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Short communication

Inhibition of the classical pathway of the complement system by saliva of *Amblyomma cajennense* (Acari: Ixodidae)

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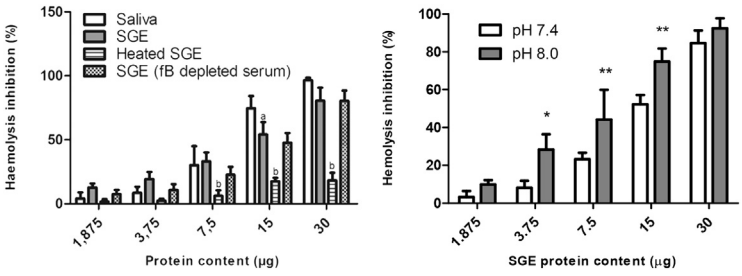
HIGHLIGHTS

- Saliva and salivary gland extracts of *A. cajennense* inhibit the classical pathway.
- Salivary anti-complement activity is present in fasting ticks.
- Salivary anti-complement activity is similar in ticks from different body weights.
- Salivary anti-complement activity is similar in ticks fed on mice and horses.
- Salivary anti-complement activity is more efficient in pH 8.0 than in pH 7.4

GRAPHICAL ABSTRACT



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ABSTRACT

Inhibition of the complement system during and after haematophagy is of utmost importance for tick success in feeding and tick development. The role of such inhibition is to minimise damage to the intestinal epithelium as well as avoiding inflammation and opsonisation of salivary molecules at the bite site. Despite its importance, the salivary anti-complement activity has been characterised only in species belonging to the *Ixodes ricinus* complex which saliva is able to inhibit the alternative and lectin pathways. Little is known about this activity in other species of the Ixodidae family. Thus, the aim of this study was to describe the inhibition of the classical pathway of the complement system by the saliva of *Amblyomma cajennense* at different stages of the haematophagy. The *A. cajennense* saliva and salivary gland extract (SGE) were able to inhibit the complement classical pathway through haemolytic assays with higher activity observed when saliva was used. The anti-complement activity is present in the salivary glands of starving females and also in females throughout the whole feeding process, with significant higher activity soon after tick detachment. The SGE activity from both females fed on mice or horses had no significant correlation ($p > 0.05$) with tick body weight. The pH found in the intestinal lumen of *A. cajennense* was 8.04 ± 0.08 and haemolytic assays performed at pH 8.0 showed activation of the classical pathway similarly to what occurs at pH 7.4. Consequently, inhibition could be necessary to protect the tick enterocytes. Indeed, the inhibition observed by SGE was higher in pH 8.0 in comparison

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to pH 7.4 reinforcing the role of saliva in protecting the intestinal cells. Further studies should be carried out in order to identify the inhibitor molecule and characterise its inhibition mechanism.

1. Introduction

The complement system is an important effector mechanism participating in both innate and acquired vertebrate immune responses (Farries and Atkinson, 1991). The inhibition of the host complement system by haematophagous animals seems to be of utmost importance and has been described in distinct and distant phylogenetic species (reviewed by Schroeder et al., 2009). In blood-sucking arthropods, this inhibition is directly linked to success in feeding, development and reproduction (Wikel and Allen, 1977).

The major function of the complement inhibitors seems to be the protection of the intestinal epithelium from the attack of the complement system during and after the blood ingestion (Barros et al., 2009). Complement inhibitors are present in the saliva as well as in the midgut (Barros et al., 2009; Mendes-Sousa et al., 2013). As saliva is ingested with the blood during feeding (Soares et al., 2006) the anti-complement activity present in the gut is likely to be performed by intestinal and salivary molecules. Furthermore, during feeding, the activation of the complement system would lead to opsonisation of salivary molecules by products of the C3 cleavage and the formation of inflammatory anaphylatoxins such as C3a and C5a (Dunkelberger and Song, 2010; Ribeiro and Spielman, 1986). The inhibition of these phenomena is useful to prevent or delay the onset of an immune response against salivary proteins and prevent inflammation at the feeding site.

The importance of complement inhibition was shown by previous studies. In guinea pigs, complement depletion reduced the resistance to *Dermacentor andersoni* larvae (Wikel and Allen, 1977). *In vitro* studies to uncover the vaccine antigen BM86 mechanism of action against *Rhipicephalus (Boophilus) microplus* demonstrated more damage to the intestinal epithelium in the presence of active complement system (Kemp et al., 1989). The lack of the anti-complement molecule ISAC in the saliva of *Ixodes scapularis* led to a significant body weight reduction on fed ticks (Soares et al., 2005).

Despite its importance among Ixodid ticks, only species of the *Ixodes ricinus* complex had in-depth studies on the presence and activity of anti-complement molecules in their saliva, as shown for *Ixodes dammini* (Ribeiro, 1987) *I. hexagonus, I. uriae* (Lawrie et al., 1999), *I. ricinus* (Couvreur et al., 2008; Daix et al., 2007; Lawrie et al., 2005, 1999) and *I. scapularis* (Tyson et al., 2007, 2008; Valenzuela et al., 2000). Interestingly, the anticomplement inhibition described for most *Ixodes* species were only for the alternative pathway, one exception is the lectin-pathway inhibitor found in *I. scapularis*' salivary glands (Schuijt et al., 2011).

There is a lack of studies in the literature on the inhibition of the complement system by tick saliva from genera other than *Ixodes*. In an isolated work, the AV422 peptide identified in *Amblyomma americanum* was shown to inhibit the formation of terminal complement complexes by the classical pathway (Mulenga et al., 2013). This inhibition of the classical pathway, firstly described for Ixodid ticks, suggests that *Amblyomma* may provide a mechanism of inhibition different from that observed for the genus *Ixodes*.

In order to generate a better understanding on the inhibition of vertebrate complement system by ticks, this study investigated the inhibition of the classical pathway present in the salivary glands of the tick *A. cajennense* at different physiological stages. *A. cajennense* is of great importance in Brazil since it has a wide distribution and low host specificity, especially during immature stages (Estrada-Peña et al., 2014). It also causes economic losses in livestock and is the main vector of the Brazilian Spotted Fever, being strongly associated with humans in Brazil (Galvão et al., 2005; Labruna et al., 2002).

2. Materials and methods

2.1. Experimental ticks

Specimens of *A. cajennense* were obtained from the colony (second generation) kept at the Department of Parasitology - UFMG or collected from naturally infested horses kept on the UFMG Experimental Farm, located in the municipally of Pedro Leopoldo, MG, Brazil. Horses were adult males and females (3–10 years old) of mixed breed not treated with acaricides during the last two months. The UFMG colony was originated from ticks collected at this same location.

2.2. Colony maintenance and feeding of the experimental groups

Ticks were kept in an incubator at 28 ± 2 °C and 85 ± 5% relative humidity. All feedings were performed in Swiss mice using feeding chambers described by Bouchard and Wikel (2005). During all procedures, mice were maintained in appropriate cages (30 × 19 × 13 cm L x W x H, maximum 5 animals/cage) and kept in a room with controlled temperature (25 ± 2 °C).

To obtain females at different physiological stages, feeding chambers were assembled into seven groups containing six mice each (females aged 6–8 week old) which were used as feeding source for the ticks (one couple with 20–30 days of fasting per mouse). The ticks were examined for attachment (6 h after being placed in contact with the host) and every 2 days when females were removed, weighed and dissected to obtain the salivary gland extract (henceforth called SGE) which was stored at −80 °C. Groups contained ticks with 2, 4, 6, 8 and 10 days of feeding, in addition to the fasting group and one with ticks that spontaneously detached from the host.

All procedures involving animals were in accordance and approved by the Ethics Committee on Animal Experimentation (CETEA/UFMG) under the protocol number 137/2011.

2.3. Saliva collection and preparation of salivary gland extracts (SGE)

Females were washed with distilled water, attached dorsally to a double face tape placed in a piece of cardboard and injected directly on the haemocoel with 3–5 μL of 2% pilocarpine (Sigma) in PBS (pH 7.4). Females were kept in a moistened chamber at 37 °C until the end of salivation (approximately 2 h) and the collected saliva was transferred to 1.5 mL tubes (each tube contained saliva from up to three females) and kept at −80 °C until use.

To obtain the SGE, females were washed with distilled water and their salivary glands were individually dissected in saline (0.9% NaCl). Each pair of glands were transferred to 1.5 mL tubes