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Short communication

Vaccination of chickens with a chimeric DNA vaccine encoding *Eimeria tenella* TA4 and chicken IL-2 induces protective immunity against coccidiosis

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Abstract

A fusion DNA vaccine co-expressed *Eimeria tenella* TA4 and chicken IL-2 (chIL-2) was constructed and its efficacy against *E. tenella* challenge was observed. TA4 gene of *E. tenella* and chIL-2 gene were cloned into expression vector pcDNA3.1 and pcDNA4.0c in different forms, producing vaccines pcDNA3.1-TA4-IL-2, pcDNA3.1-TA4 and pcDNA4.0c-IL-2. The expression of aim genes *in vivo* was detected by RT-PCR and western blot. Animal experiment was carried out to evaluate the immune efficacy of the vaccines. Results indicated these DNA vaccines were successfully constructed and the antigen genes could be expressed effectively *in vivo*. The animal experimental results showed that DNA vaccines could obviously alleviate cecal lesions, body weight loss and increase oocyst decrease ratio. The ACI of pcDNA3.0-TA4-IL-2 group was 192, higher than that of pcDNA3.1-TA4 group. The results suggested that TA4 was an effective candidate antigen for vaccine and co-expression of cytokine with antigen was an alternative method to enhance DNA vaccine immunity.

Keywords: *E. tenella*; TA4; DNA vaccine; chIL-2

1. Introduction

Eimeria tenella is an obligate intracellular apicomplexan (coccidian) parasite that infects chicken and causes a severe form of enteritis, resulting significant economic losses especially to the poultry industry. Current means of control rely primarily on the prophylactic use of coccidiostats included in the feed. The development of resistance to these drugs and the public demand for chemical-free agricultural practices has limited the application of anti-coccidial drugs and driven the need for development of anti-coccidial vaccines (Williams, 2002).

Immunity to avian coccidiosis is largely dependent on cellular immunity (Rose and Hesketh, 1982). It was reported that DNA vaccines could successfully induce protective cellular and humoral immune responses against coccidiosis (Song et al., 2001). Administration of cytokines, such as IFN-γ, IL-1, IL-2 and IL-15, could enhance host immune responses induced by DNA vaccines to *E. tenella* and *E. acervulina* (Kim et al., 1999; Song et al., 2001; Xu et al., 2006). TA4 antigen is located on the surface of *E. tenella* sporozoites (Brothers et al., 1988). Subcutaneous immunization of chickens

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with *Escherichia coli*-expressed TA4 revealed that the protein was immunogenic (Thomas et al., 2003).

Here, we reported the construction of a fusion DNA vaccine co-expressed *E. tenella* TA4 and chicken IL-2 (chIL-2) and its efficacy against coccidiosis.

2. Materials and methods

2.1. Antiserum, plasmids, parasites and animals

Rabbit antiserum against recombinant TA4 protein, plasmids pET28a-TA4 containing the full length of TA4 gene and pMDT-18-IL-2 with the complete chIL-2 gene were provided by the Laboratory of Veterinary Molecular and Immunological Parasitology, Nanjing Agricultural University, China. Sporulated oocysts of *E. tenella* isolated from Jiangsu Province of China (JS) were stored in 2.5% potassium dichromate solution at 4 °C and passed through chickens at least every 3 months. New-hatched Chinese Yellow chickens were raised in a sterilized room under coccidia-free conditions until the end of the experiment. Food and water without anti-coccidia drugs were available ad libitum.

2.2. Construction of DNA vaccine pcDNA3.1b-TA4

pET28a-TA4 and pcDNA3.1 (Invitrogen) were both digested with BamHI and EcoRI. After electrophoresis in 1% agarose gel, the pcDNA3.1 and TA4 fragments were excised and purified separately using the Agarose Gel DNA Purification Kit Ver.2.0 (TaKaRa). The above two DNA fragments were ligated together using a DNA Ligation Kit (TaKaRa). The subsequent product was transformed into *E. coli* BL21 competent cells (Invitrogen). The recombinant plasmid pcDNA3.1-TA4 was confirmed by polymerase chain reaction (PCR) amplification and endonuclease digestion.

2.3. Construction of DNA vaccine pcDNA3.1b-TA4-IL2 and pcDNA4.0c-IL-2

TA4 gene primers were designed to get rid of the stop codon. BamHI and EcoRI sites were added at the 5′ and 3′ ends of TA4 gene primers, respectively. The resultant primers were: forward, 5′-GGATCCGATGAACAAGCTGA-3′; reverse, 5′-GAATTCAAAGAGAGCGAAAGCGGA-3′. ChIL-2 primers were designed to add thrombin sequence at 5′ end of chIL-2 primers. EcoRI and NotI sites were added at the 5′ and 3′ ends of chIL-2 primers, respectively. The sequences of the primers were: forward, 5′-CTAGAATTCCCTACCCTCGATATGTGCAAAGTACTGATCT-3′; reverse, 5′-TTAGCGGCCGCATTGCAGATATCTCACAAAGTT-3′. PCR amplification of TA4 (without stop codon) was performed using the gene-specific primers with pET28a-TA4 as template. After purification, the amplified TA4 (without stop codon) was digested with BamHI and EcoRI and cloned into the BamHI/EcoRI sites of pcDNA3.1b. The recombinant plasmid pcDNA3.1b-TA4 (without stop codon) was confirmed by PCR amplification and endonuclease cleavage.

Using the same method above, amplified chIL-2 product was ligated into pcDNA3.1b-TA4 (without stop codon) and pcDNA4.0c (Invitrogen) to construct pcDNA3.1b-TA4-IL2 and pcDNA4.0c-IL-2.

2.4. Detection of the transcription of constructed DNA vaccines in vivo by RT-PCR

Chickens were vaccinated with the recombinant DNA vaccines pcDNA3.1b-TA4, pcDNA3.1b-TA4-IL-2 and pcDNA4.0c-IL-2 by intramuscular injection. Seven days later, the injected muscle was cut for total mRNA extraction. After digestion of plasmid DNA vaccines by adding DNase I (TaKaRa) into the mRNA, reverse transcription-polymerase chain reaction (RT-PCR) was performed with the product RNA as template and electrophoresis in 1% agarose gel was performed to examine the transcription of the antigen gene. Meanwhile the same site muscle from non-injected chickens was selected as control.

2.5. Detection of the expression of TA4 gene in vivo by western blot

Seven days post-vaccination, the injected muscle was grinded and treated with RIPA solution (50 mmol/L Tris–HCl, 150 mmol/L NaCl, 1% Nonnidet P-40, 0.1% SDS) for 3 h. After centrifugation at 13,000 rpm/m, the supernatant was collected for use. Meanwhile the muscle from the same site of non-injection was selected as control. Then western blot was done to detect the aim proteins with the 1:30 dilution of rabbit antiserum against recombinant TA4 protein as first antibody (Wallach et al., 1989).

2.6. Evaluation of immune protection

Efficacy of immunization was evaluated on the basis of survival rate, lesion score, body weight gain, oocyst decrease ratio and the anti-coccidial index (ACI). Survival rate was estimated by the number of surviving chickens divided by the number of initial chickens. Body weight gain of the chickens in each group was