1. ABSTRACT

The genus *Anaplasma* includes a diverse group of tick-borne pathogens found exclusively within membrane-bound vacuoles in host cells. While *A. marginale*, *A. centrale* and *A. ovis*, vectored by *Dermacentor* and *Rhipicephalus* ticks, are host-specific for ruminants, *A. phagocytophilum*, vectored by *Ixodes* spp., infects a wide range of hosts. In ticks *Anaplasma* undergoes a developmental cycle that is coordinated with the tick feeding cycle. Although research at the tick/*Anaplasma* interface is in its infancy, recent studies have provided evidence that *Anaplasma* infection and transmission is mediated by a molecular mechanism involving both tick cell and pathogen genes. Application of a growing array of molecular approaches, such as RNA interference, genomics and proteomics, are rapidly expanding our knowledge of the tick/pathogen interface. Targeting key tick cell molecules required for pathogen development in vaccine strategies may compromise the vector capacity of ticks for *Anaplasma*, thus reducing transmission and infection of vertebrates. Collectively, this information will likely lead to the development of dual target vaccines designed to protect vertebrates against tick infestations and prevent the transmission of pathogens.

2. INTRODUCTION

Pathogens classified within the family *Anaplasmataceae* develop in two very different hosts, vertebrates and ticks, and the co-evolution of pathogens with both of these hosts has insured their mutual survival. While pathogens remain dormant in ticks that are off the host, their multiplication, development and subsequent transmission to vertebrate hosts are perfectly coordinated with the tick feeding cycle.

The family *Anaplasmataceae* is one of two families in the Order *Rickettsiales*, along with the family *Rickettsiaceae* (1). Organisms within the family *Anaplasmataceae* are obligate intracellular organisms that are found exclusively within membrane-bound vacuoles in the vertebrate or tick host cell cytoplasm. Within the family *Anaplasmataceae*, phylogenetic analyses consistently supported the formation of four distinct genetic groups of the organisms: (1) *Anaplasma* with a 96.1% similarity, (2) *Ehrlichia* with a 97.7% similarity, (3) *Wolbachia* with a minimum of 95.6% similarity and (4) *Neorickettsia* with a minimum of 94.9% similarity (1). After a recent reclassification, the genus *Anaplasma* was expanded from three pathogens of ruminants, *A. marginale*, *A. centrale* an
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Table 1. Current Classification of the Genus Anaplasma

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<th>Order Rickettsiales</th>
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<td>Anaplasmataceae</td>
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<td>A. phagocytophilum</td>
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<td>Ehrlichia phagocytophilum, E. equi</td>
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A. ovis, to also include A. bovis (formerly Ehrlichia bovis), A. phagocytophilum (formerly E. phagocytophila, E. equi and the HGE agent), and A. platys (formerly E. platys). Aegyptianella, also included in this genus, was retained as a genus incertae sedis due to lack of sequence information (Table 1).

A. marginale, A. centrale and A. ovis are host-specific for ruminants (primarily cattle, sheep, goats and deer) and the main tick vectors are Dermacentor and Rhipicephalus (Boophilus). The type species of the genus Anaplasma, A. marginale, is distributed worldwide in tropical and subtropical regions of the New World, Europe, Africa, Asia and Australia where it causes bovine anaplasmosis (2). Many strains of A. marginale have been identified worldwide which differ in morphology, protein sequence, antigenic characteristics and their ability to infect and be transmitted by ticks (3, 4, 5, 6, 7, 8, 9, 10). While some strains have been shown not to be transmissible by ticks (Illinois, Okeechobee, Florida, Brazil; Mississippi, Canadian) (3, 4, 11, 12, 13, 14), ticks appear to have maintained vector competence for most other strains despite the diverse geographic locations of tick species and A. marginale strains (15, 16, 17). The closely related A. ovis infects sheep, goats and wild ruminants (18, 19, 20, 21, 22, 23, 24, 25) and was recently reported in mule deer in the U.S. (26). The less pathogenic A. centrale for cattle is used as a live cattle vaccine in Europe, Australia and Africa (27, 28, 29).

A. phagocytophilum infects a wide range of host animals including rodents, ruminants, birds, felids, horses and donkeys, dogs and humans and is transmitted by ticks of the Ixodes ricinus complex (1, 30). A. phagocytophilum is the causative agent of tick-borne fever (TBF) in ruminants and human, equine and canine granulocytic anaplasmosis (1, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40). A. phagocytophilum causes an emerging disease of public health concern in many areas of the world (1, 40) and was recently listed as the most widespread tick-borne infection of animals in Europe (30). Clinical manifestations of human granulocytic anaplasmosis (HGA) include fever, headache, myalgia, anemia, leukopenia, thrombocytopenia, and elevated liver enzymes (41, 42, 43, 44, 45, 46). The white-footed mouse, Peromyscus leucopus, is considered to be the main reservoir host of A. phagocytophilum in North America, but raccoons, gray squirrels, and cottontail rabbits become naturally infected with the pathogen and may also serve as reservoir hosts, thus contributing to the maintenance of the natural cycle of infection (47, 48). The list of wildlife hosts that may serve as reservoir hosts continues to expand (49, 50, 51, 52). The wide and expanding host range of this pathogen will most likely ensure increasing distribution of A. phagocytophilum worldwide and the continued emergence of granulocytic anaplasmosis in domestic animals and humans (30).

Anaplasma spp. share many common features. Vertebrate hosts develop persistent infections with Anaplasma spp., which, in turn, allows them to serve as reservoirs of infection. Anaplasma are transmitted horizontally by ixodid ticks while transovarial transmission does not appear to occur. Transstadial transmission occurs from stage to stage (larvae-nymphs, nymphs-adults and larvae-adults) and therefore every tick generation must obtain infection by feeding on reservoir hosts. For the ruminant host-specific Anaplasma, intrastadial transmission is effected by male ticks which can both acquire infection and, during a second feeding, transmit the pathogen (53, 54, 55), while intrastadial transmission of A. phagocytophilum by male ticks has not been well characterized. The developmental cycles of A. ovis and A. centrale and most extensively A. marginale have been described and shown to be coordinated with the tick feeding cycle (13, 55, 56, 57, 58). The midgut is the first site of infection in which large membrane-bound vacuoles or colonies first contain reticulated forms that divide by binary fission and then subsequently transform into dense forms. After attaching and feeding on a second host, the salivary glands become infected and the pathogen undergoes development similar to the midgut cycle (2, 54, 56). In addition to midgut and salivary gland sites, other tick tissues can become infected. Male ticks develop persistent A. marginale infections and can serve as a reservoir of infection (57). While A. phagocytophilum has been detected in ticks by molecular technologies and more recently by microscopy (Figure 2 a, b; unpublished data), the tick cycle has not been described by use of microscopy and the role of male ticks, similar to that in the transmission of A. marginale, has not been reported. Characterization of the tick development cycle in ticks would be a fundamental contribution toward advancing research on the molecular mechanism of A. phagocytophilum in tick-pathogen interactions.

Limited information has historically been available on the molecular interactions of Anaplasma and ticks, due at least in part to the recent availability of research tools, including a tick cell culture system, molecular technologies and genomic information. The development of tick cell culture system has provided in vitro model systems for both A. marginale and A. phagocytophilum tick-cell-pathogen interactions. In addition, molecular technologies in ticks, such as the use of RNA interference (RNAi) for the study gene function by silencing expression (59), genomics and proteomics are rapidly expanding the scope of tick/pathogen interface research. The recent report of the transfection of A.
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3. MOLECULAR INTERACTION OF ANAPLASMA SPP. HOST-SPECIFIC FOR RUMINANTS AND THE TICK VECTOR

A characteristic of the ruminant-specific Anaplasma spp, is that these pathogens are all found exclusively within membrane-bound vacuoles in the host erythrocytes and in the cytoplasm of tick cells. Because of the extensive research conducted on A. marginale over the years, more information is currently available for A. marginale than the other two species.

3.1. Tick vectors of ruminant host-specific Anaplasma

In the United States, A. marginale and A. ovis are vectored primarily by Dermacentor ticks (D. andersoni, D. variabilis and D. albipictus) (1, 2), while the one-host cattle ticks, Rhipicephalus (Boophilus) microplus and R. annulatus, are the main vectors of A. marginale in tropical and subtropical regions of the world. R. simus was also identified as a tick vector of A. marginale in South Africa (63). Recent research has demonstrated that although the A. centrale vaccine strain infects and develops in ticks, this species is not transmitted by ticks (Kocan, unpublished data; 13). In general, Anaplasma tick infections are higher when acquired from acutely infected cattle, but ticks that feed on persistently infected cattle also become infected and the biological replication of the organism in ticks appears to make up for initial differences in the infecting dose (64, 65, 66).

3.2. Anaplasma tick developmental cycle

The tick life cycle of the ruminant-specific Anaplasma is complex and coordinated with tick feeding cycle (2, 54, 56, 57). Bovine erythrocytes infected with A. marginale (Figure 1 a, b) are ingested by ticks in the infancy, definition of these interactions is fundamental to the formulation of novel control measures. The dual approach of reducing the vector capacity of ticks by interrupting the pathogen development cycle and transmission and by interfering with tick biology, feeding and reproduction would be an approach that would most certainly contribute to reduction of both tick infestations and tick-borne infections in vertebrates (61, 62). With the exception of A. marginale, which is frequently transmitted mechanically by any means of transferring infective blood from infected to susceptible animals, transmission of the other Anaplasma spp. are dependent on the respective tick vectors.

Herein, the Anaplasma host-specific for ruminants, namely A. marginale, A. ovis and A. centrale, will be reviewed together because of their similarities, while A. phagocytophilum will be considered separately because of its differences when compared to the other species. A. bovis, A. platys, and Aegyptianella will not be covered because information about these organisms in the tick vector(s) is minimal. The authors hope that this review will point to the importance of focusing research and the development of control measures on the tick-pathogen interface and thus stimulate interest in conducting research on the molecular mechanisms of the tick-pathogen interaction.

Although research on the molecular interactions between Anaplasma spp. and their tick vectors is in its...
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bloodmeal and the first site of infection in ticks occurs in gut cells (Figure 1, c, d), after which many other tick tissues become infected. Infection of salivary glands occurs during a subsequent feeding from where transmission is effected (Figure 1 e, f). The first form of *A. marginale* seen within colonies at each site of development is the reticulated (vegetative) form that divides by binary fission and results in formation of large colonies that may contain hundreds of organisms. The reticulated form then transforms into the dense or infective form which can survive for a short time outside of cells. The tick cell line, IDE8, supports the growth and propagation of *A. marginale* and the large colonies that form within these cells is similar to those described in naturally infected ticks (Figure 1 g, h).

### 3.3. Phylogenetic diversity of *A. marginale* and tick-pathogen co-evolution

The worldwide phylogenetic diversity of *Anaplasma* strains in cattle, as characterized by study of the major surface proteins (MSPs) MSP1a and MSP4, is quite remarkable and most likely has increased as a result of the long-term movement of cattle (67, 68, 69). This genetic heterogeneity has been observed among *A. marginale* strains within endemic regions such as (70), Oklahoma (11) and Kansas (70) in the United States, Mexico (67, 71), Argentina (29, 67, 72), Minas Gerais and Paraná in Brasil (73), Castilla – La Mancha in Spain (74), and Sicily, Italy (75, 76, 77). This strain diversity could be explained by cattle movement and maintenance of different genotypes by independent transmission events, due to the mechanism of infection exclusion of *A. marginale* in cattle (7, 70, 78). Recent studies on *A. marginale*-infected cattle in endemic areas demonstrated that multiple msp1a genotypes were present, but that generally only one genotype was found per individual bovine (7, 78). In contrast, sequences of Australian *A. marginale* strains were the only ones to cluster together, further supporting a more genetically homogeneous *A. marginale* population in this region in which minimal cattle introductions had occurred (68, 79, 80). Recently, Futse et al. (81) showed that cattle superinfection may be a driver of genomic diversification in *A. marginale*. These results provide additional support for the generation of genetic diversity in endemic areas and advance the question of whether a similar mechanism also exists in ticks where MSP2 antigenic variants arise during pathogen multiplication (82).

The genetic variation of *A. ovis* MSP4 has been analyzed in the United States for sheep, bighorn sheep and mule deer strains, and in Sicily, Italy, for sheep strains (24, 25, 67, 76). The results of these studies demonstrated that, while *A. ovis* msp4 genotypes may vary among geographic regions, the variation observed thus far is less than that observed with *A. marginale* and *A. phagocytophilum*. This finding may have resulted from the restricted movement of infected animals. Additionally, the limited host range of *A. ovis* as compared with *A. phagocytophilum* may have also contributed to the lack of genetic diversity of this pathogen.

The genetic diversity of MSP1a sequences complicates the study of tick-pathogen co-evolution. Analysis of MSP1a repeated sequences provided evidence of the presence of common sequences in strains from different geographic regions, a finding consistent with the existence of the same vector tick species in these regions (80). Furthermore, as suggested by sequence analysis of MSP1a repeats (80), tick-pathogen interactions could influence the presence of unique MSP1a repeats in strains of *A. marginale* from particular geographic regions. However, mechanical transmission of *A. marginale* strains that are not transmissible by ticks could also play an important role in the evolution of *A. marginale* (2).

### 3.4. *A. marginale* molecules involved in interactions at the tick/pathogen interface

Most studies of molecular events currently described to be involved in *Anaplasma* interactions with host cells have been on *A. marginale* (2, 83), with a greater emphasis on vertebrate host-pathogen interactions than at the tick-pathogen interface. Several major surface proteins (MSPs) have been identified in *Anaplasma* spp., which
have been characterized most extensively in *A. marginale* (2, 9, 77, 84, 85). These MSPs are likely to evolve more rapidly than other genes because they are subjected to selective pressures exerted by host immune systems. Limited information is available for *A. centrale* and *A. ovis* MSPs, and reports have not been published for *A. bovis* and *A. platys* (reviewed by 1, 77).

One of the MSPs studied extensively in tick/*Anaplasma* interactions is the MSP1 complex (5, 82, 86, 87). The MSP1 complex described in *A. marginale* is a heterodimer of two structurally unrelated polypeptides, MSP1a and MSP1b, which are both encoded by genes that have proved to be stable genetic markers of geographic strains of *A. marginale* throughout the developmental cycle in cattle and ticks (88, 89). MSP1a, encoded by the gene *msp1a*, has only been identified thus far in *A. marginale* despite attempts to clone this gene from *A. centrale* and *A. ovis* (90, de la Fuente unpublished data). MSP1b, encoded by two genes, *msp1β1* and *msp1β2*, has also only been identified in *A. marginale* (88, 89). However, only a single MSP1b protein, MSP1b1, was identified within the MSP1 complex (89). The MSP1a of *A. marginale* geographic strains differs in molecular weight because of a variable number of these tandem 23-31 amino acid repeats, and has been used for identification of geographic strains (5, 10, 11, 79).

MSP1a was shown to participate in infection and transmission of *A. marginale* by *Dermaecentor* spp. ticks (8), and to be involved in immunity to *A. marginale* infection in cattle (91, 92). Additional studies demonstrated the critical role of the 20th amino acid of the repeat in the interaction of MSP1a with host cell receptors (10, 11). However, analysis of tandemly repeated MSP1a peptides of several geographic isolates of *A. marginale* revealed a complex relationship between the *msp1a* genotype and the tick-transmissible phenotype of the isolate and suggested that both the sequence and conformation of the repeated peptides influenced the adhesive properties of MSP1a (11). The adhesive properties of MSP1a may also involve the glycosylated portion of the protein as described by Garcia-Garcia et al. (93). Notably, MSP1a was found to be differentially regulated in bovine erythrocytes and tick cells (94). Down regulation of MSP1a was observed in *A. marginale* in tick salivary glands and cultured ticks cells while this MSP was upregulated by *A. marginale* in bovine erythrocytes.

The MSP2 protein superfamily of *A. marginale* contains immunodominant MSPs with orthologues in all *Anaplasma* spp. studied thus far (95). The *A. marginale* (strain St. Maries) genome is reported to have 56 genes belonging to this superfamily, including eight *msp2*, eight *msp3*, two *msp3* remnants, one *msp4*, three *opag*, 15 *omp-1*, 12 *orfX* and seven *orfY* (83). The most completely characterized representative of this superfamily, MSP2, is encoded by several genes in *A. marginale*, *A. centrale* and *A. ovis* (83, 96, 97). Antigenic variation of MSP2 occurs during persistent *A. marginale* infections in cattle and ticks (82, 83). This mechanism of antigenic variation has been posited to allow *A. marginale* to evade the host immune response, thus contributing to the maintenance of persistent infections (83). Multiple antigenic variants of MSP2, MSP3 of *A. marginale* arise during the multiplication of the pathogen in mammals and ticks as the result of combinatorial gene conversion into expression sites (83). However, differences between ruminant-specific *Anaplasma* (*A. marginale*, *A. centrale*, and *A. ovis*) and the *A. phagocytophilum* MSP2 suggest that the function(s) of MSP2 may vary within *Anaplasma* spp. *A. marginale* *omp-1* genes are differentially regulated in bovine erythrocytes and tick cells but show a high degree of conservatism during pathogen life cycle in the mammalian host and the tick vector (87). Although the function of MSP4 is presently unknown, the sequence of this gene is genetically stable during the multiplication of *A. marginale* in mammalian and tick cells (72, 88). The *A. marginale* MSP2s are capable of inducing strong T-cell responses and contain antigenically variable B-cell epitopes in the hypervariable region that are recognized by the immune system of *A. marginale*-infected cattle, which results in the selection of new variants that most likely allow the pathogen to establish persistent infections (77, 98). The role of this molecule in establishing persistent infection in the tick is unknown but may be involved in pathogen infection of tick cells.

3.5. Tick molecules involved in interactions at the tick/pathogen interface

Tick-pathogen co-evolution likely involves genetic traits of the vector, as well as those of the pathogen. While recent reports have confirmed the presence of tick receptors for tick-borne pathogens (99), the tick receptor for *A. marginale* and the other ruminant-specific *Anaplasma* spp. has not been identified. In addition, expression of tick genes/proteins that facilitate the infection and multiplication of these pathogens have not been well described (61).

Clearly, isolates of *A. marginale* have been shown to vary in their infectivity for ticks. As mentioned previously, some isolates are refractive to establishing infections in ticks, some appear to infect midguts, while *A. centrale* was recently shown to infect both midguts and salivary glands but was unable to be transmitted by ticks to cattle (13). While Scoles *et al.* (16) demonstrated significant variation in *D. andersoni* midgut susceptibility to the pathogen, Futse *et al.* (15) took a different approach to study tick-*A. marginale* evolutionary adaptations and demonstrated that ticks and *A. marginale* strains retain competence for tick transmission in the absence of vector-pathogen interaction.

Recently, the role of the tick protective protein, subolesin, in the infection and transmission of *A. marginale* was characterized (62). Subolesin was shown in vaccine and RNAi studies to protect against tick infestations and to affect tick feeding, reproduction, and development, as well as infection of host cells by *Anaplasma marginale* and *A. phagocytophilum* (62, 100, 101, 102, 103). Additional experiments provided evidence that infection of tick cells with these two pathogens modified subolesin gene expression and suggested a role for subolesin in
The global tick cell response to infection with *A. marginale* was characterized at the mRNA and protein levels, and RNAi was used to study the function of these molecules during *A. marginale* infection and multiplication in ticks and cultured IDE8 tick cells (105). In these experiments, four genes (encoding for putative GST, salivary selenoprotein M, vATPase and ubiquitin) coincided with significantly lower *A. marginale* infection levels after RNAi in *D. variabilis* guts and/or salivary glands. Six genes (encoding for putative selenoprotein W2a, hematopoietic stem/progenitor cells protein-like, proteasome 26S subunit, ferritin, GST and subolesin control) affected *A. marginale* infection levels in IDE8 tick cells after RNAi. These experiments demonstrated that some of tick genes had different expression patterns in tick guts and salivary glands and affected the *A. marginale* life cycle at different sites in the tick, thus providing additional evidence of the distinct role that guts and salivary glands play on *Anaplasma* infection and transmission by ticks (13). Collectively these data support the hypothesis that *A. marginale* traffics through ticks by means of a molecular mechanism, and the pathogen’s subsequent transmission is mediated by tick cell gene expression. Another influence on pathogen development in ticks is the tick innate immune system. Tick-borne pathogens have apparently co-evolved with ticks for their mutual survival because, while pathogens undergo considerable multiplication in ticks, these infections do not appear to be detrimental to tick feeding or biology (57, 106, 107). Antimicrobial peptides are a component of the innate immune system in ticks that have been shown to provide immunity against both gram-negative and gram-positive bacteria. One component of innate immune systems of eukaryotic organisms are small cationic peptides, defensins, which have been identified in a wide range of species ranging from the simplest invertebrates to mammals, as well as in plants (108). Among invertebrates, the most completely characterized defensins contain 6 cysteines and provide immunity against gram-positive bacteria (109, 110). Defensins have also been identified in a variety of ixodid ticks, including *D. variabilis* (111, 112), *Ixodes scapularis* (113), *Amblyomma americanum* (114), *A. hebraeum* (115) and *R. microplus* (116, 117). Upregulation of defensin occurred in response to challenge-exposure of *D. variabilis* with the gram-negative rickettsia, *Rickettsia montanensis*, fed to ticks via capillary tubes (118). A defensin varisin, identified in *Dermaentor variabilis*, was shown to be expressed primarily in hemocytes but transcript levels were also upregulated in midguts and other tick cells. We recently studied the role of varisin in the immunity of ticks to *A. marginale* (119). Expression of the varisin gene was silenced by RNAi in which male ticks were injected with varisin dsRNA and then allowed to feed and acquire *A. marginale* infection on an experimentally-infected calf. Silencing of varisin occurred in hemocytes, midguts and salivary glands after RNAi. While we expected that silencing of varisin would increase *A. marginale* infections in ticks, the results demonstrated that tick infections, as determined by an *A. marginale msp4* quantitative PCR, were significantly reduced in the varisin-silenced ticks. Furthermore, colonies of *A. marginale* in ticks used for RNAi were morphologically abnormal from those seen in buffer injected control ticks. The colony shape was irregular and in some cases the *A. marginale* appeared to be free in the cytoplasm of midgut cells. Some ticks were found to be systemically infected with a microbe that may have been affected by the silencing of varisin. Surprisingly, silencing of this defensin in ticks resulted either directly or indirectly in reduced rather than increased pathogen infections in ticks and therefore defensin may be used by the pathogen during its developmental cycle. In addition, this unexpected effect of defensin silencing on *A. marginale* may have resulted because of its intracellular location and development within parasitophorous vacuoles. However, defensin may play a yet unknown function during *A. marginale* infection and may not be active against this pathogen in the natural tick vector but rather acts to limit the infection of other pathogens such as those discovered in tick with varisin gene knockdown.

4. MOLECULAR INTERACTIONS OF *A. PHAGOCYTOPHILUM* AND THE TICK VECTOR

While ticks are known to become infected with *A. phagocytophilum* and transmit the pathogen, the developmental cycle has not been described morphologically in ticks. Furthermore, little is known about the pathogen and tick molecules involved in tick-pathogen interactions.

4.1. Tick vectors and developmental cycle

Tick vectors of *A. phagocytophilum*, *Ixodes* spp. of the *I. ricinus* complex, are commonly found in regions of the United States, Europe, and Asia (120, 121, 122, 123, 124, 125, 126). *I. scapularis* and *I. pacificus* are the main vectors of *A. phagocytophilum* in North America (127). While *I. ricinus* appears to be the main vector of *A. phagocytophilum* in Europe (128), several other ticks may also be vectors, including *Haemaphysalis punctata*, *Ixodes persulcatus*, *I. trianguliceps* and *Rhipicephalus sanguineus* (as reviewed by 30). Although the developmental cycle of *A. phagocytophilum* has not been described in ticks, the pathogen was recently described in ticks (Reichard and Kocan, unpublished data; Figure 2 a, b). In addition, *A. phagocytophilum* has been propagated in the ISE6 and IDE8 tick cell lines derived originally from embryos of *I. scapularis* (Figure 2 c-f), and the basic developmental cycle in these cell lines is similar to that described for *A. marginale* (129, 130, 131). Large colonies form within the cell cytoplasm and the first form seen within the colonies were reticulated forms which subsequently transforms into
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dense forms. However, the morphology of *A. phagocytophilum* in cultured tick cells was found to be considerably more diverse than *A. marginale* (Figure 2 d, f) (129).

4.2. Evidence of tick-*A. phagocytophilum* co-evolution

The tick-pathogen co-evolution has not been as well characterized in *A. phagocytophilum* as compared with *A. marginale*. However, initial evidence suggests a process of co-evolution between *A. phagocytophilum* and the tick vector. For example, as with *A. marginale*, differences in vector competence have been reported among tick species and strains of *A. phagocytophilum* (132, 133). However, it is not known if these differences in vector competence are regulated at the tick-pathogen interface.

4.3. *A. phagocytophilum* molecules involved in interactions at the tick/pathogen interface

A comparative genomic analysis revealed differences between *A. marginale* and *A. phagocytophilum* with important evolutionary implications (95). For example, genes for cell wall biosynthesis are present in *A. marginale* but absent in *A. phagocytophilum*, which may represent an evolutionary adaptation to be important in the ability of *A. phagocytophilum* to infect vertebrate immune cells without the activation of leukocytes which occurs after binding of peptidoglycan to Toll-like receptor 2. Dunning Hotopp et al. (95) also identified one ortholog gene cluster containing conserved hypothetical proteins with homology to the patatin family of phospholipases that is present in rickettsia such as *A. marginale* that infect animals but has been lost from *A. phagocytophilum* and other human pathogens, possibly being related to establishment of infection in humans. However, *A. phagocytophilum* shares some common genes and MSPs with other *Anaplasma* spp. The *A. phagocytophilum* (strain HZ) genome has one msp2, two msp2 homologs, one msp4, 113 p44 and three omp-1, for a total of 121 genes that belong to the MSP2 superfamily (95).

Gene differential expression has been less characterized in *A. phagocytophilum* than in *A. marginale*. In *A. phagocytophilum*, diverse p44 paralogs are expressed in mammals and ticks, a regulatory mechanism that confers environmental adaptations especially during tick transmission (95). Multiple antigenic variants of P44 arise during the multiplication of the pathogen in mammals and ticks as the result of combinatorial gene conversion into expression sites (83, 95). These results indicate that p44 genes and apxR are specifically up-regulated in the mammalian host environment and suggest that ApxR not only is positively regulated but also acts as a transcriptional regulator of p44E (134). Other surface proteins have been identified in *A. phagocytophilum* but their interaction with tick cells has not been reported (95, 135).

4.4. Tick molecules involved in interactions at the tick/pathogen interface

An excellent example of the molecular tick/pathogen interface was reported by Sukumaran et al. (136, 137) for the *I. scapularis* salp16 protein which is expressed during feeding by tick salivary glands in response to infection with *A. phagocytophilum*. While several salp proteins were identified that affect tick feeding, the salp16 was the only one that was upregulated in response to *A. phagocytophilum* infection. When salp16 was silenced in ticks by RNAi, the infection levels in tick salivary glands were greatly reduced. Therefore, there is a molecular relationship between the tick and *A. phagocytophilum* and the pathogen uses this protein to enhance its uptake from the host and infect salivary glands (136, 137). Other proteins of the same family such as *I. scapularis* Salp14 affect tick feeding but not pathogen infection (138).

Additional evidence of tick-pathogen interactions were provided in experiments that demonstrated the effect of pathogen infection on the molting success of *I. scapularis* larvae (139). On the other hand, some results discussed above with *A. marginale* suggest the evolution of mechanisms to limit pathogen infection levels in ticks and thus prevent deleterious effects of infection.

5. SUMMARY AND PERSPECTIVE

This review of the molecular interactions of *Anaplasma* and tick cells points to the complexity of tick-pathogen co-evolution relationships and suggested that genetic loci of both the vector and the pathogen are required for infection and transmission of pathogens by ticks (140). Importantly, recent research has demonstrated that a molecular mechanism occurs by which *Anaplasma* infects tick midgut cells, establishes infection, traffic to salivary glands, establish a second major site of infection and then are transmitted to the vertebrate host during a second feeding. This concept is supported by the finding that infection of ticks may develop only midguts or that infection of salivary glands does not always infer that the pathogen will be transmitted to vertebrates during feeding.

Genetic factors have been associated with intraspecific variation in vector competence for *A. marginale* (10, 15, 16). These results illustrate the complexity of tick-pathogen co-evolution relationships and suggested that genetic loci of both the vector and the pathogen are required for infection and transmission of pathogens by ticks (140).

Although there are notable differences between the ruminant host-specific *Anaplasma* and *A. phagocytophilum* which are infective for a wide host range, tick infections of both pathogens are maintained from the interaction between pathogen tick cell molecules. The overall results are that pathogens are only infective for the tick species with which they have co-evolved and the ticks have evolved a mechanism to limit infections which ensures survival of both tick and pathogens. *Anaplasma* spp are unique among tick-borne pathogens because they develop persistent infections in both the tick and vertebrate hosts which both serve as reservoirs of infection along. Targeting research on both key tick and pathogen antigens will likely contribute to control strategies through reduction of the vector capacity of ticks and may even prevent transmission of *Anaplasma* pathogens to the vertebrate.
hosts, thus reducing the challenge-exposure of vertebrate hosts immunized against the pathogen. However, use of pathogens harvested from tick cell lines as vaccine antigens may be less effective because of the differential expression of key surface antigens as demonstrated by the differential expression of the A. marginale adhesion protein, MSP1a. Molecular characterization of the tick/pathogen interface will be fundamental toward the design of dual target vaccines that will be protective against tick infestations and prevent or reduce the transmission of pathogens.

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