A DNA Vaccine Containing PPE68 Induces Humoral Response in Cattle

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ABSTRACT

In this study, we used an approach known to stimulate Th1 immune responses, DNA vaccination, to produce PPE68, a RD1 antigen absent in BCG. We found that after four intramuscular doses (1/every 2 weeks), the production of IFN-γ by vaccinated cattle was not different with respect to saline- or empty vector-vaccinated animals, but sera from animals receiving PPE68 DNA vaccine was able to detect, in M. bovis BCG Pasteur whole-cell extracts, two protein bands between 37 and 50 KDa (PPE68 expected size of 37 KDa), which were absent in saline- or empty-vector vaccinated animals.This, therefore, suggested a humoral response towards a protein that might cross-react with PPE68 was raised in pVAX1-PPE68 vaccinated cattle.

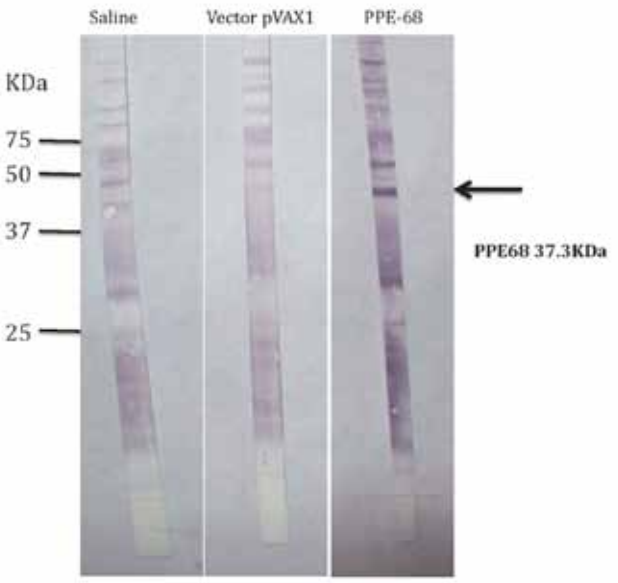
INTRODUCTION

Bovine tuberculosis (bTB), caused by Mycobacterium bovis, is an infectious disease that continues to be endemic in some developing countries,[1] including Mexico, where due to its prevalence, is likely to favor natural transmission in cattle. In developed countries, experimental infection with this pathogen is feasible due both to the availability of facilities and the possibility of sacrificing infected bovines without affecting farmers, circumstances that are difficult to meet in some instances in less developed areas. In this work, we constructed a DNA vaccine based on an eukaryotic expression vector (pVAX1, Invitrogen, USA), from where we produced PPE68, a protein encoded in a gene present in RD1, a region absent from M. bovis BCG,[2] a strain commonly used as vaccine in humans and in experimental bovine models. This strain

contributes to cross-reactivity and confusion between vaccinated and infected animals when tested with PPD. pVAX1-PPE68 was used to vaccinate Holstein bovines and to determine its capacity to induce production of IFN-γ in vitro as well as to raise antibodies in vaccinated animals.

DNA vaccines are known to promote a Th1 profile,[3-5] which is required to control tuberculosis. Some DNA vaccines have already been tested in cattle, showing a strong proliferative response to MPB836 and somewhat less effective against MPB707; vaccination with a mixture of antigens encoded in DNA vaccines has proven to protect against experimental M. bovis infection8 and reduced pulmonary loads when used to treat already infected cows.[9] Considering that PPE68 has already been shown to promote IFN-γ production in C57BL/6 mice,[10] its absence in BCG, and that it has thus far not been tested in cattle, we decided to assess its potential to raise immune responses in these animals.

Figure 1. *Western blot analysis of 20 μg Mycobacterium bovis BCG Pasteur 1173P2 whole cell lysate. A 1:40 dilution of sera from cattle vaccinated with saline, empty vector or pVAX1-PPE68 was used as indicated in each lane.*



MATERIALS AND METHODS

Animals

Holstein bovines (6-12 months old, median 8.5 months-old) were used in this study. Animals used were from "Rancho la Cofradía" belonging to Universidad de Guadalajara, a herd that has been declared bTB-free since 3 years ago.

PCR and vaccine construction

We elected to PCR-amplify the PPE68 gene using high-fidelity enzyme Phusion (Finnizymes, USA), and M. bovis AN5 genomic DNA as template, and primer pair MbPPE68-5FH3 (5´- GGAGAAGCTT-GTCACCATGCTGTGG-3) + MbPPE68-3RXb (5´- GATCCGCTCTAGATTACCT-GCCTCCTG-3), obtaining a PCR product of 1,278 bp. This was digested with HindIII and XbaI (New England Biolabs, USA), and then ligated into pVAX1 vector using the same restriction sites. Cloning was confirmed by restriction digestion, and identity/fidelity of the inserted gene was verified by sequencing performed at Laboratorio de Genómica para la Biodiversidad (LANGE-BIO, Cinvestav Irapuato, Mexico). Expression of genes cloned into pVAX1 depends on its CMV-promoter, as well as Kozak and ATG sequences that are incorporated in the primers given their absence in pVAX1.

Vaccination

After DNA sequence confirmation, we formed three groups that were vaccinated with:

- saline
- empty vector
- pVAX1-PPE68.

For DNA vaccinated groups, 500 μg of

plasmid DNA were used to intramuscularly-inject animals, once every 2 weeks during a 2-month period of time. Each group consisted of three bovines, with a median age of 8.5 months old (range 6-12 months-old).

RESULTS

We collected blood after each one of the four times bovines were injected, as well as 2 and 4 weeks after the last dose. Whole blood was obtained, heparanized, and tested for their capacity to produce IFN-γ upon in vitro stimulation with bovine and avian PPD, using the commercially available kit Bovigam® (Prionics, Switzerland). We found no difference among groups (data not shown). On the other hand, when sera were obtained from animals after the last DNA immunization, pooled sera from the animals within each group (diluted 1:40) was used to determine their capacity to recognize M. bovis Pasteur 1173P2 proteins in immunoblot experiments (20 μg of total protein) as already described,11 we found that animals vaccinated with pVAX1-PPE68 were able to recognize two protein bands between 37 and 50 KDa (Fig.1), the former close to the expected size of PPE68. These bands were not detected when using sera from saline- or pVAX1 vaccinated animals, strengthening the notion of their detection being due to a specific response.

DISCUSSION

Vaccination with BCG in neonatal calves reduces bacterial burden in M bovis experimentally infected animals, and IFN-γ production correlated significantly with protection,12 although another study indicated that a similar vaccine dose (106 UFC) did not protect calves against M bovis challenge (Buddle et al, 2011).

Both studies used within their groups' vaccination with BCG Danish, even though animals were of different age (1-27 days in Hope et al. 2011, 6 months in Buddle et al, 2011), and the strains used to challenge them were also different (AF2122/97 in Hope et al, 2011, WAg202 in Buddle et al, 2011). This might account for the observed discrepancy. On the other hand, vaccination with a mixture of antigens encoded in DNA vaccines has proven to protect against experimental M bovis infection,[8] and reduced pulmonary loads when used to treat already infected cows[9].

Using a DNA vaccine (pVAX1-PPE68), we were not able to distinguish IFN-γ response above background levels, therefore, indicating either the vaccination scheme used was not successful to trigger this cytokine induction, or that PPE68 is not abundantly present in the antigenic preparation used (PPD). Perhaps we should have tested with purified PPE68. PPE68 has previously been shown to, promote antigen-specific splenocyte proliferation in DNA vaccinated BALB/c mice [13] and a recombinant BCG expressing PPE68 was able to promote proliferation upon PPE68 stimulation, including high IFN-γ response and reduced IL-4 secretion in mice.[14]

The same pVAX1-PPE68 was able to induce humoral response in cattle, which was not detected in control (vector or saline mock-vaccinated) animals, leading to detection of two protein bands. Such detection could be the consequence of cross-reactivity with similar proteins present in M bovis Pasteur 1173P2, given the absence of PPE68 from this bacterium. The use of anti-PPE68 antibodies, as well as probing with M bovis protein extract would be helpful in determining whether or not either or both of these bands corresponds a cross-reacting antigen. Recently, a protein fusion ESAT6-CFP10-PPE68 improved humoral responses in vaccinated mice as compared to PPD and ESAT-CFP1015. Further experiments testing Th1 response in cattle vaccinated with PPE68, using purified protein as antigen should shed light on whether or not our vaccine is able to induce this profile.

CONCLUSIONS

The DNA vaccine pVAX1-PPE68 constructed and evaluated in Holstein bovines was not able to induce IFN-γ production in vaccinated animals above background levels, but was able to induce antibody production

directed against proteins that cross-react in Mycobacterium bovis BCG Pasteur 1173P2 whole-cell extracts. Such cross-reaction might be useful given the abundance of PPE-family proteins in pathogenic Mycobacteria. Experimental vaccination and challenge, either in mice or bovines, would help us determine the capacity of our vaccine to prevent or treat M. bovis infection.

CONFLICT OF INTEREST STATEMENT

The authors of this paper acknowledge that they do not have any relationship with other people or organizations that could influence the results of this work.

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