Vaccine 33 (2015) 2764–2770

Contents lists available at ScienceDirect

Vaccine

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

Induction of protective immunity against *Eimeria tenella*, *Eimeria necatrix*, *Eimeria maxima* and *Eimeria acervulina* infections using multivalent epitope DNA vaccines

Xiaokai Song, Zhe Ren, Ruofeng Yan, Lixin Xu, Xiangrui Li*

College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu 210095, P.R. China

ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form 8 April 2015
Accepted 13 April 2015
Available online 25 April 2015

Keywords:
Eimeria
T-cell epitope
DNA vaccine
Cross-species protection

ABSTRACT

Avian coccidiosis is mostly caused by mixed infection of several *Eimeria* species under natural conditions and immunity to avian coccidiosis is largely dependent on T-cell immune response. In this study, 14 T-cell epitope fragments from eight antigens of *Eimeria tenella* (*E. tenella*), *Eimeria necatrix* (*E. necatrix*), *Eimeria maxima* (*E. maxima*) and *Eimeria acervulina* (*E. acervulina*) were ligated with pVAX1 producing 14 monovalent DNA vaccines, respectively. Protective immunity of the monovalent DNA vaccines was assessed by in vivo challenge experiments and then four most protective fragments of each species were chosen to construct multivalent epitope DNA vaccines with or without chicken IL-2 as genetic adjuvant. Protective efficacies of the epitope DNA vaccines on chickens against *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina* were evaluated. The results showed that the constructed multivalent epitope DNA vaccines significantly increased body weight gain, alleviated enteric lesions and reduced oocyst output of the infected birds. Especially, the multivalent epitope DNA vaccines of pVAX1-NA4-1-TA4-1-LDH-2-EMCDPK-1 and pVAX1-NA4-1-TA4-1-LDH-2-EMCDPK-1-IL-2 not only significantly increased body weight gain, alleviated enteric lesions and reduced oocyst output of the infected birds, but also resulted in anti-coccidial index (ACI) more than 170 against *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina*, which indicated they could induce protective immunity against *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina*. Our findings suggest the constructed multivalent epitope DNA vaccines are the potential candidate multivalent vaccines against mixed infection of *Eimeria*.

1. Introduction

Avian coccidiosis, caused by multiple species of protozoan *Eimeria*, is the major parasitic intestinal disease of chicken worldwide [1,2]. Domestic chickens can be infected by seven *Eimeria* species and four species are mainly responsible for global disease burden and economic impact, namely *E. tenella* (*Et*), *E. necatrix* (*En*), *E. maxima* (*Em*) and *E. acervulina* (*Ea*) [3–5]. Infection by coccidial parasites has an enormous impact on poultry production due to the morbidity, mortality, inefficient feed conversion ratios and reduced body weight gain in broilers, as well as a reduction of egg production in layers [6–8]. The estimated annual economic loss caused by avian coccidiosis is more than US$3 billion worldwide [2,9]. Conventional control strategy is achieved by careful husbandry combined with in-feed anticoccidial drugs or vaccination with live or attenuated parasites [2]. However, alternative control strategies are urgent to be developed due to the rise of drug resistance, the increasing legislation restrictions on the use of coccidiostat, the high costs of new drug development and the potential reversion of pathogenicity, as well as the limited cross-protection among *Eimeria* species [2,8,10–12]. Particularly, it is very urgent to develop safe and effective multivalent vaccine against mixed infection of all the economically important species of *Eimeria* [8,13].

Progress has been made toward development of DNA vaccines against coccidia and many reports show effective protection [6,12,14–19], however, these DNA vaccines are monovalent vaccines and not practical to prevent the simultaneous infection of multiple *Eimeria* species under natural conditions [8,20,21]. Therefore, effective vaccines against coccidiosis should induce protective immunity against several economically important species of *Eimeria* [8,13]. Furthermore, T cells play a critical role in protective immunity against *Eimeria* infection [6,12]. Hence, an effective vaccine against coccidiosis also should contain a greater amount of protective T-cell epitopes and could induce effective cellular immune response [6,10,12,14]. Basing on the comprehensive consideration of the two aspects, we integrate T-cell epitopes from

* Corresponding author. Tel.: +86 25 8439 9000; fax: +86 25 8439 9000.
E-mail address: lixiangrui@njau.edu.cn (X. Li).

http://dx.doi.org/10.1016/j.vaccine.2015.04.052
0264-410X/

different *Eimeria* species into one plasmid to construct multivalent epitope DNA vaccines which could provide effective protection against mixed infection of several *Eimeria* species.

In our lab, eight antigen genes of *Eimeria* species have been cloned, i.e. TA4 (GenBank: M21004.1), MIC4 (GenBank: AJ306453), 5401 (GenBank: AY819649) and pEtk2 (GenBank: AY389513.1) of *Et*, 3-1E (GenBank: AF113613.1) and LDH (GenBank: FJ617009) of *Ea*, NA4 (GenBank: EU523548.1) of *En* and EMCDPK (GenBank: Z71756.1) of *Em*, which have been demonstrated to be effective against *Eimeria* [16,18]. In this study, T-cell epitopes of the eight antigens were predicted using DNAStar software, and then 14 T-cell epitope fragments of the eight antigens were chosen and cloned into eukaryotic expression vector pVAX1. Protective efficacies of T-cell epitope fragments were evaluated. T-cell epitope fragments with the best protective efficacy of each species were selected to construct multivalent epitope DNA vaccines. Protective efficacies of the multivalent epitope DNA vaccines were evaluated by challenge with *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina*.

2. Materials and methods

2.1. Plasmids, parasites and animals

Plasmids pET-28a-TA4 (pETA4), pMD18-T-MIC4 (pTMIC4), pMD18-T-pEtK2 (pTpEtK2), pMD18-T-5401 (pT5401), pET-28a-NA4 (pENA4), pET-28a-LDH (pELDH), pMD18-T-3-1E (pTE), pMD18-T-EMCDPK (pTEK) and pVAX1-IL-2 (pVIL-2) were provided by the Laboratory of Veterinary Molecular and Immunological Parasitology, Nanjing Agricultural University, China. *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina* were isolated from Jiangsu Province of China. The purities of single species *Eimeria* isolates were determined with ITS1-PCR [22–24]. New-hatched Hy-Line layer chickens (commercial breed W-36) were obtained from Tangquan chicken farm, a local farm nearby Nanjing, and reared under coccidian-free conditions in wire cages until the end of experiment. Food and water without anti-coccidia drugs were available ad libitum. All experiments were approved according to the Animal Care and Use Committee of the Jiangsu Province Animal Care Ethics Committee.

2.2. Analysis and selection of T-cell epitope fragments from the eight antigens of Eimeria

T-cell epitopes of TA4, MIC4, pEtK2, 5401, NA4, LDH, 3-1E, and EMCDPK were analyzed with Rothbard–Taylor method and AMPHI method of DNAStar software. Rothbard–Taylor method locates a common sequence motif which is of 3–4 residues consisting of glycine followed by hydrophobic residues [25]. The AMPHI method assumes T-cell antigenic sites are composed of amphipathic helices [26]. Subsequently 14 fragments with concentrated T-cell epitopes were selected. Fragment from TA4 was designated as TA4-1. Similarly, Fragments from MIC4, pEtK2, 5401, NA4, LDH, 3-1E and EMCDPK were designated as MIC4-1, MIC4-2, pEtK2-1, pEtK2-2, 5401-1, NA4-1, NA4-2, LDH-1, LDH-2, 3-1E-1, 3-1E-2, EMCDPK-1 and EMCDPK-2, respectively. Characteristics of the 14 T-cell epitope fragments were shown in Table 1.

2.3. Cloning of the selected T-cell epitope fragments

Using the corresponding primers (Table 1), PCR amplifications were performed with pETA4, pTMIC4, pTpEtK2, pT5401, pENA4, pELDH, pTE or pTEK as templates, respectively. After purification, PCR products were cloned into pMD18-T vector producing recombinant plasmids pMD18-T-TA4-1 (pTTA4-1), pTMIC4-1, pTMIC4-2, pTpEtK2-1, pTpEtK2-2, pT5401-1, pMD18-T-NA4-1 (pTNA4-1), pMD18-T-NA4-2, pMD18-T-LDH-1, pMD18-T-LDH-2 (pTLDH-2), pTE-1, pTE-2, pTEK-1 and pTEK-2.

2.4. Constructions of monovalent epitope DNA vaccines containing one fragment

Recombinant plasmids pTTA4-1 and eukaryotic expression vector pVAX1 (Invitrogen) were digested with *Hin*dIII and *Kpn*I. After electrophoresis in 1% agarose gel, TA4-1 and pVAX1 fragments were purified and ligated together to produce pVAX1-TA4-1. Similarly, DNA vaccines pVAX1-MIC4-1, pVAX1-MIC4-2, pVAX1-pEtK2-1, pVAX1-pEtK2-2, pVAX1-5401-1, pVAX1-NA4-1, pVAX1-NA4-2, pVAX1-LDH-1, pVAX1-LDH-2, pVAX1-3-1E-1, pVAX1-3-1E-2, pVAX1-EMCDPK-1 and pVAX1-EMCDPK-2 were constructed. Subsequently, the recombinant plasmids were confirmed by endonuclease digestion and DNA sequencing.

2.5. Determination of the most protective T-cell epitope fragments of each Eimeria species

Animal experiments were carried out following the animal experimental design (Table 2) and evaluation of immune protection described in Sections 2.10 and 2.11. The determined four T-cell epitope fragments with best protection of each *Eimeria* species were provided for the construction of multivalent epitope DNA vaccines against *Eimeria*.

2.6. Constructions of multivalent epitope DNA vaccines

Based on determination result of Section 2.5, four T-cell epitope fragments, NA4-1 of *En*, TA4-1 of *Et*, LDH-2 of *Ea* and EMCDPK-1 of *Em*, were selected to construct multivalent epitope DNA vaccines. Chicken IL-2 (chIL-2) was used as genetic adjuvant in combination of the multivalent epitope DNA vaccine.

2.6.1. Cloning of T-cell epitope fragments

Corresponding endonuclease sites were added on primers of the four fragments (Table 1). Primers of chIL-2 were also designed and synthetized as following: forward, 5′-ATTTGCGGCC-GCAATGATGTGCAAAGTA-3′, reverse, 5′-TGCTCTAGATTATTTT-TGCAGATATCTCAC-3′. PCR amplifications were performed using plasmids pENA4, pETA4, pELDH, pTEK and pVIL-2 as templates. After purification, PCR products were cloned into pMD18-T vector producing pTNA4-1, pTTA4-1, pTLDH-2, pTEK-1 and pMD18-T-IL-2 (pTIL-2).

2.6.2. Constructions of multivalent epitope DNA vaccines

Plasmid pTNA4-1 and pVAX1 vector were digested with *Hin*dIII and *Kpn*I, and then fragments NA4-1 and pVAX1 were ligated together producing pVAX1-NA4-1 (pV-N1). Hereafter, plasmid pTTA4-1 and pV-N1 vector were digested with *Kpn*I and *Eco*RI and fragments TA4-1 and pV-N1 were ligated together producing pVAX1-NA4-1-TA4-1 (pV-N1-T1). Subsequently, plasmids pTLDH-2 and pV-N1-T1 vector were digested with *Eco*RI and *Eco*RV and fragments LDH-2 and pV-N1-T1 were ligated together producing pVAX1-NA4-1-TA4-1-LDH-2 (pV-N1-T1-L2). Plasmid pTEK-1 and pV-N1-T1-L2 vector were digested with *Eco*RV and *Not*I and fragments EMCDPK-1 and pV-N1-T1-L2 were ligated together to producing pVAX1-NA4-1-TA4-1-LDH-2-EMCDPK-1 (pV-N1-T1-L2-E1). Plasmids pTIL-2 and pV-N1-T1-L2-E1 vector were digested with *Not*I and *Xba*I and fragments IL-2 and pV-N1-T1-L2-E1 were ligated together producing DNA vaccines pVAX1-NA4-1-TA4-1-LDH-2-EMCDPK-1-IL-2 (pV-N1-T1-L2-E1-IL2). With the similar methods, DNA vaccines pV-N1-T1, pVAX1-NA4-1-TA4-1-LDH-2 and pV-N1-T1-L2-E1 were constructed. Subsequently, the