Veterinary Parasitology 221 (2016) 30–38

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

Design of the ATAQ peptide and its evaluation as an immunogen to develop a *Rhipicephalus* vaccine

André de Abreu Rangel Aguirre[a,e,*], Francisco Pereira Lobo[b], Rodrigo Casquero Cunha[a,c], Marcos Valério Garcia[c,d], Renato Andreotti[c]

[a] *Programa de Pós-graduação em Ciência Animal, FAMEZ, Universidade Federal de Mato Grosso do Sul, Av. Felinto Müller, No. 2443, Vila Ipiranga, CEP 79074-460 Campo Grande, MS, Brazil*
[b] *Embrapa Informática Agropecuária, Av. André Tosello, No. 209 Campus Unicamp, Barão Geraldo, CEP 13083-886 Campinas, SP, Brazil*
[c] *Embrapa Gado de Corte, Av. Rádio Maia, No. 830, Zona Rural, CEP 79106-550 Campo Grande, MS, Brazil*
[d] *Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil*
[e] *Fundação Oswaldo Cruz, Fiocruz Rondônia, Rua da Beira, No. 7671, Bairro Lagoa, CEP 76812-245 Porto Velho, RO, Brazil*

ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form 21 February 2016
Accepted 27 February 2016

Keywords:
Reverse vaccinology
ATAQ
Immunization
Rhipicephalus microplus
Rhipicephalus sanguineus
Synthetic peptide

ABSTRACT

Tick infestation may cause several problems including affecting domestic animal health and reducing the production of meat and milk, among others. Resistance to several classes of acaricides have been reported, forcing researchers to search for alternative measures, such as vaccines against ticks, to ensure tick control while having no or at least low negative impacts on the environment and public health. However, the current commercially available vaccines in different strains of *Rhipicephalus microplus* are reported to be of low efficacy. Fortunately, reverse vaccinology approaches have shown positive results in the new generation of vaccines. On this basis, a synthetic peptide from the ATAQ protein, which is present in the gut and Malpighi tubes of *R. microplus*, was synthesized. The ATAQ proteins were isolated, characterized and sequenced from several species of the genus *Rhipicephalus*. The alignment showed 93.3% identity among DNA sequences of ATAQs from these species. Because of this, immunization trials with this peptide were conducted on mice, rabbits and cattle to evaluate the humoral immune response and the efficacy against *Rhipicephalus sanguineus* in addition to *R. microplus*. Based on recent results, we conclude that reverse vaccinology is a promising approach because it is more accurate and faster than conventional methods in the detection of potential antigens to use in anti-tick vaccines. It is not only applicable against *R. microplus* but also against tick species that play important roles in spreading other diseases. ATAQ proteins should be considered as the antigen in new trials to develop a multi-antigenic vaccine. Although these peptides behave as hapten and are not able to be recognized by the immune system on its own, using carriers and adjuvants helps its presentation and induces strong immune responses. Furthermore, an efficiency of 35% reduction in overall life cycle parameters was reported for *R. microplus* (98% for ELISA responder animals) and 47% for *R. sanguineus*. Although not yet enough to prevent the environment to infestation of ticks, this still constitutes a promising strategy that could be applied to integrated measures on tick control and in new research that develops anti-tick vaccines.

1. Introduction

Rhipicephalus microplus control has been conducted mainly by acaricide application, resulting in the selection of resistant ticks and contributing to environmental pollution (De La Fuente and Kocan, 2006). Because of this, studies have started to focus on vaccine development. The current main antigen used for anti-tick vaccines is the glycoprotein Bm86 (Willadsen and Kemp, 1988; Willadsen et al., 1988), which is predominantly located in the membrane of gut cells of ticks (Gough and Kemp, 1993).

Recombinant Bm86 is the basis for the commercial vaccines TickGARD and Gavac™ (Rodriguez et al., 1995; De La Fuente et al., 2007). Several vaccine formulations have been developed with this protein (Rand et al., 1989; Rodriguez et al., 1995; De La Fuente et al., 1999; Patarroyo et al., 2002). However, variable percentages of efficacy against strains of *R. microplus* were found in different geo-

* Correspondence to: Fundação Oswaldo Cruz, Fiocruz Rondônia, Rua da Beira, No. 7671, Bairro Lagoa, CEP 76812-245 Porto Velho, RO, Brazil.
E-mail address: andreaguirrevet@hotmail.com (A.d.A.R. Aguirre).

http://dx.doi.org/10.1016/j.vetpar.2016.02.032
0304-4017/

graphical locations, which may be due to natural allelic variations in the gene Bm86 (De La Fuente and Kocan, 2006).

A difference of 3.4% in Bm86 sequence among different strains could be enough to cause inefficient immune response against the strains (Garcia-Garcia et al., 1999). The percentage of efficacy of TickGARD and Gavac™ in a stall test with a regional isolate of *R. microplus* was 46.4% and 49.2%, respectively (Andreotti, 2006). These results are lower than what was observed in other regions of the world (Rand et al., 1989; Richardson et al., 1993; De La Fuente et al., 1999; Patarroyo et al., 2002). There is a difference in Bm86: the hydrophobic sites of CG (Campo Grande strain) have the potential to interfere with antibody binding, which can explain the low efficacy of Gavac™ to prevent tick infestation in cattle from the Campo Grande region (Andreotti, 2006).

Due to recent reports about the low efficacy of commercial vaccines in different strains of *R. microplus*, studies for new potential antigens have increased over recent years. Several vaccines have used whole proteins as antigens, such as trypsin inhibitors, subolesin, akirin, kallikrein, elastase inhibitors and peptides designed by reverse vaccinology approaches in post genomic studies. Interest has also been shown in strategies of compound vaccines (Tanaka et al., 1999; Sasaki and Tanaka, 2008; Prudencio et al., 2010; Andreotti et al., 2012; Carreón et al., 2012).

A recent study described the ATAQ protein, a putative Bm86 homolog with high similarity in primary and secondary structures. Despite the RNAi experiments showing a very weak phenotype for both Bm86 and ATAQ, Bm86 caused strong protection when used as an antigen (Nijhof et al., 2006). We hypothesized that the ATAQ protein is also a potential antigen for vaccine development because it is present in the gut and Malpighian tubes of all tick instars from *R. microplus*, *Rhipicephalus annulatus*, *Rhipicephalus decoloratus* and *Rhipicephalus evertsi evertsi* (Nijhof et al., 2010).

Reverse vaccinology is a promising strategy to discover new immunogen candidates for vaccines and is gaining increasing attention (Rappouli, 2000). The novel feature of this methodology is a bottom-up approach to identify potential antigens before animal testing, which is enabled by bioinformatics tools that can process huge amount of data from parasite genomics. Reverse vaccinology analysis usually searches for proteins and domains present in the host-parasite molecular interface (extracellular portion) and is capable of being recognized by the host immune system (presence of linear B and T cell epitopes). Great advances using the reverse vaccinology approach have been made in several disease agents that, up until now, lack effective means of control and whose vaccine development has not been successful using traditional methods. These diseases include meningitis B, malaria, tuberculosis, syphilis and hepatitis C (Rappouli, 2000; Adu-Bobie et al., 2003).

In the case of tick vaccines, one study targeting the Bm86 glycoprotein found a specific domain (Bm7462) and had 81.05% efficacy against *R. microplus* (Patarroyo et al., 2002). Maritz-Olivier et al. (2012) performed the transcriptome analysis of *R. microplus* and used the VaxiJen algorithm to analyze properties of the translated proteins to find 25 peptides with high antigenic properties. Among them all, three peptides were recognized by polyclonal serum against *R. microplus* gut protein extract.

Despite the fact that the ATAQ protein has not been detected in *Rhipicephalus sanguineus* (e.g., NCBI BioSystems Database, Geer et al., 2010), our *in silico* analysis identified sequences similar to ATAQ in other *Rhipicephalus* species, suggesting that this protein is likely to exist in these species.

This study aims to find an antigenic peptide from the ATAQ protein of *R. microplus* described in GenBank using a reverse vaccinology approach and characterize the immune response against such peptide. Using distinct adjuvants and testing in different host species, we have surveyed the vaccine effectiveness of this peptide against *R. sanguineus* in rabbits and *R. microplus* in cattle.

2. Materials and methods

2.1. Animals

Eighteen female BALB/c mice, aged between six to eight weeks, were used to test the immune response against the peptide. They were kept in cages (three mice per cage) with individual ventilation, controlled room temperature (22 °C), an exhaustion system and photoperiod (12 h of light and 12 h of dark). They were fed with sterilized commercial ration and sterile water *ad libitum*.

Nine female white cross-breed rabbits, aged between 12 to 20 weeks and weighing around two kilograms, were used. The animals were kept in individual cages and fed with commercial ration and sterile water *ad libitum*.

We selected eight male cattle Holstein-Friesan breed from a squad maintained at Embrapa Beef Cattle, aged between twelve to eighteen months old. These animals were kept in stalls and were fed with corn silage supplemented with mineral salt and water *ad libitum*.

All animals were maintained at Embrapa's biotery during the trials. All procedures with animals were approved by the ethical committee of animal use from Universidade Federal de Mato Grosso do Sul under protocol number 595/2014 and were carried out in accordance with the International Guiding Principles for Biomedical Research Involving Animals issued by the Council for the International Organizations of Medical Sciences.

2.2. Bioinformatics analyses and peptide synthesis

To detect potentially immunogenic peptides from the ATAQ protein of *R. microplus* (access number GenBank: ADR01301.1) we used various bioinformatics programs that added biological information about the potential immunogenicity of a peptide targeting the humoral immune response. The task of identifying immunogenic peptides can be defined as detecting protein peptides that have features that potentially increase the immunogenicity of this peptide. We used tools for the prediction of linear B-cell epitopes to detect properties that increase the probability of immunogenicity of a peptide (Bepipred program, IEDB Analysis Resource, cutoff 1.0 for 9 consecutive amino acids), the prediction of extracellular regions (signalp, tmhmm and GPI programs, cutoff of 0.5 for all) and the prediction of intrinsically unstructured proteins (IUPs, program IUPred, cutoff of 0.3 for 9 consecutive amino acids). These were intended to predict regions that could be constantly exposed in the whole protein at the tertiary structure level. This kind of feature is desirable because it indicates that the peptide is in a region of the protein that could be exposed to the host immune system. For the prediction of properties that diminish the immunogenicity, we used the BLAST program for the detection of possible regions of the ATAQ protein that have high similarity with *Bos taurus* proteins (e-value less than 0.5) in order to avoid possible cross-reaction with host proteins and intracellular portions as predicted by the tmhmm software. These regions would be less exposed to the humoral immune response (Fig. 1).

Other analyses of the ATAQ protein were also performed using algorithms that already existed. The software Geneious Pro 4.8.5 (Biomatters, Auckland, New Zeland) includes tools that predict antigenic properties from the primary protein structure, considering features such as hydrophobic and linear regions, and predict the secondary structure (alpha helix, beta strand and coils). The probability of an amino acid sequence region to be exposed on the surface of the protein was calculated by the Emini Surface Acces-