Experimental Parasitology 126 (2010) 224–231

Contents lists available at ScienceDirect

Experimental Parasitology

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

Efficacy of DNA vaccines carrying *Eimeria acervulina* lactate dehydrogenase antigen gene against coccidiosis

Hongyan Song, Ruofeng Yan, Lixin Xu, Xiaokai Song, Muhammad Ali A Shah, Huili Zhu, Xiangrui Li *

College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu 210095, PR China

ARTICLE INFO

Article history:
Received 8 March 2009
Received in revised form 22 April 2010
Accepted 21 May 2010
Available online 26 May 2010

Keywords:
Eimeria acervulina
Lactate dehydrogenase
DNA vaccine

ABSTRACT

The efficacies of DNA vaccines encoding either *Eimeria acervulina* lactate dehydrogenase (LDH) antigen or a combination of LDH antigen and chicken IL-2 or IFN-γ were evaluated against chicken coccidiosis. Three vaccine plasmids pVAX-LDH, pVAX-LDH-IFN-γ and pVAX-LDH-IL-2 were constructed using the eukaryotic expression vector pVAX1. Expressions of proteins encoded by plasmids DNA *in vivo* were detected by reverse transcription-polymerase chain reaction (RT-PCR) and western blot assay. Average body weight gain, oocyst output, survival rate and lesion scores were measured to evaluate the protective effects of vaccination on challenge infection. The results showed that DNA vaccines could obviously alleviate body weight loss, duodenal lesions, oocyst output and enhance oocyst decrease ratio. Anti-coccidial indexes (ACIs) of pVAX-LDH-IFN-γ and pVAX-LDH-IL-2 groups were higher than that of other groups. Flow cytometric analysis of T lymphocytes in spleen and cecal tonsil demonstrated that DNA vaccines had significantly increased percentages of $CD3^+$ T cells compared with pVAX1 alone or TE buffer. The results provided the first proof that DNA vaccine carrying *E. acervulina* LDH antigen gene induced protective immunity against homologous infection and its effect could be enhanced by co-expression of chicken IL-2 or IFN-γ.

1. Introduction

Eimeria acervulina is an intestinal protozoon, which causes chicken coccidiosis and results in intense damage to duodenum accompanied by severe weight loss and poor feed conversion (Lillehoj et al., 1990). A variety of strategies are used to control coccidiosis, including medication with anti-coccidial drugs and immunization of chickens with live vaccines (Chapman, 1993; Hong et al., 2006a). Unfortunately, drug resistance and societal pressure against use of anti-coccidial drugs are impacting chemotherapeutic control (Martin et al., 1997; Shirley et al., 2007; Swinkels et al., 2007). In addition, live vaccines consisting of virulent and attenuated *Eimeria* parasites have drawbacks including the potential reversion to virulence and high production expenses (Vermeulen, 1998; Du and Wang, 2005). The above-stated issues have prompted the development of alternative control strategies against coccidiosis.

Recent efforts to clone *Eimeria* genes as potential recombinant vaccines are directed towards the goal of developing novel coccidiosis control approaches (Ding et al., 2005). To this end, several promising vaccine candidates containing immunogenic *Eimeria* surface antigens or internal antigens have been described (Song et al., 2001; Hong et al., 2006b). It was reported that vaccination with recombinant 3-IE or EtMIC2 proteins of *Eimeria* species (spp.) induced protective intestinal immunity against coccidiosis (Ding et al., 2004, 2005). Recently published work revealed that DNA vaccines such as pcDNA3–5401, pcDNA-TA4 and pcDNA-TA4-IL-2 could induce protective immunity against coccidial challenge (Du and Wang, 2005; Xu et al., 2008; Song et al., 2009). The efficiency of DNA vaccination could be enhanced by cytokines as adjuvants. It was reported that injection of cDNA encoding chicken IL-2 enhanced vaccine response to *Eimeria* parasites, while administration of recombinant chicken IFN-γ improved body weight gain following challenge with *Eimeria* (Lowenthal et al., 1997; Lillehoj et al., 2000).

Lactate dehydrogenase (LDH) of *E. acervulina* was first identified by Schaap and co-workers and they reported that this protein could produce partial protection against *E. acervulina* challenge infection (Schaap et al., 2004). Here, we report the immune effects of DNA vaccines encoding either *E. acervulina* LDH antigen or a combination of LDH antigen and chicken IL-2 or IFN-γ.

2. Materials and methods

2.1. Proteins, antiserum, animals and parasites

Recombinant *E. acervulina* LDH protein expressed in *Escherichia coli* BL21 (DE3), inclusion bodies of expressed LDH isolated from *E. coli* and rat antiserum against recombinant LDH protein were

* Corresponding author. Fax: +86 25 8439 8669.
E-mail address: lixiangrui@njau.edu.cn (X. Li).

0014-4894/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2010.05.015

provided by the Laboratory of Veterinary Molecular and Immunological Parasitology, Nanjing Agricultural University, China. Newhatched Chinese Yellow chickens were reared under coccidian-free conditions in wire colony cages. Chicks were fed ad libitum and free water without anti-coccidial drugs, and constant light was provided during the entire experimental period. Oocysts of *E. acervulina* isolated from Jiangsu Province of China (JS) were propagated and maintained in our laboratory. Oocysts were purified from feces by floating in sucrose solution and enumerated by hemocytometer.

2.2. Cloning of E. acervulina LDH gene

First generation *E. acervulina* schizonts were obtained from the duodenum of 5-week-old chickens as described (Schaap et al., 2004). The open reading frame (ORF) of LDH (GenBank accession No. AY143388) was amplified from total RNA of first generation schizonts by reverse transcription-polymerase chain reaction (RT-PCR) with the following restriction enzyme-anchored (underlined) primers: *Bam*HI anchored forward primer, 5′-GGATCCATGGC GGTCTTCGAGAA-3′; *Eco*RI anchored reverse primer, 5′-GAATTC GCTGCTGCTTACTTGGAT-3′. PCR products were cloned into pMD18-T vector (TaKaRa) and transformed into *E. coli* DH5α competent cells (Invitrogen). Recombinant pMD18-T-LDH clones were identified by PCR amplification and endonuclease digestion. Three positive clones were further confirmed by sequence analysis. The complete nucleotide sequence data inserted in recombinant plasmid was analyzed for homology to known sequences in GenBank databases using a basic alignment search tool (BLAST) (<http://www.ncbi.nlm.nih.gov/BLAST/>).

2.3. Vaccine plasmid construction

Three vaccine plasmids pVAX-LDH, pVAX-LDH-IFN-γ and pVAX-LDH-IL-2 were generated using the eukaryotic expression vector pVAX1. *E. acervulina* LDH gene fragment was excised from previously constructed pMD18-T-LDH by *Bam*HI and *Eco*RI (TaKaRa) digestion and ligated into pVAX1 vector (Invitrogen) at the same enzymes sites to construct pVAX-LDH. To construct vaccine plasmids of pVAX-LDH-IFN-γ and pVAX-LDH-IL-2, the reverse primer of LDH gene was redesigned to get rid of stop codon and to add thrombin sequence at 3′ end of LDH primer. The sequence of the redesigned primer was: 5′-GAATTCCCTACCCTCGATCTTGGATGCAT CAA-3′. PCR amplification of LDH gene (without stop codon) was performed using above described forward primer and redesigned reverse primer for LDH gene with pMD18-T-LDH plasmid as template. The PCR products were, then, inserted into pVAX1 vector (Invitrogen) to acquire pVAX-LDH (without stop codon). PVAX-IFN-γ and pVAX-IL-2 were provided by Laboratory of Veterinary Molecular and Immunological Parasitology, Nanjing Agricultural University, China. The IFN-γ gene fragment was excised by *Eco*RI and *Apa*I (TaKaRa) digestion from pVAX-IFN-γ and ligated with large fragment from pVAX-LDH (without stop codon) excised by the same enzymes. The resulting plasmid was named as pVAX-LDH-IFN-γ. Similarly, recombinant plasmid pVAX-LDH-IL-2 was also constructed. Gene sequence encoding IL-2 or IFN-γ was fused downstream to sequence encoding LDH and the expression of LDH-IL-2 or LDH-IFN-γ was driven by one promoter (CMV promoter). Three recombinant plasmids were then confirmed by PCR amplification, endonuclease cleavage and sequence analysis. The structures of eukaryotic expression plasmids are shown schematically in Fig. 1.

2.4. Detection of the expressions of proteins encoded by plasmids DNA in vivo by RT-PCR assay and western blot analysis

Chickens were injected intramuscularly (IM) in leg muscle with 100 μg of recombinant plasmids pVAX-LDH, pVAX-LDH-IFN-γ and pVAX-LDH-IL-2, respectively. One week post-inoculation, injected tissues were collected and total RNA was extracted. To remove contaminating genomic DNA or plasmids injected, all RNA samples were treated with RNase-free DNase I (TaKaRa). RT-PCR assays were performed with cloning primer pairs of LDH, IFN-γ and IL-2 genes, respectively. The primers for IFN-γ and IL-2 genes were provided by Laboratory of Veterinary Molecular and Immunological Parasitology, Nanjing Agricultural University, China. Meanwhile, the same site muscles from non-injected and pVAX1 plasmid injected chickens were collected as controls. The PCR products were detected by electrophoresis on 1% agarose gel.

Detection by western blot analysis was performed as described (Xu et al., 2008). Briefly, seven days after vaccination, injected muscles were grinded and treated with ice-cold RIPA solution (0.1 M phenylmethylsulfonyl fluoride PMSF, 150 mM sodium chloride, 1% Nonnidet P-40, 0.5% SDS, 50 mM Tris–HCl, pH8.0). Meanwhile, the same site muscles from non-injected and pVAX1 plasmid injected chickens were collected as controls. Proteins were separated by sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred to a nitrocellulose membrane (Bio-Rad). The membrane was incubated with rat anti-LDH polyclonal antibody as primary antibody and horseradish peroxidase (HRP)-conjugated rabbit anti-rat IgG (Sigma) as secondary antibody. The bound antibody was detected using 3,3′-diaminobenzidine (DAB).

2.5. Immunization and challenge infection

Two-week-old chickens were randomly divided into eight groups of 30 each as shown in Table 1. Experimental group chickens were inoculated with 100 μg plasmids pVAX-LDH, pVAX-LDH-IFN-γ, pVAX-LDH-IL-2, recombinant LDH protein and inclusion bodies by leg intramuscular injection, respectively. 100 μg of pVAX1 plasmid alone was given to chickens as plasmid control. Challenged control group and unchallenged control chickens were injected with sterile TE buffer at the same injection site. A booster immunization was given 1 week later with the same amount of components as the first immunization. All chickens except the

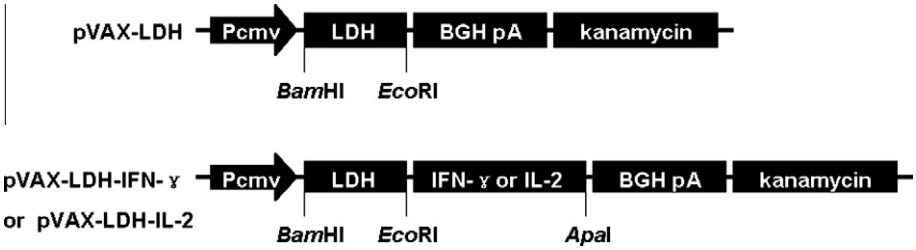

Fig. 1. Schematic representation of DNA vaccine constructs. pVAX-LDH: the expression of LDH was driven by one promoter (CMV promoter); pVAX-LDH-IFN-γ and pVAX-LDH-IL-2: Gene sequence encoding IL-2 or IFN-γ was fused downstream to the sequence encoding LDH and the expression of LDH-IL-2 or LDH-IFN-γ was driven by one promoter (CMV promoter).