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Ehrlichia: Avances en vacunas, diagnóstico y patobiología

(*Ehrlichia:* Advances in vaccines, diagnostics and pathobiology)

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Resumen

Ehrlichia spp. es responsable de una zoonosis humana emergente y una enfermedad veterinaria importante en las Américas. *Ehrlichia chaffeensis* emerge en Norte América en 1986 y nuevas erlichias asociadas con enfermedad humana continúan emergiendo junto con la identificación reciente de una *E. muris*-like en Minnesota y Wisconsin en el 2011. *E. canis* es prevalente en toda América en los perros y ha sido asociada con enfermedad humana en América del Sur. La erlichiosis humana causada por todas estas erlichias junto con *E. ewingii*, es un importante problema de salud pública. En años recientes se han descrito importantes avances en el desarrollo de la vacuna, inmuno-diagnóstico y la pato biología de estas enfermedades. Sin embargo es necesario entender por completo el mecanismo de la enfermedad producida por estos patógenos emergentes trasmitidos por garrapatas, tanto en humanos como a nivel de medicina veterinaria.

Descriptores: *Ehrlichia,* vacunas, diagnóstico, patobiología

Abstract

Ehrlichia spp. is responsible emerging human zoonoses and diseases of veterinary importance in the Americas. *Ehrlichia chaffeensis* emerged in North America in 1986 and new *Ehrlichia* spp. associated with human disease continue to emerge with the recent identification of an *E. muris*-like agent in Minnesota and Wisconsin in 2011. *E. canis* is prevalent throughout the Americas in dogs and has been associated with human disease in South America. The human ehrlichioses caused by all these erlichias together with *E. ewingii*, is an important public health problem. In the last years have been important advanced in vaccine development, immunodiagnostic and pathobiology of these diseases. However, is necessary the understanding of disease mechanisms of these emerging tick-transmitted pathogens in human and veterinary medicine.

Keywords: *Ehrlichia,* vaccine, diagnostic, pathobiology

Ehrlichia spp. is responsible emerging human zoonoses and diseases of veterinary importance in the Americas. *Ehrlichia chaffeensis* emerged in North America in 1986[1] and new *Ehrlichia* spp. associated with human disease continue to emerge with the recent identification of an *E. muris*-like agent in Minnesota and Wisconsin in 2011.[2] *E. canis* is prevalent throughout the Americas in dogs and has been associated with human disease in South America.[3] The emergence of human ehrlichioses caused by multiple agents including *E. chaffeensis, E. ewingii*, *E. canis* and most recently the *E. muris*-like agent (**EMLA**) is an important public health problem. The need for broadly effective countermeasures, diagnostics and understanding of disease mechanisms of these emerging tick-transmitted pathogens in human and veterinary medicine is needed.

Vaccine development

Recent progress in the immunomolecular characterization of *Ehrlichia* spp. has substantially improved understanding of the molecular basis of antigenicity of *Ehrlichia.* Effective immunity

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to ehrlichiae involves humoral and cell-mediated immunity against antigens that have recently been molecularly identified in *E. chaffeensis* including a group of tandem repeat protein (TRP) effectors,[4,5,6,7] and a paralogous outer membrane protein family (OMP-1).[8,9] Several TRPs in *Ehrlichia* have been completely molecularly characterized, and major continuous species-specific antibody epitope(s) have been mapped to the acidic serine-rich TRs of *E. chaffeensis* and *E. canis* TRPs, [5,6,7] and passive transfer of antibodies against *E. chaffeensis* TRPs provides protection against challenge in mice.[10]

Transcriptional analysis of ehrlichial gene expression in mammalian and tick cells has also revealed that TRPs are highly upregulated in the mammalian host.[11] In addition, many genes were found to be differentially expressed, most of which have unknown function,[11] suggesting that proteins encoded by these genes should be considered as vaccine candidates. The development of animal models that faithfully reproduce human disease,[12,13] delineating protective and pathologic innate and adaptive immune mechanisms,[10,14,15] and defining the ehrlichial phenotypic profile in both mammalian hosts and arthropod vectors[11] collectively offers new opportunities for rational development of an effective multivalent subunit vaccine for this group of tick-transmitted pathogens.

Immunodiagnostics

Clinical diagnosis of human ehrlichosis is usually confirmed retrospectively by detection of *Ehrlichia*-specfic antibodies in sera using an indirect fluorescent-antibody assay (IFA).[16] IFA is the current gold standard, but has limitations that include lack of standardization between laboratories, false positive interpretations due to autoantibodies or antibodies directed at conserved bacterial proteins, and cross-reactive antibodies produced by related organisms that can make identification of the specific etiologic agent difficult.[17] Furthermore, IFA requires expensive microscopy equipment and highly skilled technicians to produce the antigen and interpret results. Molecular diagnostic methods such as PCR are useful for specific and sensitive detection of *E. chaffeensis* prior to development of reactive antibodies,[18] but PCR is not useful after antibiotic therapy is initiated, and the clinical sensitivity of PCR in the primary care setting has not been unequivocally determined. Therefore, PCR is currently considered a valuable adjunct to IFA for diagnosis.

Advances in the immunomolecular characterization of *E. chaffeensis* have provided new opportunities to dramatically improve the sensitivity, specificity and standardization of immunodiagnostics for the ehrlichioses. Species-specific continuous epitopes have been identified in the tandem repeats (TRs) of *E. chaffeensis* TRP32, TRP47, and TRP120[5,6,7] The *E. chaffeensis* TRP32 has two to six nonidentical 30-amino acid TRs, and two major species-specific antibody epitopes (continuous and discontinuous) have been identified in the tandem repeats.[7] Single major molecularly distinct continuous antibody epitopes (18 to 22 amino acids) have also been identified in the TRP47 and TRP120, and corresponding orthologs of *E. canis*.[6] These peptide epitopes can be used for development of solid phase high throughput assays. Synthetic peptides can be produced consistently in highly pure forms and can be produced quickly and efficiently without costly and laborious purification procedures and need for defined expression vectors and hosts. The development of standardized and commercially available assays will be advanced by a molecularly defined polypeptide epitopes that provide comparable or better sensitivity than IFA and analytical and clinical specificity that is much higher than IFA. Hence, the future of immunodiagnostics for the ehrlichioses can be substantially improved through the development of peptide-based immunodiagnostics.

Pathobiology

Substantial progress in defining *Ehrlichia*-host interactions and effector proteins involved has been advanced by recent studies on the role of ehrlichial TRPs as effector proteins in reprogramming host cell through interactions with host cell targets and DNA. Early immunoelectron microscopy studies identified TRP120 extracellularly, associated with the morular fibrillar matrix and the morula membrane.[19] It is now known that *E. chaffeensis* TRPs are secreted by the type 1 secretion systems and are related to the repeats-in-toxin exoprotein family.[20] Generally, TRPs in pathogenic bacteria have been associated with host-pathogen interactions such as adhesion and internalization,[19,21] actin nucleation[22] and immune evasion.[23] In *Ehrlichia* spp., long period tandem repeats are distributed in intergenic and coding regions of *Ehrlichia* and appear to have evolved after divergence of the species, through active locally occurring independent events that create and delete TRs through a mechanism compatible with DNA slippage.[24]

Studies have determined that three *E. chaffeensis* TRPs (TRP47, 120, & 32) are involved in a diverse array of interactions with the host cell.[25,26,27] Host cell proteins that are targeted by TRPs include proteins involved in signaling, vesicle trafficking, and transcriptional regulation. The interactions between TRPs and host targets cause the redistribution of some host proteins to ehrlichial morula or cytoplasm adjacent to the morulae in *E. chaffeensis*-infected cells, further indicating the profound effects of TRPs on host cell protein recruitment. Experiments using RNA interference to knockdown genes encoding host proteins such as CD63, DAZAP2 and FTL that interact with *E. chaffeensis* TRP32 resulted in decreased bacterial load in infected cells demonstrating an important functional significance for intracellular survival of *Ehrlichia*.[27] The reduction of a single target protein could not abolish the ehrlichial growth completely; therefore, further study is needed to understand the importance of each specific TRP-host protein interaction in ehrlichial pathobiology.

Nuclear effector proteins (nucleomodulins) have been identified in several intracellular human bacterial pathogens including *Ehrlichia, Anaplasma, Shigella* and *Yersinia*. It is well documented that *E. chaffeensis* significantly alters the transcriptional levels of approximately 5% of host genes within 24 hr of infection.[28] Genes that are modulated include those coding for apoptosis inhibitors, regulation of cell cycle and differentiation, signal transduction, proinflammatory cytokines, biosynthetic and metabolic proteins, and membrane trafficking

proteins. How *Ehrlichia* regulate host cell gene transcription is not known, but two nucleomodulins have recently been described including *E. chaffeensis* TRP120 and Ank200. These nucleomodulins interact with the host cell chromatin through distinct DNA motifs, targeting many host cell genes.[29,30] We have determined the *E. chaffeensis* TRP120 directly binds a defined host cell DNA motif through a novel tandem repeat DNA binding domain that has never been described previously in a human pathogen.[30] Using a direct DNA sequencing approach, we found that TRP120 has a large number of binding sites throughout the genome, but binds strongly to a defined group of host cell process genes involved in transcriptional regulation, protein modification, signaling, and apoptosis. TRP120 directly activated host cell target genes, supporting the conclusion that *E. chaffeensis* TRP120 acts as transcriptional activator of eukaryotic host genes.[30] These new findings have broad implications related to prokaryotic-eukaryotic interactions that include mechanisms of transcriptional modulation of the host cell, the characteristics of bacterial effector proteins, structure and function of prokaryote DNA binding proteins, functional and non functional eukaryote *cis*-regulatory code, and alternative mechanisms for immune escape through direct transcriptional regulation of host defense genes by obligately intracellular pathogens.

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