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Targeting the Tick/Pathogen Interface for Developing New Anaplasmosis Vaccine Strategies

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Abbreviations: dsRNA, double stranded RNA; MSP1a, major surface protein 1a; PCV, packed cell volume; RNAi, RNA interference

ABSTRACT

Bovine anaplasmosis is a tick-borne hemolytic disease of cattle that occurs worldwide caused by the intraerythrocytic rickettsiae *Anaplasma marginale*. Control measures, including use of acaricides, administration of antibiotics and vaccines, have varied with geographic location. Our research is focused on the tick-pathogen interface for development of new vaccine strategies with the goal of reducing anaplasmosis, tick infestations and the vectorial capacity of ticks. Toward this approach, we have targeted (1) development of an *A. marginale* cell culture system to provide a non-bovine antigen source, (2) characterization of an *A. marginale* adhesion protein, and (3) identification of key tick protective antigens for reduction of tick infestations. A cell culture system for propagation of *A. marginale* was developed and provided a non-bovine source of *A. marginale* vaccine antigen. The *A. marginale* adhesion protein, MSP1a, was characterized and use of recombinant MSP1a in vaccine formulations reduced clinical anaplasmosis and infection levels in ticks that acquired infection on immunized cattle. Most recently, we identified a tick-protective antigen, subolesin, that reduced tick infestations, as well as the vectorial capacity of ticks for acquisition and transmission of *A marginale*. This integrated approach to vaccine development shows promise for developing new strategies for control of bovine anaplasmosis.

INTRODUCTION

Control measures for bovine anaplasmosis, targeted primarily toward reduction of clinical symptoms, have not changed markedly over the past 60 years. These control measures have varied with geographic location and have included arthropod control by

application of acaricides, administration of antibiotics and vaccination (as reviewed by Kocan et al., 2003). Development of new vaccine strategies for bovine anaplasmosis is a challenging undertaking because the pathogen is transmitted both biologically by ticks and mechanically by biting flies or blood-contaminated fomites. In addition, both cattle and ticks become persistently infected and serve as reservoirs of *A. marginale*. Male ticks can transmit *A. marginale* to multiple cattle and persistently infected cattle are of infection for mechanical transmission (Kocan et al., 2004). Chemotherapy, probably used more often for prevention of anaplasmosis in the United States than in other areas of the world, is expensive, often not applicable to range cattle and the intensive use of tetracyclines is accompanied by the risk of causing selection of resistant microbes which may limit its use in the future.

Vaccination has been used widely as a more economical and effective way to control bovine anaplasmosis worldwide (Kocan et al., 2003). Live and killed vaccines have used erythrocyte-derived *A. marginale* to stimulate the bovine immune response, thus reducing rickettsemia and the associated anemia characteristic of bovine anaplasmosis. To date, reduction of clinical signs has been the only realistic vaccine goal; prevention of infection has not been proven to be possible. In addition, immunized animals that are challenge-exposed develop persistent infections and can therefore serve as a source of infection. Further complicating the challenge of anaplasmosis vaccine development is the increasing numbers of *A. marginale* field strains that occur in a given geographic area, probably resulting from cattle transport (as reviewed by de la Fuente et al., 2005). Because of the phenomena of infection-exclusion that results in the maintenance of individual *A. marginale* strains in nature by independent transmission events (de la Fuente et al., 2002a; 2003a), *A. marginale* erythrocyte-derived vaccine formulations are not likely to be cross-protective against genetically diverse field strains.

Herein we describe an integrated approach to anaplasmosis vaccine development which targets the tick vector, bovine host and pathogen.

THE TICK-*A. marginale* INTERFACE

Understanding the *A. marginale*/tick interface and the mechanism(s) involved in infection and transmission will likely provide new targets for anaplasmosis vaccine development. While erythrocytes appear to be the only site of infection in cattle, *A. marginale* undergoes a complex developmental cycle in ticks (as reviewed by Kocan et al., 2004) that begins by infection of midgut cells. Toward the end of acquisition feeding, single organisms develop within parasitophorous vacuoles, first as reticulated forms which subsequently transform into large colonies of dense or infective forms. After a second feeding, *A. marginale* infects the salivary glands, the site of transmission to cattle. The developmental cycle of *A. marginale* is therefore well coordinated with the tick feeding cycle.

Factors that mediate the infection and movement of *A. marginale* through ticks are not well defined, but most likely involve both pathogen and tick cellular pathways. We have shown that infection of ticks with *A. marginale* is dependent on the adhesive properties of MSP1a. The adhesive qualities of MSP1a serve as a predictor of tick transmissibility

or the ability to propagate *A. marginale* isolates in tick cell culture (Blouin et al., 2002). MSP1a most likely interacts with a receptor protein, although presently an *A. marginale* receptor protein has not been identified. Both adhesion and receptor proteins would be likely infection-blocking vaccine targets. Undoubtedly there remains to be discovered tick cell pathways that mediate the passage of *A. marginale* from gut to salivary cells and that promote transmission of the pathogen, the interruption of which could greatly increase the repertoire of anaplasmosis vaccine targets.

DEVELOPMENT OF A NON-BOVINE SOURCE OF *A. Marginale* FOR USE AS A VACCINE ANTIGEN

A cell culture system was developed for *A. marginale* in which the rickettsia was propagated in a continuous culture in a cell line, IDE8, derived from embryos of the tick *Ixodes scapularis* (as reviewed by Blouin et al., 2002; Kocan et al., 2004). The developmental cycle of *A. marginale* in cultured tick cells was similar to that described previously in naturally infected ticks. *A. marginale* harvested from cell culture were infective for both cattle and ticks. The six MSPs characterized on *A. marginale* from bovine erythrocytes were found to be conserved on the cell culture-derived organisms and the antigenic composition of *A. marginale* remained the same after successive passage in cell culture or after passage through ticks. The *A. marginale* isolate antigenic identity, as determined by the molecular weight of the MSP1a, was retained in culture. *A. marginale* derived from the cultured tick cells was tested as an immunogen for cattle. In two trials, cattle immunized with the cell culture-derived *A. marginale* developed protective immunity and cattle did not develop clinical signs of anaplasmosis after challenge-exposure by infected blood or feeding infected ticks (Kocan et al., 2001; de la Fuente et al., 2002b). Nevertheless, the protection was partial and the disease is not prevented. The main effect of the vaccine is similar to the effect observed with erythrocyte-derived *A. marginale*, resulting predominantly in a less pronounced reduction in the levels of PCV which directly correlate with the anemia produced by *A. marginale* infection. However, cell culture derived *A. marginale* may be important to include in a vaccine formulation in order to include the full repertoire of major surface proteins.

VACCINE STRATEGY TARGETING MAJOR SURFACE PROTEIN 1A, THE *A. marginale* ADHESION PROTEIN FOR TICK CELLS

Of the six MSPs, MSP1a, MSP1b, MSP2, MSP3, MSP4, & MSP 5 described on *A. marginale* (as reviewed by Kocan et al., 2003), MSP1a was shown to be an adhesin for bovine erythrocytes and tick cells, to be involved in infection and transmission of *A. marginale* by *Dermacentor* spp. ticks and to contribute to immunity to *A. marginale* infection in cattle. MSP1a, although variable in the number of repeated peptides, induces strong T cell responses (Brown et al., 2001) and contains conserved B-cell epitopes n the repeated peptides that are recognized by immunized and protect cattle (Garcia-

Garcia et al., 2004a). These results, together with the biological significance of the MSP1a function in infection and transmission of *A. marginale*, suggest that MSP1a may be a good candidate for inclusion in vaccines for the control of bovine anaplasmosis. Experiments conducted to evaluate the protection capacity of recombinant MSP1a alone or in combination with whole *A. marginale* antigens from infected cultured tick IDE8 cells demonstrated that a preferential antibody response to MSP1a correlated with lower percent reductions in PCV and thus reduce clinical disease (Garcia-Garcia et al., 2004a). Although cattle infected with *A. marginale* mount an immune response against MSP1a, it is likely directed to the MSP1 complex in which MSP1a is covalently linked to MSP1b, perhaps masking some of the MSP1a protective epitopes otherwise exposed in the recombinant antigen. This hypothesis was further supported by the finding that immunization of cattle with *A. marginale* derived from infected erythrocytes in which MSP1a is upregulated with respect to MSP1b and probably exists uncoupled to MSP1b, provided a level of protection comparable to that observed in cattle immunized with recombinant MSP1a and *A. marginale* derived from infected ticks cells, in which MSPla is downregulated and more likely exists on in the MSP1 complex with MSP1b (Garcia-Garcia et al., 2004a). Another factor to be considered for the induction of a protective immune response against *A. marginale* is the role of sugar moieties of MSP1a in the protection properties, which may also contribute to the generation of a neutralizing response in cattle (Garcia-Garcia et al., 2004b). Although transmission-blocking antigens have not been identified from the tick vector or the pathogen, recent results suggest that antibodies to recombinant MSP1a may reduce infectivity for *D. variabilis* (de la Fuente et al., 2003b), in accordance with results obtained in neutralization studies in vitro (Blouin et al., 2002; 2003). The results of our research suggest that rMSP1a may be an important component of an improved vaccine for anaplasmosis by inducing protective immunity in cattle and reducing *A. marginale* infections in ticks.

VACCINE STRATEGIES FOR *A. marginale* TARGETING A KEY TICK-PROTECTIVE PROTEIN, SUBOLESIN

Subolesin was recently shown by both gene silencing and immunization with the recombinant protein to protect against tick infestations, and to cause reduced tick survival and degeneration of gut and salivary tissues (as reviewed by de la Fuente and Kocan, 2006). In further research, we tested whether targeting subolesin by RNAi or vaccination interfered with the ability of ticks to become infected with two *Anaplasma* spp., *A. marginale* and *A. phagocytophilum* (de la Fuente et al., 2006). For the *A. marginale* studies, *Dermacentor variabilis* males were injected with subolesin dsRNA or saline and then were allowed to feed on cattle with ascending rickettsemias, while for the *A. phagocytophilum* studies, mice were immunized with the recombinant subolesin protein, infected with the pathogen and then infested with larval *Ixodes scapularis*. Tick infections, as determined by quantitative PCR on gut and salivary glands, were significantly reduced and the results suggest that subolesin appears to be candidate antigen that may contribute to control of *A. marginale*, as well as tick infestations.

CONCLUSIONS

Our research has focused on three major strategies for anaplasmosis vaccine development (1)) to develop a non-bovine source of *A. marginale* antigen for vaccine development (2) to characterize the functional portion of the *A. marginale* adhesion protein MSP1a, most notably as the adhesion protein for tick cells and to demonstrate the role of MSP1a antibodies in a protective immune response, and (3) to identify tick-protective antigens that, when used as vaccine antigens, will block or reduce the vectorial capacity of ticks, as well as reduce tick infestations. By use of an integrated approach to anaplasmosis vaccine development, our research shows promise for development of a recombinant vaccine that targets both the pathogen and tick by containing key antigens from both hosts to effect bovine immunity and reduction of capacity of ticks to serve as biological vectors and reservoirs. The ideal anaplasmosis vaccine would be the one that induces protective immunity and prevents infection and transmission of the pathogen. Current vaccines do not prevent infection and persistently infected cattle are a major reservoir of infection for mechanical and biological transmission by ticks.

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