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Dermacentor variabilis: Characterization and modeling of macrophage migration inhibitory factor with phylogenetic comparisons to other ticks, insects and parasitic nematodes

Nalinda B. Wasala, Deborah C. Jaworski *

Department of Entomology & Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK 74078, United States

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ABSTRACT

We have identified and characterized the full length cDNA sequence of macrophage migration inhibitory factor (MIF) from the American dog tick, *Dermacentor variabilis*. The nucleotide and putative amino acid sequences from this study shared a high level of sequence conservation with other tick MIFs. The bioinformatics analysis showed across species conservation of the MIF amino acid sequence in ticks, insects and nematodes. The multiple sequence alignment identified Pro 1, 3, 55; Thr 7, 112; Asn 8, 72; Ile 64, 96; Gly 65, 110, Ser 63 and Leu 87 amino acids to be highly conserved among the sequences selected for this study. Tick MIF does not have the oxidoreductase domain as found in MIFs from other animals suggesting that tick MIF is not capable of performing as an oxidoreductase. The phylogenetic analysis revealed that tick MIFs share a closer evolutionary proximity to parasitic nematode MIFs than to insect MIFs.

1. Introduction

The American dog tick, *Dermacentor variabilis*, is the primary vector of Rocky Mountain spotted fever and bovine anaplasmosis in North America (Kocan et al., 1981; Lankester et al., 2007; Torres, 2007). It has also been shown to transmit *Cytauxzoon felis* and *Ehrlichia canis* (Blouin et al., 1984; Johnson et al., 1998). Also, *Ehrlichia chaffeensis* and *Ehrlichia ewingii* have been identified in *D. variabilis*, but transmission has not been tested (Steiert and Gilfoy, 2002). In addition, *D. variabilis* has been implicated in the transmission of *Franciscella tularensis* and recently ticks acquiring the infection as nymphs and molting to the adult stage have been shown to efficiently transmit the bacteria (Reese et al., 2011). Given the importance of *D. variabilis* as a vector in these disease systems, learning more about the molecular aspects of its blood feeding phase are critical for developing strategies to control the tick and inhibit the transmission of tick-transmitted bacteria.

Ticks consume milliliters of blood and remain embedded in the skin for several days to weeks, where they elicit an inflammatory response. The blood meal is not only necessary for the ixodid tick's sustenance, it is required for development to the next life stage—larva to nymph, nymph to adult, and adult to thousands of eggs. Transmission of a variety of pathogens also depends on a blood meal by the tick. Tick saliva factors that aid in tick feeding are likely to provide an advantage to the pathogen as well (reviewed by Hovius et al., 2008). Host reactions to tick feeding can include blood clotting, platelet aggregation and vasoconstriction. The tick appears to require an arsenal of active secretions to evade the host response and achieve full engorgement. The tick–host interaction represents a dynamic process where tick products can modulate or mediate the host innate and immune defenses.

Previously, we identified macrophage migration inhibitory factor (MIF) from *Amblyomma americanum* ticks (Jaworski et al., 2001), and others characterized an MIF homolog in *Haemaphysalis longicornis* (Umemiya et al., 2007). In both studies, tick MIF was found to be as active as human MIF in its ability to inhibit macrophages. With *A. americanum*, we have some data to suggest that tick MIF is secreted into the host and may be involved in producing inflammation at the tick feeding site (Jaworski et al., 2009). In feeding female *A. americanum* ticks, we found that expression of MIF in the tick midgut rose up to five days, while the rise in MIF expression in the salivary glands was not as dramatic (Bowen et al., 2010). In the first four days of female *D. variabilis* feeding, MIF gene expression was gradually elevated in the salivary glands and was higher than what we observed for *A. americanum* (Wasala et al.,

* Corresponding author. Fax: +1 405 744 6039.
E-mail address: Deborah.jaworski@okstate.edu (D.C. Jaworski).

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unpublished). MIF expression in the midgut tissue jumped to levels similar to what we observed in *A. americanum* by four days. Our work clearly demonstrates a temporal pattern for MIF gene expression in early tick feeding. MIF is classified as a pro-inflammatory cytokine and is a multifunctional protein that is predominantly involved in the regulation of glucocorticoids in macrophages. It was named for its ability to inhibit the movement of monocytes in Boyden assays. This protein has been identified from many organisms including mammals, nematodes, arthropods, jawed/jawless fish, plants, cyanobacteria and parasites. Here, we sequenced and cloned MIF from the American dog tick, *D. variabilis*. Using the putative amino acid sequence for dog tick MIF, we produced a molecular model for tick MIF. Finally, we utilized our sequence data to draw phylogenetic comparisons between tick MIFs and MIFs from insects and parasitic nematodes.

2. Materials and methods

2.1. Ticks

Adult *D. variabilis* ticks were purchased from the National Tick Research and Education Facility at Oklahoma State University. They were maintained at 96% relative humidity with saturated K_2SO_4 solution and 12:12 (light:dark) photoperiod prior to use. Standard tick rearing procedures were utilized for the production of American dog ticks on sheep and rabbit hosts (Patrick and Hair, 1975).

2.2. RNA isolation

Whole partially fed females from each feeding interval (0, 8, 24, 48, 72 and 96 h) were pooled to create a template that contained all the cDNAs from every tissue for female ticks that were not undergoing rapid engorgement (slow feeding phase). Our final template for these studies was a cDNA expression library of whole feeding *D. variabilis* females taken at different times during the early feeding phase. For rapid amplification of cDNA ends (RACE), pooled ticks were lyophilized with liquid nitrogen and macerated to a fine powder. The powder was re-suspended in 500 µl Tri-Reagent (Molecular Research Center, Cincinnati, OH) for RNA isolation. Working RNA solutions were standardized to250 ng/µl.

2.3. Primers

Gene Specific Primers (GSPs) for rapid amplification of cDNA ends (RACE) were designed using *Amblyomma americanum* MIF sequence (Jaworski et al., 2001) and the initial sequences obtained for *D. variabilis* MIF (complete cDNA sequence for *D. variabilis* MIF is shown in Fig 1). All the primers were generated by Integrated DNA Technologies (IDT) (IDT DNA, Coralville, IA) and were used at the concentration of 10 µM (Table 1). Amplifications were performed using the PTC-100™ thermocycler (MJ Research Inc.).

2.4. Rapid amplification of cDNA ends (RACE)

Marathon® cDNA Amplification Kit and Advantage™ 2 Polymerase Mix (Clontech Laboratories Inc. Mountain View, CA) were used for the RACE reactions. First strand synthesis was performed using 1 µg of total RNA at 42 °C for 1 h immediately followed by the second strand synthesis. Adaptor ligation was performed at 16 °C overnight. Adaptor-ligated cDNA was diluted 1:50 with Tricine-EDTA buffer and stored at −20 °C. 5′ and 3′ RACE reactions were performed at optimized conditions using adaptor-ligated cDNA, GSP primers and adaptor primers. Amplification conditions for 5′ RACE were 94 °C for 30 s, 35 cycles of 94 °C for 5 s, 70 °C for 2 min then 4 °C and 3' RACE amplification conditions were, 94 °C for 30 s, 35 cycles of 94 °C for 5 s, 68 °C for 2 min then 4 °C. Amplification products from each reaction were analyzed on 1.5% agarose gels with 0.1% ethidium bromide, recovered from the gel, cloned into a pGEM®-T Vector System, transformed into *Escherichia coli* JM109 competent cells and plated on 1.5% agar in Luria–Bertoli media with 0.2 µg/µl ampicillin and incubated overnight. Plates were screened for the presence of white colonies and a single colony was transferred to 50 ml of Luria broth with 0.2 µg/µl ampicillin and incubated overnight. Eppendorf Fast Plasmid® Mini Prep kit was used to plasmid extraction from bacteria. Insert was sequenced with T7 primers using ABI 3730 DNA Analyzer. The full length cDNA sequence for *D. variabilis* MIF was deduced by performing sequence alignments of 3′ and 5′ RACE sequences with known tick MIF nucleic acid sequences individually and manually editing them using BioEdit Version 7.0.9.0 sequence editing software (Hall, 1999).

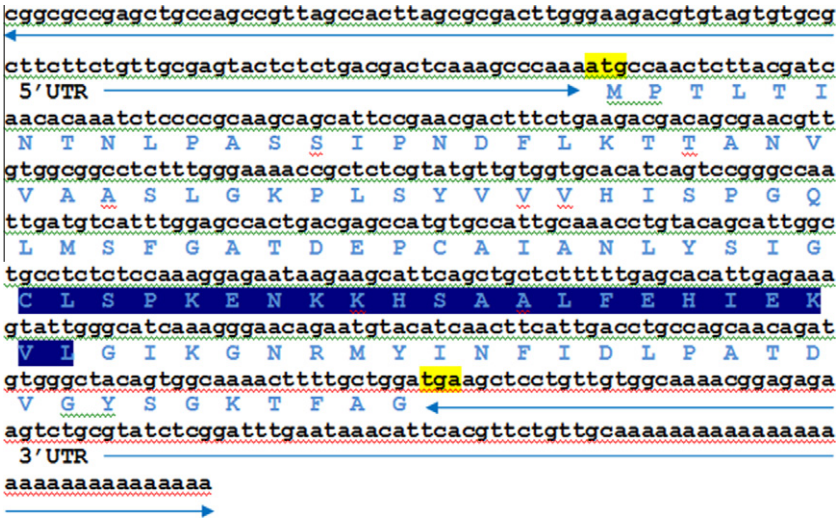

Fig. 1. *D. variabilis* MIF nucleotide sequence and putative amino acid sequence. The nucleotide sequence is shown in simple letters and the encoding amino acid sequence is shown right below the respective codon (shown in blue block capitals). The start codon and stop codon are highlighted in the nucleotide sequence in yellow. Specific antibody to detect tick MIF has been raised using the peptide highlighted in blue. The 5′ and 3′ UTRs span in the regions shown by horizontal arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)