Developmental and Comparative Immunology 59 (2016) 1–14

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Exploring the immune signalling pathway-related genes of the cattle tick *Rhipicephalus microplus*: From molecular characterization to transcriptional profile upon microbial challenge

Rafael D. Rosa [a, 1], Janaína Capelli-Peixoto [a], Rafael D. Mesquita [b], Sandra P. Kalil [a], Paula C. Pohl [a], Glória R. Braz [b], Andrea C. Fogaça [a], Sirlei Daffre [a, *]

[a] Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, SP, Brazil
[b] Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro, 21941-909 Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form
21 December 2015
Accepted 22 December 2015
Available online 24 December 2015

Keywords:
Innate immunity
Tick
Signalling pathway
Rhipicephalus microplus
Anaplasma marginale

ABSTRACT

In dipteran insects, invading pathogens are selectively recognized by four major pathways, namely Toll, IMD, JNK, and JAK/STAT, and trigger the activation of several immune effectors. Although substantial advances have been made in understanding the immunity of model insects such as *Drosophila melanogaster*, knowledge on the activation of immune responses in other arthropods such as ticks remains limited. Herein, we have deepened our understanding of the intracellular signalling pathways likely to be involved in tick immunity by combining a large-scale *in silico* approach with high-throughput gene expression analysis. Data from *in silico* analysis revealed that although both the Toll and JAK/STAT signalling pathways are evolutionarily conserved across arthropods, ticks lack central components of the *D. melanogaster* IMD pathway. Moreover, we show that tick immune signalling-associated genes are constitutively transcribed in BME26 cells (a cell lineage derived from embryos of the cattle tick *Rhipicephalus microplus*) and exhibit different transcriptional patterns in response to microbial challenge. Interestingly, *Anaplasma marginale*, a pathogen that is naturally transmitted by *R. microplus*, causes downregulation of immune-related genes, suggesting that this pathogen may manipulate the tick immune system, favouring its survival and vector colonization.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Ticks (phylum Arthropoda; subphylum Chelicerata; class Arachnida; subclass Acari; family Ixodidae) are blood-feeding arthropods that transmit a wide variety of bacteria, protozoa, viruses and helminths to human and animals (Sonenshine and Roe, 2014). Rickettsial diseases are among the most severe diseases transmitted by ticks and are caused by various species of gram-negative obligate intracellular bacteria (Dantas-Torres et al., 2012; Parola et al., 2005). Bovine anaplasmosis is a tick-borne rickettsial disease caused by *Anaplasma marginale* (order Rickettsiales: family Anaplasmatacea). Various tick species can transmit this bacterium to bovines around the world, but in South America, including Brazil, its main tick vector is *Rhipicephalus microplus* (Aubry and Geale, 2011; Kocan et al., 2010). *R. microplus* is a one-host tick and one of the most important ectoparasites of cattle, being responsible for severe economic impact on agricultural systems globally, transmitting an extensive list of etiological agents of bovine diseases, including babesiosis (caused by intraerythrocytic protozoa in the genera *Babesia*, such as *Babesia bovis* and *Babesia bigemina*) and anaplasmosis (caused by the intraerythrocytic bacteria *A. marginale*) (Kocan et al., 2010). Clinical manifestations of babesiosis are frequently characterized by fever, lethargy, anemia, hemoglobinemia, hemoglobinuria, and jaundice (Pérez de Leon et al., 2014). Anaplasmosis clinical manifestations are similar to the babesiosis disease but without hemoglobinemia and hemoglobinuria (Gaff et al., 2014). The economic losses are associated with reductions in milk and meat production, temporary infertility (and abortion), treatment costs and high animal mortality in enzootic areas (Kocan et al., 2010). In Brazil, the economic losses

* Corresponding author.
E-mail address: sidaffre@icb.usp.br (S. Daffre).
[1] Present address: Laboratory of Immunology Applied to Aquaculture, Department of Cell Biology, Embryology and Genetics, Federal University of Santa Catarina, 88040-900 Florianópolis, SC, Brazil.

http://dx.doi.org/10.1016/j.dci.2015.12.018
0145-305X/© 2015 Elsevier Ltd. All rights reserved.

due to *R. microplus* are highly significant, being estimated at around USD 3.24 billion per year (Grisi et al., 2014).

When a pathogen is acquired from an infected host by a tick during blood feeding, it has to overcome several tissue barriers (midgut, haemocoel, salivary glands) and resist the tick's innate immune responses to be successfully transmitted to another host (Hajdusek et al., 2013; Kopacek et al., 2010). Indeed, the innate immune response is the first line of defence against microbial infections in animals (Hoffmann et al., 1999). The tick's innate immune system, like in other invertebrates, is composed of both cellular and humoral immune responses that recognize pathogens and act against them in an orchestrated manner. The cellular responses are mostly represented by phagocytosis, encapsulation and nodulation of foreign particles, while the humoral responses include a variety of pattern-recognition proteins and effector molecules such as the complement-like system [composed of thioester-containing proteins (TEP)], lectins, antimicrobial peptides (AMPs), and reactive oxygen species (ROS) (Hajdusek et al., 2013; Kopacek et al., 2010; Smith and Pal, 2014; Sonenshine and Hynes, 2008).

Transcription of AMPs and other effectors that compose the immune system of several insects of the order Diptera is selectively triggered by microorganisms through activation of the Toll, IMD (immune deficiency), JNK (Jun-N-terminal kinase) or JAK/STAT (Janus kinase/signalling transducer and activator of transcription) pathway. For instance, the Toll pathway is triggered by gram-positive bacteria and fungi in *Drosophila melanogaster* (Lemaitre, 2004) and by viruses in the mosquito *Aedes aegypti* (Xi et al., 2008), culminating in the expression of specific AMPs. The IMD pathway is activated by diaminopimelic acid (DAP)-type peptidoglycan (PGN), which is present in the cell wall of most gram-negative bacteria and some gram-positive bacteria, triggering the synthesis of specific AMPs (Kleino and Silverman, 2014). The activation of one specific complex in the IMD pathway, the TAK1/TAB2 complex, can alternatively lead to the activation of the JNK pathway (Silverman et al., 2003). Apparently, this pathway does not induce the transcription of AMP coding genes. In *Drosophila*, JNK signalling has been shown to be involved in a wide range of biological processes including embryonic development, apoptosis, stress response, cell proliferation and differentiation, and immunity (Kockel et al., 2001). Conversely, the JAK/STAT pathway can be induced by microbial or viral stimulation in mosquitoes and has also been implicated in the control of *Plasmodium* (Bahia et al., 2011; Gupta et al., 2009).

Even though the immune signalling pathways are well understood in insects, very little is known about them in ticks (Hajdusek et al., 2013; Severo et al., 2013; Smith and Pal, 2014). Elucidation of the genome of *Ixodes scapularis* allowed some components of tick immune pathways to be identified (Severo et al., 2013; Smith and Pal, 2014), but functional data about the activation of immune signalling pathways and the control of tick-borne pathogens are scarce. It was previously shown that the JAK/STAT pathway from the tick *I. scapularis* is likely involved in the control of infection by both *Anaplasma phagocytophilum* (Liu et al., 2012) and *Borrelia burgdorferi* (Narasimhan et al., 2014). In addition, it was hypothesized that transcription of the factor Relish/NF-κB from the IMD pathway may be involved in regulating the expression of subolesin (orthologue of insect akirins), which was tested as a vaccine against ticks (de la Fuente et al., 2011). Interestingly, the importance of ubiquitination during colonization of *I. scapularis* by *A. phagocytophilum* has been shown, but the link between ubiquitination and immune signalling pathways is not completely clear (Severo et al., 2013).

In the current study, we used an *in silico* large-scale approach to identify components from the tick Toll, IMD and JAK/STAT signalling pathways. In addition, we analysed the phylogenetic relationships of the identified components with homologous sequences from other arthropods. We also determined the gene expression profile of *R. microplus* immune signalling pathway-associated genes in BME26 cells (Esteves et al., 2009, 2008) exposed to microbial challenges, including one naturally transmitted pathogen, *A. marginale*. The acquired knowledge about the biology of the tick and its interaction with pathogens may be useful in controlling ticks and tick-borne diseases.

2. Materials and methods

2.1. In silico identification and annotation of immune signalling pathways in ticks

The information on coding sequences of *R. microplus* that is publicly available is very limited. There are only 52,901 ESTs deposited in Gene Bank (Benson et al., 2013) (http://www.ncbi.nlm.nih.gov/genbank/), and most of them are un-annotated fragments. In relation to annotated proteins, there are no more than 962 sequences deposited in Gene Bank so far. As this scenario is not favourable for finding complete coding sequences of *R. microplus*, in the current study, we used unpublished data from an Illumina-based transcriptome databank from seven organs (ovary, synganglion, salivary gland, fat body, digestive cells) of naïve R. microplus, acquired at various stages of development (embryo, semi-engorged and engorged female). The resulting reads were deposited in NCBI (Biosample SAMN02463642 and Bioproject PRJNA232001). These reads were then assembled into contigs using a series of programs mastered by in-house software designed by J.M.C. Ribeiro (NIH/NIAID) (Ribeiro et al., 2011). These contigs were further annotated, and a databank, which will be made public in a forthcoming publication, is available to our associates. Sequences of other tick species (*Amblyomma cajennense*, *Amblyomma maculatum*, *Amblyomma variegatum*, *Ixodes ricinus*, *I. scapularis*, and *Rhipicephalus pulchellus*) deposited in Gene Bank were also obtained and used to generate a tick databank.

Protein sequences of components of the *D. melanogaster* immune signalling pathways were obtained through the downloadable hyperlinked spreadsheet “List of *Drosophila* genes potentially involved in the immune response” available at http://lemaitrelab.epfl.ch/page-7767-en.html. The conserved domains present in those proteins were used by FAT software (Seabra-Junior et al., 2011) to select potentially homologous sequences from the tick databank and automatically annotate them. FAT uses both sequence similarity and presence of conserved domains for annotation based on the results of BLAST against both the nr and Swiss-prot uniprot databases and also on HMMSCAN against the Pfam database (Finn et al., 2014). As non-identified immune-related genes could still be in the transcriptome as fragments or truncated proteins without any conserved domain, we performed tblastn searches. Analyses were performed using sequences from tick-related organisms (insects, including *D. melanogaster*, chelicerates and crustaceans) as queries to improve the chances of finding tick immune-related genes. Sequences identified by the tblastn analyses were extracted using a custom script, and automatic annotation was performed as described above. All results were further manually inspected.

2.2. Molecular cloning

Sequences encoding Rel/NF-κB transcription factor 1 (Dorsal/Rel1) and inhibitor of NF-κB (IκB/Cactus) of *R. microplus* were obtained by conventional PCR using primers (Appendix A) designed to match the consensus nucleotide sequences of *I. scapularis* (Dorsal: XP_002399379; Cactus: EEC10897), *I. ricinus* (Dorsal: JAC92732)

Download English Version:

https://daneshyari.com/en/article/2428877

Download Persian Version:

https://daneshyari.com/article/2428877

Daneshyari.com