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Molecular characterization of *Ehrlichia* interactions with tick cells and macrophages

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1. ABSTRACT

Several tick-transmitted *Anaplasmataceae* family rickettsiales of the genera *Ehrlichia* and *Anaplasma* have been discovered in recent years. Some species are classified as pathogens causing emerging diseases with growing health concern for people. They include human monocytic ehrlichiosis, human granulocytic ewingii ehrlichiosis and human granulocytic anaplasmosis which are caused by *Ehrlichia chaffeensis, E. ewingii* and *Anaplasma phagocytophilum*, respectively. Despite the complex cellular environments and defense systems of arthropod and vertebrate hosts, rickettsials have evolved strategies to evade host clearance and persist in both vertebrate and tick host environments. For example, *E. chaffeensis* growing in vertebrate macrophages has distinct patterns of global host cell-specific protein expression and differs considerably in morphology compared with its growth in tick cells. Immunological studies suggest that host cell-specific differences in *Ehrlichia* gene expression aid the pathogen, extending its survival. Bacteria from tick cells persist longer when injected into mice compared with mammalian macrophage-grown bacteria, and the host response is also significantly different. This review presents the current understanding of tick-*Ehrlichia* interactions and implications for future research in devising effective control methods.

2. BACKGROUND

2.1. Tick-borne illnesses caused by *Anaplasmataceae* family pathogens

The family *Anaplasmataceae* within alpha-proteobacteria contains several species of obligate, intracellular pathogens of the genera *Ehrlichia* and *Anaplasma* that infect a wide range of vertebrate host species (1-3). In recent years, several new *Ehrlichia* and *Anaplasma* species responsible for causing potentially fatal human diseases have been reported (4-6). These species include *E. chaffeensis,* the causative agent of human monocytic ehrlichiosis (HME) in 1987 (7-9), *A. phagocytophilum,* the human granulocytic anaplasmosis (HGA) agent (previously known as the HGE agent) in 1994 (10) and *E. ewingii,* the agent of human ewingii *(*granulocytic) ehrlichiosis in 1999 (11). *E. ewingii* is first identified as the canine pathogen responsible for granulocytic ehrlichiosis (3,5,12). *Ehrlichia phagocytophilum* and *Ehrlichia equi* have long been known as the causal agents of bovine and equine ehrlichiosis, respectively (1-3,13). Following the discovery that human granulocytic ehrlichiosis (HGE) is caused by a pathogen highly homologous to bovine and equine ehrlichiosis pathogens in 1993 and subsequent molecular reevaluation, the HGE agent together with *E. phagocytophilum* and *E. equi* are grouped as one pathogen with a newly assigned name,

Anaplasma phagocytophilum (1). Infections in people with *E. canis*, the canine monocytic ehrlichiosis agent (3,5), are also reported from Venezuela (14,15). *E. ruminantium* is the agent of an economically important disease, heartwater, in domestic and wild ruminants in sub-Saharan Africa and in some Caribbean islands (16-18). Infections in ruminant populations with *E. ruminantium* can reach upto 80% mortality, particularly when the pathogen is introduced into a nonendemic area (18). Reports from South Africa suggest that *E. ruminantium* may be associated with infections and disease in people (19,20). A novel *Amblyomma americanum* tick-transmitted *Ehrlichia* is identified in Panola Mountain State Park, Georgia, USA (21). This *Ehrlichia*, referred to as the Panola Mountain *Ehrlichia* (PME), is closely related to *E. ruminantium*. Infections with this agent are originally reported in goats and white-tailed deer, *Odocoileus virginianus (*21,22). Human infections with PME have also been documented (23). However, the potential for PME to cause severe and potentially fatal infections in people remains unclear.

2.2. *E. chaffeensis*, an emerging pathogen

In 1987, monocytic infection with an *Ehrlichia* organism highly homologous to the canine ehrlichiosis agent, *E. canis,* is reported in a clinically ill patient with a history of tick bites (7,8,24). Subsequent molecular characterization confirmed that the human infection is the result of a new species, *E. chaffeensis (*24). Later, the transmitting tick vector, *A. americanum*, and the natural reservoir host of the pathogen, white-tailed deer, are identified (25-27). *E. chaffeensis* is now well recognized as the agent of HME (24). Initially, HME cases are reported from southeastern United States where the *A. americanum* tick is the most abundant species (28,29). Later, widespread distribution of the disease is documented from all parts of the USA and also from many regions of the world (30,31). As of 2006, there are approximately 600 documented cases in the USA per year (32). However, the true incidence is likely significantly more than reported because active surveillance studies revealed much higher infections rates. For example, a study conducted in the state of Missouri revealed 0.02%- 0.06% *E. chaffeensis* infection rates in people and a similar study in the state of Tennessee revealed even a higher incidence of 0.3%-0.4% (30). Together, these numbers translate to ~25,000 HME cases in Missouri and Tennessee alone. *A. americanum* has a much broader distribution than the foci of the disease indicates, spanning several southern and midwestern states. Consistent with this hypothesis, HME cases have been documented from many regions of the south central, southeastern and mid-Atlantic states, where *A. americanum* tick and white-tailed deer are prevalent (31).

E. chaffeensis is maintained in nature by transmission between its reservoir host and tick vectors, white-tailed deer and *A. americanum,* respectively. *E. chaffeensis* infections have also been identified in several other vertebrate animals such as dogs, coyotes, and raccoons (33-35). The availability of additional hosts for *E. chaffeensis* may contribute to the maintenance of this pathogen in nature. Ticks acquire an *E. chaffeensis* infection after feeding on an infected animal during their larval or nymphal stages. The bacteria acquired at larval or nymphal stage remain in the ticks as they transform (transstadial transmission) to nymphal or adult stages, respectively. Infected nymphs and adult ticks can then transmit *E. chaffeensis* to people and other vertebrates during blood feeding on a host (31,36) Disease manifestation of HME in people varies significantly from an asymptomatic infection to a severe, life-threatening disease with about 3% mortality rate (31). HME patients are typically more than 50 years old (37) and exhibit clinical symptoms that may include fever, headache, myalgia and malaise, thrombocytopenia, leucopenia, and hepatic injury (37-40).

2. 3. Rickettsial pathgen persistence

Tick-borne pathogenic bacteria reside in the complex cellular environments of ticks and vertebrates in spite of the sophisticated systems of defense found in these environments. The *Anaplasmataceae* pathogens evolved strategies to persist in both tick and vertebrate hosts in support of their lifecycle. Persistence in a host is particularly important for a pathogen, such as *E. chaffeensis,* which cannot be transovarially transmitted from an infected adult female tick to its offspring (41-43). Because ticks have a low probability of finding a host for a blood meal, a pathogen transfer from a tick to a vertebrate host would be significantly low. It is, therefore, crucial for a tick-transmitted pathogen to survive in its vertebrate host for extended periods of time. Understanding how tick-transmitted *rickettsiales,* such as *E. chaffeensis*, remain in tick and vertebrate hosts for long periods is important for devising effective methods of controlling the disease spread. There are many potential evasion mechanisms that allow tick-borne pathogens to persist in vertebrate hosts altering host responses, host protein mimicry, varying expressed antigens relative to time postinfection in a vertebrate host and vector- and host-specific changes in protein expression. Ticks are heterothermic, and vertebrate hosts are homeothermic, thus it is likely that the pathogens have evolved to express unique proteins and other macromolecules in support of their growth in dual-host environments.

Tick-transmitted *Ehrlichia* and *Anaplasma* species establish persistent infections in the vertebrates they infect (26,41-49). Molecular evidence on *A. marginale* and *A. phagocytophilum* suggest that the pathogens vary their antigens during persistent infection in vertebrate hosts, a possible mechanism for evading host clearance (50-53). To date, no evidence has been presented about antigenic variation in *Ehrlichia* species as one of the strategies employed in support of evading host clearance. Differential protein expression may be an important adaptation mechanism evolved by several members of the arthropod-borne pathogens to support their growth and persistence in invertebrate and vertebrate host-cell environments. For example, differential antigen expression is reported for tick-transmitted pathogens such as *A. marginale* and *Borrelia burgdorferi (*54-59). Differential expression in *B. burgdorferi* aids in the adaptation of the pathogen transition between the arthropod vector and mammalian hosts (55,56,58,59). *Ehrlichia* species may have evolved common strategies of host-pathogen adaptation that may be distinct

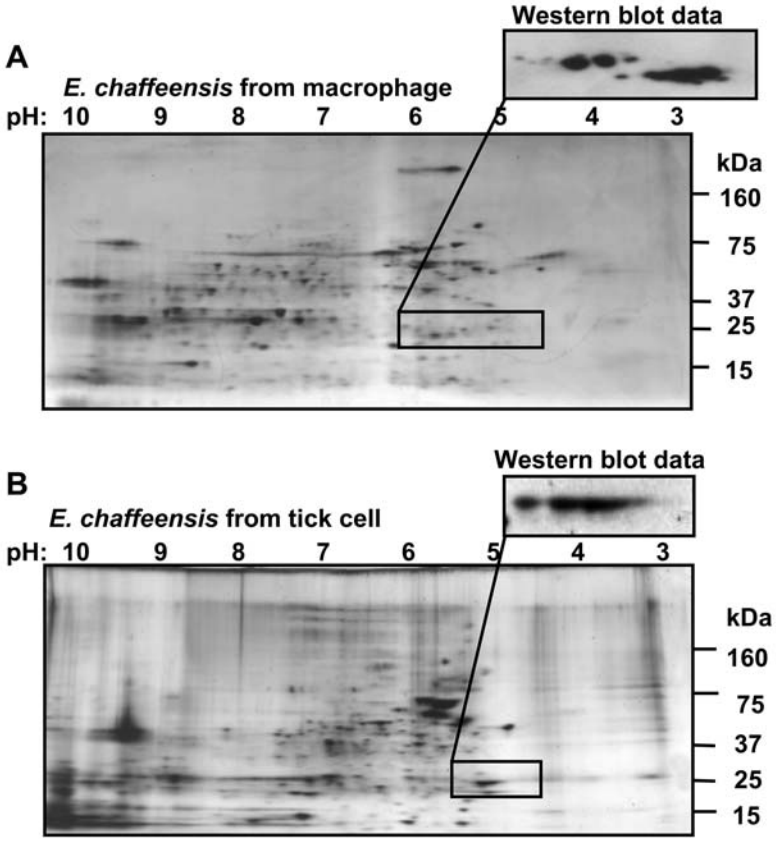

Figure 1. *E. chaffeensis* total proteins resolved on 2DE gels. The approximate pH values of the protein migration and the protein molecular weight standards are presented on the top and right side of the gel images, respectively. The insets represent Western blot data for the proteins spanning 28-30 kDa in size, identified using the *E. chaffeensis* polyclonal sera. [Reproduced with permission from (61) American Society for Microbiology.]

from those of *Anaplasma* species. *Ehrlichia* species share extensive genetic homology and have several expressed proteins with similar immunogenic epitopes (10,60). We reported that *Ehrlichia* species vary many expressed proteins in a host cell-specific manner. We also presented evidence suggesting that mice take longer to clear *E. chaffeensis* originating from a tick cell compared with those grown in a vertebrate host environment (described in detail in the following section).

3. MOLECULAR CHARACTERIZATION OF *EHRLICHIA* INTERACTIONS WITH TICK CELLS AND MACROPHAGES

3.1. Host specific differences in *E. chaffeensis* protein expression assessed at protein and RNA levels

We utilized proteomic approaches to demonstrate novel host cell-specific protein expression differences in *E. chaffeensis* (61-63). Two-dimensional gel electrophoresis (2DE) analysis revealed protein expression differences between *E. chaffeensis* cultivated in macrophages and tick cells (Figure 1) (61). *E. chaffeensis* grown in macrophages expresses numerous proteins that are resolved on pH gradient between 7 and 9, whereas bacteria grown in tick cells expressed considerably more proteins that resolved between pH 5 and 7. Immunoblots of 2DE proteome gels revealed host cell-specific differences in *E. chaffeensis* expressed proteins (Figure 1 inset). The majority of the immunoreactive proteins in *E. chaffeensis* grown in macrophage cells resolved in two rows between 28 to 30 kDa with pH ranging from 4.5 to 5.5. The immunodominant proteins of the tick cell-grown *E. chaffeensis* resolved as one row in the 30 kDa region within the same pH range. Mass spectrometric analysis by LC-MS/MS established these immunodominant proteins as the products of p28-Omp multigene locus genes 19 and 20 in macrophage-derived *E. chaffeensis* and p28-Omp14 in tick cell-derived *E. chaffeensis* (61). The expression of p28-Omp14 protein by *E. chaffeensis* growing in tick cells *in vitro* is consistent with the gene expression in *E. chaffeensis*-infected *A. americanum* ticks (45).

The *E. chaffeensis* p28-Omp multigene locus contains 22 tandemly arranged paralogous genes that encode for immunodominant 28 kDa outer membrane proteins. *E. canis* and *E. ruminantium*, two other closely related species of *E. chaffeensis*, also have multigene loci that are homologous to the p28-Omp locus of *E. chaffeensis* (Figure 2) (64-71). Recently, Kumagai *et al.* (72) reported that the p28-Omp gene products form porin-like structures and activities on the membrane of *E. chaffeensis*. Macrophage-

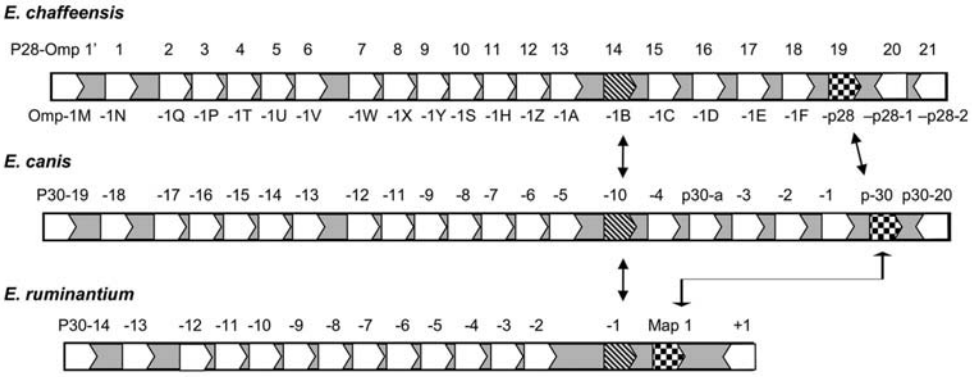

Figure 2. A cartoon representing the p28-Omp loci of *E. chaffeensis, E. canis* and *E. ruminantium* with identified expressed proteins from genes in vertebrate macrophages (slanted line boxes) and tick cells (checker board boxes) are presented.

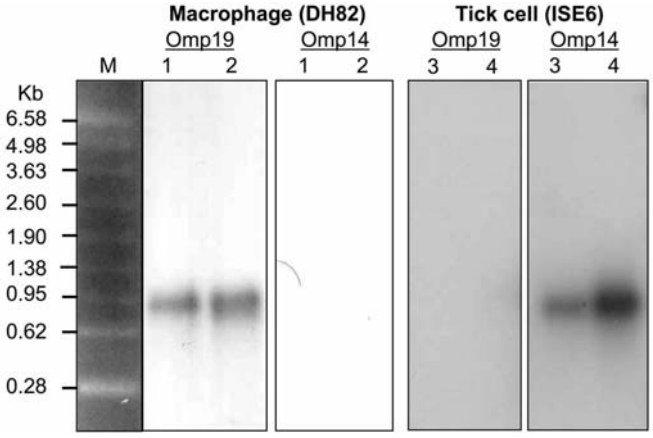

Figure 3. Northern blot analysis. About 10 micro grams each of *E. chaffeensis* RNA isolated from cultures grown in macrophage cell line, DH82 (lanes 1 and 2) and tick cell line, ISE6 (lanes 3 and 4) were resolved in duplicate sets on a denaturing RNA gel and assessed by Northern blot analysis using p28-Omp 14 or 19 gene-specific 32P-labeled probes. Gene 19 transcript of the size 0.9 kb is detected only in macrophage-derived RNA. Similarly, the tick cell-derived RNA contained 0.9 kb transcript for gene 14. RNA molecular weight markers were resolved in lane M. [Reproduced with permission from (62) Wiley-Blackwell Publishing.]

and tick cell-specific expression has also been documented from the p28/p30-Omp multigene loci from several *E. chaffeensis* isolates and in *E. canis (*61,62). Macrophage-specific expression in *E. chaffeensis* is primarily from p28-Omp 19 and from its paralog, p30, in *E. canis*. In *E. ruminantium* infected ruminants, the host induced B-cell response is against the protein made from major antigenic protein 1 (MAP1) gene (71,73-75) which is located at the 3' end of the alpha-region of the multigene locus similar to p28-Omp 19 and p30 of *E. chaffeensis* and *E. canis*, respectively (Figure 2). In tick cells, *E. canis* expresses only one protein, p30-10, from the p30-Omp multigene locus (62). This *E. canis* gene is positioned at the 5' end of the alpha-region, similar to the p28-Omp 14 gene in *E. chaffeensis (*70). The protein expression data of p28-Omp 14 and p30-10 in *E. chaffeensis* and *E. canis*, respectively, are consistent with the RT-PCR data for gene expression of *E. chaffeensis* and *E. canis* in infected ticks (45,76). Bekker *et al.* (77) presented evidence for the predominant expression of the *E. ruminantium* MAP1-1 gene in infected tick cells. This gene is also positioned within the multigene locus homologous to the p28-Omp locus of *E. chaffeensis* (71). The *E. ruminantium* MAP1-1 is located at the 5' end of alpha-region similar to that reported for p28-Omp 14 and p30-10 in *E. chaffeensis* and *E. canis*, respectively (Figure 2) (71).

Transcriptional analysis data of the p28-Omp 14 and 19 genes by Northern blot and diplex real time RT-PCR are in agreement with the protein expression data for these genes (Figures 3 and 4). The presence of multiple p28-Omp gene transcripts using non-quantitative RT-PCR methods have also been reported (45,78,79). One possible explanation for the presence of transcripts from multiple genes is that there may have been quantitative differences in the protein expression from the p28-Omp locus. For example, *Ehrlichia* species may induce higher levels of transcription leading to the synthesis of detectable protein from a subset of genes, whereas the gene activity from other genes is limited only to minimal levels of transcription that may be difficult to

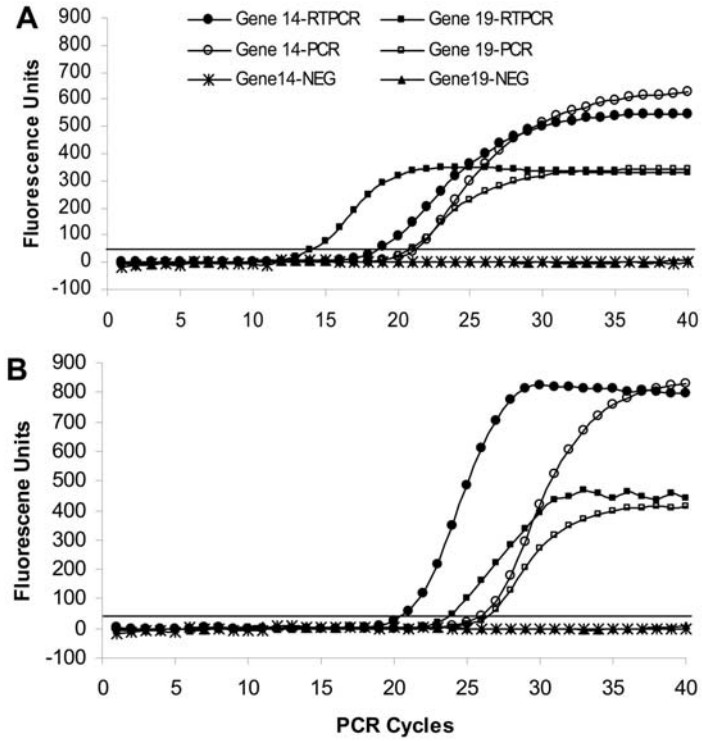

Figure 4. Diplex real-time RT-PCR assay. TaqMan-based diplex real time RT-PCR analysis was performed using total RNA isolated from infected macrophages (DH82) (panel A) or A. americanum tick cells; AAE2 (panel B). The amplification cycles were plotted against fluorescence emission for the analysis performed in the presence or absence of reverse transcriptase. Fluorescence emission crossing the threshold fluorescence line (horizontal solid line) at an amplification cycle is regarded as the Ct value. Gene 14-NEG and Gene 19-NEG represent data derived for reaction negative control diplex assay that included all assay components but no template; Gene 14-RTPCR and Gene 19-RTPCR represent data derived for a reaction containing RNA in presence of reverse transcriptase; Gene 14-PCR and Gene 19-PCR represent data generated for RNA from the diplex assay that did not include reverse transcriptase.) [Reproduced with permission from (62) Wiley-Blackwell Publishing.]

visualize with direct RNA detection methods. Diplex real time RT-PCR and large scale mass-spectrometric analysis of the total protein repertoire support this hypothesis (62,63). Using real time RT-PCR analysis, we noted minor expression of transcripts from gene 14 relative to gene 19 in macrophage-grown *E. chaffeensis* and similar minor expression of gene 19 relative to gene 14 in the bacteria derived from tick cells (Figure 4) (62). *E. chaffeensis* total protein expression is assessed using a shotgun proteomic approach. The analysis revealed the expression of 18 of the 22 p28-Omp proteins in macrophage-grown *E. chaffeensis* and p28-Omp 1 and 14 in the tick cell-grown pathogen (63). Nevertheless, the major expressed proteins in macrophage and tick cells are still the p28-Omp 19 and 14 gene products, respectively *(*63). These data are consistent with the identification of multiple p28-Omp proteins being expressed by *E. chaffeensis* by Ge and Rikihisa (80) and the identification of immunoglobulins specific for all 22 proteins of the p28-Omp locus in dogs experimentally infected with *E. chaffeensis (*81). It is not clear why *E. chaffeensis* expresses nearly all 22 proteins when it is grown in macrophages with dominant expression by one or two genes. One hypothesis is that the dominant expression of p28-Omp 19 is critical for the pathogen's survival in vertebrate hosts and the expression of other genes at low levels may serve as non-essential decoy targets to confuse the host immune system. This strategy allows the pathogen to evade host defenses and survive longer in the vertebrate. Conservation of host cell-specific protein expression from similar genomic regions of three different *Ehrlichia* species suggest that the pathogens have conserved this decoy strategy by inducing the expression of multiple proteins in support of their growth in vertebrate cells.

Expressed p28-Omp proteins in *E. chaffeensis* and *E. canis* have post-translational modifications: phosphorylation and glycosylation (61,62). Phosphorylation is reported for the first time in an *Ehrlichia* species, whereas glycosylation has been documented for several membrane-expressed proteins of both *Ehrlichia* and *Anaplasma* species (82-84). Prokaryotic glycoproteins may be involved in maintaining cell shape, protein stability, protection against proteolysis and/or adherence to host cells (82,83,85). MSP1a is a glycosylated outer surface protein of *A. marginale.* It appears to be involved in adhesion of the bacteria to host cells because chemical deglycosylation significantly reduces the bacteria's adhesive properties (82). As with p28-Omp proteins in *Ehrlichia* species, MSP1a is differentially

Table 1. Membrane immunogenic proteins of *E. chaffeensis* analyzed by mass-spectrometry (MOLDI-TOF)

	Protein identification [1]	GenBank Numbers	Theoretical MW (Da)	Protein identified [2]	
1	*Phage minor structural protein, N-terminal domain protein*	ECH_0568	171,570	M	
2	Hypothetical protein ECH_0488	ECH_0488	158,757		T
3	DNA-directed RNA polymerase beta' subunit	ECH_0951	157,560		T
4	*Ankyrin repeat protein*	ECH_0684	156,542	M	
5	Putative proline dehydrogenase/pyrroline-5-carboxylate	ECH_0667	116,167	M	
6	*AcrB/AcrD/AcrF family protein*	ECH_0561	116,001	M	
7	Hypothetical protein ECH_0176	ECH_0176	97,292	M	T
8	*ATP-dependent protease La*	ECH_0899	90,079	M	T
9	Polyribonucleotide nucleotdyltransferase	ECH_0726	86,972	M	
10	*Type IV secretion system protein VirD4*	ECH_0040	82,702		T
11	Glycyl-tRNA synthetase, beta subunit	ECH_0024	81,148		T
12	Elongation factor G	ECH_0961	76,112		T
13	Primosomal protein N'	ECH_0483	74,453		T
14	*Heat shock protein 90*	ECH_0853	72,751	M	T
15	*Ribonucleoside-diphosphate reductase, alpha subunit*	ECH_0459	67,749	M	T
16	Thiamin biosynthesis protein ThiC	ECH_0798	62,309	M	
17	Putative monovalent cation/H+ antiporter subunit D	ECH_0474	59,124	M	
18	*Hypothetical protein ECH_0526*	ECH_0526	55,364		T
19	Magnesium chelatase, subunit D/I family, ComM subfamily	ECH_0532	55,266		T
20	Phage uncharacterized protein	ECH_0665	54,842		T
21	F0F1 ATP synthase subunit beta	ECH_0573	54,722		T
22	Hypothetical protein ECH_0159	ECH_0159	54,222		T
23	Argininosuccinate lyase	ECH_0937	52,927		T
24	Dihydrolipoamide dehydrogenase	ECH_0509	49,647		T
25	*Prolyl-tRNA synthetase*	ECH_0740	48,633	M	
26	Type IV secretion system virB10	ECH_0042	48,373	M	
27	*Serine hydroxymethyltransferase*	ECH_0311	45,626	M	
28	Cell division protein FtsA	ECH_1090	44,895		T
29	ATP-dependent protease ATP-binding subunit (clpx)	ECH_0900	44,721	M	
30	*Elongation factor Tu*	ECH_0407	43,256	M	T
31	Hypothetical protein ECH_0116	ECH_0116	42,411		T
32	NADH dehydrogenase H subunit	ECH_0617	40,681	M	T
33	Hypothetical protein ECH_0635	ECH_0635	40,203		T
34	Aspartate-semialdehyde dehydrogenase	ECH_0016	37,072	M	
35	Type IV secretion system VirB11	ECH_0041	36,698	M	
36	Octaprenyl-diphosphate synthase	ECH_0088	36,312	M	
37	Putative dehydrogenase subunit beta	ECH_0149	36,281	M	
38	Prolipoprotein diacylglyceryl transferase	ECH_1101	32,539	M	T
39	Hypothetical protein ECH_0895	ECH_0895	31,573		T
40	*p28-omp 14 (Omp-1B)*	ECH_1136	30,886	M	T
41	*p28-omp 19 (Omp-p28)*	ECH_1143	30,212	M	
42	*Putative lipoprotein*	ECH_0128	29,973	M	
43	*p28-Omp 20 (Omp-p28-1)*	ECH_1144	29,919	M	
44	Putative pyrroline-5-carboxylate reductase	ECH_0013	29,554		T
45	Putative competence protein ComL	ECH_1005	29,288		T
46	Hydrolase, alpha/beta fold family	ECH_0326	29,248	M	
47	*Isoprenoid biosynthesis protein with amidotransferase-like domain*	ECH_0012	24,872		T
48	Hypothetical protein ECH_0988	ECH_0988	24,160	M	
49	Hypothetical protein ECH_0199	ECH_0199	23,748		T
50	*Superoxide dismutase, Fe*	ECH_0493	23,357	M	
51	*Hypothetical protein ECH_0663*	ECH_0663	23,297	M	
52	GTP-binding protein EngB	ECH_0595	22,767		T
53	Peptidyl-tRNA hydrolase	ECH_0141	22,495		T
54	*Deoxycytidine triphosphate deaminase*	ECH_0296	21,363	M	
55	Hypothetical protein ECH_0578	ECH_0578	21,339	M	
56	*Hypothetical protein ECH_0660*	ECH_0660	21,301	M	
57	Cytochrome c oxidase assembly protein CtaG	ECH_1055	19,880		T
58	Phosphoribosylcarboxyaminoimidazole carboxylase, catalytic subunit	ECH_0160	17,561	M	
59	*Iron-sulfur cluster assembly accessory protein*	ECH_0631	16,994	M	T
60	Iron-sulfur cluster binding protein	ECH_0634	12,721		T
61	*Thioredoxin*	ECH_0218	11,933		T
62	Hypothetical protein ECH_0181	ECH_0181	11,699	M	
63	*Hypothetical protein ECH_1043*	ECH_1043	10,885	M	

[1] Italic text, proteins identified from immuno blot analysis; [2]M, macrophage and T, tick cell

expressed when it is grown in vertebrate and tick cells (57). Phosphorylation of proteins in bacteria can affect a wide variety of cellular activities ranging from activation of membrane protein transport to alteration of a protein's function (86). However, little is known about the contributions of either glycosylation or phosphorylation to the growth and persistence of *Ehrlichia* species in vertebrate and tick hosts. It is not clear how those different protein forms modified bacterial function or recognition by a host.

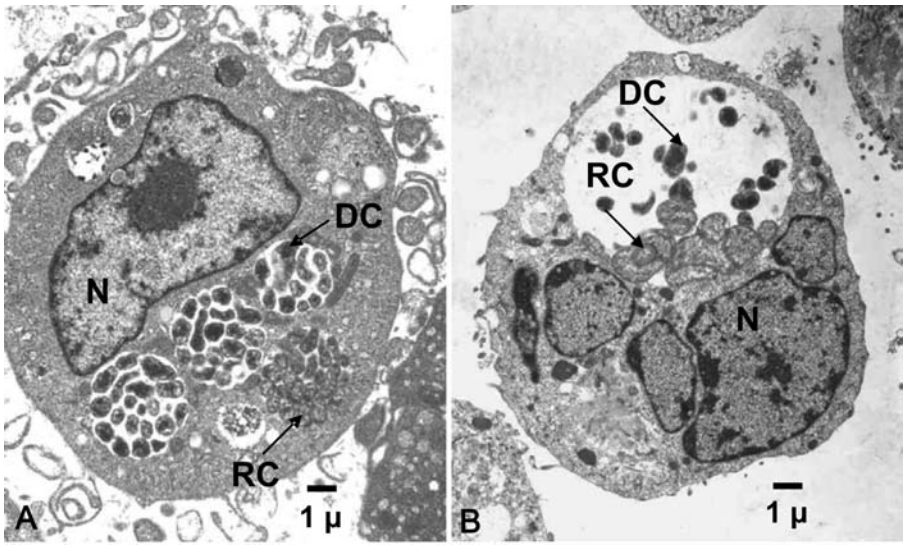

Figure 5. Transmission electron microscopy analysis was performed on *E. chaffeensis*-infected macrophages (A) and tick cells (ISE6) (B). (N, nucleus; DC, densed core bodies of *Ehrlichia* in phagosomes; RC, reticulate bodies of *Ehrlichia* in phagosomes)

This is clearly a nascent field for *Ehrlichia* and *Anaplasma* research.

To identify the expressed proteins, we analyzed total, membrane and immunogenic proteomes of *E. chaffeensis* grown in macrophage and tick cell cultures (63). Total proteins resolved by one-dimensional gel electrophoresis and individually picked immunogenic membrane and total membrane proteins subjected to mass-spectrometry. These analyses identified 191 and 128 proteins from macrophage- and tick cell-derived *E. chaffeensis*, respectively (63). These represent a total of 278 distinct *E. chaffeensis* proteins. Genes encoding for normal physiological functions of a cell, (e.g., those involved in protein synthesis, energy metabolism and biosynthesis of building blocks (amino acids, nucleic acids and lipids.)) are among the commonly expressed proteins (63). Macrophage- and tick cell-specific proteins also included many hypothetical, cell envelope proteins and proteins with unknown function. Proteins identified in the unknown function group included many novel proteins such as ankyrin repeat proteins, GTP-binding proteins, zinc finger-like domain proteins and metallopseudopeptide glycoprotease. The ankyrin-repeat protein homologue from *A. phagocytophilum* has recently been described as a secretory protein (87,88) and may play an important role in modulating host resistance, possibly by interfering with host gene expression (89). GTP-binding proteins and zinc finger proteins play important roles in regulating cell function and gene expression (90,91). Expression of these proteins in *E. chaffeensis* suggests that they may allow the bacteria to control cellular processes within macrophage and tick cells.

The outer membrane is the primary contact between *Ehrlichia* and the parasitized cell. Membrane proteins, therefore, are likely targets of the host response (65,92,93). It would be to a pathogen's advantage to alter its membrane makeup to avoid clearance and to support its adaptation to host environments. *E. chaffeensis* membrane protein analysis by mass-spectrometry revealed the identity of 63 expressed proteins (63) (Table 1). Macrophage-derived *E. chaffeensis* membrane proteins included several that are not detected in tick cell-derived bacteria. Mass-spectrometric analysis of total and immunogenic membrane proteins from tick cell-derived *E. chaffeensis* also confirmed the expression of host cell-specific p28-Omp protein expression reported earlier (61,62). In addition, total membrane protein analysis aided in identifying several more membrane-associated proteins (Table 1). These novel data suggest that the *E. chaffeensis* membrane is very complex and differs considerably in bacteria originating from macrophage and tick cells.

3.2. Host specific differences in *E. chaffeensis* morphology assessed by transmission electron microscopy

Transmission electron microscopic analysis revealed considerable differences in *E. chaffeensis* originating from macrophages and tick cells (DeDonder and Ganta, unpublished results). An important difference noted is that the pathogen in macrophage phagosomes typically grows in a synchronized form, whereas in tick cells both reticulate and dense core bodies are seen (Figure 5). Also, the reticulate bodies in tick cells are pleomorphic and larger in size than those observed in macrophages.

3.3. Tick cell-derived *E. chaffeensis* clearance by mice is delayed and associated with an altered immune response

Borrelia burgdorferi, a tick-transmitted spirochete, varies protein expression between tick and vertebrate hosts and the differences in protein expression appear to contribute to the spirochete's dual host adaptation (55,56,58). For example, *B. burgdorferi*, expresses outer surface protein A (OspA) when it infects the tick, *Ixodes scapularis*, and

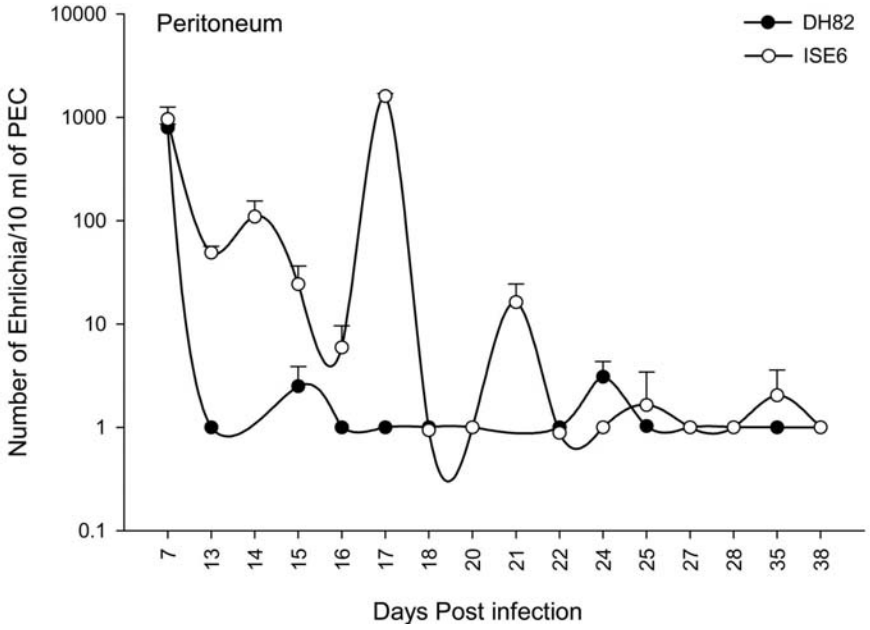

Figure 6. Kinetics of wildtype, C57BL6 (B6), mouse infection after i.p. inoculation of *E. chaffeensis* cultures grown in macrophage cells (DH82) (●) or tick cells (ISE6) (○). *E. chaffeensis* clearance was assessed by 16S rRNA-based real time RT-PCR assay. The number of bacteria were estimated by extrapolation of the RT-PCR cycles at which the samples tested positive (Ct-values) compared to Ct values of known numbers of bacteria in a standard curve. Number of bactaria were plotted against days post infection on a semi log graph. The data represent the median values±Sd from five independent experiments with 4-6 mice per group per each time point. [Reproduced with permission from (95) American Society for Microbiology.]

continues to produce abundant quantities of OspA while growing in the resting tick (references in (55)). During the transmission from tick to mammals and while it is growing in the vertebrate the predominant outer membrane protein expression is switched from OspA to OspC. To address whether host-specific differences in protein expression by *E. chaffeensis* similarly contribute to the pathogen's adaptation, we conducted experimental infection studies in C57Bl/6J mice.

E. chaffeensis grown in macrophages is cleared by mice in about two weeks, and optimal resolution requires macrophage activation, MHCII molecules, $CD4^+$ helper T-cell responses and antibody production (94-96). The rapid clearance of *Ehrlichia* in mice contrasts persistent infections in hosts infected from a tick bite (42,48,49). Therefore, because *Ehrlichia* protein expression is influenced by the parasitized cell, and possibly by the way the bacteria are spread, we assessed the effect of the origin of the bacteria on host resistance and immune function (95). Although the bacteria grown in tick cells or macrophages are cleared by mice, the infection by *E. chaffeensis* grown in tick cells takes longer to cure (Figure 6). Those mice exhibited higher rickettsemia and resolved peritoneal bacteremia about 9 days later than mice injected with bacteria grown in macrophages (Figure 6). This appears to be, in part, a result of the poorer macrophage response as judged from the nitric oxide and IL-6 secretion by the peritoneal macrophages (95). *E. chaffeensis* grown in macrophages and tick cells induced a similar range of macrophage and T-cell cytokines. However, the concentrations of cytokines made in response to infection with tick cell-derived *E. chaffeensis* were lower than those induced by macrophage-derived bacteria (95). The host response against bacteria originating from tick cells also included a steady rise in antibody production with distinct antibody specificities compared with that observed for macrophage-grown bacteria (Figure 7) (95). The suppressed cytokine responses in mice challenged with tick cell-grown bacteria along with their distinct humoral responses have significant implications. Importantly, the host cell environment (macrophage vs. tick cells) plays a role in activating the host immune response. The transition of host cell-specific protein expression (e.g. p28-Omp 14 to Omp19) is a slow process for pathogens moving from tick cells to mammalian macrophages under both *in vitro* and *in vivo* conditions (Figure 8) (95). Therefore, the delayed clearance of tick cell-derived *E. chaffeensis* with distinct immune responses may reflect the host's inability to alter its immune response to the changing antigenic makeup of the bacteria. The differential protein expression might be one of the important virulence mechanisms used by *E. chaffeensis* to persist longer in vertebrate hosts.

4. CONCLUSIONS AND PERSPECTIVES

In summary, studies aimed at understanding the evasion strategies employed by pathogens are particularly important in devising effective intervention measures. We discovered that *E. chaffeensis* protein expression for the pathogen grown in tick cells differs considerably from that of the pathogen grown in macrophages (61-63). These differences caused a delay in curing the infection and a

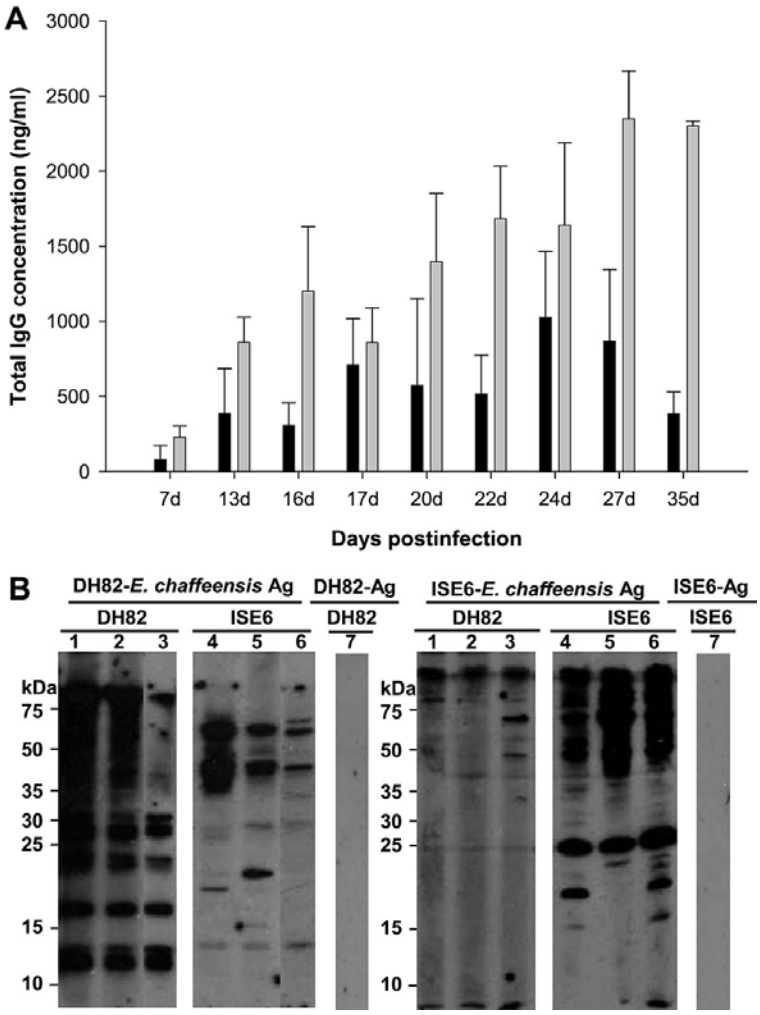

Figure 7. A) Total IgG made by B6 mice infected with *E. chaffeensis* cultivated DH82 or ISE6 Cells. Plasma samples were analyzed by quantitative ELISA. The data are presented by days post-infection and are pooled from 8-12 mice for all post-infection dates. Each bar represents the mean±sd IgG concentration determined from samples analyzed. Data in solid bars represent total IgG concentration assessed for macrophage-derived plasma using antigens derived from *E. chaffeensis* cultured in DH82 cells, while shaded bars represent data for plasma from mice infected with ISE6 culture derived- *E. chaffeensis* using purified homologous antigen. B) Western blot profile showing response to *E. chaffeensis* whole cell antigens. Representative data for three mice each for 28 days post-infection were presented. Lanes 1 to 3 had plasma for three mice infected with DH82 culture-derived *E. chaffeensis*; lanes 4 to 6 had plasma for three mice infected with ISE6 culture-derived *E. chaffeensis*. Lane 7 had plasma from one of the mice infected with DH82 cultures derived *E. chaffeensis*. Similarly, lane 8 had plasma from one of the mice infected with ISE6 culture derived *E. chaffeensis*. First set of six samples contained antigens derived from DH82 grown *E. chaffeensis*. Second set of six lanes included purified *E. chaffeensis* antigens from ISE6 cultures. Lanes 7 and 8 had antigens derived from uninfected DH82 and ISE6 cultures which were subjected to similar purification protocol as used for *E. chaffeensis* from infected cultures. [Reproduced with permission from (95) American Society for Microbiology.]

subdued immune response to tick cell-derived *E. chaffeensis (*95). The data suggest that *E. chaffeensis* protein expression pattern differences in tick cells and macrophages influence the way animals respond to infection. Although, some of the differences in protein expression are confirmed *in vivo*, whether the proteomic differences observed *in vitro* for macrophage and tick cell-grown *Ehrlichia* reflect those in the bacteria in ticks and vertebrate hosts remains to be determined. Importantly, *Ehrlichia* protein expression changes during the pathogen developmental cycle in a vertebrate host and in a tick at salivary glands and midgut may differ considerably and the differences may play crucial role in the pathogen's survival. Nonetheless, our novel findings of host-specific differences in the pathogen proteomes and morphology, and differences in host responses point out that the pathogen emerging from the tick cell environment has evolved a natural ability to evade the host response. Importantly, the early host immune response to *Ehrlichia* growing in tick cells is likely to be significantly different than that for bacteria growing in macrophages.

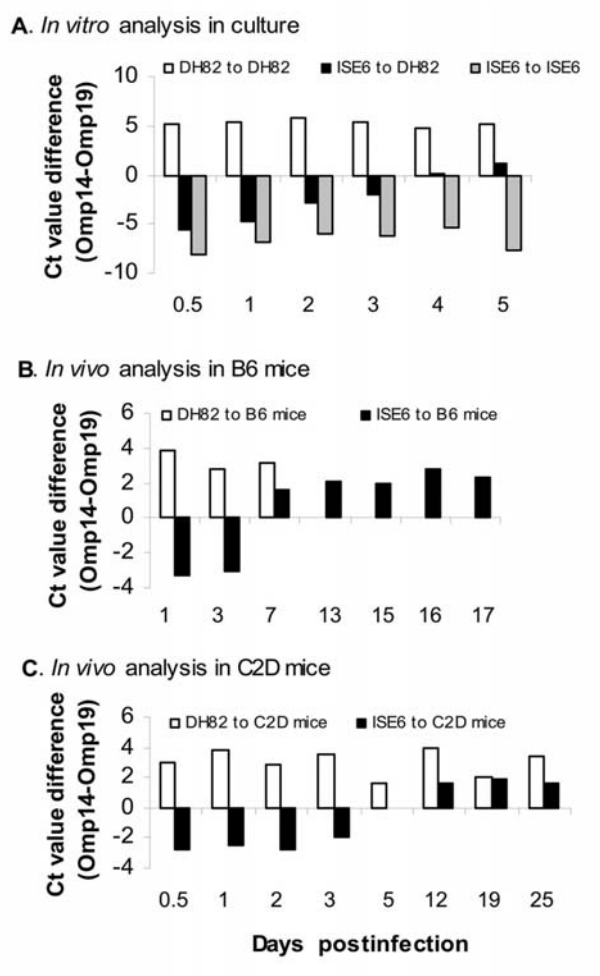

Figure 8. *In vitro* and *in vivo* gene expression of p28-Omp locus genes 14 and 19. A TaqMan-based, diplex real time RT-PCR analysis was performed to assess the expression for p28-Omp multigene locus genes 14 and 19 of *E. chaffeensis.* RNA was isolated from *in vitro* cultures infected with tick cell- or macrophage culture-derived *E. chaffeensis* (panel A). The RNA analysis was also performed on the RNAs isolated from the B6 and C2D mice (panels B and C, respectively) infected with macrophage- or tick cell-derived *E. chaffeensis*. The Ct value differences (gene 14 Ct value-gene 19 Ct value) in the amplification cycles were plotted for analyzed RNA samples collected at different times after infection. The negative values refer to high level transcription of gene 14 relative to gene 19, whereas the positive values indicate that the transcription of gene 19 was higher. (In panel A, DH82 to DH82 refers to *E. chaffeensi*s organisms grown in the macrophage cell line and used to infect DH82 cells; ISE6 to DH82 indicates that ISE6 tick cell-grown bacteria were used to infect DH82 cells, whereas ISE6 to ISE6 represents ISE6 culture-derived *E. chaffeensis* that was used to infect ISE6 cells. Panel B, DH82 to B6 mice indicates that DH82-grown bacteria were used as the inoculum to infect B6 mice; ISE6 to B6 represents B6 mice infected with ISE6 culture-derived *E. chaffeensis*. In panel C, DH82 to C2D mice (mice deficient in MHC class II expression) and ISE6 to C2D mice are similar to the descriptions for the captions in panel B except that the C2D mice were used for infection. [Reproduced with permission from (95) American Society for Microbiology.]

Because the antigenic make up on *Ehrlichia* originating from tick cells is likely to represent antigens that a mammalian host sees during the early stages of a natural infection from an infected tick, future studies should focus on understanding differences in *Ehrlichia* in ticks and the importance of host-specific, differentially expressed proteins to the pathogen's growth and how they allow for the pathogen's survival in response to vertebrate host immunity. Such studies are important for learning what actually happens in nature and how to develop effective methods of controlling tick-borne illnesses.

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6. REFERENCES

1. 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* 51, 2145-2165 (2001)

2. Ristic M, Huxsoll DL: Tribe II. *Ehrlichieae* Philip 1957, 948^{AL}. In *Bergey's Manual of Systematic Bacteriology, Vol.1*. Krieg NR, Holt JG, Eds. Baltimore, Williams and Wilkins, 1984, p. 704-711

3. Rikihisa Y. The tribe *Ehrlichieae* and Ehrlichial diseases. *Clin Microbiol Rev* 4, 286-308 (1991)

4. Dumler JS, Bakken JS. Human granulocytic ehrlichiosis in Wisconsin and Minnesota: a frequent infection with the potential for persistence. *J Infect Dis* 173, 1027-1030 (1996)

5. McDade JE. Ehrlichiosis - A disease of animals and humans. *J Infect Dis* 161, 609-617 (1990)

6. Schaffner W, Standaert SM. Ehrlichiosis- in pursuit of an emerging infection. *N Engl J Med* 334, 262-263 (1996)

7. Maeda K, Markowitz N, Hawley RC, Ristic M, Cox D, McDade JE. Human infection with *Ehrlichia canis*, a leukocytic rickettsia. *N Engl J Med* 316, 853-856 (1987)

8. Dawson JE, Anderson BE, Fishbein DB, Sanchez CY, Goldsmith CY, Wilson KH, Duntley CW. Isolation and characterization of an *Ehrlichia* sp. from a patient diagnosed with human ehrlichiosis. *J Clin Microbiol* 29, 2741-2745 (1991)

9. Paddock CD, Sumner JW, Shore GM, Bartley DC, Elie RC, McQuade JG, Martin CR, Goldsmith CS, Childs JE. Isolation and Characterization of *Ehrlichia chaffeensis* Strains from Patients with Fatal Ehrlichiosis. *J Clin Microbiol* 35, 2496-2502 (1997)

10. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol* 32, 589-595 (1994)

11. Buller RS, Arens M, Hmiel SP, Paddock CD, Sumner JW, Rikhisa Y, Unver A, Gaudreault-Keener M, Manian FA, Liddell AM, Schmulewitz N, Storch GA. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. *N Engl J Med* 341, 148-155 (1999)

12. Ewing SA, Roberson WR, Buckner RG, Hayat CS. A new strain of *Ehrlichia canis*. *J Am Vet Med Assoc* 159, 1771-1774 (1971)

13. Dumler JS, Asanovich KM, Bakken JS, Richter P, Kimsey R, Madigan JE. Serologic Cross-reactions among *Ehrlichia equi, Ehrlichia phagocytophila,* and human granulocytic *Ehrlichia*. *J Clin Microbiol* 33, 1098-1103 (1995)

14. Perez M, Rikihisa Y, Wen B. *Ehrlichia canis*-Like Agent Isolated from a Man in Venezuela: Antigenic and Genetic Characterization. *J Clin Microbiol* 34, 2133-2139 (1996)

15. Perez M, Bodor M, Zhang C, Xiong Q, Rikihisa Y. Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. *Ann N Y Acad Sci* 1078:110-7., 110-117 (2006)

16. Kock ND, VanVliet AHM, Charlton K, Jongejan F. Detection of *Cowdria ruminantium* in blood and bone marrow samples from clinically normal, free-ranging Zimbabwean wild ungulates. *J Clin Microbiol* 33, 2501-2504 (1995)

17. Perreau P, Morel PC, Barre N, Durand P. (Cowdriosis (Heartwater) by *Cowdria ruminantium* in ruminants of French Indies (Guadeloupe) and Mascarene Islands (La Reunion and Mauritius)). *Rev Elev Med Vet Pays Trop* 33, 21-22 (1980)

18. Uilenberg G. Heartwater (*Cowdria ruminantium* infection): current status. *Adv Vet Sci Comp Med* 27, 427-480 (1983)

19. Allsopp MT, Louw M, Meyer EC. *Ehrlichia ruminantium*: an emerging human pathogen? *Ann N Y Acad Sci* 1063:358-60., 358-360 (2005)

20. Louw M, Allsopp MT, Meyer EC. *Ehrlichia ruminantium*, an emerging human pathogen--a further report. *S Afr Med J* 95, 948, 950 (2005)

21. Loftis AD, Reeves WK, Spurlock JP, Mahan SM, Troughton DR, Dasch GA, Levin ML. Infection of a goat with a tick-transmitted *Ehrlichia* from Georgia, U.S.A., that is closely related to *Ehrlichia ruminantium*. *J Vector Ecol* 31, 213-223 (2006)

22. Yabsley MJ, Loftis AD, Little SE. Natural and experimental infection of white-tailed deer (*Odocoileus virginianus*) from the United States with an *Ehrlichia* sp. closely related to *Ehrlichia ruminantium*. *J Wildl Dis* 44, 381-387 (2008)

23. Reeves WK, Loftis AD, Nicholson WL, Czarkowski AG. The first report of human illness associated with the Panola Mountain *Ehrlichia* species: a case report. *J Med Case Reports* 2:139., 139 (2008)

24. Anderson BE, Dawson JE, Jones DC, Wilson KH. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. *J Clin Microbiol* 29, 2838-2842 (1991)

25. Anderson BE, Sims KG, Olson JG, Childs JE, Piesman

JF, Happ CM, Maupin GO, Johnson BJ. *Amblyomma americanum*: a potential vector of human ehrlichiosis. *Am J Trop Med Hyg* 49, 239-244 (1993)

26. Dawson JE, Childs JE, Biggie KL, Moore C, Stallknecht D, Shaddock J, Bouseman J, Hofmeister E, Olson JG. White-tailed deer as a potential reservoir of *Ehrlichia* spp. *J Wildl Dis* 30, 162-168 (1994)

27. Lockhart JM, Davidson WR, Stallknecht DE, Dawson JE, Howerth EW. Isolation of *Ehrlichia chaffeensis* from wild white-tailed deer (*Odocoileus virginianus*) confirms their role as natural reservoir hosts. *J Clin Microbiol* 35, 1681-1686 (1997)

28. Dumler JS, Bakken JS. Human ehrlichioses: newly recognized infections transmitted by ticks. *Annu Rev Med* 49, 201-213 (1998)

29. Whitlock JE, Fang QQ, Durden LA, Oliver JH, Jr. Prevalence of *Ehrlichia chaffeensis* (Rickettsiales: Rickettsiaceae) in *Amblyomma americanum* (Acari: Ixodidae) from the Georgia coast and Barrier Islands. *J Med Entomol* 37, 276-280 (2000)

30. Demma LJ, Holman RC, McQuiston JH, Krebs JW, Swerdlow DL. Epidemiology of human ehrlichiosis and anaplasmosis in the United States, 2001-2002. *Am J Trop Med Hyg* 73, 400-409 (2005)

31. Paddock CD, Childs JE. *Ehrlichia chaffeensis*: a prototypical emerging pathogen. *Clin Microbiol Rev 2003 Jan ;16 (1):37 -64* 16, 37-64

32. McNabb SJ, Jajosky RA, Hall-Baker PA, Adams DA, Sharp P, Worshams C, Anderson WJ, Javier AJ, Jones GJ, Nitschke DA, Rey A, Wodajo MS. Summary of notifiable diseases--United States, 2006. *MMWR Morb Mortal Wkly Rep* 55, 1-92 (2008)

33. Comer JA, Nicholson WL, Paddock CD, Sumner JW, Childs JE. Detection of antibodies reactive with *Ehrlichia chaffeensis* in the raccoon. *J Wildl Dis* 36, 705-712 (2000)

34. Dawson JE, Ewing SA. Susceptibility of dogs to infection with *Ehrlichia chaffeensis*, causative agent of human ehrlichiosis. *Am J Vet Res* 53, 1322-1327 (1992)

35. Kocan AA, Levesque GC, Whitworth LC, Murphy GL, Ewing SA, Barker RW. Naturally occurring *Ehrlichia chaffeensis* infection in coyotes from Oklahoma. *Emerg Infect Dis* 6, 477-480 (2000)

36. Ewing SA, Dawson JE, Kocan AA, Barker RW, Warner CK, Panciera RJ, Fox JC, Kocan KM, Blouin EF. Experimental transmission of *Ehrlichia chaffeensis* (Rickettsiales: Ehrlichieae) among White-tailed deer by *Amblyomma americanum* (Acari: Ixodidae). *J Med Entomol* 32, 368-374 (1995)

37. Fishbein DB, Dawson JE, Robinson LE. Human ehrlichiosis in the United States, 1985 to 1990. *Ann Intern Med* 120, 736-743 (1994)

38. Dumler JS, Dawson JE, Walker DH. Human ehrlichiosis: hematopathology and immunohistologic detection of *Ehrlichia chaffeensis*. *Hum Pathol* 24, 391-396 (1993)

39. Walker DH, Dumler JS. Human monocytic and granulocytic ehrlichioses. Discovery and diagnosis of emerging tick-borne infections and the critical role of the pathologist. *Arch Pathol Lab Med* 121, 785-791 (1997)

40. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. *Clin Infect Dis* 45 Suppl 1, S45-S51 (2007)

41. Andrew HR, Norval RA. The carrier status of sheep, cattle and African buffalo recovered from heartwater. *Vet Parasitol* 34, 261-266 (1989)

42. Dumler JS, Sutker WL, Walker DH. Persistent Infection with *Ehrlichia chaffeensis*. *Clin Infect Dis* 17, 903-905 (1993)

43. Zaugg JL, Stiller D, Croan ME, Lincoln SD. Transmission of *Anaplasma marginale* Theiler by males of *Dermacentor andersoni* Stiles fed on an Idaho field infected, chronic carrier cow. *Am J Vet Res* 47, 2269-2271 (1986)

44. Zhang XF, Zhang JZ, Long SW, Ruble RP, Yu XJ. Experimental *Ehrlichia chaffeensis* infection in beagles. *J Med Microbiol* 52, 1021-1026 (2003)

45. Unver A, Rikihisa Y, Stich RW, Ohashi N, Felek S. The omp-1 major outer membrane multigene family of *Ehrlichia chaffeensis* is differentially expressed in canine and tick hosts. *Infect Immun* 70, 4701-4704 (2002)

46. Stuen S, Engvall EO, Artursson K. Persistence of *Ehrlichia phagocytophila* infection in lambs in relation to clinical parameters and antibody responses (In Process Citation). *Vet Rec* 143, 553-555 (1998)

47. French TW, Harvey JW. Serologic diagnosis of infectious cyclic thrombocytopenia in dogs using an indirect flourescent antibody test. *Am J Vet Res* 44, 2407-2411 (1983)

48. Dawson JE, Biggie KL, Warner CK, Cookson K, Jenkins S, Levine JF, Olson JG. Polymerase chain reaction evidence of *Ehrlichia chaffeensis*, an etiologic agent of human erlichiosis, in dogs from southeast Virginia. *Am J Vet Res* 57, 1175-1179 (1996)

49. Davidson WR, Lockhart JM, Stallknecht DE, Howerth EW, Dawson JE, Rechav Y. Persistent *Ehrlichia chaffeensis* infection in white-tailed deer. *J Wildl Dis* 37, 538-546 (2001)

50. Barbet AF, Yi J, Lundgren A, McEwen BR, Blouin EF, Kocan KM. Antigenic variation of *Anaplasma marginale*: major surface protein 2 diversity during cyclic transmission

between ticks and cattle. *Infect Immun* 69, 3057-3066 (2001)

51. Lohr CV, Brayton KA, Shkap V, Molad T, Barbet AF, Brown WC, Palmer GH. Expression of *Anaplasma marginale* major surface protein 2 operon-associated proteins during mammalian and arthropod infection. *Infect Immun* 70, 6005-6012 (2002)

52. Rurangirwa FR, Stiller D, French DM, Palmer GH. Restriction of major surface protein 2 (MSP2) variants during tick transmission of the *Ehrlichia Anaplasma marginale*. *Proc Natl Acad Sci U S A* 96, 3171-3176 (1999)

53. Barbet AF, Meeus PF, Belanger M, Bowie MV, Yi J, Lundgren AM, Alleman AR, Wong SJ, Chu FK, Munderloh UG, Jauron SD. Expression of multiple outer membrane protein sequence variants from a single genomic locus of *Anaplasma phagocytophilum*. *Infect Immun* 71, 1706-1718 (2003)

54. Jauron SD, Nelson CM, Fingerle V, Ravyn MD, Goodman JL, Johnson RC, Lobentanzer R, Wilske B, Munderloh UG. Host cell-specific expression of a p44 epitope by the human granulocytic ehrlichiosis agent. *J Infect Dis* 184, 1445-1450 (2001)

55. Schwan TG, Piesman J, Golde WT, Dolan MC, Rosa PA. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc Natl Acad Sci U S A* 92, 2909-2913 (1995)

56. de Silva AM, Telford SR, III, Brunet LR, Barthold SW, Fikrig E. *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. *J Exp Med* 183, 271-275 (1996)

57. de la Fuente J, Garcia-Garcia JC, Blouin EF, Kocan KM. Differential adhesion of major surface proteins 1a and 1b of the *Ehrlichia*l cattle pathogen *Anaplasma marginale* to bovine erythrocytes and tick cells. *Int J Parasitol* 31, 145-153 (2001)

58. Fikrig E, Pal U, Chen M, Anderson JF, Flavell RA. OspB antibody prevents *Borrelia burgdorferi* colonization of *Ixodes scapularis*. *Infect Immun* 72, 1755-1759 (2004)

59. Pal U, Li X, Wang T, Montgomery RR, Ramamoorthi N, Desilva AM, Bao F, Yang X, Pypaert M, Pradhan D, Kantor FS, Telford S, Anderson JF, Fikrig E. TROSPA, an *Ixodes scapularis* receptor for *Borrelia burgdorferi*. *Cell* 119, 457-468 (2004)

60. Rikihisa Y, Ewing SA, Fox JC. Western immunoblot analysis of *Ehrlichia chaffeensis, E. canis,* or *E. ewingii* infections in dogs and humans. *J Clin Microbiol* 32, 2107-2112 (1994)

61. Singu V, Liu H, Cheng C, Ganta RR. *Ehrlichia chaffeensis* expresses macrophage- and tick cell-specific 28-kilodalton outer membrane proteins. *Infect Immun* 73, 79-87 (2005)

62. Singu V, Peddireddi L, Sirigireddy KR, Cheng C, Munderloh UG, Ganta RR. Unique Macrophage and Tick Cell-specific Protein Expression from the p28/p30 Omp Multigene Locus in *Ehrlichia* Species. *Cell Microbiol 8:1475-87* (2006)

63. Seo GM, Cheng C, Tomich J, Ganta RR. Total, membrane, and immunogenic proteomes of macrophage- and tick cell-derived *Ehrlichia chaffeensis* evaluated by LC-MS/MS and MALDI-TOF methods. *Infect Immun (*2008) (Epub ahead of print; 2008 Aug 18).

64. Reddy GR, Sulsona CR, Harrison RH, Mahan SM, Burridge MJ, Barbet AF. Sequence heterogeneity of the major antigenic protein 1 genes from *Cowdria ruminantium* isolates from different geographical areas. *Clin Diag Lab Immunol* 3, 417-422 (1996)

65. Ohasi N, Zhi N, Zhang Y, Rikihisa Y. Immunodominant major outer membrane proteins of *Ehrlichia chaffeensis* are encoded by a polymorphic multigene family. *Infect Immun* 66, 132-139 (1998)

66. Reddy GR, Sulsona CR, Barbet AF, Mahan SM, Burridge MJ, Alleman AR. Molecular characterization of a 28 kDa surface antigen gene family of the tribe *Ehrlichia*e. *Biochem Biophys Res Commun* 247, 636-643 (1998)

67. Reddy GR, Streck CP. Variability in the 28-kDa surface antigen protein multigene locus of isolates of the emerging disease agent *Ehrlichia chaffeensis* suggests that it plays a role in immune evasion. *Molecular Cell Biology Research Communications* 1, 167-175 (1999)

68. Yu X, McBride JW, Zhang X, Walker DH. Characterization of the complete transcriptionally active *Ehrlichia chaffeensis* 28 kDa outer membrane protein multigene family. *Gene* 248, 59-68 (2000)

69. Gusa AA, Buller RS, Storch GA, Huycke MM, Machado LJ, Slater LN, Stockham SL, Massung RF. Identification of a p28 gene in *Ehrlichia ewingii*: evaluation of gene for use as a target for a species-specific PCR diagnostic assay. *J Clin Microbiol* 39, 3871-3876 (2001)

70. Ohashi N, Rikihisa Y, Unver A. Analysis of Transcriptionally Active Gene Clusters of Major Outer Membrane Protein Multigene Family in *Ehrlichia canis* and *E. chaffeensis*. *Infect Immun* 69, 2083-2091 (2001)

71. van Heerden H, Collins NE, Brayton KA, Rademeyer C, Allsopp BA. Characterization of a major outer membrane protein multigene family in *Ehrlichia ruminantium*. *Gene* 330:159-68., 159-168 (2004)

72. Kumagai Y, Huang H, Rikihisa Y. Expression and porin activity of P28 and OMP-1F during intracellular *Ehrlichia chaffeensis* development. *J Bacteriol* 190, 3597-3605 (2008)

73. Kakono O, Hove T, Geysen D, Mahan S. Detection of antibodies to the *Ehrlichia ruminantium* MAP1-B antigen in

goat sera from three communal land areas of Zimbabwe by an indirect enzyme-linked immunosorbent assay. *Onderstepoort J Vet Res* 70, 243-249 (2003)

74. Semu SM, Peter TF, Mukwedeya D, Barbet AF, Jongejan F, Mahan SM. Antibody responses to MAP 1B and other *Cowdria ruminantium* antigens are down regulated in cattle challenged with tick-transmitted heartwater. *Clin Diagn Lab Immunol* 8, 388-396 (2001)

75. Mahan SM, Semu SM, Peter TF, Jongejan F. Evaluation of the MAP-1B ELISA for cowdriosis with field sera from livestock in Zimbabwe. *Ann N Y Acad Sci* 849:259-61., 259-261 (1998)

76. Unver A, Ohashi N, Tajima T, Stich RW, Grover D, Rikihisa Y. Transcriptional analysis of p30 major outer membrane multigene family of *Ehrlichia canis* in dogs, ticks, and cell culture at different temperatures. *Infect Immun* 69, 6172-6178 (2001)

77. Bekker CP, Bell-Sakyi L, Paxton EA, Martinez D, Bensaid A, Jongejan F. Transcriptional analysis of the major antigenic protein 1 multigene family of *Cowdria ruminantium*. *Gene* 285, 193-201 (2002)

78. Long SW, Zhang XF, Qi H, Standaert S, Walker DH, Yu XJ. Antigenic variation of *Ehrlichia chaffeensis* resulting from differential expression of the 28-kilodalton protein gene family. *Infect Immun* 70, 1824-1831 (2002)

79. Cheng C, Paddock CD, Reddy GR. Molecular Heterogeneity of *Ehrlichia chaffeensis* Isolates Determined by Sequence Analysis of the 28-Kilodalton Outer Membrane Protein Genes and Other Regions of the Genome. *Infect Immun* 71, 187-195 (2003)

80. Ge Y, Rikihisa Y. Surface-exposed proteins of *Ehrlichia chaffeensis*. *Infect Immun* 75, 3833-3841 (2007)

81. Zhang JZ, Guo H, Winslow GM, Yu XJ. Expression of members of the 28-kilodalton major outer membrane protein family of *Ehrlichia chaffeensis* during persistent infection. *Infect Immun* 72, 4336-4343 (2004)

82. Garcia-Garcia JC, de la FJ, Bell-Eunice G, Blouin EF, Kocan KM. Glycosylation of *Anaplasma marginale* major surface protein 1a and its putative role in adhesion to tick cells. *Infect Immun* 72, 3022-3030 (2004)

83. McBride JW, Yu XJ, Walker DH. Glycosylation of homologous immunodominant proteins of *Ehrlichia chaffeensis* and *Ehrlichia canis*. *Infect Immun* 68, 13-18 (2000)

84. Sarkar M, Troese MJ, Kearns SA, Yang T, Reneer DV, Carlyon JA. *Anaplasma phagocytophilum* MSP2 (P44)-18 predominates and is modified into multiple isoforms in human myeloid cells. *Infect Immun* 76, 2090-2098 (2008)

85. Moens S, Vanderleyden J. Glycoproteins in prokaryotes. *Arch Microbiol* 168, 169-175 (1997)

86. Ostrovsky PC, Maloy S. Protein phosphorylation on serine, threonine, and tyrosine residues modulates membrane-protein interactions and transcriptional regulation in *Salmonella typhimurium*. *Genes Dev* 9, 2034-2041 (1995)

87. 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* 9, 2644-2657 (2007)

88. Ijdo JW, Carlson AC, Kennedy EL. *Anaplasma phagocytophilum* AnkA is tyrosine-phosphorylated at EPIYA motifs and recruits SHP-1 during early infection. *Cell Microbiol* 9, 1284-1296 (2007)

89. Park J, Kim KJ, Choi KS, Grab DJ, Dumler JS. *Anaplasma phagocytophilum* AnkA binds to granulocyte DNA and nuclear proteins. *Cell Microbiol* 6, 743-751 (2004)

90. Chou AY, Archdeacon J, Kado CI. *Agrobacterium* transcriptional regulator Ros is a prokaryotic zinc finger protein that regulates the plant oncogene ipt. *Proc Natl Acad Sci U S A* 95, 5293-5298 (1998)

91. Collazo CM, Yap GS, Hieny S, Caspar P, Feng CG, Taylor GA, Sher A. The function of gamma interferon-inducible GTP-binding protein IGTP in host resistance to *Toxoplasma gondii* is Stat1 dependent and requires expression in both hematopoietic and nonhematopoietic cellular compartments. *Infect Immun* 70, 6933-6939 (2002)

92. Brown WC, McGuire TC, Mwangi W, Kegerreis KA, Macmillan H, Lewin HA, Palmer GH. Major histocompatibility complex class II DR-restricted memory CD4 (+) T lymphocytes recognize conserved immunodominant epitopes of *Anaplasma marginale* major surface protein 1a. *Infect Immun* 70, 5521-5532 (2002)

93. Macmillan H, Norimine J, Brayton KA, Palmer GH, Brown WC. Physical linkage of naturally complexed bacterial outer membrane proteins enhances immunogenicity. *Infect Immun* 76, 1223-1229 (2008)

94. Ganta RR, Wilkerson MJ, Cheng C, Rokey AM, Chapes SK. Persistent *Ehrlichia chaffeensis* infection occurs in the absence of functional major histocompatibility complex class II genes. *Infect Immun* 70, 380-388 (2002)

95. Ganta RR, Cheng C, Miller EC, McGuire BL, Peddireddi L, Sirigireddy KR, Chapes SK. Differential clearance and immune responses to tick cell-derived versus macrophage culture-derived *Ehrlichia chaffeensis* in mice. *Infect Immun* 75, 135-145 (2007)

96. Winslow GM, Yager E, Shilo K, Volk E, Reilly A, Chu FK. Antibody-mediated elimination of the obligate intracellular bacterial pathogen *Ehrlichia chaffeensis* during active infection. *Infect Immun* 68, 2187-2195

(2000)

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