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Vaccination of calves using the BRSV nucleocapsid protein in a DNA prime–protein boost strategy stimulates cell-mediated immunity and protects the lungs against BRSV replication and pathology

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ABSTRACT

Respiratory syncytial virus (RSV) is a major cause of respiratory disease in both cattle and young children. Despite the development of vaccines against bovine (B)RSV, incomplete protection and exacerbation of subsequent RSV disease have occurred. In order to circumvent these problems, calves were vaccinated with the nucleocapsid protein, known to be a major target of $CD8^+$ T cells in cattle. This was performed according to a DNA prime–protein boost strategy. The results showed that DNA vaccination primed a specific T-cell-mediated response, as indicated by both a lymphoproliferative response and IFN-γ production. These responses were enhanced after protein boost. After challenge, mock-vaccinated calves displayed gross pneumonic lesions and viral replication in the lungs. In contrast, calves vaccinated by successive administrations of plasmid DNA and protein exhibited protection against the development of pneumonic lesions and the viral replication in the BAL fluids and the lungs. The protection correlated to the cell-mediated immunity and not to the antibody response.

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1. Introduction

RSV is a major cause of hospitalization of infants less than 2 years of age [1]. Like its human counterpart, bovine respiratory syncytial virus (BRSV) is a major cause of calf morbidity and mortality [2]. Calves less than 1 year of age are particularly susceptible to the disease [3]. Maternal antibodies against respiratory syncytial virus do not prevent infection in calves but antibodies seem to decrease the severity of infection [4–6].

Furthermore, BRSV is one of the viruses contributing to bovine respiratory disease complex [7]. Protection against BRSV afforded by vaccination has been described and numerous vaccines are licensed and widely used in the field [8–10]. However, exacerbation of respiratory disease has been induced in calves and in infants vaccinated with formalin-inactivated BRSV vaccine [11,12]. In mice, enhanced disease has an immunopathological basis, characterized by polarized type 2-helper (TH2) response. Increased IL-4 production has been associated with pulmonary histopathology and eosinophilia [13–15]. In calves, enhanced pathology has been associated with elevated IgE titers and marked eosinophilia, suggesting a TH2-mediated immune response [16–18]. In addition, some authors have reported high mortality in calves subsequent to the use of inactivated vaccine in the field [19,20]. There is thus a need for protective vaccines, able to confer protection in neonates, even in the presence of maternal antibodies and lacking disease exacerbating side effects.

It has previously been shown that the F and G surface glycoproteins, and the N protein, are the major protective antigens of BRSV [21]. Neutralizing antibodies to the F protein can mediate protection [22]. However, serum antibodies provide greater contribution in reducing BRSV induced lower respiratory disease than upper respiratory tract virus shedding [4,23]. The cell-mediated immunity is also a critical parameter in the outcome of RSV infection [24,25]. The F, P, G, M, M2 and N proteins are antigenic targets recognized by bovine $CD8^+$ T cells, the latter two proteins being also specific targets for murine (M2 protein) or murine and human (N protein) $CD8^+$ T cells [26–29]. These cells constitute the major lymphocyte subpopulation in the respiratory tract of calves recovering from BRSV infection and depletion of those cells also induces delayed virus clearance from the lungs and nasopharynges in calves [23,30,31].

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In mice, diminished CTL activation and migration to the lungs by treatment with anti-LFA-1 delayed viral clearance [32]. Furthermore, priming of the RSV-specific $CD8^+$ response suppressed the eosinophilia induced after challenge of animals vaccinated with the G glycoprotein [33].

DNA vaccination is an effective way of generating humoral and cell-mediated response [34]. Intramuscular vaccination of mice with plasmid DNA expressing the F or G proteins of RSV has shown to induce a strong TH1 response and to reduce the viral load in the lungs after RSV challenge, without inducing enhanced pulmonary inflammatory response or eosinophilia [35,36]. However, it is generally recognized that DNA vaccines are often less effective in large animals than in mice [37]. Improvement of the immune response can be achieved by boosting animals previously vaccinated with plasmid DNA with inactivated vaccine. This approach has successfully been used to protect calves against bovine herpesvirus-1 (BoHV-1) challenge [38]. This strategy was also successfully used in a previous BRSV experiment. Calves vaccinated with codon-optimized plasmid DNA expressing the F and N BRSV proteins and boosted with an inactivated commercial vaccine were protected against clinical signs, gross pneumonic lesions and virus replication [39]. In that study, the humoral and cell-mediated immune responses were primed by DNA injection and enhanced by the protein boost. They reduced the clinical signs and the virus replication after BRSV challenge. The use of the F protein of RSV in a prime–boost protocol also represents an interesting strategy for early life immunization [40].

The purpose of the work presented here was to investigate the protection of calves against BRSV, by focusing mainly on the stimulation of the BRSV cell-mediated immunity. Therefore, a combination of plasmid and protein vaccination was performed with the N protein as immunogen. The protection conferred by this vaccination scheme was evaluated after BRSV challenge.

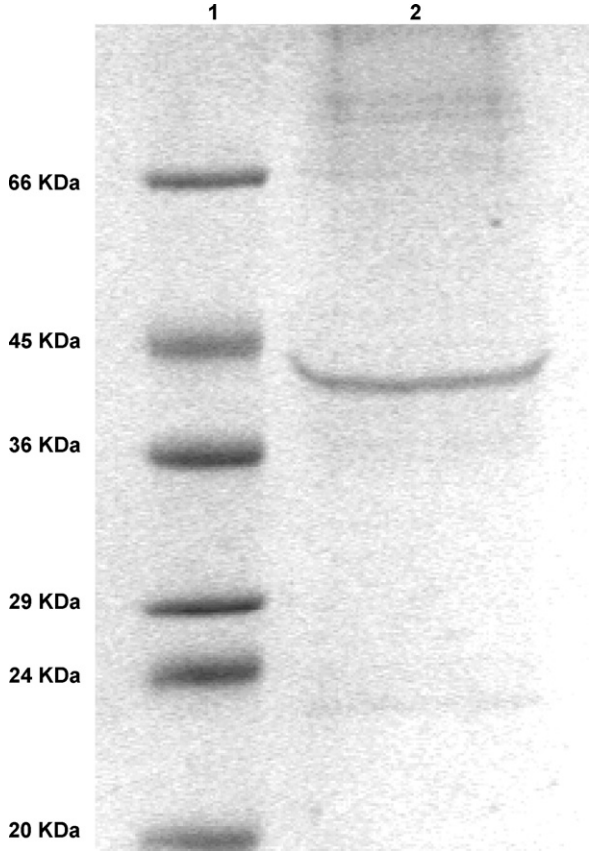

Fig. 1. Polyacrylamide gel electrophoresis of the N protein, purified from recombinant baculovirus infected Sf9 insect cells (lane 2) and a molecular weight marker (Sigma, lane 1). The proteins were stained with Coomassie blue.

2. Materials and methods

2.1. Expression of the N protein

The pcDNA3 plasmid expressing synthetic DNA sequence coding for the ORF of the N protein of BRSV (pNsyn) has been described elsewhere [39]. Large scale plasmid DNA was purified from transformed TOP 10 bacteria cells by affinity chromatography on anion-exchange resin (Plasmid Giga kit, Qiagen).

The purified N protein was obtained from a recombinant baculovirus expressing the coding sequence of the N protein of BRSV strain RB94. Briefly, the N gene was cloned in the pBlueBacHis2A vector (Invitrogen). Recombinant baculovirus expressing the N protein (BacN) was obtained after transfection of Sf9 insect cells with the recombinant plasmid and purification of the β-galactosidase-expressing plaques as recommended by the manufacturer. Sf9 cells were infected with BacN at a moi of 2. Cells were harvested 48–72 h pi, when at least 95% of the cells were lysed, washed with PBS and resuspended in Tris–HCl 20 mM pH 7.9, NaCl 0.5 M, Imidazole 5 mM buffer. The 6His-tagged N protein was purified on Ni-NTA resin (Qiagen) in batch, as recommended by the manufacturer. The protein concentration was determined by BCA protein assay (Pierce) according to the manufacturer's recommendations. As expected, a protein of 43 kDa was purified and analyzed by PAGE (Fig. 1) [41]. Prior to immunization, QuilA adjuvant was added to the N protein at a final concentration of 1 mg/ml.

2.2. Cell culture and virus

The BRSV RB90 virus was grown on calf primary kidney cells in Minimal Eagle medium (MEM, GIBCO BRL) supplemented with 10% fetal calf serum, 10 mg/ml gentamycin and 10^6 IU penicillin.

Challenge inoculum's consisted in lung lavage fluid of a calf collected after intratracheal inoculation with the BRSV strain VRS3761, as described previously [39,42]. This inoculum contained $10^{3.4}TCID_{50}$/ml and was free of bovine viral diarrhea virus, bovine herpesvirus-1, bovine parainfluenza virus-3, bovine coronavirus, bovine adenovirus-5, endotoxins, bacteria and mycoplasmas. Back titration confirmed the amount of virus inoculated.

2.3. Study design

Fourteen cross-bred dairy calves between 4 and 6 weeks of age were randomly allocated in 4 experimental groups of 3 or 4 animals and housed in 3 identically conditioned stables. All calves were shown to be seronegative for BRSV, BoHV-1 and Bovine viral diarrhea virus at the start of the experiment.

As shown in Table 1, 7 calves were vaccinated twice at a 4 weeks interval (day 0 and 29) with 500 μg of pNsyn plasmid DNA in saline. The first vaccine dose was injected intramuscularly and the second dose was administrated intradermally in 4 sites in the neck. Seven other calves were subjected to the same protocol, but with the pcDNA3 control plasmid. Four weeks after the second DNA administration (day 60), respectively, 4 and 3 calves injected with the pNsyn and the control plasmids were subjected to intramuscular injection of 300 μg of purified N protein, adjuvanted with Quil A in a 2 ml volume. The other calves were treated with saline. Three weeks after the third vaccination (day 80), all the calves were challenged with the BRSV 3761 strain by intratracheal (10 ml) injection combined to intranasal nebulization (5 ml). The calves were examined

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