Veterinary Parasitology 176 (2011) 132–138

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Soluble parasite antigens from *Babesia canis* do not directly activate the kallikrein system in dogs infected with *Babesia canis*

A.-L. Finizio[a], J.A.G.M. Kleuskens[b], J. Van De Crommert[b], A. Gorenflot[a], B. Carcy[a], Th.P.M. Schetters[a,b,*]

[a] *Laboratoire de Biologie Cellulaire et Moléculaire, UMR5235 DIMNP, ERT 1038 Vaccination antiparasitaire, UFR des Sciences Pharmaceutiques et Biologiques, Université Montpellier I, 15 avenue Charles Flahault, BP 14491, 34093 Montpellier Cedex 5, France*
[b] *Department of Microbiology, Intervet/Schering-Plough Animal Health, P.O. Box 31, 5830 AA Boxmeer, The Netherlands*

ARTICLE INFO

Article history:
Received 27 July 2010
Received in revised form 29 October 2010
Accepted 4 November 2010

Keywords:
Babesia canis
Soluble parasite antigens
Kallikrein
Pathology
Dogs

ABSTRACT

Soluble parasite antigens (SPA) from *Babesia canis* have been shown to induce protective immunity when used as vaccine. In order to explain the immune mechanisms of vaccination, the precise role of SPA in the pathogenesis of canine babesiosis is under investigation. Earlier studies suggested that the plasma kallikrein system is central in the pathogenesis of babesiosis, malaria and trypanosomosis, and significant plasma kallikrein activation during acute *B. bovis* and *P. knowlesi* infections has been described. In the studies presented here dogs were experimentally infected with *B. canis* to investigate whether the plasma kallikrein system is activated during babesiosis infection. Results showed that prekallikrein levels decreased during episodes of peak parasitaemia. No effect was found on the kallikrein levels. In order to determine whether *B. canis* SPA could activate plasma kallikrein, dogs were infused with variable amounts of *B. canis* SPA and plasma samples were taken for (pre-) kallikrein determination. The results indicated that *B. canis* SPA did not affect plasma (pre-) kallikrein levels. In addition, the effect of *B. canis* SPA on (pre-) kallikrein levels in normal dog plasma was determined *in vitro*. Again, no effect on (pre-) kallikrein levels was found. The results suggest that, although the kallikrein pathway may be involved in *B. canis*-associated pathology, the system is not directly activated by *B. canis* SPA. Furthermore, infusion of *B. canis* SPA as well as stroma of normal dog erythrocytes triggered the production of the acute phase reactant, C-reactive protein. This suggests that the inflammatory response that is triggered during *B. canis* infection could be in part due to the release and exposure of self molecules. The implications of these findings are discussed.

1. Introduction

Canine babesiosis is a world-widespread parasitic infection (reviewed by Irwin, 2009). In Europe, this disease is mainly caused by the Apicomplexan haemoprotozoan parasite *Babesia canis* and transmitted to susceptible canine hosts by the tick *Dermacentor reticulatus*. The typical infection is characterized by a syndrome associated with fever, anemia, depression, weakness, and anorexia. In most cases, the disease may evolve in an acute phase where infected animals develop severe clinical complications. During this acute phase, systemic inflammation and life-threatening hypotension, associated with non-haemolytic decrease of packed cell volume (PCV), followed by both disturbance of coagulation system and multiple organ failures has been described (Schetters et al., 1998, 2009; Matijatko et al., 2007, 2009). Without chemotherapeutic treatment,

* Corresponding author at: Department of Microbiology, Intervet/Schering-Plough Animal Health, P.O. Box 31, 5830 AA Boxmeer, The Netherlands. Tel.: +31 485 587348; fax: +31 485 585313.
E-mail addresses: theo.schetters@sp.intervet.com, Theo.Schetters@intervet.com (Th.P.M. Schetters).

0304-4017/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2010.11.005

this acute phase often leads to death of infected animals (Freeman et al., 1994; Bourdoiseau, 2006; Máthé et al., 2006; Schetters et al., 2009). Commercially available vaccines, which protect dogs against the severe clinical signs of canine babesiosis contain soluble parasite antigens (SPA) of *Babesia* parasites (Moreau et al., 1988; Schetters et al., 1995). These SPA can be recovered from the supernatants of *in vitro* cultures of *Babesia* parasites and are also naturally released in the plasma of infected dogs during the erythrocyte life cycle of the parasite. The precise role of *B. canis* SPA in the sequence of events that lead to development of clinical signs in infected dogs and how the host immune system might control the infection remain largely unknown. Recently, a potential role of *B. canis* SPA in the triggering of inflammatory response of infected dogs was suggested by Schetters et al. (2009). Results from a detailed haematological study of dogs that were experimentally infected with low, moderate or high numbers of *B. canis*-infected red blood cells (iRBC) showed that hypotension is induced, the onset of which was dependent on the infectious dose. As an explanation of the decrease in blood pressure, these authors hypothesized that *B. canis* SPA might induce the release of vasoactive molecules, including kinin through the activation of kallikrein pathway in the plasma of infected dogs. Indeed, plasma kallikrein (EC 3.4.21.34; http://www.brenda-enzymes.org/) and kinins are extremely potent vasoactive mediators that belong to a system of blood proteins that might interact with complement pathway, the inflammatory system and coagulation cascade (contact phase). The involvement of SPA in the plasma kallikrein activation was clearly demonstrated to play a critical role in the clinical signs in *B. bovis*-infected cattle such as chemotaxis, PCV decrease, vascular disturbances (which are characterized by vasodilatation and enhanced capillary permeability), hypotension, and shock syndrome (Wright and Mahoney, 1974; Wright, 1975, 1979, 1981; Wright and Goodger, 1988). This suggested a correlation between the biological activities of *Babesia* SPA, the kallikrein–kinin pathway, and clinical signs leading to acute phase babesiosis.

The objective of the present study was to clarify the role of *B. canis* SPA in the clinical disorders of canine babesiosis with an emphasis on the role of the kallikrein system. In these studies, the effect of experimental infection of dogs with *B. canis*-infected red blood cells (iRBC) and infusion of dogs with variable amounts of *B. canis* SPA purified from *in vitro* cultures on the plasma levels of (pre-) kallikrein was determined. In addition, the potential of SPA to activate plasma kallikrein *in vitro* was assessed

2. Materials and methods

2.1. Animals

Beagle dogs of either sex of approximately 6 months of age were used. They were obtained from a commercial breeder (Harlan, The Netherlands). The animals were clinically healthy and had no history of babesiosis. They received standard dog feed daily, and drinking water was supplied *ad libitum*. Dogs had a unique number as a tattoo in the ear to allow identification.

2.2. Seric and aseric in vitro cultures of B. canis strain A parasites

B. canis strain A was cultured *in vitro* essentially as previously described (Schetters et al., 1997). In short, the culture was started at 1% (v/v) parasitized erythrocytes (PE) with canine RBC at 2% haematocrit in culture medium supplemented with 10% (v/v) normal dog serum (nds; ABPM-Australia). Twenty-four hours later, the culture medium was replaced by fresh culture medium without nds for aseric culture (nds−) or supplemented with 10% nds for seric culture (nds+). Every 48 h, subcultures were performed at 1% (v/v) PE in a fresh culture medium supplemented with 10% nds for each culture type.

Parasitaemia was determined daily by evaluation of blood smears that were stained with May-Grünwald/Giemsa solutions and expressed as the log number of *B. canis*-PE per 10^5 erythrocytes (Schetters et al., 1994).

2.3. Preparations of supernatant-SPA fractions from B. canis-iRBC and control supernatant fractions from uninfected erythrocytes cultures

SPA of the *B. canis* strain A were produced in large scale *in vitro* cultures in cell factories 10 (CF10, Nunc). The *in vitro* culture conditions were essentially similar as described in Section 2.2. SPA were collected every 48 h from supernatants of seric (SPA_{nds+}) and aseric (SPA_{nds-}) cultures. Supernatants with SPA were concentrated about seven times on 10 kDa, 2000 cm^2 hollow fibre column (GE Healthcare), aliquoted, freeze-dried and stored at −20 °C. Supernatants from seric and aseric cultures of canine uninfected erythrocytes, performed in the same conditions as *B. canis*-iRBC, were similarly processed and used as controls (C_{nds+} and C_{nds-}, respectively).

2.4. Preparations of stroma-SPA fractions from B. canis-iRBC and stroma fractions from uninfected erythrocytes

The stroma-SPA fractions (SPA_{str}) were prepared from CF10 *in vitro* seric cultures of *B. canis*-iRBC (strain A). In short, iRBC were collected every 48 h and pelleted by centrifugation (3000 × *g*, 30 s, RT). They were washed three times with sterile isotonic 0.4 M phosphate buffered saline (PBS), pH 7.2. Then, the pellet of iRBC was suspended in 100 mL sterile isotonic PBS in a 175 cm^2 flat-bottom tissue culture flask and incubated overnight at 37 °C in 5% CO_2 humidified atmosphere according to Bate et al. (1989, 1992). During this incubation time, parasites continuing their life cycle naturally come out from iRBC thus releasing stroma-SPA and erythrocytic stroma in the supernatants. Cellular contaminants were removed from the stroma-derived preparations of SPA (SPA_{str}) by centrifugation (3000 × *g*, 10 min, RT). Subsequently, SPA_{str} preparations were aliquoted, freeze-dried and stored at −20 °C.

As a control, an equal amount of red blood cell lysate (based on haemoglobin content) was added to supernatant from uninfected cultures, centrifuged (3000 × *g*, 10 min, RT), aliquoted and freeze dried as for the SPA_{str} preparations. The SPA_{str} and RBC_{str} preparations were sterilised by