettsiae. New perspectives and opportunities offered by unusual intracellular parasites. Microbes Infect 2002;4:625–633.
31. Dumler JS, Walker DH. Rocky Mountain spotted fever—changing ecology and persisting virulence. N Engl J Med 2005;353:551–553.
32. Sexton DJ, Kanj SS, Wilson K, et al. The use of a polymerase chain reaction as a diagnostic test for Rocky Mountain spotted fever. Am J Trop Med Hyg 1994;50:59–63.
33. Demma LJ, Traeger MS, Nicholson WL, et al. Rocky Mountain spotted fever from an unexpected tick vector in Arizona. N Engl J Med 2005;353:587–594.
34. Paddock CD, Greer PW, Ferebee TL, et al. Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. J Infect Dis 1999;179:1469–1476.
35. Paddock CD, Childs JE. *Ehrlichia chaffeensis*: a prototypical emerging pathogen. Clin Microbiol Rev 2003;16:37–64.
36. Barnewall RE, Rikihisa Y, Lee EH. *Ehrlichia chaffeensis* inclusions are early endosomes which selectively accumulate transferrin receptor. Infect Immun 1997;65:1455–1461.
37. Lee EH, Rikihisa Y. Anti-*Ehrlichia chaffeensis* antibody complexed with *E. chaffeensis* induces potent proinflammatory cytokine mRNA expression in human monocytes through sustained reduction of IkappaB-alpha and activation of NF-kappaB. Infect Immun 1997;65:2890–2897.
38. Lin M, Rikihisa Y. *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* lack genes for lipid A biosynthesis and incorporate cholesterol for their survival. Infect Immun 2003;71:5324–5331.
39. Paparone PW, Ljubich P, Rosman GA, Nazha NT. Ehrlichiosis with pancytopenia and ARDS. NJ Med 1995;92:381–385.
40. Marty AM, Dumler JS, Imes G, et al. Ehrlichiosis mimicking thrombotic thrombocytopenic purpura. Case report and pathological correlation. Hum Pathol 1995;26:920–925.
41. Walker DH. Ehrlichia under our noses and no one notices. Arch Virol Suppl 2005:147–156.
42. Safdar N, Love RB, Maki DG. Severe *Ehrlichia chaffeensis* infection in a lung transplant recipient: a review of ehrlichiosis in the immunocompromised patient. Emerg Infect Dis 2002;8:320–323.
43. Patel RG, Byrd MA. Near fatal acute respiratory distress syndrome in a patient with human ehrlichiosis. South Med J 1999;92:333–335.
44. Smith Sehdev AE, Sehdev PS, Jacobs R, Dumler JS. Human monocytic ehrlichiosis presenting as acute appendicitis during pregnancy. Clin Infect Dis 2002;35:e99–e102.
45. Ismail N, Walker DH. Balancing protective immunity and immunopathology: a unifying model of monocytotropic ehrlichiosis. Ann NY Acad Sci 2005;1063:383–394.
46. Zhang JZ, McBride JW, Yu XJ. L-selectin and E-selectin expressed on monocytes mediating *Ehrlichia chaffeensis* attachment onto host cells. FEMS Microbiol Lett 2003;227:303–309.
47. Popov VL, Yu X, Walker DH. The 120 kDa outer membrane protein of *Ehrlichia chaffeensis*: preferential expression on dense-core cells and gene expression in *Escherichia coli* associated with attachment and entry. Microb Pathog 2000;28:71–80.
48. Rikihisa Y. *Ehrlichia* subversion of host innate responses. Curr Opin Microbiol 2006;9:95–101.

49. Zhang JZ, Sinha M, Luxon BA, Yu XJ. Survival strategy of obligately intracellular *Ehrlichia chaffeensis*: novel modulation of immune response and host cell cycles. Infect Immun 2004;72:498–507.
50. Dierberg KL, Dumler JS. Lymph node hemophagocytosis in rickettsial diseases: a pathogenetic role for CD8 T lymphocytes in human monocytic ehrlichiosis (HME)? BMC Infect Dis 2006;6:121.
51. Ismail N, Stevenson HL, Walker DH. Role of tumor necrosis factor alpha (TNF-alpha) and interleukin-10 in the pathogenesis of severe murine monocytotropic ehrlichiosis: increased resistance of TNF receptor p55- and p75-deficient mice to fatal ehrlichial infection. Infect Immun 2006;74: 1846–1856.
52. Stone JH, Dierberg K, Aram G, Dumler JS. Human monocytic ehrlichiosis. JAMA 2004;292:2263–2270.
53. Winslow GM, Bitsaktsis C, Yager E. Susceptibility and resistance to monocytic ehrlichiosis in the mouse. Ann NY Acad Sci 2005;1063:395–402.
54. Ganta RR, Cheng C, Wilkerson MJ, Chapes SK. Delayed clearance of *Ehrlichia chaffeensis* infection in $CD4^+$ T-cell knockout mice. Infect Immun 2004;72:159–167.
55. Ganta RR, Wilkerson MJ, Cheng C, et al. Persistent *Ehrlichia chaffeensis* infection occurs in the absence of functional major histocompatibility complex class II genes. Infect Immun 2002;70:380–388.
56. Sotomayor EA, Popov VL, Feng HM, et al. Animal model of fatal human monocytotropic ehrlichiosis. Am J Pathol 2001;158:757–769.
57. Yager E, Bitsaktsis C, Nandi B, et al. Essential role for humoral immunity during *Ehrlichia* infection in immunocompetent mice. Infect Immun 2005;73:8009–8016.
58. Stevenson HL, Jordan JM, Peerwani Z, et al. An intradermal environment promotes a protective type-1 response against lethal systemic monocytotropic ehrlichial infection. Infect Immun 2006;74:4856–4864.
59. Winslow GM, Yager E, Shilo K, et al. Infection of the laboratory mouse with the intracellular pathogen *Ehrlichia chaffeensis*. Infect Immun 1998;66:3892–3899.
60. Li JS, Chu F, Reilly A, Winslow GM. Antibodies highly effective in SCID mice during infection by the intracellular bacterium *Ehrlichia chaffeensis* are of picomolar affinity and exhibit preferential epitope and isotype utilization. J Immunol 2002;169:1419–1425.
61. Winslow GM, Yager E, Li JS. Mechanisms of humoral immunity during *Ehrlichia chaffeensis* infection. Ann NY Acad Sci 2003;990:435–443.
62. Winslow GM, Yager E, Shilo K, et al. Antibody-mediated elimination of the obligate intracellular bacterial pathogen *Ehrlichia chaffeensis* during active infection. Infect Immun 2000;68:2187–2195.
63. Dumler JS, Dawson JE, Walker DH. Human ehrlichiosis: hematopathology and immunohistologic detection of *Ehrlichia chaffeensis*. Hum Pathol 1993;24:391–396.
64. Dawson JE, Paddock CD, Warner CK, et al. Tissue diagnosis of *Ehrlichia chaffeensis* in patients with fatal ehrlichiosis by use of immunohistochemistry, in situ hybridization, and polymerase chain reaction. Am J Trop Med Hyg 2001;65:603–609.
65. Dunning Hotopp JC, Lin M, Madupu R, et al. Comparative genomics of emerging human ehrlichiosis agents. PLoS Genet 2006;2:e21.
66. Long SW, Whitworth TJ, Walker DH, Yu XJ. Overcoming barriers to the transformation of the genus *Ehrlichia*. Ann NY Acad Sci 2005;1063:403–410.
67. Rachek LI, Hines A, Tucker AM, et al. Transformation of *Rickettsia prowazekii* to erythromycin resistance encoded by the *Escherichia coli ere*B gene. J Bacteriol 2000;182: 3289–3291.