Chapter 12
Epidemiology, Molecular Biology, and Pathogenic Mechanisms of *Ehrlichia* Infections

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1 Introduction

Ehrlichia are tick-borne, obligately intracellular Gram negative bacteria. The diseases caused by *Ehrlichia* are called ehrlichioses, which are zoonotic and are transmitted through tick bite. Ehrlichioses have been recognized as veterinary diseases much earlier than as human diseases. *Ehrlichia canis* is the first bacterium that was named as *Ehrlichia* in 1935 (Donatien and Lestoquard 1935), but the first *Ehrlichia* organism *Ehrlichia ruminantium* was discovered in 1925. At the time, it was named *Rickettsia ruminantium* (Allsopp 2010). Human ehrlichiosis caused by *E. chaffeensis* was discovered in 1980s (Maeda et al. 1987). Since then, other human ehrlichioses have been discovered including ewingii ehrlichosis and *E. muris* ehrlichiosis. The recognized *Ehrlichia* species include *E. canis*, *E. chaffeensis*, *E. muris*, *E. ewingii*, and *E. ruminantium*. All these organisms cause animal and human infections.

2 Definition and Phylogeny of *Ehrlichia*

Despite more than a century of research on *Ehrlichia* and in this era of molecular biology, the definition of *Ehrlichia* is not well-delineated. New species of *Ehrlichia* are continually designated, even though there are no criteria for a new species.

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Table 12.1 Percentage similarity of sequences of 16S rRNA gene of recognized *Ehrlichi*a species

		1	2	3	4	5	6	7	8	9	10	11
1.	*E. chaffeensis* AF416764	***	99.9	99.1	99.1	98.2	98.1	98.2	97.9	98.9	97.2	97.5
2.	*E. chaffeensis* U60476		***	99.2	99.2	98.2	98.2	98.2	97.9	98.9	97.3	97.6
3.	*E. muris* CP006917			***	100	98.3	98.2	98.3	98.6	98.8	97.8	97.9
4.	*E. muris* EMU15527				***	98.3	98.2	98.3	98.6	98.8	97.8	97.9
5.	*E. canis* EF011110					***	99.9	100	97.6	98.5	97.1	97.3
6.	*E. canis* AF162860						***	99.9	97.5	98.4	97.1	97.2
7.	*E. ovina* AF318946							***	97.6	98.5	97.1	97.3
8.	*E. ewingii* EEU96436								***	99.8	97	97.1
9.	*E. ewingii* NR_044747									***	98.2	98.3
10.	*E. ruminantium* NR_074513										***	99.7
11.	*E. ruminantium* X62432											***

Currently, *Ehrlichia* are classified using their 16S rRNA gene (*rrs*) sequence homology. Genetic analysis of the organisms in the genus *Ehrlichia* indicates that different strains of the same species have 99–100 % homology in the 16S rRNA gene sequence. Comparison of the sequence of 16S rRNA gene of the recognized species of *Ehrlichia* indicates that the closest homology among *Ehrlichia* species except for *E. canis* and *E. ovina* is 99.1 % between *E. chaffeensis* and *E. muris* (Table 12.1), which can be a criterion for classification of *Ehrlichia* organism. The sequence homology between *E. canis* and *E. ovina* is 99.9–100 %, indicating that these two organisms should be a single species, i.e., *E. ovina* is a strain of *E. canis* because *E. canis* was discovered much earlier than *E. ovina. E. canis* and *Candidatus* Ehrlichia regneryi are phylogenetically in the same cluster (Fig. 12.1) and share 99 % *rrs* DNA sequence homology (Table 12.1).

When more strains of *Ehrlichia* are added for comparison, the species boundaries become less distinct (Table 12.2). For example, *Ehrlichia* sp. HF (DQ647318) from an *Ixodes ricinus* tick in France is 99.2–100 % homologous to *E. muris*, and 99 % homologous to *E. chaffeensis* by *rrs* homology (Table 12.2). In this case, we think DNA sequence homology and phylogeny should be considered together. A genogroup contains *Ehrlichia* sp. HF (DQ647318) and several uncultured *Ehrlichia* species that are phylogenetically closely related to *E. muris*.

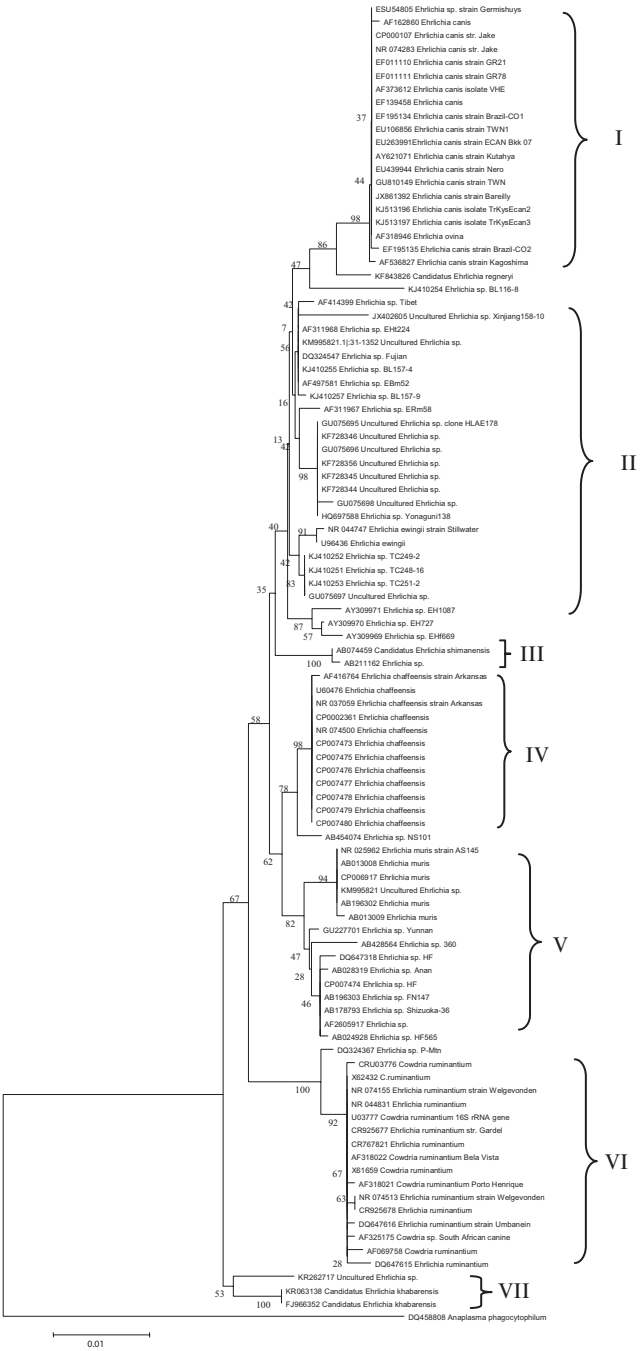

Fig. 12.1 Phylogenetic analysis of *Ehrlichia* species uisng the sequences of 16S rRNA gene. *Ehrlichia* species are classified into 7 geneotypes/species.

Table 12.2 Percent similarity of sequences of 16S rRNA gene of recognized *Ehrlichia* species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. AB196302 *E. muris*	***	98.6	98.6	98.5	98.7	99.2	98	97.7	97.9	97.2	97.1	97.2	97.2	97.5	97.1	96.8	97.1	97.9	97.8	97.6
2. AB024928 *Ehrlichia* sp.		***	98.8	99.8	99.9	99.8	98.6	98.3	98.3	97.7	97.5	97.6	97.6	97.8	97.2	96.9	97	98.3	98.3	97.8
3. AB428564 *Ehrlichia* sp.			***	98.8	98.9	99.2	98.2	97.9	97.9	97.2	97.3	97.3	97.6	97.8	96.9	97	96.8	97.9	97.9	97.8
4 AB028319 *Ehrlichia* sp.				***	99.9	99.8	98.5	98.3	98.3	97.7	97.5	97.5	97.5	97.7	97.1	96.9	96.9	98.2	98.3	97.7
5. AB178793 *Ehrlichia* sp.					***	99.8	98.7	98.4	98.4	97.8	97.6	97.7	97.7	97.8	97.3	97	97.1	98.4	98.4	97.9
6. DQ647318 *Ehrlichia* sp.						***	99	98.7	98.8	98.1	98	98.1	98.2	98.5	97.7	97.7	98	98.8	98.6	98.6
7. NR074500 *E. chaffeensis*							***	99.6	99	98.3	98.3	98.4	98.4	98	97.7	97.1	97.5	98.2	98.4	98.3
8. AF147752 *E. chaffeensis*								***	99.1	98.5	98.5	98.5	98.5	98.1	97.3	97.4	97.3	98.6	98.7	98.2
9. AF497581 *Ehrlichia* sp.									***	99.3	98.8	98.9	98.8	98.6	97.8	97.9	97.8	99.3	99.1	98.9
10. JX402605 *Ehrlichia* sp.										***	98.1	98.2	98.1	98	97.1	97.2	97.1	98.7	98.5	98.3
11. EF195135 *E. canis*											***	99.9	99	98.2	97.5	97.4	96.8	98.5	98.6	97.8
12. AF318946 *E. ovina*												***	99.1	98.1	97.4	97.4	96.9	98.5	98.5	97.9
13. KF843826 *E. regneryi*													***	98.2	97.2	97.3	97	98.2	98.5	97.9
14. U96436 *E. ewingii*														***	97.4	97.4	96.7	98.3	98.4	98.3
15. NR044831 *E. ruminantium*															***	96.2	97.1	97.7	98	97.6
16. KJ410254 *Ehrlichia* sp.																***	96.6	97.4	97.6	97.4
17. KR262717 *Ehrlichia* sp.																	***	97.2	97.4	97.5
18. KF728346 *Ehrlichia* sp.																		***	99	98.6
19. AY309970 *Ehrlichia* sp.																			***	98.6
20. AB074459 *E. shimanensis*																				***

Ehrlichia organisms can be classified into 7 genotypes/species (Fig. 12.1). Each species/genotype contains core species and satellite species. The first genotype is *E. canis* and *E. ovina* as core species (99.5–100 % *rrs* identity) and *Candidatus Ehrlichia regneryi* as satellite species (99 % *rrs* identity). Both *E. ovina* and *Candidatus Ehrlichia regneryi* may be strains of *E. canis*. The second genotype has *E. ewingii* as species and many uncultured *Ehrlichia* from ticks and animals as satellite species. These satellite species are mainly from Asia, especially from China. The classification of these species needs to be determined. The third genotype is *Candidatus Ehrlichia shimanensis* from ticks collected in Japan, which is most closely related to the uncultured *Ehrlichia* from China in the *E. ewingii* group (98.4–99.1 % *rrs* homology, Table 12.2). The fourth genotype is *E. chaffeensis*. The fifth genotype is *E. muris*, which can be further divided into two subtypes. The sixth genotype is *E. ruminantium*. The seventh genotype is *Candidatus* Ehrlichia khabarensis from ticks in the Russian Far East.

3 Biphasic Life Cycle Inside Host Cells

Electron microscopy showed that *E. chaffeensis* are polymorphic bacteria (0.2–2.0 μm in diameter), but mainly consists of two morphologic forms: a small dense-core cell (DC) and a large reticulate cell (RC) (Popov et al. 1995). A biphasic developmental cycle has been demonstrated. In the biphasic life cycle, the small DC is infectious, binds to, and is internalized into host cells, and then develops into a larger replicating RC inside a membrane-lined compartment that resembles late endosomes. After replication in expanding inclusions, the mature RCs transform into DCs and are released from the host cells (Zhang et al. 2007). DCs are more resistant to oxidative stress than RCs (Cheng et al. 2011).

4 Molecular Biology

4.1 Genome Reduction

Inside the host cell, *Ehrlichia* are evolved to obtain nutritional components from the host cell rather than expend energy to synthesize them. For a long period of intracellular life, *Ehrlichia* genes involved in metabolism have mutated, lost functions that are not required to synthesize molecules that are available from the host cell, and were eventually deleted. Therefore, *Ehrlichia* evolved a small genome through genome reduction process. The size of the *Ehrlichia* genome (approximately 1.2 MKbs) is only a quarter of the size of the genome of a free living bacterium *Escherichia coli*. All organisms in *Rickettsiales* including *Ehrlichia* have a diminished ability to synthesize amino acids compared to their closest free-living relatives (Yu et al. 2009). However, unlike members of the Rickettsiaceae family, *Ehrlichia* and *Anaplasma* are capable of producing all major vitamins, cofactors, and nucleotides, which could confer a beneficial role in the invertebrate vector or the vertebrate host (Dunning Hotopp et al. 2006).

4.2 Tandem Repeat and Ankyrin Repeat Proteins

The *Ehrlichia* genome encodes several surface proteins that are involved in host–pathogen interactions, including tandem repeat proteins and ankyrin repeat containing proteins (Dunning Hotopp et al. 2006; Mavromatis et al. 2006). The major immunodominant proteins of *E. chaffeensis* contain acidic serine-rich tandem repeats, including P120 (Yu et al. 1997), TRP47, and TRP32 (Doyle et al. 2006; Luo et al. 2008, 2009). *Ehrlichia chaffeensis* P120 and TRP47 are associated with dense-core ehrlichiae, and P120 has been demonstrated to be an adhesin (Popov et al. 2000).

Ankyrin (Andrić) repeat, one of the most widely existing protein motifs in nature, appearing as repeat units in *Ehrlichia* proteins, consists of 30–34 amino acid residues and exclusively functions to mediate protein–protein interactions, involved in a multitude of host processes including cytoskeletal motility, tumor suppression, and transcriptional regulation (Li et al. 2006; Mosavi et al. 2004). Ank consists of two anti-parallel α-helices connected to the next repeat unit via a loop region (Mosavi et al. 2004). Ank is very common in eukaryotes, but *ank* genes encoding heterogeneous Ank proteins are present in facultative or obligate intracellular bacteria, including *Ehrlichia* and *Anaplasma. Ehrlichia chaffeensis* Ank protein is a 200 kDa protein (Ank200), which is translocated to the nuclei of *Ehrlichia*-infected mononuclear phagocytes where it interacts with an adenine-rich motif in promoter and intronic *alu* elements (Zhu et al. 2009). The association of Ank200 with *alu* elements suggests that Ank200 could affect gene transcription globally through *alu*-mediated transcriptional control mechanisms. The global analysis of binding sites of Ank200 demonstrated that this protein binds to multiple regions distributed on nearly every chromosome via direct DNA interaction or with other DNA-binding proteins (Wakeel et al. 2010). *Ehrlichia chaffeensis* Ank200 interacts with apoptosis, ATPase, and transcriptional regulatory genes, and genes associated with pathogenesis and immune evasion including TNF-α, Jak2, and CD48 (Lee and Rikihisa 1996).

5 Epidemiology and Clinical Manifestations of Ehrlichioses

5.1 E. canis *Infection*

Ehrlichia canis was discovered in Algeria in 1935. The first case in the United States was reported in 1963. It was not until about 1968–1970, during the Vietnam war, when the full pathologic potential of *E. canis* was first recognized. A severe epizootic episode of ehrlichiosis occurred among U.S. military dogs resulting in hundreds of cases of morbidity and mortality.

Ehrlichia canis is transmitted through the bite of the brown dog tick *Rhipicephalus sanguineus*. However, *E. canis* is not transmitted transovarially in *R. sanguineus* (Groves et al. 1975). *Ehrlichia canis* organisms can infect and multiply in the midgut and salivary gland, but not in the ovary of ticks (Smith et al. 1976).

Adult brown dog ticks efficiently transmitted *E. canis* to susceptible dogs for 155 days after detachment as engorged nymphs from a dog in the acute phase of ehrlichiosis. Adult ticks that had similarly engorged on a dog in the chronic phase of ehrlichiosis failed to transmit *E. canis* to susceptible dogs, suggesting that acutely infected dogs are more important than chronically infected dogs in transmission of *E. canis* (Lewis et al. 1977). The brown dog tick is the most widespread tick, is more commonly found in warmer climates, and is associated with human habitations and domestic dogs in urban, suburban, and rural environments. The brown dog tick can be found in most populated areas in the United States and is rarely associated with uninhabited wild or forested areas (Faherty and Maurelli 2008). The brown dog ticks feed on a variety of hosts, but domestic dogs are the preferred host in the United States (Dantas-Torres 2010). *Rhipicephalus sanguineus* is unique among tick species as it can complete its entire life cycle indoors; therefore, infestations in homes or kennels can become established rapidly (Lord 2001). *Dermacentor variabilis* has been experimentally demonstrated to successfully transmit *E. canis* to dogs after transstadial ehrlichial passage after molting (Johnson et al. 1998).

Ehrlichia canis primarily infects dogs and occasionally infects humans and felines (Bowman et al. 2009). Antibody to *E. canis* has been detected in 64 of 250 patients suspected to have vector-borne diseases in Montenegro (Andrić 2014). Human infection with *E. canis* has been occasionally reported in South America. Two studies reported asymptomatic infection by *E. canis* in humans in Venezuela and Mexico (Perez et al. 1996). A third study reported that six human patients with clinical signs compatible with human monocytic ehrlichiosis and admitted to the emergency clinic in Lara State, Venezuela, were identified as infected by detection of *E. canis* 16S rRNA by gene-specific polymerase chain reaction (PCR) (Perez et al. 2006). These studies showed that *E. canis* can cause asymptomatic to severe disease in humans. This was the first report of *E. canis* infection of human patients with clinical signs of HME. Compared with the U.S. strains, 16S rRNA gene sequences from all six patients had the same base mutation as the sequence of the *E. canis* Venezuelan human *Ehrlichia* (VHE) strain previously isolated from an asymptomatic human.

Clinical manifestations of E. canis: In dogs experimentally infected with *E. canis*, incubation periods ranged between 17 and 22 days (mean = 19 days). Clinical signs typical of ehrlichiosis included mucopurulent ocular discharge, lymphadenopathy and malaise with accompanying pyrexia, leukopenia, and thrombocytopenia. Pyrexia, thrombocytopenia, erythrophagocytosis, and vacuolation of the cytoplasm of monocytic cells were observed 1–4 days prior to detection of morulae. *Ehrlichia canis* morulae in peripheral blood lymphocytes can be detected at 30 days post-exposure.

5.2 Human Monocytic Ehrlichiosis

In April 1986, a medical intern scanning the peripheral blood smear of a presumed spotless Rocky Mountain spotted fever patient discovered morulae inside monocytes of the patient (Paddock and Childs 2003; Fishbein et al. 1987).

These inclusions resembled morulae of *E. canis* previously known in the United States solely as veterinary pathogens (Maeda et al. 1987; Paddock and Childs 2003). The pathogenic bacterium formally named *E. chaffeensis* was used in cell culture in 1991 (Anderson et al. 1991; Dawson et al. 1991). The disease caused by *E. chaffeensis* was named human monocytic ehrlichiosis because the major target cells of *E. chaffeensis* are monocytes. Since then, epidemiologic studies indicated that human monocytic ehrlichiosis is a widespread and a significant public health problem in the United States. Human monocytic ehrlichiosis became a reportable disease in 1999 (Centers for Disease Control and Prevention). The number of ehrlichiosis cases due to *E. chaffeensis* reported to CDC has increased steadily since the disease became reportable; from 200 cases in 2000 to 961 cases in 2008, but the annual case fatality rate has declined (Centers for Disease Control and Prevention). The lone star tick, *Amblyomma americanum,* is considered the vector of *E. chaffeensis* (Anderson et al. 1993; Ewing et al. 1995). Ehrlichiosis is most frequently reported from the southeastern and south-central United States, from the eastern seaboard extending westward to Texas. The areas from which cases are reported correspond with the known geographic distribution of the lone star tick. These states include Oklahoma, Missouri, and Arkansas, which reported 30 % of *E. chaffeensis* infections (Paddock and Childs 2003).

5.3 Human Ewingii Ehrlichiosis

Ehrlichia ewingii causes granulocytic ehrlichiosis in dogs and was discovered to cause human infection in 1999. *Ehrlichia* DNA detected in leukocytes from four patients from Missouri by a broad-range *rrs* PCR assay matched that of *E. ewingii*, but not *E. chaffeensis* (Buller et al. 1999). *Ehrlichia ewingii* morulae were identified in neutrophils (Buller et al. 1999). *Ehrlichia ewingii* is also transmitted by the lone star tick in the US (Anziani et al. 1990).

The clinical manifestations of *E. ewingii* infection in humans include fever, headache, and thrombocytopenia, with or without leukopenia. Although previous literature suggests *E. ewingii* primarily affects those who are immunocompromised (Buller et al. 1999) or infected with HIV (Paddock et al. 2001), a recent study showed that most cases occurred among immunocompetent patients; among 55 cases between 2008 and 2012, only 26 % were those for whom immune status were immunosuppressed (Heitman et al. 2016). *Ehrlichia ewingii* infections are impossible to distinguish from *E. chaffeensis* infections based on clinical signs alone, and some proportion of cases currently reported as *E. chaffeensis* infection may actually be due to *E. ewingii*. There is no currently available serologic test that can distinguish these agents, and surveillance for *E. ewingii* is currently based on detection of the organism through molecular-based diagnostic tests. A total of 55 cases of *E. ewingii* were reported to CDC from 2008 to 2012 (Centers for Disease Control and Prevention) and none was reported to be fatal.

5.4 E. muris *Infection in Humans*

Ehrlichia muris was first described from the vole *Eothenomys kageus* as well as from murid rodents in Japan (Kawahara et al. 1993; Wen et al. 1995) and *E. muris*-like (EML) organism was described to infect humans in the US in 2009 (Pritt et al. 2011). Since its initial identification, at least 69 persons exposed in Wisconsin and Minnesota have been confirmed to have been infected with the EML organism (Johnson et al. 2015). EML infection in humans causes fever, malaise, headache, lymphopenia, thrombocytopenia, and elevated liver-enzyme levels. All recovered after receiving doxycycline treatment. EML organism has been detected from the deer tick *Ixodes scapularis* collected in Minnesota and Wisconsin (Pritt et al. 2011; Telford et al. 2011).

6 Pathogenic Mechanisms

The pathogenesis of severe ehrlichial infections is mediated principally by the immune response. Immunocompetent patients who die with HME have few ehrlichiae in their tissues, suggesting host-mediated pathogenic mechanisms. In contrast, severely immunocompromised patients with HME such as those with human immunodeficiency virus-acquired immunodeficiency syndrome have overwhelming *E. chaffeensis* bacterial loads, suggesting that the organisms themselves also can damage the host directly. Extensive studies in mice experimentally infected with highly virulent *Ixodes ovatus* ehrlichia (IOE) and *E. muris*, which causes persistent subclinical infection, have elucidated some unique immunopathologic mechanisms. IOE induces a pathogenic CD8 T cell response with tissue damaging overproduction of TNF-α and suppression of protective immunity by overproduction of IL-10 by CD4 T cells and FoxP3 T regulatory cells (Ismail et al. 2004, 2006, 2007). These immune responses to *Ehrlichia* infection are mediated by type I interferon (IFN-1), inflammasome activation, an early neutrophil response, NK cells, production of numerous chemokines and chemokine receptors, and Nod-2 activation. IFN-1 activation appears to lead to noncanonical inflammasome activation, decreased autophagic ehrlichial clearance, and immunosuppressive decreased IFN-γ/IL-10 ratio with fewer protective CD4 T cells and NKT cells, which are important sources of anti-ehrlichial IFN-γ (Yang et al. 2015; Zhang et al. 2014). The early neutrophil response results in an increased bacterial load and immunopathology associated with increased chemokines that may be the mechanism of increased NK cells and CD8 T cells in the lesions (Yang et al. 2013). Nod-2 activation results in decreased protection and increased bacterial load associated with increased CD8 T cells and decreased NKT cells, CD4 T cells, and IFN-γ. IL-18 and IL-1β derived from inflammasome activation play roles in mediating the decreased protective immunity and occurrence of severe immunopathology (Chattoraj et al. 2013).

7 Manipulating Host Immune System

Ehrlichia are obligate intracellular Gram negative bacteria, which invade and multiply inside host phagocytes including macrophages, monocytes, and neutrophils, and for some organisms, endothelial cells. Phagocytes are the result of highly evolved host defenses to destroy invading pathogens. However, *Ehrlichia* organisms have evolved multiple strategies to subvert host innate immune responses to create an environment that is suitable for the organisms to reside and multiply inside the host cells.

7.1 Invading the Host Cell

Ehrlichia has a special ability to enter and replicate inside host cells (macrophages/monocytes, neutrophils, and endothelium). The P120 surface protein of *E. chaffeensis* is able to mediate recombinant *E. coli* to enter Vero cells and is expressed only on the infectious dense-core cell of *E. chaffeensis*, but not on the non-infectious reticulate form of *E. chaffeensis* (Popov et al. 2000). In a recent study, another *E. chaffeensis* outer-surface protein, EtpE, was shown to bind to a specific host cell-surface protein, DNase X, and this ligand-receptor interaction is required to induce the entry of *E. chaffeensis* into its host cells. Mice immunized with the recombinant EtpE protein are resistant to *E. chaffeensis* challenge. Mice lacking DNase X are resistant to *E. chaffeensis* infection (Kumar et al. 2013).

7.2 Evading Lysosomal Destruction

Once inside the cytoplasm, the pathogen prevents *Ehrlichia*-containing vacuole (ECV) fusion with lysosomes, an essential condition for *Ehrlichia* to survive inside phagocytes, but the mechanism of inhibiting the fusion of the phagosome with lysosomes is unclear. *Ehrlichia chaffeensis*-containing vacuoles contain the late endosomal marker Rab7 and are acidified at approximately pH 5.2, suggesting that the *E. chaffeensis* vacuole is an acidified late endosomal compartment. *Ehrlichia chaffeensis* inhibits phagosome-lysosome fusion by modifying its vacuolar membrane composition, rather than by regulating the expression of host genes involved in trafficking (Cheng et al. 2014).

7.3 Evading Host Cell Pattern Recognition Receptors

Pattern recognition receptors (PRRs) are a primitive part of the innate immune responses because they evolved before other innate immune system components and adaptive immunity were evolutionarily developed. Toll-like receptors (TLRs)

are a type of PRR that recognize pathogen-associated molecular patterns (PAMPs). There are a total of 10 human and 12 mouse TLRs named from TLR1 to TLR13 and each recognizes and is activated by a particular pathogen molecule. TLR2 recognizes Gram-positive bacterial lipoproteins, and peptidoglycan activates TLR2 in conjunction with either TLR1 or TLR6; lipopolysaccharide (LPS) is detected by TLR4; flagellin is detected by TLR5; poly I:C, a double-stranded RNA (dsRNA) analog, is detected by TLR3; unmethylated DNA and CpG-oligodeoxynucleotides (CpG-DNA) are detected by TLR9; and single-stranded RNA and its synthetic analogs resiquimod, imiquimod, and loxoribine activate TLR7 (Takeda et al. 2003; Takahashi 2008).

In the evolution of its intracellular life cycle, *Ehrlichia* organisms have deleted genes encoding cell wall components that strongly stimulate PRRs including LPS and peptidoglycan. Due to the lack of these cell wall components, *Ehrlichia* organisms are not recognized by PRRs and do not stimulate proinflamatory cytokines via these components.

7.4 Inhibiting Host Cell Apoptosis

Apoptosis is a process of programmed cell death that occurs in multicellular organisms (Green 2011). Apoptosis is characterized by DNA fragmentation, chromatin condensation, cytoplasmic shrinkage, and cell death without lysis or damage to neighboring cells. Bacteria, viruses, and parasites can either induce or prevent apoptosis to augment infection. Many bacterial pathogens that cause apoptosis target immune cells such as macrophages and neutrophils because these cells would otherwise kill the pathogens (Faherty and Maurelli 2008). In contrast, because they multiply inside host cells, *Ehrlichia* have evolved a mechanism to inhibit apoptosis of infected host cells in order to prolong the opportunity for intracellular pathogen growth that requires host cell survival. *Ehrlichia chaffeensis* and *E. muris* inhibit apoptosis through up-regulating NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) (Mathema et al. 2013; Zhang et al. 2004). NF-kB is found in almost all animal cell types and is involved in cellular stress responses such as that caused by bacterial or viral infection, cytokine stimulation, free radicals, ultraviolet irradiation, and oxidized low-density lipoprotein stimulation (Gilmore 2006). NF-kB controls DNA transcription, cytokine production, and cell survival and plays a key role in regulating the immune response to infection. When the cell is not stimulated, the NF-kB dimers are sequestered in the cytoplasm by inhibitors of kB (IkBs). IkB contains multiple copies of ankyrin sequence repeats, which mask the nuclear localization signals (NLS) of NF-kB proteins to sequester NF-kB in the inactive state in the cytoplasm (Jacobs and Harrison 1998). In *Ehrlichia muris*-infected cells, IkB is degraded resulting in activation of NF-kB. NF-kB complex is then freed to enter the nucleus where it can "turn on" the expression of specific genes that have DNA-binding sites for NF-kB nearby. The activation of these genes by NF-kB then leads to a cell survival response, or cellular proliferation.

Ehrlichia ewingii infects neutrophils. Neutrophils generally have a short life span and naturally undergo apoptosis within 6–12 h after release into the peripheral blood from the bone marrow (Akgul et al. 2001), which is even shorter than the time for *Ehrlichia* to replicate and mature into dense core cells (Zhang et al. 2007). To overcome the short life of the neutrophil, *E. ewingii* infection delays canine neutrophil spontaneous apoptosis by maintaining the mitochondrial membrane potential in neutrophils (Xiong et al. 2008).

7.5 Dampening the Host Cell Immune Response

Both cellular and humoral immunity are important in elimination of *Ehrlichia* infection. Resistance of mice to sublethal challenge of *Ixodes ovatus* ehrlichia (IOE) is CD4-, but not CD8-, dependent and requires the IL-12p40-dependent cytokines, IFN-γ, and TNF-α, but not IL-4. In response to IOE antigens, CD4 T cells purified from infected mice proliferate in vitro and produce IFN-γ, which can rescue IFN-γ-deficient mice from fatal infection (Bitsaktsis et al. 2004). Wild-type, C57BL/6J mice are resistant to *E. chaffeensis*, but major histocompatibility complex class II (MHCII) knockout mice lacking helper T cells develop prolonged infections,$CD4^+$ T-cell-deficient mice clear the infection, but the clearance requires 2 weeks longer than in wild-type mice. These data suggest that the cell-mediated immunity orchestrated by $CD4^+$ T cells is critical for conferring efficient clearance of *Ehrlichia* (Ganta et al. 2004).

Although major roles are clearly played by T cells, antibodies can also control *Ehrlichia* infection in both normal and immunocompromised SCID mice and can protect the latter from lethal infection. Much of the humoral immune response is directed at the bacterial outer membrane proteins (OMPs). The antibodies (mostly IgG2a) can mediate bacterial clearance from tissues as early as 24 h after administration and require host Fc receptors for their function(s). One possible mechanism is that antibodies or immune complexes trigger microbicidal activities in infected macrophages that lead to the elimination of bacteria residing inside host macrophages. Alternatively, it is proposed that antibodies opsonize bacteria exposed during intercellular transfer. This notion is supported by studies that have demonstrated the presence of bacteria in the extracellular milieu during infection and suggests that our understanding of the behavior of the bacterium in the host may be key to our understanding of its susceptibility to antibody-mediated host defenses (Winslow et al. 2003). Survival of mice infected with highly virulent *Ixodes ovatus* ehrlichia requires both CD4 and CD8 T cells as well as antibodies (Ismail et al. 2004).

To survive inside animal hosts, *Ehrlichia* down-regulates host immune responses. *Ehrlichia chaffeensis* infection does not stimulate host cell cytokines that activate innate and adaptive immunity to intracellular bacteria. *Ehrlichia chaffeensis* does not stimulate IL-12, IL-15, and IL-18 production (Zhang et al. 2004). These cytokines play fundamental roles in stimulating NK cells and T helper 1 cells to produce gamma interferon (IFN-γ), which then activates macrophages to kill phagocytosed bacteria. IL-12 and IL-15 also activate NK cells and cytotoxic T lymphocytes to kill cells infected with intracellular bacteria. Thus, deficient production of IL-12, IL-15, and IL-18 may help *E. chaffeensis* to evade host innate and adaptive immunity.

Another intracellular bacterium, *Mycobacterium tuberculosis* (Nau et al. 2002), the intracellular protozoan *Leishmania major* (Carrera et al. 1996), and fungus *Histoplama capsulatum* (Marth and Kelsall 1997) inhibit IL-12 production. Thus, intracellular pathogens may have convergently evolved the ability to survive inside the macrophage by repressing IL-12 production.

MHC class II receptors are found on the surface of antigen presenting cells (B cells, macrophages, dendritic cells), but may be expressed by other cells (e.g., epithelial and endothelial) within inflammatory lesions (Day 2011). MHC class II molecules, first identified as antigen-presenting elements, interact with CD4 inflammatory (Th1) and helper (Th2) T-cells and are recognized as signal transduction molecules that regulate macrophage function (Day 2011). The expression of the MHC class II molecules is also necessary for CD4 T cell maturation. *Ehrlichia canis* infection downregulates MHC class II receptor expression in DH82 cells, suggesting a possible mechanism by which *E. canis* evades the immune system (Harrus et al. 2003).

Ehrlichia chaffeensis can survive by inhibiting critical signaling in monocyte activation pathways linked to pattern recognition receptors. *Ehrlichia chaffeensis* infection downregulates the expression of several pattern recognition receptors, including CD14, TLR2, TLR4, and transcription factor PU.1. *Ehrlichia chaffeensis* inhibits the activation of ERK 1/2 and p38 MAPK by LPS treatment in monocytes, suggesting that the inhibition of p38 MAPK by *E. chaffeensis* is involved in the suppression of several downstream signaling pathways (Harrus et al. 2003).

8 Conclusion

Ehrlichia are tick-borne, Gram negative intracellular bacteria, including *E. chaffeensis*, *E. canis*, *E. muris*, *E. ewingii*, and *E. ruminantium*. Human infections have been reported by all the *Ehrlichia* species. Human ehrlichioses have been mainly reported in the United States and have not yet reported in Asia, despite the existence of *Ehrlichia* in the area. Due to their obligately intracellular lifestyle, *Ehrlichia* reside in a stable environment with plenty of nutrients; therefore, *Ehrlichia* can afford to mutate and delete genes involved in cell wall components such as LPS and peptidoglycan and genes involved in metabolism, which resulted in a small genome. Without typical bacterial cell wall components, *Ehrlichia* become stealthy to the host cell innate immune system that recognizes pathogen-associated molecules. In addition to a stealthy cell wall, *Ehrlichia* have also evolved other strategies to subvert the host innate immune system such as inhibition of apoptosis and inhibition of fusion of *Ehrlichia*-containing vacuole with lysosome.

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