Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Prime-booster vaccination of cattle with an influenza viral vector *Brucella abortus* vaccine induces a long-term protective immune response against *Brucella abortus* infection

Kaissar Tabynov*, Bolat Yespembetov, Sholpan Ryskeldinova, Nadezhda Zinina, Zhailaubay Kydyrbayev, Yerken Kozhamkulov, Dulat Inkarbekov, Abylai Sansyzbay

The Research Institute for Biological Safety Problems, Zhambulskaya oblast, Kordaiskiy rayon, Gvardeiskiy 080409, Kazakhstan

ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form 4 December 2015
Accepted 8 December 2015
Available online xxx

Keywords:
Brucella abortus
Influenza viral vector
Vaccine
Antibody and T-cell-mediated immune response
Protectiveness
Cattle

ABSTRACT

This study analyzed the duration of the antigen-specific humoral and T-cell immune responses and protectiveness of a recently-developed influenza viral vector *Brucella abortus* (Flu-BA) vaccine expressing *Brucella* proteins Omp16 and L7/L12 and containing the adjuvant Montadine Gel01 in cattle. At 1 month post-booster vaccination (BV), both humoral (up to 3 months post-BV; GMT IgG ELISA titer 214 ± 55 to 857 ± 136, with a prevalence of IgG2a over IgG1 isotype antibodies) and T-cell immune responses were observed in vaccinated heifers ($n = 35$) compared to control animals ($n = 35$, injected with adjuvant/PBS only). A pronounced T-cell immune response was induced and maintained for 12 months post-BV, as indicated by the lymphocyte stimulation index (2.7 ± 0.4 to 10.1 ± 0.9 cpm) and production of IFN-γ (13.7 ± 1.7 to 40.0 ± 3.0 ng/ml) at 3, 6, 9, and 12 months post-BV. Prime-boost vaccination provided significant protection against *B. abortus* infection at 3, 6, 9 and 12 months (study duration) post-BV (7 heifers per time point; *alpha* = 0.03–0.01 vs. control group). Between 57.1 and 71.4% of vaccinated animals showed no signs of *B. abortus* infection (or *Brucella* isolation) at 3, 6, 9 and 12 months post-BV; the severity of infection, as indicated by the index of infection ($P = 0.0003$ to <0.0001) and rates of *Brucella* colonization ($P = 0.03$ to <0.0001), was significantly lower for vaccinated diseased animals than appropriate control animals. Good protection from *B. abortus* infection was also observed among pregnant vaccinated heifers (*alpha* = 0.03), as well as their fetuses and calves (*alpha* = 0.01), for 12 months post-BV. Additionally, 71.4% of vaccinated heifers calved successfully whereas all pregnant control animals aborted (*alpha* = 0.01). Prime-boost vaccination of cattle with Flu-BA induces an antigen-specific humoral and pronounced T cell immune response and most importantly provides good protectiveness, even in pregnant heifers, for at least 12 months post-BV.

1. Introduction

Bovine brucellosis is mainly caused by the bacterium *Brucella abortus*, and represents a major problem to livestock industry development worldwide and is also a threat to human health in many developing and underdeveloped countries [1]. Vaccination against bovine brucellosis, along with other anti-epidemic measures, is an effective tool in the battle against this dangerous zoonotic disease. However, the existing commercial vaccines *B. abortus* S19 and RB51 can cause abortions in pregnant cows, are secreted into milk and are pathogenic to humans [2,3]; therefore, the antibrucellar activities with these vaccines are insufficient or their use can be associated with considerable economic losses. Therefore, over a number of years, scientists around the world have conducted research aimed at improving existing vaccines, as well as developing new types of *Brucella* vaccines based on DNA, subunit, vector vaccines and *B. abortus* recombinant mutants [4].

Our research group has developed a novel vaccine candidate (Flu-BA) based on the recombinant influenza virus subtypes H5N1 and H1N1 expressing the *Brucella* L7/L12 or Omp16 proteins from the *NS1* open reading frame (ORF). Preliminary studies in laboratory animals [5] and numerous previous studies in cattle [6–9] demonstrated the high efficiency of this vaccine, which has been confirmed to be significantly safer and offer equivalent protectiveness to the commercial vaccines *B. abortus* S19 and RB51. Additionally, we also established that the Flu-BA vaccine induces good cross-protection against *Brucella melitensis* infection in pregnant heifers [10]. The next important step in our research

* Corresponding author. Tel.: +7 72636 72228; fax: +7 72636 72228.
E-mail addresses: tabynov.81@mail.ru, tabynov.81@biosafety.kz (K. Tabynov).

http://dx.doi.org/10.1016/j.vaccine.2015.12.028
0264-410X/

is to determine the duration of the protective immune response against *B. abortus* infection in vaccinated cattle, including pregnant heifers; which was the aim of this study.

2. Materials and methods

2.1. Generation of influenza viral vectors

All influenza viral vectors (IVV) were generated by a standard reverse genetics method using eight bidirectional plasmids pHW2000. The detailed procedure by which the IVV were generated has been reported previously [5]. A total of four IVV of the subtypes H5N1 or H1N1 expressing the *Brucella* L7/L12 or Omp16 proteins from the ORF of the *NS1* gene were generated: H5N1 (Flu-NS1-124-L7/L12-H5N1, Flu-NS1-124-Omp16-H5N1) and H1N1 (Flu-NS1-124-L7/L12-H1N1 and Flu-NS1-124-Omp16-H1N1).

2.2. Vaccine preparation

Vaccines were prepared from the IVV Flu-NS1-124-L7/L12-H5N1, Flu-NS1-124-Omp16-H5N1, Flu-NS1-124-L7/L12-H1N1 and Flu-NS1-124-Omp16-H1N1; the vaccines were accumulated in 10-day-old chicken embryos (CE; Lohmann Tierzucht GmbH, Cuxhaven, Germany) at 34 °C for 48 h. The titer of the IVV was determined in CE, as previously described [11,12]. The detailed procedure by which the Flu-BA vaccine was prepared has been previously described [9]. Immediately before administration, the lyophilized vaccine was resuspended (1 ml per ampoule) in a 10% solution of the adjuvant Montanide Gel01 (Seppic, Puteaux, France) in PBS.

2.3. Vaccination and study design

A total of 70 Kazakh white breed heifers aged 17–19 months-old were used in this study. These animals were randomly divided into two equal-sized groups: vaccinated and control. Heifers in the vaccinated group (Flu-BA vaccine; $n = 35$) were immunized twice via the subcutaneous route at an interval of 28 days with vaccines generated from the IVV subtypes H5N1 (prime vaccination; 6.2–6.5 $\log_{10}$ EID_{50}/animal) and H1N1 (booster vaccination; 6.1–6.3 $\log_{10}$ EID_{50}/animal). This immunization strategy effectively overcomes the immune background elicited against the viral vector during prime vaccination. Animals in the control group ($n = 35$) were subcutaneously injected with 1.0 ml of 10% Montanide Gel01 adjuvant in PBS. To determine the antigen-specific humoral (IgG, IgG2a, IgG1) and T-cell (stimulation index and IFN-γ production) immune responses to the *Brucella* L7/L12 and Omp16 proteins, serum (10 ml per Becton Dickinson Vacutainer tube) and whole blood (50–70 ml in tubes coated with EDTA/citrate) were collected at 1 ($n = 35$ animals/group), 3 ($n = 28$ animals/group), 6 ($n = 28$ animals/group), 9 ($n = 14$ animals/group), and 12 ($n = 7$ animal/group) months post booster vaccination (BV). To determine the duration of the protective immune response against *B. abortus* infection in heifers, seven heifers from each group were challenged with the virulent strain *B. abortus* 544 at 3, 6, 9, and 12 months post-BV. At 7 months post-BV, seven heifers from each group were artificially inseminated and challenged with *B. abortus* 544 at 12 months post-BV (approximately 5 months of pregnancy). All animals challenged with *B. abortus* 544 were housed under ABSL-3 conditions.

This study was carried out in compliance with national and international laws and guidelines on animal handling. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Research Institute for Biological Safety Problems of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Permit Number: 0314/97).

2.4. ELISA

Ninety-six well microtiter plates (Nunc, Roskilde, Denmark) were coated overnight with L7/L12 (2 μg/ml) protein and Omp16 (2 μg/ml) protein in PBS, blocked for 1 h using PBS containing 1% ovalbumin (PBS-OVA; 200 μl/well), and washed with PBS containing 0.05% Tween-20 (PBS/Tw). Serial twofold dilutions of the serum samples from the immunized cattle (100 μl/well) were diluted in PBS/OVA, added to the plates and incubated for 1 h at room temperature. Horseradish peroxidase-conjugated mouse anti-bovine IgG monoclonal antibody (mAb; clone IL-AR; Serotec, Raleigh, NC, USA) and sheep anti-bovine IgG1 and IgG2 polyclonal antibodies (Novus Biologicals, Littleton, CO, USA) were used for detection. After a 90 min incubation at 37 °C and washing, specific reactivity was determined by the addition of an enzyme substrate, ABTS [2,2-azinobis(3-ethylbenzthiazolinesulfonic acid)] diammonium (Moss, Inc., Pasadena, CA, USA) at 100 ml/well. The absorbance values were measured at 415 nm. The cut-off value for titer determination was calculated based on the mean OD values of wells containing only buffer (blank) ± three standard deviations. Endpoint serum ELISA titers are presented as geometric mean titers (GMT) ± standard error (SE).

2.5. Preparation of PBMC for lymphocyte proliferation assay

Peripheral blood mononuclear cells (PBMC) were enriched by density centrifugation using a Ficoll-sodium diatrizoate gradient (DNA-Technology, Moscow, Russia). Further studies were conducted as previously described [8]. Cell number was adjusted to 10^7 viable cells per ml, as determined by trypan blue dye exclusion, and 50 μl of each cell suspension (containing 5×10^5 cells), was added to each of eight separate flat-bottomed wells of 96-well microtiter plates that contained 100 μl of RPMI-1640 medium only or RPMI-1640 medium containing 8.0 μg of purified *B. abortus* proteins L7/L12 and Omp16 per well. The cell cultures were incubated for 7 days at 37 °C under 5% CO_2. After 7 days of incubation, cell cultures were pulsed with 1.0 μCi of [^{3}H] thymidine per well for 18 h. Cells were harvested onto glass filter mats and counted for radioactivity in a liquid scintillation counter. Cell proliferation results were converted to stimulation index (counts per minute [cpm] of wells containing antigens/cpm in the absence of antigens) for statistical comparisons.

2.6. IFN-γ production

PBMC from each animal were adjusted to 10^7 viable cells per ml as described previously. Aliquots (50 μl) of each cell suspension containing 5×10^5 cells were added to the flat-bottomed wells of 96-well microtiter plates that contained 100 μl of RPMI-1640 medium only or RPMI-1640 medium containing 8.0 μg of purified *B. abortus* proteins L7/L12 or Omp16 per well. Cell cultures were incubated at 37 °C under 5% CO_2, and the supernatants were removed 72 h later and assayed for IFN-γ using a commercially available kit (RayBio® Bovine IFN-gamma ELISA Kit; RayBiotech, Inc., Norcross, GA, USA). Antigen-specific net IFN-γ was determined for each individual animal by subtracting the concentration of IFN-γ in wells without antigen from the IFN-γ concentration in wells with antigen.

2.7. Assessment protectiveness of vaccine in heifers

At 3, 6, 9 and 12 months post-BV, heifers from the vaccinated and control groups were subcutaneously challenged with a virulent strain of *B. abortus* 544 at a dose of 5×10^8 CFU/animal. On day 30 after challenge, all animals after euthanized by intravenous injection of 100 mg/kg sodium pentobarbital (Euthasol; Le Vet. Pharma

Please cite this article in press as: Tabynov K, et al. Prime-booster vaccination of cattle with an influenza viral vector *Brucella abortus* vaccine induces a long-term protective immune response against *Brucella abortus* infection. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.12.028