Published in final edited form as:

Semin Immunopathol. 2015 May ; 37(3): 289–299. doi:10.1007/s00281-015-0480-x.

The Role of CD8 T Lymphocytes in Rickettsial Infections

David H. Walker, M.D.^{1,*} and J. Stephen Dumler, M.D.²

¹Department of Pathology, Director, UTMB Center for Biodefense and Emerging Infectious Disease, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0609

²Department of Pathology and Department of Microbiology and Immunology, University of Maryland School of Medicine, Health Sciences Facility-I, Room 322D, 685 W Baltimore St. Baltimore, MD 21201

Abstract

Arthropod-borne obligately intracellular bacteria pose a difficult challenge to the immune system. The genera *Rickettsia*, *Orientia*, *Ehrlichia*, and *Anaplasma* evolved mechanisms of immune evasion, and each interacts differently with the immune system. The roles of CD8 T cells include protective immunity and immunopathology. In *Rickettsia* infections, CD8 T cells are protective mediated in part by cytotoxicity toward infected cells. In contrast, TNF α overproduction by CD8 T cells is pathogenic in lethal ehrlichiosis by induction of apoptosis/necrosis in hepatocytes. Yet, CD8 T cells, along with CD4 T cells and antibodies, also contribute to protective immunity in ehrlichial infections. In granulocytic anaplasmosis, CD8 T cells impact pathogen control modestly but could contribute to immunopathology by virtue of their dysfunction. While preliminary evidence indicates that CD8 T cells are important in protection against *Orientia tsutsugamushi*, mechanistic studies have been neglected. Valid animal models will enable experiments to elucidate protective and pathologic immune mechanisms. The public health need for vaccines against these agents of human disease, most clearly *O. tsutsugamushi*, and the veterinary diseases, canine monocytotropic ehrlichiosis (*E. canis*), heartwater (*E. ruminantium*) and bovine anaplasmosis (*A. marginale*) requires detailed immunity and immunopathology investigations, including the roles of CD8 T lymphocytes.

Keywords

Rickettsia; *Ehrlichia*; *Anaplasma*; *Orientia*; cytotoxic lymphocytes; CD8 T lymphocytes; TNF- α

Introduction

The order Rickettsiales comprises two families of alpha-proteobacteria. All are vector-borne obligately intracellular gram-negative bacteria [1,2]. The family Rickettsiaceae contains two genera, *Rickettsia* and *Orientia*, which include many human pathogens. The family Anaplasmataceae consists of five genera, *Ehrlichia*, *Anaplasma*, *Neorickettsia*,

*Corresponding author Office: (409) 772-3989, Fax: (409) 772-1850, dwalker@utmb.edu.

Neoehrlichia, and *Wolbachia*. Members of the genera range from endosymbiotes (*Wolbachia*) to agents of veterinary diseases and emerging human infectious diseases.

Rickettsiaceae

Rickettsia

Rickettsia species are small (0.3 x 1.0 µm) bacteria with atypical gram-negative cell wall structure including lipopolysaccharide, peptidoglycan, lipoprotein, and autotransporter outer membrane proteins. They reside free in the cytoplasm from which they obtain amino acids and other molecules by their active transporter systems. They are facultative ATP parasites that obtain ATP via rickettsial transport systems as well as by biosynthesis. As a result of their evolution to take advantage of much of the host cell's metabolic machinery, *Rickettsia* have small genomes, generally 1.1–1.5 Mb. Rickettsial ligands bind to host cell receptors, induce their endocytosis, and rapidly escape into the cytosol [3]. Spotted fever group rickettsiae move within the cell and between cells by actin-based motility triggered by specific rickettsial proteins. Typhus group rickettsiae lack actin-based motility and replicate intracellularly until the host cell bursts releasing the bacteria.

Rickettsial diseases include some of the most lethal infections of previously healthy immunocompetent persons such as Rocky Mountain spotted fever (*R. rickettsii*) and epidemic louse-borne typhus (*R. prowazekii*) with case-fatality rates ranging from 15–65% if not treated with an appropriate antibiotic such as doxycycline. Some other rickettsial diseases include life-threatening Mediterranean spotted fever (*R. conorii*) and murine typhus (*R. typhi*). African tick bite fever (*R. africae*) is a frequent disease occurring in tourists returning from safari or other activities in sub-Saharan Africa.

Rickettsiae that infect vascular endothelial cells throughout the body can cause life-threatening encephalitis and interstitial pneumonia/acute respiratory distress syndrome in severe cases. Endothelial injury is associated with the most important pathophysiologic effect, increased vascular permeability. The characteristic histopathologic lesion is vasculitis with vascular and perivascular infiltration of a predominantly CD4 and CD8 T lymphocytes and macrophages [4].

Infection of dermal dendritic cells and macrophages is very likely an early event in the skin where rickettsiae-infected human body louse or flea feces (*R. prowazekii* or *R. typhi* typhus group) or saliva of the feeding tick or mite (spotted fevers) are scratched or deposited in the into the skin [5]. A substantial portion of rickettsiae in dendritic cells are retained in phagosomes associated with entry [6,7]. Thus, rickettsial antigens are presented by both the cytosolic MHC class I and MHC class II pathways and effectively present antigens to both CD8 and CD4 T cells. Rickettsiae-infected dendritic cells activate naïve CD8 T cells *in vitro* in the absence of CD4 T lymphocyte help. *In vivo* cutaneous transfer of *R. conorii*-infected dendritic cells protects mice against a rickettsial dose that is lethal for mice receiving uninfected dendritic cells when challenged 24 hours after transfer. The crucial effects of this innate immunity are associated with interferon-γ [IFN-γ] produced by NK cells and triggered by TLR-4 stimulation [8].

An overview of adaptive immunity would emphasize the coordinated effects of CD4 and CD8 T lymphocytes and cytokines in elimination of intracellular rickettsiae. Antibodies to autotransporters OmpA and OmpB are protective against reinfection but appear too late to contribute to recovery from primary infection [9]. Infected endothelial cells, mononuclear phagocytes, and hepatocytes kill intracellular rickettsiae after activation by cytokines (most importantly IFN- γ and tumor necrosis factor- α [TNF- α]). The cytokine-activated rickettsiacidal mechanisms include production of nitric oxide produced by nitric oxide synthase 2 (NOS2), tryptophan degradation to kyurenin by indoleamine 2, 3-dioxygenase (IDO), and reactive oxygen species [10].

A critical role for CD8 T lymphocytes in the clearance of rickettsial infections was demonstrated by CD8 T cell depletion, immune CD8 T cell adoptive transfer, and experiments in mice with knockout of selected immune response genes [11,12].

The mouse model of spotted fever rickettsioses that most faithfully represents the disseminated endothelial cell infection, clinical course, and pathologic lesions is intravenous *R. conorii* -inoculated C3H/HeN mice [13]. All mice infected with 0.07 median lethal doses (LD₅₀) become ill after five days. Sham-depleted and CD4 T-cell-depleted mice all recover on days 10 or 11. Mice depleted of CD8 T lymphocytes either die (71%) or have a substantially delayed recovery and persistent infection of lungs, liver, and brain through days 10 and 15. The pathologic lesions in CD8 T cell-depleted mice on day 15 are exceptionally severe including myocardial cell necrosis, necrotizing vasculitis of the great vessels, and marked interstitial pneumonia, meningitis and vasculitis in the brain and meninges. Sham-depleted and CD4 T-cell-depleted mice had cleared infection by day 10 [12].

Rickettsial antigen-stimulated splenocytes from CD 4 T cell-depleted mice produce markedly less IFN- γ than splenocytes from CD8 T cell-depleted mice on days 5, 10, and 15 after infection, suggesting that decreased IFN- γ production is not the mechanism of increased severity of infection in CD8 T cell-depleted mice. In these studies, infiltration of CD4 and CD8 T lymphocytes was in a perivascular distribution adjacent to the vascular endothelial cells in the brain of sham-depleted mice on day 10, the time of rickettsial elimination from the infected cerebral endothelium. Mice that received adoptive transfer of nonimmune CD4 T lymphocytes died seven days after infection whereas those that received immune CD4 T cells all survived a 2.5 LD₅₀ challenge. Mice that were adoptively transferred nonimmune CD8 T cells died on day 6 and 7. In contrast, recipients of immune CD8 T lymphocytes survived challenge with 2.5 and 3.6 LD₅₀ but not 5.0 LD₅₀. These studies determined that clearance of rickettsiae from endothelium requires CD8 T cells [12].

The concept that infected cells are a target of T lymphocyte-mediated immunity was demonstrated by Rollwagen, who showed that MHC class I-, but not MHC class II-matched *R. typhi* infected fibroblasts are targets of immune T lymphocyte-mediated cytotoxicity [14]. Similarly splenocytes from mice immune to *R. conorii* effect cytotoxicity on specific MHC class I-matched *R. conorii*-infected SVEC-1 endothelial cells *in vitro* with peak activity on day 10, and splenocytes from *R. australis*-immune C57BL/6 mice demonstrated specific

cytotoxic T lymphocyte activity against an *R. australis*-infected macrophage-like cell line [11].

Evaluation of the LD₅₀ of various gene-targeted knockout C57BL/6 mice with *R. australis*, the only *Rickettsia* to which the strain of mouse is susceptible, revealed that IFN- γ knockout mice are more than 100-fold more susceptible than wild type mice [11]. MHC class I gene knockout mice are greater than 50,000-fold more susceptible than wild type mice, indicating that cytotoxic activity of CD8 T cells is more important than the IFN- γ that they produce. The LD₅₀ of perforin gene knockout mice is 1,000-fold greater than that of wild type mice, suggesting that other mechanisms of CTL activity such as granulysin or Fas/Fas ligand interactions could also be active in clearing rickettsiae from endothelium. Adoptive transfer of immune CD8 T lymphocytes from IFN- γ gene knockout mice into IFN- γ gene knockout mice reduced the infective *R. australis* bacterial burden in lungs, spleen, and liver greater than transfer of naïve wild type CD8 T cells, indicating that cytotoxic activity is an effective mechanism of rickettsial elimination. The occurrence of substantially more apoptotic cells in mice that were adoptively transferred immune CD8 T cells than nonimmune CD8 T cells suggests that clearance of *R. australis* by CD8 T cells is mediated at least in part by cytotoxic apoptotic elimination of *R. australis*-infected endothelial cells [11].

A novel vaccine discovery strategy involving nucleofection of antigen-presenting cells targeting the MHC class I pathway with clones expressing *R. prowazekii* genes was used to immunize mice [15]. Rickettsial challenge with an ordinarily lethal dose of *R. typhi* revealed that *R. prowazekii* gene RP884 stimulated cross protection reducing the bacterial load in liver. This single rickettsial gene, which is annotated as a ferrochelatase provided significant cross-protection and stimulated immune effector and memory CD8 T cells that produced IFN- γ , an effector of anti-rickettsial immunity. This is further evidence supporting the importance of CD8 T lymphocytes in rickettsial immunity.

Orientia tsutsugamushi

Orientia tsutsugamushi, another member of the family Rickettsiaceae, has a larger genome (2.1 Mb) that contains many repetitive sequences [16]. Its cell wall differs tremendously from that of *Rickettsia*, lacking lipopolysaccharide and possessing distinct cell wall proteins. *Orientia* ligands include the major 56 kDa surface protein, ScaC (an autotransporter), and a 47 kDa surface protein. Binding to host fibronectin and host cell receptors integrin $\alpha 5 \beta 1$ and syndecan-4 is followed by induced phagocytosis mediated by clathrin. Orientiae then escape rapidly into the cytosol where they are moved by the host's microtubules to the perinuclear microtubule organizing center [17].

Scrub typhus caused by *O. tsutsugamushi* has the highest incidence of any of the rickettsioses, 1 million cases per year [18]. It is a life-threatening disease with 7–15% case fatality rate in immunocompetent persons. The bacteria are maintained in nature by transovarian transmission in trombiculid mites, and the infection is transmitted to humans by their feeding larval stage (chiggers) in Asia, northern Australia, and islands of the Western Pacific and Indian oceans.

Orientiae infect mainly dermal dendritic cells in the skin at the site where the chigger feeds. Hematogenous dissemination leads to widespread infection of endothelial cells and macrophages throughout the body leading to acute respiratory distress, meningoencephalitis, gastrointestinal bleeding, hypotensive shock, acute renal failure, and coagulopathy in severe cases [19].

Most immunologic studies of *O. tsutsugamushi* infections were performed prior to the development of contemporary concepts of immunology and were descriptive in nature. Adoptive transfer of macrophage-depleted immune splenocytes provided cross-protection against lethal challenge that was abrogated by depletion of T cells in the mouse model of infection with a heterologous *O. tsutsugamushi* strain inoculated intraperitoneally [20]. This model targets the peritoneal macrophages and mesothelial cells and results in death due to peritonitis rather than disseminated endothelial infection and vasculitis characteristic of human scrub typhus. Only recently were mouse models developed that result in disseminated endothelial infection and the appropriate pathologic lesions, namely vasculitis leading to interstitial pneumonia and encephalitis.

Adoptive transfer of antigen-specific IFN- γ producing T cells, presumably Th1 lymphocytes, protects mice in the peritonitis model, suggesting that IFN- γ is an important immune effector [21]. In a disseminated endothelial infection model following intravenous inoculation of orientiae, a strong Th1 response contributed to the severity of illness that showed angiopoietin 1 and 2 levels indicative of endothelial injury [22]. Current studies in the disseminated endothelial infection model revealed that 100% of CD8 T cell deficient mice died after receiving a dose of orientiae that killed only 50% of wild type mice (Guang X and Walker DH unpublished data). Further investigation of mechanisms of immunity to scrub typhus including the role of CD8 T cells should be a high priority.

Anaplasmataceae

Ehrlichia

Ehrlichiae are small gram-negative obligately intracellular bacteria that reside as a microcolony in a cytoplasmic vacuole of monocytes (*Ehrlichia chaffeensis*), neutrophils (*E. ewingii*), or as yet undetermined target cells (*E. muris* -like agent) [23,24]. Ehrlichiae have small (1.2–1.5 Mb) genomes and a cell wall that lacks lipopolysaccharide and peptidoglycan, and contain cholesterol obtained from the host. These bacteria secrete several proteins containing multiple tandem repeat units that pass through the vacuole membrane and bind host cytoplasmic proteins. Some of them also are nucleomodulins that are transported into the nucleus and bind to the chromatin. Ehrlichiae thus modify the host response decreasing expression of host defense proteins (IL-15, IL-18, chemokine receptors 2, 3, and 4 and MHC class II), inhibiting the JAK/STAT pathway and the IFN- γ anti-rickettsial iron starvation mechanism, degrading p22^{phox} and inhibiting superoxide generation. Ehrlichiae manipulate the host to their advantage by inhibiting phagolysosomal fusion, inducing apoptosis inhibitors, decreasing expression of apoptosis inducers, and redirecting transferrin receptor iron to the ehrlichial vacuole [25–27].

Ehrlichiae have a 72 hour developmental cycle with distinct ultrastructural forms that express characteristic surface proteins [28]. Dense core cells are infective and do not replicate; reticulate cells replicate by binary fission and are not infectious. Thus, dense core cells attach to the host cell, induce phagocytosis, transform into reticulate cells that undergo multiple rounds of growth and convert into dense core cells that are released from the host cell and spread to infect new host cells.

Human monocytotropic ehrlichiosis is caused by *E. chaffeensis* transmitted by *Amblyomma americanum* ticks, and is a life-threatening acute febrile disease characterized by leukopenia, thrombocytopenia, elevated hepatic enzymes, and in severe cases, interstitial pneumonitis and meningoencephalitis. Illness caused by *E. ewingii* is observed mainly in immunocompromised persons. Most recently human infections with the novel *E. muris*-like agent transmitted by *Ixodes scapularis* ticks were identified in Wisconsin and Minnesota.

Canine monocytic ehrlichiosis caused by *E. canis* transmitted by *Rhipicephalus sanguineus* ticks is a major infectious disease of dogs leading to a chronic phase with bone marrow failure. Heartwater caused by *E. ruminantium* is an important pathogen in sub-Saharan Africa where it results in severe losses of livestock.

CD8 T lymphocytes contribute significantly to the protective immunity to ehrlichiae and to the pathogenesis of immune-mediated injury in ehrlichiosis. Knowledge of the immune mechanisms in ehrlichiosis has been generated by experiments employing mouse models of infection with *E. muris* and an unnamed *Ehrlichia* species isolated from *Ixodes ovatus* ticks (IOE). Mice infected with *E. muris* develop a subclinical lifelong persistent infection similar to that of natural vertebrate hosts (e.g., white tailed deer infected with *E. chaffeensis*) [29]. Mice infected intraperitoneally with all but the very lowest doses of IOE succumb to a toxic shock-like illness [30]. In contrast, mice inoculated intradermally with all but the very highest doses of IOE control the infection and survive [31].

The importance of CD8 T cells and immune control of *E. muris* by mice is demonstrated in lethality of the infection for 81% of MHC class I-knockout C3H/HeN mice, and 80% of mice depleted of both CD8 and CD4 T cells compared with no deaths in wild type C3H/HeN mice and only 44% of CD4 T-cell knockout mice [32]. Moreover, immune CD8 T lymphocytes exert cytotoxic activity against *E. muris*-infected target cells. Adoptive transfer of protective immunity to *E. ruminantium* requires CD8 but not CD4 T cells [33]. Although depletion of either IFN- γ or TNF- α alone does not have a significant effect on infection of C3H/HeN mice with *E. muris*, depletion of both cytokines results in a lethal outcome in 75% of the animals. It is unclear whether cytotoxicity or IFN- γ or both are critical mechanisms of CD8 T cell-mediated protection.

The observation that persistent infection of C57BL/6 mice with *E. muris* confers protective immunity against an ordinarily lethal IOE challenge enabled the study of protective immune mechanisms against this highly lethal infection [34]. Mice inoculated intraperitoneally with a high dose of IOE develop progressive disease with weight loss, hypoglycemia, and pancytopenia and die on day 8–10 of infection with multiorgan inflammatory pathology including hepatocellular necrosis and apoptosis. When infected with 100-fold lower dose

inoculum of IOE, mice become ill on day 8 and die between days 15 and 17. Death is associated with extremely high serum levels of TNF- α (900 pg/mL) and an elevated serum concentration of IL-10. ELISPOT assays reveal that CD8 T cells comprise 60% of the TNF- α producing cells in the spleen, representing the major source of excessive TNF- α . Indeed, CD8 T-cell knockout mice are less susceptible than wild type mice to both IOE and *E. ruminantium* infection [35,36].

Survival of mice persistently infected with *E. muris* when challenged with IOE is associated with expansion of IFN- γ -producing CD4 and CD8 T lymphocytes but not TNF- α -producing CD8 T cells, a high titer of IgG_{2a} antibodies to ehrlichiae, and a low concentration of TNF- α [34].

Transfer of immune CD4 and CD8 T cells and polyclonal anti-*Ehrlichia* antibodies protect naïve mice against an ordinarily lethal IOE challenge, but only when given all together. Thus, CD8 T lymphocytes contribute to cross-protective immunity [34].

Intradermal infection of mice with IOE results in 100 to 1,000-fold lower bacterial loads in the spleen, a greater quantity of IFN γ -producing CD4 T cells, higher concentrations of TNF- α in the spleen, but lower serum concentrations of TNF- α (TNF α) and IL-10, reinforcing that IFN- γ and TNF are protective in the sites of infection and that high serum concentrations of TNF- α and IL-10 have pathologic effects [31].

The immunopathologic effects of TNF- α are evident in studies of IOE infections of TNF receptor p55- and p75- (*Tnfr I/II*) knockout mice [37]. *Tnfr I/II* knockout mice survive longer and have only a small amount of hepatic necrosis and apoptosis, but have greater bacterial loads than similarly infected wild type mice, indicating that TNF- α largely produced by CD8 T cells plays a key role in the hepatic pathology and immune control of IOE.

Compared with wild type C57BL/6 mice, β 2 microglobulin knockout mice and TAP knockout mice infected with IOE have increased survival. Infected β 2 microglobulin-deficient mice also have significantly less hepatic injury and bacterial burden associated with maintenance rather than loss of CD4 T lymphocytes, increased *Ehrlichia*-specific CD4 T cells, and did not have systemic or local overproduction of TNF- α and IL-10. Toxic shock appears to be caused by uncontrolled activation of pathogenic CD8 T cells that mediate apoptosis of infected and uninfected host cells including CD4 T lymphocytes, possibly by the high concentration of IL-10 produced. CD8 T cells are the source of overproduction of TNF- α . Perforin plays an important role in both controlling bacterial infection and in mediating tissue injury in the IOE model [37].

Anaplasma

Anaplasma species are phylogenetically-related to the genus *Ehrlichia*, and are tick-transmitted, small gram-negative obligately intracellular bacteria that reside as a microcolony in a cytoplasmic vacuole of several cells types, including granulocytes (*Anaplasma phagocytophilum*), erythrocytes (*A. marginale* and *A. centrale*), platelets (*A. platys*), and in monocytes (*A. bovis*) [2,38]. Anaplasmae have small genomes (1.1–1.5 Mb) and their cell walls lack lipopolysaccharide and peptidoglycan, and contain host-derived

cholesterol [39–41]. Their genomes encode from less than 10 (*A. marginale*) to more than 100 copies (*A. phagocytophilum*) of genes or pseudogenes encoding outer surface proteins in the PFAM01617 Surface_Ag_2 family, each expressed from a single genomic transcriptional site after gene conversion [39,42]. These gene conversion events are critical for generating antigenic diversity and immune evasion that promotes survival and persistence in naturally-infected mammalian hosts [42,43]. *A. phagocytophilum* also encodes several proteins that are secreted to interact with the pathogen-containing vacuolar membrane that direct vacuolar fusion events by recruiting membrane trafficking proteins [44–49], or several nucleomodulins that translocate into the nucleus, including AnkA which exerts transcriptional regulating effects on genes critical for host microbicidal activity by altering chromatin structure and perhaps DNA methylation [50–56]. Other changes in host transcription with infection include a marked upregulation in chemokine and proinflammatory cytokine expression [57,58]. Blocking chemokine receptor interactions *in vivo* results in lower pathogen loads and lower transmission fitness potential [59,60]. Delayed apoptosis among infected neutrophils promotes bacterial fitness, and results from either *A. phagocytophilum* effector proteins interacting with signaling pathways to stabilize mitochondria [61,62], or via effects on transcription of pro-apoptotic and anti-apoptotic transcriptional programs [57,62,63]. The transcriptional control programs are unlikely to impact those species which survive with anucleate cells – erythrocytes and platelets [64]. As in *Ehrlichia* species, the *Anaplasma* species developmental cycle includes a dense core cell that expresses a surface protein almost not present on metabolically active, non-infectious reticulate cells [65,66].

Two major diseases are associated with *A. phagocytophilum* and *A. marginale*/*A. centrale* infections. The former is the cause of granulocytic anaplasmosis, a zoonosis that is a significant cause of febrile disease in animals and humans [67,68]. Human disease is characterized by sudden onset of high fever, headache, myalgia, and gastrointestinal manifestations, with leukopenia, thrombocytopenia and moderate inflammatory liver injury. Severe complications are infrequent but include interstitial pneumonitis with acute respiratory distress syndrome, a sepsis- or toxic shock-like syndrome with or without renal failure sometimes associated with a hemophagocytic or macrophage activation syndrome [67,69].

In contrast, *A. marginale* and *A. centrale* are erythrocytic veterinary pathogens that persist for long intervals in their ruminant hosts by the generating waves of antigenic variants [70]. Each recurrent episode occurs with the expansion of a new antigenic variant that escapes host immune control. The first episode is generally most severe and is characterized by fever and hemolysis with considerable loss of productivity in the infected animals; subsequent episodes have fewer clinical signs, but low productivity persists.

A. phagocytophilum controls its mammalian host neutrophil to increase microbial fitness by: i) increasing inflammatory recruitment of new neutrophils [58–60,71], ii) delaying neutrophil apoptosis for hours to days to enhance propagation [57,61–63,72,73], and iii) regulating host antimicrobial mechanisms [53,54,74,75]. In the absence of pathogen control, the net result is increased inflammatory injury, the major mechanism of disease in humans and animals. Control of *A. phagocytophilum* depends on adequate immunity, as SCID mice

sustain infection for long intervals compared to wild type mice [76,77]. A major factor in this control is dependent on the production of IFN- γ , presumably related to its activation of macrophages and their intracellular killing [77–80]. IFN- γ knockout mice infected by *A. phagocytophilum* develop 5–100 fold higher bacterial loads in spleen than do wild type or IL-10 knockout mice [78,71]. In contrast, histologic inflammatory injury is inhibited in IFN- γ knockout mice but significantly worsen in IL-10 knockout mice [78], demonstrating the dependence of inflammatory histopathology on pathogen-induced inflammatory and innate immune signaling, even in the absence of high pathogen loads. Despite a lack of IFN- γ , all infected mice survive, implying the presence of other protective mechanisms [77]. Similarly, infection of mice devoid of several critical innate immune signaling mechanisms (nitric oxide synthase, gp91^{phox}, TNF, MyD88, and TLR2) do not contribute to pathogen control [77,81], but reduced inflammatory histopathology with infection in mice devoid of these functional host defense pathways demonstrates that their activation is critical for disease but not control [79].

The cellular sources of IFN- γ after *A. phagocytophilum* infection are not established, yet infection of SCID or separately NKT-deficient mice does not significantly impair its production [77,82] or production of downstream effectors such as IDO, IFN gamma induced GTPase (IGTP), or LRG-47 (immunity-related GTPase family M member 1) [77]. Populations of NK, NKT, CD4 and CD8 T lymphocytes expand during infection with *A. phagocytophilum*, with NK and NKT cells showing the greatest increases early [77,82], followed by expansions of CD4 and CD8 T lymphocytes later [77]. While the majority of early IFN- γ production comes from NK and/or NKT cells, IFN- γ production is only completely inhibited in RAG2^{-/-} γ_c ^{-/-} mice that lack T, B, NKT and NK cells [77]. Passive immunization with immune serum has a moderate protective effect against challenge [83], and B lymphocyte-deficient mice still develop increased pathogen loads early [77]; however, these animals do control infection, suggesting a potential role for B cells in immune regulation. Infection in both CD4- and CD8-depleted mice results in increased pathogen loads in blood and tissues, yet mice deficient in MCH-I but not MHC-II molecules control infection [77]. In agreement with these observations, perforin and Fas knockout mice are able to control infection, suggesting that CD4 T cell responses are the most critical for *A. phagocytophilum* control [77]. A role for dendritic cell maturation is likely since DC depletion and infection of CD40 knockout mice leads to incomplete elimination of the infection [77].

Notwithstanding the lack of *A. phagocytophilum* control by CD8 T cells, the development of severe disease manifestations in humans is associated with hypercytokinemia and macrophage activation or hemophagocytic syndromes (MAS/HPS) [58,67,69]. MAS/HPS is genetically-determined or acquired, and is driven to a great extent by persistent stimulation of cytokine production, in particular production of macrophage activating IFN- γ (figure) [84–88]. As observed with *A. phagocytophilum*-infected mice, lack of IFN- γ resolves inflammatory tissue injury [78]; similarly, dampening of IFN- γ production in *A. phagocytophilum*-infected horses by the use of glucocorticoids diminishes clinical and laboratory signs of disease severity [89]. The biological basis for the genetic forms derives from mutations that result in defective cytotoxic granule exocytosis and cytotoxicity such as

would occur with mutations in perforin (*PRF1*), vesicular trafficking and degranulation (*RAB27A*, *UNC13D*, and *LYST*), and genes involved in the development of cytotoxic lymphocytes (*SH2D1A*, *BIRC4*) [90,91]. As APCs present their cognate epitopes in the context of MHC1 to CD8 cells (or by CD1d to NKT cells), their interaction with cytotoxic lymphocytes should ordinarily result in APC cytotoxicity and contraction of the immune response, including reduced expression of proinflammatory and macrophage-activating cytokines and their toxic effectors. The underlying pathogenesis of acquired MAS/HPS is poorly understood, but is frequently associated with defective function or depletion of cytotoxic cells, including NK, NKT and CD8 T lymphocytes [84,86,90,91]. NKT and CD8 T cells from *A. phagocytophilum*-infected mice demonstrate defects in degranulation when stimulated by ionomycin-C *ex vivo* [67]. Such cytotoxic failures could account for the lack of immune control by these cell subsets and the association of immune response and inflammatory tissue injury. One hypothesis is that subtle hypomorphic mono- biallelic or single nucleotide polymorphic mutations in critical genes could be unmasked by microbes that persist or achieve high antigenic densities [92]. The nature of the defect is unclear, but these data demonstrate the dichotomy of protective and pathological immune responses.

Immune control of *A. marginale* has been extensively studied owing to the pathogen's enormous capacity to generate antigenic variants through segmental gene conversion that promotes evasion of host immune response [93–96]. The vast majority of published work in this domain focuses on the development of antibody responses, although memory CD4 T cells are studied extensively. Infection induces production of IgG1 and IgG2 [97,98], a predominant CD4 T cell expansion [97], and eventual loss of antigen-specific CD4 T cell responses [97,99,100]. These responses do not eliminate infection in part owing to T cell exhaustion/apoptosis with high antigen load, although extensive study of CD8 T cells has not been conducted.

Conclusions

The vast majority of investigations of immunity to the Rickettsiales studied the role of the immune response in recovery from infection. As a byproduct, evidence of participation of the immune response as a pathogenic mechanism has been observed to varying degrees for *Ehrlichia*, *Anaplasma*, *Orientia*, and *Rickettsia* (Table). Fewer studies addressed correlates of protection and mechanisms of protection required for effective vaccines against these diseases. A valid consideration is the avoidance of developing a vaccine that would set up triggering an immunopathologic response upon encountering the pathogen from which protection was intended.

There is evidence that CD8 T cells contribute to both protective immunity and lethal immunopathology in animal models of ehrlichioses. The CD8 T-cell overproduction of TNF is a novel immunopathologic mechanism. Targeting CD8 T cell immunity is a valid strategy to be included in development of a vaccine against *Rickettsia*, particularly for broad cross protection that is not achieved by species-specific antibodies. The study of immunity to Rickettsiales is a fertile field for elucidation of mechanisms of immunity and immunopathology with outstanding unique animal models from which the field of immunology could learn important concepts that studies of model antigens cannot unlock.

Acknowledgments

DHW was supported in part by grants R01 AI021242, R21 AI102304, and U54 AI057156 from the National Institute of Allergy and Infectious Diseases. JSD support was provided in part by grants R01-AI044102 and R21-AI096062 from the National Institutes of Allergy and Infectious Diseases.

References

1. Yu, X-J.; Walker, DH. Family I. Rickettsiaceae. In: Garrity, GM.; Brenner, DJ.; Krieg, NR.; Staley, JT., editors. *Bergey's Manual of Systematic Bacteriology*, vol Volume Two: The *Proteobacteria*. vol Part C: The *Alpha-, Beta-, Delta-, and Epsilonproteobacteria*. 2. Springer; New York, N.Y: 2005. p. 96-116.
2. Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *Int J Syst Evol Microbiol*. 2001; 51:2145–2165. [PubMed: 11760958]
3. Walker DH, Ismail N. Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events. *Nature Reviews Microbiology*. 2008; 6:375–386.
4. Herrero-Herrero JJ, Walker DH, Ruiz-Beltran R. Immunohistochemical evaluation of the cellular immune response to *Rickettsia conorii* in *taches noires*. *J Infect Dis*. 1987; 155:802–805. [PubMed: 3493304]
5. Walker DH, Hudnall SD, Szaniawski WK, Feng H-M. Monoclonal antibody-based immunohistochemical diagnosis of rickettsialpox: the macrophage is the principal target. *Mod Pathol*. 1999; 12:529–533. [PubMed: 10349992]
6. Jordan JM, Woods ME, Feng H-M, Soong L, Walker DH. Rickettsiae-stimulated dendritic cells mediate protection against lethal rickettsial challenge in an animal model of spotted fever rickettsiosis. *J Infect Dis*. 2007; 196:629–638. [PubMed: 17624851]
7. Fang R, Ismail N, Soong L, Popov VL, Whitworth T, Bouyer DH, Walker DH. Differential interaction of dendritic cells with *Rickettsia conorii*: impact on host susceptibility to Murine spotted fever rickettsiosis. *Infect Immun*. 2007; 75:3112–3123. [PubMed: 17403875]
8. Jordan JM, Woods ME, Soong L, Walker DH. Rickettsiae stimulate dendritic cells through TLR4, leading to enhanced NK cell activation *in vivo*. *J Infect Dis*. 2009; 199:236–242. [PubMed: 19072551]
9. Feng H-M, Olano JP, Whitworth T, Popov VL, Walker DH. Fc-dependent polyclonal antibodies and antibodies to outer membrane proteins A and B, but not to lipopolysaccharide, protect SCID mice against fatal *Rickettsia conorii* infection. *Infect Immun*. 2004; 72:2222–2228. [PubMed: 15039346]
10. Feng H-M, Walker DH. Mechanisms of intracellular killing of *Rickettsia conorii* in infected human endothelial cells, hepatocytes, and macrophages. *Infect Immun*. 2000; 68:6729–6736. [PubMed: 11083788]
11. Walker DH, Olano JP, Feng H-M. Critical role of cytotoxic T lymphocytes in immune clearance of rickettsial infection. *Infect Immun*. 2001; 69:1841–1846. [PubMed: 11179362]
12. Feng H-M, Popov VL, Yuoh G, Walker DH. Role of T-lymphocyte subsets in immunity to spotted fever group rickettsiae. *J Immunol*. 1997; 158:5314–5320. [PubMed: 9164951]
13. Walker DH, Popov VL, Wen J, Feng H-M. *Rickettsia conorii* infection of C3H/HeN mice A model of endothelial-target rickettsiosis. *Lab Invest*. 1994; 70:358–368. [PubMed: 7511715]
14. Rollwagen FM, Bakun AJ, Dorsey CH, Dasch GA. Mechanisms of immunity to infection with typhus rickettsiae: infected fibroblasts bear rickettsial antigens on their surfaces. *Infect Immun*. 1985; 50:911–916. [PubMed: 2415459]
15. Gazi M, Caro-Gomez E, Goetz Y, Cespedes MA, Hidalgo M, Correa P, Valbuena G. Discovery of a protective *Rickettsia prowazekii* antigen recognized by CD8+ T cells, RP884, using an *in vivo* screening platform. *PloS one*. 2013; 8:e76253. [PubMed: 24146844]

16. Cho N-H, Kim H-R, Lee J-H, Kim S-Y, Kim J, Cha S, Darby AC, Fuxelius H-H, Yin J, Kim JH, Kim J, Lee SJ, Koh Y-S, Jang W-J, Park K-H, Andersson SGE, Choi M-S, Kim I-S. The *Orientia tsutsugamushi* genome reveals massive proliferation of conjugative type IV secretion system and host-cell interaction genes. *Proc Natl Acad Sci*. 2007; 104:7981–7986. [PubMed: 17483455]
17. Kim, I-S.; Walker, DH. Scrub Typhus. In: Guerrant, RL.; Walker, DH.; Weller, PF., editors. *Tropical Infectious Diseases, Principles, Pathogens and Practice*. 3. Elsevier Saunders; London: 2011. p. 334-338.
18. Paris DH, Shelite TR, Day NP, Walker DH. Unresolved problems related to scrub typhus: a seriously neglected life-threatening disease. *Am J of Trop Med Hyg*. 2013; 89:301–307. [PubMed: 23926142]
19. Moron CG, Popov VL, Feng H-M, Wear D, Walker DH. Identification of the target cells of *Orientia tsutsugamushi* in human cases of scrub typhus. *Mod Pathol*. 2001; 14:752–759. [PubMed: 11504834]
20. Shirai A, Catanzaro PJ, Phillips SM, Osterman JV. Host defenses in experimental scrub typhus: role of cellular immunity in heterologous protection. *Infect Immun*. 1976; 14:39–46. [PubMed: 820646]
21. Kodama K, Kawamura S, Yasukawa M, Kobayashi Y. Establishment and characterization of a T-cell line specific for *Rickettsia tsutsugamushi*. *Infect Immun*. 1987; 55:2490–2495. [PubMed: 2443453]
22. Soong L, Wang H, Shelite TR, Liang Y, Mendell NL, Sun J, Gong B, Valbuena GA, Bouyer DH, Walker DH. Strong type 1, but impaired type 2, immune responses contribute to *Orientia tsutsugamushi*-induced pathology in mice. *PLoS Neg Trop Dis*. 2014; 8:e3191.
23. McBride JW, Walker DH. Molecular and cellular pathobiology of *Ehrlichia* infection: targets for new therapeutics and immunomodulation strategies. *Expert Reviews in Molecular Medicine*. 2011; 13:e3–e21. [PubMed: 21276277]
24. Pritt BS, Sloan LM, Johnson DKH, Munderloh UG, Paskewitz SM, McElroy KM, McFadden JD, Binnicker MJ, Neitzel DF, Liu G, Nicholson WL, Nelson CM, Franson JJ, Martin SA, Cunningham SA, Steward CR, Bogumill K, Bjorgaard ME, David JP, McQuiston JH, Warshauer DM, Wilhelm MP, Patel R, Trivedi VA, Ereemeeva ME. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. *New Engl J Med*. 2011; 365:422–429. [PubMed: 21812671]
25. Zhang J-Z, Sinha M, Luxon BA, Yu X. Survival strategy of obligately intracellular *Ehrlichia chaffeensis*: novel modulation of immune response and host cell cycles. *Infect Immun*. 2004; 72:498–507. [PubMed: 14688131]
26. Lee EH, Rikihisa Y. Protein kinase A-mediated inhibition of gamma interferon-induced tyrosine phosphorylation of janus kinases and latent cytoplasmic transcription factors in human monocytes by *Ehrlichia chaffeensis*. *Infect Immun*. 1998; 66:2514–2619. [PubMed: 9596710]
27. Lin M, Rikihisa Y. *Ehrlichia chaffeensis* downregulates surface toll-like receptors 2/4, CD14 and transcription factors PU.1 and inhibits lipopolysaccharide activation of NF- κ B, ERK 1/2 and p38 MAPK in host monocytes. *Cell Microbiol*. 2004; 6:175–186. [PubMed: 14706103]
28. Zhang J-Z, Popov VL, Gao S, Walker DH, Yu X-J. The developmental cycle of *Ehrlichia chaffeensis* in vertebrate cells. *Cellular Microbiology*. 2007; 9:610–618. [PubMed: 16987329]
29. Olano JP, Wen G, Feng H-M, McBride JW, Walker DH. Histologic, serologic, and molecular analysis of persistent ehrlichiosis in a murine model. *Am J Pathol*. 2004; 165:997–1006. [PubMed: 15331423]
30. Sotomayor E, Popov V, Feng H-M, Walker DH, Olano JP. Animal model of fatal human monocyctotropic ehrlichiosis. *American Journal of Pathology*. 2001; 158:757–769. [PubMed: 11159213]
31. Stevenson HL, Jordan JM, Peerwani Z, Wang H-Q, Walker DH, Ismail N. An intradermal environment promotes a protective Type-1 response against lethal systemic monocyctotropic ehrlichial infection. *Infect Immun*. 2006; 74:4856–4864. [PubMed: 16861674]
32. Feng H-M, Walker DH. Mechanisms of immunity to *Ehrlichia muris*: a model of monocyctotropic ehrlichiosis. *Infect Immun*. 2004; 72:966–971. [PubMed: 14742542]

33. du Plessis JL, Berche P, Van Gas L. T cell-mediated immunity to cowdria ruminantium mice: the protective role of LYT-2⁺ T cells. Onderstepoort J Vet Res. 1991; 58:171–179. [PubMed: 1923379]
34. Ismail N, Soong L, McBride JW, Valbuena G, Olano JP, Feng H-M, Walker DH. Overproduction of TNF- α by CD8⁺ type 1 cells and down-regulation of IFN- γ 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. [PubMed: 14734762]
35. Ismail N, Crossley EC, Stevenson HL, Walker DH. Relative importance of T-cell subsets in monocytotropic ehrlichiosis: a novel effector mechanism involved in *Ehrlichia*-induced immunopathology in murine ehrlichiosis. Infect Immun. 2007; 75:4608–4620. [PubMed: 17562770]
36. Byrom B, Barbet AF, Obwolo M, Mahan SM. CD8⁺ T cell knockout mice are less susceptible to *Cowdria ruminantium* infection than athymic, CD4⁺ T cell knockout, and normal C57BL/6 mice. Veterinary Parasitology. 2000; 93:159–172. [PubMed: 11035234]
37. Ismail N, Stevenson HL, Walker DH. Role of tumor necrosis factor α 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. [PubMed: 16495559]
38. Dumler, JS.; Rikihisa, Y.; Dasch, GA. Genus I. *Anaplasma*. In: Garrity, GM.; Brenner, DJ.; Krieg, NR.; Staley, JT., editors. Bergey's Manual of Systematic Bacteriology, vol Volume 2 The *Proteobacteria*; Part C The *Alpha*-, *Beta*-, *Delta*-, and *Epsilon* *proteobacteria*. 2. Springer; East Lansing, MI: 2005. p. 117-125.
39. Dunning Hotopp JC, Lin M, Madupu R, Crabtree J, Angiuoli SV, Eisen J, Seshadri R, Ren Q, Wu M, Utterback TR, Smith S, Lewis M, Khouri H, Zhang C, Niu H, Lin Q, Ohashi N, Zhi N, Nelson W, Brinkac LM, Dodson RJ, Rosovitz MJ, Sundaram J, Daugherty SC, Davidsen T, Durkin AS, Gwinn M, Haft DH, Selengut JD, Sullivan SA, Zafar N, Zhou L, Benahmed F, Forberger H, Halpin R, Mulligan S, Robinson J, White O, Rikihisa Y, Tettelin H. Comparative genomics of emerging human ehrlichiosis agents. PLoS Genet. 2006; 2:e21. [PubMed: 16482227]
40. Brayton KA, Kappmeyer LS, Herndon DR, Dark MJ, Tibbals DL, Palmer GH, McGuire TC, Knowles DP Jr. Complete genome sequencing of *Anaplasma marginale* reveals that the surface is skewed to two superfamilies of outer membrane proteins. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:844–849. [PubMed: 15618402]
41. NCBI . [February 27, 2015] Genome information by organism. 2015. <http://www.ncbi.nlm.nih.gov/genome/Anaplasma> (taxid:768) Prokaryotes [724]
42. Futse JE, Brayton KA, Knowles DP, Palmer GH. Structural basis for segmental gene conversion in generation of *Anaplasma marginale* outer membrane protein variants. Mol Microbiol. 2005; 57:212–221. [PubMed: 15948961]
43. Rejmanek D, Foley P, Barbet A, Foley J. Antigen variability in *Anaplasma phagocytophilum* during chronic infection of a reservoir host. Microbiology. 2012; 158:2632–2641. [PubMed: 22859615]
44. Truchan HK, Seidman D, Carlyon JA. Breaking in and grabbing a meal: *Anaplasma phagocytophilum* cellular invasion, nutrient acquisition, and promising tools for their study. Microbes and infection/Institut Pasteur. 2013; 15:1017–1025. [PubMed: 24141091]
45. Huang B, Troese MJ, Howe D, Ye S, Sims JT, Heinzen RA, Borjesson DL, Carlyon JA. *Anaplasma phagocytophilum* APH_0032 is expressed late during infection and localizes to the pathogen-occupied vacuolar membrane. Microb Pathog. 2010; 49:273–284. [PubMed: 20600793]
46. Huang B, Hubber A, McDonough JA, Roy CR, Scidmore MA, Carlyon JA. The *Anaplasma phagocytophilum*-occupied vacuole selectively recruits Rab-GTPases that are predominantly associated with recycling endosomes. Cell Microbiol. 2010; 12:1292–1307. [PubMed: 20345488]
47. Niu H, Rikihisa Y. Ats-1: a novel bacterial molecule that links autophagy to bacterial nutrition. Autophagy. 2013; 9:787–788. [PubMed: 23388398]
48. Niu H, Kozjak-Pavlovic V, Rudel T, Rikihisa Y. *Anaplasma phagocytophilum* Ats-1 is imported into host cell mitochondria and interferes with apoptosis induction. PLoS Pathog. 2010; 6:e1000774. [PubMed: 20174550]

49. Lin M, den Dulk-Ras A, Hooykaas PJ, Rikihisa Y. *Anaplasma phagocytophilum* AnkA secreted by type IV secretion system is tyrosine phosphorylated by Abl-1 to facilitate infection. *Cell Microbiol.* 2007; 9:2644–2657. [PubMed: 17587335]
50. Caturegli P, Asanovich KM, Walls JJ, Bakken JS, Madigan JE, Popov VL, Dumler JS. *ankA*: an *Ehrlichia phagocytophila* group gene encoding a cytoplasmic protein antigen with ankyrin repeats. *Infect Immun.* 2000; 68:5277–5283. [PubMed: 10948155]
51. Park J, Kim KJ, Choi KS, Grab DJ, Dumler JS. *Anaplasma phagocytophilum* AnkA binds to granulocyte DNA and nuclear proteins. *Cell Microbiol.* 2004; 6:743–751. [PubMed: 15236641]
52. Sinclair SHG, Garcia-Garcia JC, Dumler JS. Bioinformatic and mass spectrometry identification of *Anaplasma phagocytophilum* proteins translocated into host cell nuclei. *Front Microbiol.* 2015; 6:55. [PubMed: 25705208]
53. Garcia-Garcia JC, Barat NC, Trembley SJ, Dumler JS. Epigenetic silencing of host cell defense genes enhances intracellular survival of the rickettsial pathogen *Anaplasma phagocytophilum*. *PLoS Pathog.* 2009; 5:e1000488. [PubMed: 19543390]
54. Garcia-Garcia JC, Rennoll-Bankert KE, Pelly S, Milstone AM, Dumler JS. Silencing of host cell *CYBB* gene expression by the nuclear effector AnkA of the intracellular pathogen *Anaplasma phagocytophilum*. *Infect Immun.* 2009; 77:2385–2391. [PubMed: 19307214]
55. Rennoll-Bankert KE, Sinclair SH, Lichay MA, Dumler JS. Comparison and characterization of granulocyte cell models for *Anaplasma phagocytophilum* infection. *Pathog Dis.* 2014; 71:55–64. [PubMed: 24376092]
56. Sinclair SH, Rennoll-Bankert KE, Dumler JS. Effector bottleneck: Microbial reprogramming of parasitized host cell transcription by epigenetic remodeling of chromatin structure. *Front Genet.* 2014; 5:274. [PubMed: 25177343]
57. Borjesson DL, Kobayashi SD, Whitney AR, Voyich JM, Argue CM, Deleo FR. Insights into pathogen immune evasion mechanisms: *Anaplasma phagocytophilum* fails to induce an apoptosis differentiation program in human neutrophils. *J Immunol.* 2005; 174:6364–6372. [PubMed: 15879137]
58. Dumler JS, Triggiani ER, Bakken JS, Aguero-Rosenfeld ME, Wormser GP. Serum cytokine responses during acute human granulocytic ehrlichiosis. *Clin Diagn Lab Immunol.* 2000; 7:6–8. [PubMed: 10618268]
59. Scorpio DG, Akkoyunlu M, Fikrig E, Dumler JS. CXCR2 blockade influences *Anaplasma phagocytophilum* propagation but not histopathology in the mouse model of human granulocytic anaplasmosis. *Clin Diagn Lab Immunol.* 2004; 11:963–968. [PubMed: 15358660]
60. Akkoyunlu M, Malawista SE, Anguita J, Fikrig E. Exploitation of interleukin-8-induced neutrophil chemotaxis by the agent of human granulocytic ehrlichiosis. *Infect Immun.* 2001; 69:5577–5588. [PubMed: 11500432]
61. Ge Y, Rikihisa Y. *Anaplasma phagocytophilum* delays spontaneous human neutrophil apoptosis by modulation of multiple apoptotic pathways. *Cell Microbiol.* 2006; 8:1406–1416. [PubMed: 16922860]
62. Choi KS, Park JT, Dumler JS. *Anaplasma phagocytophilum* delay of neutrophil apoptosis through the p38 mitogen-activated protein kinase signal pathway. *Infect Immun.* 2005; 73:8209–8218. [PubMed: 16299317]
63. Lee HC, Goodman JL. *Anaplasma phagocytophilum* causes global induction of antiapoptosis in human neutrophils. *Genomics.* 2006; 88:496–503. [PubMed: 16876385]
64. Ramabu SS, Schneider DA, Brayton KA, Ueti MW, Graça T, Futse JE, Noh SM, Baszler TV, Palmer GH. Expression of *Anaplasma marginale* ankyrin repeat-containing proteins during infection of the mammalian host and tick vector. *Infect Immun.* 2011; 79:2847–2855. [PubMed: 21576345]
65. Troese MJ, Kahlon A, Ragland SA, Ottens AK, Ojogun N, Nelson KT, Walker NJ, Borjesson DL, Carlyon JA. Proteomic analysis of *Anaplasma phagocytophilum* during infection of human myeloid cells identifies a protein that is pronouncedly upregulated on the infectious dense-cored cell. *Infect Immun.* 2011; 79:4696–4707. [PubMed: 21844238]

66. Troese MJ, Carlyon JA. *Anaplasma phagocytophilum* dense-cored organisms mediate cellular adherence through recognition of human P-selectin glycoprotein ligand 1. *Infect Immun*. 2009; 77:4018–4027. [PubMed: 19596771]
67. Dumler JS. The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. *FEMS Immunol Med Microbiol*. 2012; 64:13–20. [PubMed: 22098465]
68. Bakken JS, Dumler S. Human granulocytic anaplasmosis. *Infectious Disease Clinics of North America*. 2008; 22:433–448. viii. [PubMed: 18755383]
69. Dumler JS, Barat NC, Barat CE, Bakken JS. Human granulocytic anaplasmosis and macrophage activation. *Clin Infect Dis*. 2007; 45:199–204. [PubMed: 17578779]
70. Kocan KM, de la Fuente J, Blouin EF, Coetzee JF, Ewing SA. The natural history of *Anaplasma marginale*. *Vet Parasitol*. 2010; 167:95–107. [PubMed: 19811876]
71. Martin ME, Bunnell JE, Dumler JS. Pathology, immunohistology, and cytokine responses in early phases of human granulocytic ehrlichiosis in a murine model. *J Infect Dis*. 2000; 181:374–378. [PubMed: 10608792]
72. Niu H, Rikihisa Y. Investigating interference with apoptosis induction by bacterial proteins. *Methods in Molecular Biology*. 2014; 1197:169–184. [PubMed: 25172281]
73. Ge Y, Yoshiie K, Kuribayashi F, Lin M, Rikihisa Y. *Anaplasma phagocytophilum* inhibits human neutrophil apoptosis via upregulation of bfl-1, maintenance of mitochondrial membrane potential and prevention of caspase 3 activation. *Cell Microbiol*. 2005; 7:29–38. [PubMed: 15617521]
74. Banerjee R, Anguita J, Roos D, Fikrig E. Cutting edge: infection by the agent of human granulocytic ehrlichiosis prevents the respiratory burst by down-regulating gp91phox. *J Immunol*. 2000; 164:3946–3949. [PubMed: 10754283]
75. Carlyon JA, Chan WT, Galan J, Roos D, Fikrig E. Repression of rac2 mRNA expression by *Anaplasma phagocytophilum* is essential to the inhibition of superoxide production and bacterial proliferation. *J Immunol*. 2002; 169:7009–7018. [PubMed: 12471136]
76. Bunnell JE, Triggiani ER, Srinivas SR, Dumler JS. Development and distribution of pathologic lesions are related to immune status and tissue deposition of human granulocytic ehrlichiosis agent-infected cells in a murine model system. *J Infect Dis*. 1999; 180:546–550. [PubMed: 10395880]
77. Birkner K, Steiner B, Rinkler C, Kern Y, Aichele P, Bogdan C, von Loewenich FD. The elimination of *Anaplasma phagocytophilum* requires CD4+ T cells, but is independent of Th1 cytokines and a wide spectrum of effector mechanisms. *European journal of immunology*. 2008; 38:3395–3410. [PubMed: 19039769]
78. Martin ME, Caspersen K, Dumler JS. Immunopathology and ehrlichial propagation are regulated by interferon-gamma and interleukin-10 in a murine model of human granulocytic ehrlichiosis. *Am J Pathol*. 2001; 158:1881–1888. [PubMed: 11337387]
79. Scorpio DG, von Loewenich FD, Gobel H, Bogdan C, Dumler JS. Innate immune response to *Anaplasma phagocytophilum* contributes to hepatic injury. *Clin Vaccine Immunol*. 2006; 13:806–809. [PubMed: 16829620]
80. Akkoyunlu M, Fikrig E. Gamma interferon dominates the murine cytokine response to the agent of human granulocytic ehrlichiosis and helps to control the degree of early rickettsemia. *Infect Immun*. 2000; 68:1827–1833. [PubMed: 10722570]
81. von Loewenich FD, Scorpio DG, Reischl U, Dumler JS, Bogdan C. Frontline: control of *Anaplasma phagocytophilum*, an obligate intracellular pathogen, in the absence of inducible nitric oxide synthase, phagocyte NADPH oxidase, tumor necrosis factor, Toll-like receptor (TLR)2 and TLR4, or the TLR adaptor molecule MyD88. *Eur J Immunol*. 2004; 34:1789–1797. [PubMed: 15214027]
82. Choi KS, Webb T, Oelke M, Scorpio DG, Dumler JS. Differential innate immune cell activation and proinflammatory response in *Anaplasma phagocytophilum* infection. *Infect Immun*. 2007; 75:3124–3130. [PubMed: 17403880]
83. Sun W, Ijdo JW, Telford SR 3rd, Hodzic E, Zhang Y, Barthold SW, Fikrig E. Immunization against the agent of human granulocytic ehrlichiosis in a murine model. *J Clin Invest*. 1997; 100:3014–3018. [PubMed: 9399947]

84. Menasche G, Feldmann J, Fischer A, de Saint Basile G. Primary hemophagocytic syndromes point to a direct link between lymphocyte cytotoxicity and homeostasis. *Immunol Rev.* 2005; 203:165–179. [PubMed: 15661029]
85. Janka G, zur Stadt U. Familial and acquired hemophagocytic lymphohistiocytosis. *Hematology.* 2005; 2005:82–88. [PubMed: 16304363]
86. Larroche C, Mouthon L. Pathogenesis of hemophagocytic syndrome (HPS). *Autoimmun Rev.* 2004; 3:69–75. [PubMed: 15003190]
87. Grom AA. Natural killer cell dysfunction: A common pathway in systemic-onset juvenile rheumatoid arthritis, macrophage activation syndrome, and hemophagocytic lymphohistiocytosis? *Arthritis Rheum.* 2004; 50:689–698. [PubMed: 15022306]
88. Fisman DN. Hemophagocytic syndromes and infection. *Emerg Infect Dis.* 2000; 6:601–608. [PubMed: 11076718]
89. Davies RS, Madigan JE, Hodzic E, Borjesson DL, Dumler JS. Dexamethasone-induced cytokine changes associated with diminished disease severity in horses infected with *Anaplasma phagocytophilum*. *Clin Vaccine Immunol.* 2011; 18:1962–1968. [PubMed: 21880854]
90. Janka GE, Lehmborg K. Hemophagocytic lymphohistiocytosis: pathogenesis and treatment. *ASH Education Program Book.* 2013; 2013:605–611.
91. Janka GE, Lehmborg K. Hemophagocytic syndromes — An update. *Blood Rev.* 2014; 28:135–142. [PubMed: 24792320]
92. Zhang K, Jordan MB, Marsh RA, Johnson JA, Kissell D, Meller J, Villanueva J, Risma KA, Wei Q, Klein PS, Filipovich AH. Hypomorphic mutations in PRF1, MUNC13-4, and STXBP2 are associated with adult-onset familial HLH. *Blood.* 2011; 118:5794–5798. [PubMed: 21881043]
93. Weil AA, Baron EL, Brown CM, Drapkin MS. Clinical findings and diagnosis in human granulocytic anaplasmosis: a case series from Massachusetts. *Mayo Clin Proc.* 2012; 87:233–239. [PubMed: 22386178]
94. Brown WC, Palmer GH, Brayton KA, Meeus PF, Barbet AF, Kegerreis KA, McGuire TC. CD4+ T lymphocytes from *Anaplasma marginale* major surface protein 2 (MSP2) vaccinees recognize naturally processed epitopes conserved in MSP3. *Infect Immun.* 2004; 72:3688–3692. [PubMed: 15155686]
95. Brown WC, Palmer GH, Lewin HA, McGuire TC. CD4(+) T lymphocytes from calves immunized with *Anaplasma marginale* major surface protein 1 (MSP1), a heteromeric complex of MSP1a and MSP1b, preferentially recognize the MSP1a carboxyl terminus that is conserved among strains. *Infect Immun.* 2001; 69:6853–6862. [PubMed: 11598059]
96. Brown WC, Zhu D, Shkap V, McGuire TC, Blouin EF, Kocan KM, Palmer GH. The repertoire of *Anaplasma marginale* antigens recognized by CD4(+) T-lymphocyte clones from protectively immunized cattle is diverse and includes major surface protein 2 (MSP-2) and MSP-3. *Infect Immun.* 1998; 66:5414–5422. [PubMed: 9784552]
97. Han S, Norimine J, Brayton KA, Palmer GH, Scoles GA, Brown WC. *Anaplasma marginale* infection with persistent high-load bacteremia induces a dysfunctional memory CD4+ T lymphocyte response but sustained high IgG titers. *Clin Vacc Immunol.* 2010; 17:1881–1890.
98. Palmer GH, Eid G, Barbet AF, McGuire TC, McElwain TF. The immunoprotective *Anaplasma marginale* major surface protein 2 is encoded by a polymorphic multigene family. *Infect Immun.* 1994; 62:3808–3816. [PubMed: 8063397]
99. Abbott JR, Palmer GH, Kegerreis KA, Hetrick PF, Howard CJ, Hope JC, Brown WC. Rapid and long-term disappearance of CD4+ T lymphocyte responses specific for *Anaplasma marginale* major surface protein-2 (MSP2) in MSP2 vaccinees following challenge with live *A. marginale*. *J Immunol.* 2005; 174:6702–6715. [PubMed: 15905510]
100. Brown WC. Adaptive immunity to *Anaplasma* pathogens and immune dysregulation: Implications for bacterial persistence. *Comp Immunol Microbiol Infect Dis.* 2012; 35:241–252. [PubMed: 22226382]
101. 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. [PubMed: 16859547]

Figure.

Hypothetical macrophage activation (MAS) and hemophagocytic (HPS) syndromes with *A. phagocytophilum*, *Ehrlichia* species, and possibly *Rickettsia* species. The graphic depicts the normal contraction of immune response owing to cytotoxic lymphocyte recognition and deletion of antigen presenting cells, such as dendritic cells. In genetic forms of HPS (hemophagocytic lymphohistiocytosis) and in acquired forms of MAS and HPS, defective delivery of cytotoxic lymphocyte granule contents is accompanied by increasing IFN- γ production permitting continued immune stimulation, lack of pathogen control, hypercytokinemia, and ultimately massive tissue infiltration by inflammatory cells with accompanying inflammatory tissue or organ injury.

Table
Protective and immunopathologic responses linked to CD8 T lymphocytes in infections by Rickettsiales.

Agent	Protective	Immunopathologic
<i>Rickettsia conorii</i>	Depletion of CD8 T cells exacerbates severity and results in persistent infection in survivors [12].	Adoptive transfer of immune CD8 T cells at the peak of infection appears to exacerbate disease (Feng and Walker, unpublished).
<i>Rickettsia australis</i>	Immune spleen cells are cytotoxic for infected MHC I endothelial cells [11]. Gene-deficient mice are more susceptible to infection than wild type mice for MHC-I (50,000-fold), perforin (1,000-fold) and IFN- γ (100-fold) [11].	
<i>Rickettsia prowazekii</i>	Vaccination strategy to target MHC I by nucleofection with one rickettsial gene provided demonstrable protection [15].	
<i>Orientia tsutsugamushi</i>	CD8 $^{-/-}$ mice develop more severe illness than wild type mice. (Xu and Walker, unpublished)	
<i>Ehrlichia muris</i>	Mice depleted of CD8 and CD4 T lymphocytes have greater mortality than mice depleted of CD4 T cells only [32]. Mice deficient in MHC I have 81% mortality compared with 0% of wild type mice [32]. Immune CD8 T cells are cytotoxic for <i>E. muris</i> -infected cells [32].	
<i>Ehrlichia ruminantium</i>	Adoptive transfer of immune CD8 T cells is more protective than immune CD4 T cells [33].	CD8 $^{-/-}$ mice are less susceptible than wild type mice [36].
<i>Ixodes ovatus Ehrlichia</i> (IOE)	Adoptive transfer of <i>E. muris</i> -immune CD8 T cells along with polyclonal antibodies and immune CD4 T cells confers protection against a lethal IOE challenge [34].	Lethal IOE infection is caused by extremely high levels of TNF that is apparently produced by CD8 T cells and causes hepatic apoptosis/necrosis [35,37]. CD8 T cells also appear to eliminate protective CD4 T cells [37].
<i>Ehrlichia chaffeensis</i>		Human patients with human monocyctotropic ehrlichiosis and lymph node histopathologic hemophagocytosis, have more CD8 T lymphocytes than CD4 T lymphocytes, macrophages or B lymphocytes [101].
<i>Anaplasma phagocytophilum</i>	Mice depleted of both CD4 and CD8 T cells do not control infection as compared to wild type mice, but infection is well controlled in MHC I $^{-/-}$ but not MHC II $^{-/-}$ mice [77].	<i>Ex vivo</i> day 4–7 post-infection mouse splenic CD8 and NKT cells do not degranulate as much as cells from mock-infected mice when stimulated with ionomycin C [67].