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Original article

Evaluation of the protective efficacy of *Ornithodoros moubata* midgut membrane antigens selected using omics and *in silico* prediction algorithms

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ABSTRACT

The African argasid tick *Ornithodoros moubata* transmits two important pathogens, the African swine fever virus and the spirochete *Borrelia duttoni*, the cause of human relapsing fever. To date, only conventional control measures such as widespread application of acaricides, strict control measures, and animal movement restrictions have been implemented to confine these diseases. Vaccines against tick infestations have the potential to be among the most efficacious interventions for the management of these diseases. Plasma membrane-associated proteins upregulated in tick midgut cells in response to blood feeding and digestion are thought to play vital functions in tick physiology and in the transmission of tick-borne pathogens. In addition, their antigenic extracellular regions are easily accessible to antibodies synthesised by immunised hosts, which makes them interesting targets for tick vaccine design. The mialomes (midgut transcriptomes and proteomes) of unfed *O. moubata* females and of engorged females at 48 h post-feeding have recently been obtained, providing a wealth of predicted midgut protein sequences. In the current study, these mialomes were screened using *in silico* tools to select predicted antigenic transmembrane proteins that were upregulated after feeding (516 proteins). The functionally annotatable proteins from this list (396 proteins) were then manually inspected following additional criteria in order to select a finite and easy-manageable number of candidate antigens for tick vaccine design. The extracellular antigenic regions of five of these candidates were obtained either as truncated recombinant proteins or as KLH-conjugated synthetic peptides, formulated in Freund's adjuvant, and individually administered to rabbits to assess their immunogenicity and protective potential against infestations by *O. moubata* and the Iberian species *Ornithodoros erraticus*. All candidates were highly immunogenic, but provided low protection against the *O. moubata* infestations (ranging from 7% to 39%). Interestingly, all candidates except one also protected against infestations by *O. erraticus*, achieving higher efficacies against this species (from 20% to 66%). According to their protective potential, three of the five antigens tested (Om17, Om86 and OM99) were considered little suitable for use in tick vaccines, while the other two (OM85 and OM03) were considered useful antigens for tick vaccine development, deserving further studies.

1. Introduction

The argasid tick *Ornithodoros moubata* colonises wild and anthropic habitats throughout South and East Africa, feeding on warthogs, domestic swine and humans (Vial, 2009). *O. moubata* transmits the African swine fever (ASF) virus and the human relapsing fever (TBRF) agent, *Borrelia duttoni*. The presence of this tick in anthropic environments contributes to the persistence of ASF and TBRF in endemic areas and may facilitate the spread of these diseases into surrounding areas (Cutler, 2010; Costard et al., 2013; EFSA pannel, 2014; Sánchez-Vizcaíno et al., 2015; Quembo et al., 2016). It is expected that elimination of synanthropic populations of *O. moubata* would greatly improve the prevention and control of such diseases.

Anti-tick vaccines have emerged as a cost-effective and environmentally sustainable method for the control of tick infestations and tick-borne diseases (Willadsen, 2008; de la Fuente et al., 2016; Šmit and Postma, 2016).

Our team has made substantial efforts in the development of an anti-*Ornithodoros* vaccine, testing the protective effects of two types of antigens: salivary antigens, which are naturally exposed to the host immune system during tick feeding, and concealed antigens from the tick midgut (Astigarraga et al., 1995; Díaz-Martín et al., 2015a).

The studies with salivary antigens resulted in the identification of three anti-haemostatic proteins that displayed individual vaccine

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efficacies between 27% and 44%, and up to 60% efficacy when they were administered together as a multicomponent vaccine, making them interesting vaccine candidates. The main protective effect of these antigens consisted of the partial inhibition of blood feeding and subsequent reduction in female fertility, most likely as a consequence of the antibody-mediated loss of function of the target antigen at the tick-host interface. These vaccine-induced antibodies were mainly directed to immune-dominant linear B-cell epitopes located on the surface of the target salivary antigens (Díaz-Martín et al., 2015b). Despite these promising results, a fully protective vaccine against *O. moubata* based only on salivary antigens has not been obtained, which makes it necessary to continue searching for new and more protective antigens in other tick tissues and organs (García-Varas et al., 2010; Díaz-Martín et al., 2015a,b).

O. moubata midgut antigens seem very promising as vaccine candidates because a midgut antigen, the Bm86 protein, is the basis of the only two tick vaccines marketed to date, TickGARD® and GAVAC®. The Bm86 antigen induces protective immune responses mainly mediated by host complement system and antibody interactions which damage the tick midgut wall subsequently disrupting tick survival and egg viability (de la Fuente et al., 2007; Lew-Tabor and Rodriguez Valle, 2016).

Not surprisingly, animal vaccination with crude extracts of midgut membranes from *O. moubata* — and with similar extracts from the Iberian species *Ornithodoros erraticus* — induced protective responses that reduced female feeding and fertility by up to 50% in both species and, additionally, caused up to 80% mortality to the nymphs of *O. erraticus* in the first 72 h post-feeding.

The antigens responsible for this protection were partially characterised as membrane proteins of the luminal surface of midgut epithelial cells (enterocytes), whose expression increased upon blood ingestion, peaking between 24 and 72 h post-feeding (h.p.f.). The protective immune mechanism involved the fixation and activation of the host complement system onto the membrane of enterocytes by specific vaccine-induced antibodies. This caused enterocyte lysis and midgut damage in a similar way to what was observed for the vaccines based on the Bm86 antigen (García-Varas, 2004; Manzano-Román et al., 2006, 2007).

The identity of these proteins remains unknown, but the above results confirmed the protective potential of midgut antigens from both *Ornithodoros* species, establishing this organ as a suitable source of antigens for soft tick vaccine development.

Blood digestion and the absorption of the released nutrients take place in the tick midgut; moreover, the midgut constitutes a pivotal entry point for tick-borne pathogens that determines the pathogen success in survival, vector colonisation and subsequent transmission (Narasimhan et al., 2014; Abraham et al., 2017). Accordingly, the tick midgut expresses a wide range of proteins that play vital functions in digestion-related physiological processes and in the infection and transmission of blood-borne pathogens (Kocan et al., 2004; Sojka et al., 2013, 2016). Many of these proteins are upregulated in response to the stimulus provided by host binding and blood ingestion (Sojka et al., 2013; Oleaga et al., 2015; Sojka et al., 2016; Oleaga et al., 2017a,b). According to previous observations, it may be expected that some of the midgut proteins that are upregulated upon tick feeding have protective potential (Akov, 1982; Matsuo et al., 2003; García-Varas, 2004; Manzano-Román et al., 2006, 2007); in other words, that the disruption of their function by vaccine-induced antibodies might have a significant impact on tick physiology and survival.

Among midgut proteins, transmembrane proteins expressed on the luminal side of the enterocyte plasma membrane are interesting candidates in vaccine design because their antigenic extracellular regions are easily accessible to host immune effectors — mainly antibodies — ingested in blood (Rappuoli and Bagnoli, 2011). Hence, midgut transmembrane proteins are considered first-election targets for the development of new drugs and vaccines aimed at tick control (Richards et al., 2015).

Recently, our team obtained the mialomes (midgut proteomes and transcriptomes) of *O. moubata* female ticks in two physiological conditions: before feeding and in the initial phases of the blood digestion, at 48 hp.f. These studies have provided an unprecedented number of novel nucleotide sequences coding for midgut proteins from *Ornithodoros* ticks, further allowing the identification of genes/proteins that are differentially expressed upon blood feeding (Oleaga et al., 2017a,b).

These data can be screened with suitable bioinformatics tools to identify predicted proteins with particular traits in cellular localisation, topology, antigenicity and biological function in order to select potential antigenic candidates for vaccine development. The selected candidates can then be produced, mainly as recombinant proteins, and their protective efficacy experimentally tested in animal immunisation trials (Richards et al., 2015; Lew-Tabor and Rodriguez Valle, 2016; de la Fuente et al., 2016).

Accordingly, the aim of the current work was to identify new protective antigens from the *O. moubata* mialome, with the following specific objectives: first, the *in silico* selection of potentially protective candidate antigens of *O. moubata* among transmembrane proteins upregulated in the midgut after feeding; second, the production of these candidates as recombinant proteins or synthetic peptides; and, finally, their experimental assessment and validation as vaccine antigens in animal immunisation trials.

2. Materials and methods

2.1. Ticks and tick material

The *O. moubata* and *O. erraticus* ticks used in this study were all obtained from the two colonies currently maintained at the laboratory of Animal Parasitology, IRNASA, CSIC, Spain. The colony of *O. moubata* was established from specimens obtained from the Institute for Animal Health in Pirbright (Surrey, UK) and the colony of *O. erraticus* was established from specimens captured in Salamanca, western Spain. These ticks are regularly fed on rabbits and kept in a culture chamber at 28 °C, at 85% relative humidity with a 12 h light-dark cycle. Tick feeding and animal manipulation was performed according to the regulations established by the Ethical and Animal Welfare Committee of the institution where the experiments were conducted (IRNASA, CSIC), following the corresponding EU legislation (Directive 2010/63/EU).

Midguts from engorged *O. moubata* females at 48 hp.f. were obtained as described by Oleaga et al. (2017a) and preserved in RNA-later (Ambion) for RNA extraction. Total RNA was extracted and purified using the RNeasy Mini Kit (Qiagen) following the manufacturer's instructions.

Midguts from unfed *O. moubata* and *O. erraticus* females and from engorged females at 48 hp.f. were also obtained and used to prepare extracts of soluble and membrane proteins as previously described (Oleaga et al., 2017b). Briefly, batches of 25 midguts from each species and physiological condition were homogenised and sonicated in ice-cold PBS containing proteinase inhibitors (Roche Diagnostics, Indianapolis, USA). Tissue homogenates were centrifuged at 10^4 g to remove particulate remnants, and the supernatants were fractionated by centrifugation at 10^5 g into two fractions enriched in either soluble or membrane proteins (the 10^5 g supernatants and pellets, respectively). The protein concentrations in these samples were assessed using the BCA Protein Assay Reagent kit (Thermo-Fisher, Rockford, USA), and the samples were stored at −20 °C.

Tick saliva from unfed female *O. moubata* and *O. erraticus* ticks was collected after stimulating them with 1% pilocarpine, as described previously (Díaz-Martín et al., 2013). Protein concentrations in the saliva samples were measured using the Bradford assay (Bio-Rad) and samples were stored at −20 °C.

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