Vaccine 29 (2011) 6620–6628

Contents lists available at ScienceDirect

Vaccine

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

Prime-boost immunisation against tropical theileriosis with two parasite surface antigens: Evidence for protection and antigen synergy

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ARTICLE INFO

Article history:
Received 11 February 2011
Received in revised form 5 May 2011
Accepted 28 June 2011
Available online 14 July 2011

Keywords:
Theileria annulata
Recombinant vaccine
Sporozoite
Merozoite
Surface antigen
Prime-boost immunisation

ABSTRACT

Current methods for control of tropical theileriosis in cattle suffer from several disadvantages that could be circumvented by development of an effective sub-unit vaccine. Previous work has utilised two major surface antigens (SPAG-1 and Tams1) and conventional adjuvants to provide partial protection against parasite challenge. In this study we have delivered these antigens using the prime-boost system and analysed whether a combination regime can enhance protection against lethal challenge. Delivery of the boost as recombinant protein or expressed from a recombinant MVA vector was also assessed. The results confirmed that immunisation with Tams1 alone could reduce the severity of several disease parameters compared to non-immunised controls and these effects were more marked when recombinant protein was used for boosting compared to MVA delivery. A similar outcome was obtained by immunisation with SPAG-1 alone. Significantly, delivery of SPAG-1 and Tams1 as a cocktail showed enhanced protection. This was manifest by significant improvement in a large range of clinical and parasitological parameters and, most dramatically, by the survival and recovery of 50% of the immunised animals compared to 0% of the controls. Analysis of the antibody response post-challenge showed that while there was a strong response to Tams1, no response to SPAG-1 was detected. In contrast, lymphoproliferation assays showed a significant enhancement of response at day 7 post-challenge in calves of the SPAG-1 group but a dramatic decrease of the proliferation activity in all three groups receiving Tams1. We conclude that immunisation with a cocktail of SPAG-1 and Tams1 generates a synergistic protective response that significantly improves the efficacy of recombinant vaccination against tropical theileriosis. Potential effector mechanisms that could mediate this response are discussed.

1. Introduction

Tropical theileriosis, caused by infection of cattle with the protozoan parasite *Theileria annulata*, is endemic in a region stretching from Southern Europe through North Africa and the Middle East to Asia. Some 250 million cattle are estimated to be at risk [1], and the disease represents a major constraint on livestock productivity. This is in part due to high susceptibility of *Bos taurus* cattle to infection, which in the absence of treatment can show 40–50% mortality [2]. To combat such unacceptable losses a number of control methods have been developed including acaracide treatment against the vector tick, vaccination with naturally avirulent stocks [3], drug treatment and, more recently, vaccination with infected cell lines that have been attenuated for virulence by repeated culture *in vitro* [4]. Attenuated lines have been successfully used in many endemic countries [2,5,6] and provide solid and long lasting protection against clinical disease. However, there are a number of practical constraints that limit their use, specifically: (i) a requirement for distribution in liquid nitrogen, which

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doi:10.1016/j.vaccine.2011.06.109

accounts for approximately 30% of the cost [7], (ii) the need to use the vaccine immediately after thawing, (iii) the difficulty and cost of quality control to ensure efficacy and the absence of other bovine pathogens, (iv) problems with post-vaccination reactions, which have been recorded in 3% of animals immunised with Chinese, Moroccan, Iranian or Tunisian vaccine stocks [8] and (v) the possibility of reversion to virulence. Many, if not all, of these limitations and logistical difficulties could be circumvented by development of an effective sub-unit vaccine.

A number of antigens have been identified in different life cycle-stages of *T. annulata* but, to date, two antigens, SPAG-1 located on the surface of the sporozoite [9] and Tams1 (the orthologue of the major merozoite and piroplasm surface antigen, mMPSA/MPSP characterised in a number of *Theileria* species [10,11]) located on the surface of the merozoite [12,13], have provided the best evidence that immunisation with recombinant antigen could provide protection against challenge. For example, immunisation with SPAG-1, induced protection against challenge by an infected tick stabilate with protection expressed as a reduction in mortality, a lengthening of the pre-patent period, a reduction in parasitaemia and an increase in the survival time of the animals (reviewed by Boulter and Hall [14]). Recombinant Tams1 also confers a relative level of protection, as indicated by a reduction or absence of mortality, reduced parasitaemia and a reduction in fever [15,16]. Furthermore in two trials, combination regimes of Tams1 and SPAG-1 [15] or an attenuated vaccine together with SPAG-1 [17] have provided evidence for a synergistic effect, which was particularly evident in the latter trial.

The adaptive immune response against tropical theileriosis is thought to be mediated by antibodies that neutralise parasite invasion and generation of $CD4^+$ T cells and cytotoxic $CD8^+$ T cells (reviewed in [18,19]) that target the macroschizont infected leukocyte. Innate immunity operating via activated macrophages and NK cells is also thought to play an important role, with production of nitric oxide and suppressive cytokines inhibiting establishment and proliferation of the macroschizont infected cell [20–22]. Therefore, it is likely that any sub-unit vaccine with an equivalent efficacy to the currently available live attenuated vaccines will be required to engender a response that is effective against parasite infected leukocytes. While immunity generated against SPAG-1 and Tams1 is most logically predicted to operate via neutralising antibody this has not been established conclusively. Indeed, in a DNA based vaccination regime using a recombinant Tams1 construct, the protective response was induced in the absence of a specific humoral response [16], implying involvement of an unknown effector mechanism.

In addition to protective antigens, delivery systems can have a significant impact on vaccination outcomes. The antigen delivery system provided by prime-boost has been shown to significantly enhance immunity against a range of pathogens including parasites, bacteria and viruses [23]. In this system, vaccination is primed using an antigen expressed in one delivery system, while the boost is administered by the same antigen expressed via a different, usually viral, DNA vector [24]. It has been established that prime-boost leads to additive and synergistic effects, particularly if the boost is provided using a *Pox* or *Adenovirus* vector to deliver the antigen [24]. Importantly the prime-boost approach, using heterologous delivery systems, confers a combination of cellular and humoral immune responses, and has been demonstrated to induce a $CD8^+$ T cell response and activate memory T cells [23,25]. For example, in cattle, heterologous prime-boosting has been shown to be effective in the induction of interferon secreting $CD4^+$ and $CD8^+$ T cell responses against a *Mycobacterium tuberculosis* antigen [26]. It has also been shown that following priming with plasmid DNA constructs, the boost can be delivered with purified recombinant protein. This variant of prime-boost has been used in mice with the MAP-1 antigen of *Ehrlichia* (formerly *Cowdria*) *ruminantium* [27] and in humans with the CSP protein of *Plasmodium falciparum* [28], the latter immunisation protocol generating a lasting humoral response in the presence of a strong T_h1 type cellular response, induced by the DNA immunisation.

Based on previous vaccination trials that indicated synergistic effector responses raised against antigen combinations and the possibility that a protective response engendered by SPAG-1 and Tams1 may involve an unknown cellular component, we performed this study to evaluate prime-boost delivery using these antigens both singly and in combination (i.e. a cocktail) as a means of immunisation against tropical theileriosis. The results indicate for the first time that prime-boost delivery of a SPAG-1/Tams1 cocktail provides enhanced protection against lethal challenge of *T. annulata* and that a cellular response which inhibits the establishment of proliferating parasite infected cells may be involved.

2. Materials and methods

2.1. Animals

A total of 20 Holstein–Friesian calves, aged between 3 and 6 months were used in the trial. Calves were randomly assigned into five equal groups as this allowed statistical assessment of the results using a factorial 2×2 analysis [29] and reduced the number of animals required without the loss of statistical power. The calves, sourced from a modern, tick and tick-borne infection-free farm, were examined for clinical signs of disease, housed in a tick-free experimental facility at Ecole Nationale de Médecine Vétérinaire de Sidi Thabet (Tunisia) and kept one month for adaptation and observation.

2.2. Vaccination protocol

The priming vaccination was undertaken using injections of DNA representing the genes encoding the SPAG-1 and Tams1 antigens cloned into the plasmid vector pSG2. Four milligrams of DNA was injected per animal, in total, with two injections twenty days apart. Twenty days after the second priming immunisation, calves were immunised with either the antigen genes cloned into the modified vaccinia virus Ankara (MVA) vaccine vector (5×10^8 pfu per animal) or 450 μg of purified recombinant antigen (per animal) in Montanide ISA 50 adjuvant (solution of Montanide oleate, Seppic Laboratories). All MVA and DNA injections were intradermal and DNA was injected using a Dermojet syringe in 10×0.1 ml aliquots on the left side of the neck, with appropriate measures to ensure asepsis. Injection sites were then monitored fortnightly for any bacterial infection. Recombinant SPAG-1 and Tams1 antigens were injected in a total volume of 2 ml per animal in adjuvant, prepared by dissolving the antigens in 1 ml of PBS (phosphate buffered saline) and mixing, in a syringe, with an equal volume of adjuvant until a stable emulsion was obtained.

In total, four groups of animals were vaccinated with the SPAG-1 and Tams1 antigens either singly or together as shown in Table 1. To establish whether there was an interaction between the two antigens, animals were immunised singly with each antigen and with both antigens combined ('cocktail'). The control group was injected with adjuvant alone, since results from a preliminary trial showed that neither non-recombinant MVA nor plasmid DNA induced a non-specific protective effect in animals challenged with the infective sporozoite stabilate (data not shown).

2.3. Vaccine vectors and constructs for vaccination

DNA sequences encoding the SPAG-1 and Tams1 antigens (from the Ankara strain of the parasite) were inserted into the plasmid vector pSG2 [25]. This plasmid contains an hCMV promoter