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Research paper

Rhipicephalus (*Boophilus*) *microplus* embryo proteins as target for tick vaccine

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ABSTRACT

Rhipicephalus (*Boophilus*) *microplus* is one of the most widely distributed tick in the world. The control of the parasite is based mainly on the use of chemical acaricides, which are produced from a limited set of molecules. These drugs induce selection of acaricide-resistant ticks, and are an important source of environmental pollution. An approach based on anti-tick vaccines may circumvent these obstacles. Characterization of the physiological function of tick molecules may be useful to develop new vaccines. Previously, we reported the ability of some tick proteins as inducers of protective immune response. Vaccination studies using tick proteins like native (nBYC), recombinant (rBYC) egg-yolk aspartic endopeptidase and cysteine endopeptidase (VTDCE) from *R. microplus* and glutathione S-transferase (Hl-GST) from *Haemaphysalis longicornis* demonstrated the immunogenicity and antigenicity of these proteins in bovines. Eventually, immunization with these proteins triggered a partial immune response against *R. microplus* infestation in cattle, manifested mainly as a reduction in egg fertility (7.7% and 13.9% for nBYC, 5.9% for rBYC; 4.7% for VTDCE, 7.9% for Hl-GST), and in the number of fully engorged ticks (18.2% for rBYC, 14.6% for VTDCE, 53% for Hl-GST). The data so far obtained suggest that these proteins have potential to be used as antigens in an anti-tick vaccine. Other proteins involved in tick embryogenesis also have this potential, like THAP and BmCl1, which are enzymes with key roles in vitellin and hemoglobin hydrolysis. Moreover, the identification of analogous proteins present in other tick species may bring information about the way to develop a vaccine against multiple tick species which can help to solve the problem faced by numerous countries where animals are parasitized by more than one tick species. The aim of the present review is to comprehensibly summarize the data obtained in the last few years by our collaborative research, discussing the efforts we have made to find antigens efficient enough for a cattle tick-controlling vaccine. This review discusses tick physiology studies aimed at the selection of possible targets, characterization of the selected proteins with emphasis on their biochemical and immunological aspects and results of vaccine trials on bovines.

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1. Introduction

The bovine tick (*Rhipicephalus* (*Boophilus*) *microplus*) is one of the most relevant ectoparasites in the cattle industry in tropical and sub-tropical areas (Jonsson, 2006). By far, *R. microplus* is one of the major causal agents behind low cattle productivity figures in numerous countries, due to its role as the vector of the etiologic agents of babesiosis and anaplasmosis, as well as its own spoliation action. Infestation by *R. microplus* leads to deterioration of host fitness, weight loss, lowered milk production, decrease in leather quality, and higher production costs. It is estimated that the worldwide economic impact of *R. microplus* reaches circa 10 billion US dollars per year (Grisi et al., 2002).

Controlling tick populations to levels acceptable is crucial to minimize these massive economic losses. Unfortunately, control strategies currently adopted against *R. microplus* based on conventional acaricides are becoming less efficient and increasingly expensive due to the widespread resistance to current acaricides (Willadsen, 2006; Rosario-Cruz et al., 2009). Since the large-scale use of acaricides results in potential meat, milk and environmental contamination (Davey and George, 1998), there is also an increasing social demand for new control methods.

Alternative tick control methods can be classified into four groups: (i) biological control by tick pathogens or predators (Samish and Glazer, 2001); (ii) habitat alterations by planting tick-killing or repelling vegetation (Sutherst et al., 1982); (iii) immunological control (Willadsen, 2004); and (iv) development of tick-resistant breeds (Rodriguez-Valle et al., 2010; Piper et al., 2010). Although these methods have been proved to be theoretically valuable, most of them have been forsaken, since they did not afford acceptable cost/benefit ratios under field conditions, except for vaccines (Willadsen, 2004; Vercruysse et al., 2007), which showed potential to replace, at least in part, chemical acaricides. Immunological tick control strategies have been the subject of research for more than 40 years (Roberts, 1968; Wagland, 1975; de la Fuente et al., 2007). It is well established that after successive infestations bovines acquire some resistance against ticks (Allen, 1994). In general, resistant animals reject between 80% and 99% of the larvae (Tatchell, 1987; Allen, 1994). These studies form the theoretical framework supporting the use of vaccines for tick control. Typically, acquired resistance is expressed as reduced tick engorgement, increased feeding time, lowered egg production and viability, inhibition of molting, and death of feeding ticks (Wikel, 1996; Parizi et al., 2009). Comparing to chemical acaricides, anti-tick vaccines offer a number of advantages: specificity for target species, environmental safety, absence of risks to human health, absence of residues in bovine derived foods, prevention of drug-resistance, simple administration techniques, and low cost (Willadsen, 2004).

The feasibility of an anti-tick vaccine was definitively demonstrated by the seminal study conducted by Willadsen and coworkers, who successfully developed and commercialized a recombinant anti-*R. microplus* vaccine based on a midgut-associated protein, namely Bm86 (Willadsen and Kemp, 1988). Studies like these provided crucial evidence of the reliability and practicability of anti-tick vaccines. However, the protection level achieved in Australia was not observed in other regions in the world. As a result, efforts are still being made worldwide in order to identify candidate antigens for a more effective anti-tick vaccine (de la Fuente et al., 2007).

Currently, anti-tick vaccine development is focused on the identification, molecular cloning and *in vitro* production of proteins playing key putative roles in tick physiology, such as cell signaling, modulation of host immune response, pathogen transmission, embryogenesis, digestion, and intermediary metabolism (Parizi et al., 2009). Indeed, a considerable research effort is currently under way in several laboratories worldwide in order to identify and characterize tick molecules potentially useful for an anti-tick vaccine. In this context, our group has focused our research efforts on the study of embryo proteins as a target for an anti-tick vaccine.

2. Embryogenesis

Embryo development of oviparous organisms relies on a number of different compounds, like proteins, lipids, sugars and other molecules. These components are stored in special organelles called yolk granules (Atella et al., 2005). Yolk accumulation (vitellogenesis) is the process in which ovarian and extraovarian tissues produce proteins precursors which are accumulated inside oocytes (Seixas et al., 2010b). Once vitellogenesis has finished, the egg contains all substances needed for embryo development during embryogenesis which occurs outside maternal organism, as well as the genetic material (Logullo et al., 2002).

Despite its importance, tick embryogenesis has not been studied in detail and, unfortunately, there is a paucity of reports describing the events involved in tick embryo formation. In this sense, various aspects of *R. microplus* embryogenesis has been described, with a view to finding proteins that could be useful as target to novel control methods. Briefly, tick embryogenesis begins with a non-cellular zygote and repeated divisions of the nucleus without cell division, starting from the 1st into the 4th day (Fig. 1A and B). Four days after oviposition (DAO), embryos reach the syncytial blastoderm stage (Fig. 1C), and after several other divisions the nucleus migrates to the periphery. On the 5th DAO the cellular blastoderm stage starts (Fig. 1D); on the 7th DAO, the dorsal closure stage begins, with evident segmentation (Fig. 1E), and on the 12th DAO (Fig. 1F) embryos reach the late organogenesis stage (Davis and Patel, 2002). Embryonic development at 28 °C is completed on the 21st DAO (Campos et al., 2006).

The study of yolk proteins, where they are produced, how they are gathered inside the oocyte, and how they are mobilized during embryogenesis is part of a general strategy that will determine detailed information about tick reproduction. Maternally produced yolk proteases like BYC (Logullo et al., 1998), THAP (Sorgine et al., 2000) and VTDCE (Seixas et al., 2003) accumulate in oocytes. These enzymes are responsible for yolk degradation (Fig. 2C) and are activated by acidification after the fertilization event (Abreu et al., 2004). This enzyme repertoire is an interesting object in the search for a target useful for parasite control, since one can hypothesize that disturbance