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Antibody responses induced by recombinant ALV-A gp85 protein vaccine combining with CpG-ODN adjuvant in breeder hens and the protection for their offspring against early infection

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ABSTRACT

To observe the antibody responses induced by recombinant A subgroup avian leukosis virus (ALV-A) gp85 protein vaccine plus CpG-ODN adjuvant and the protection of maternal antibodies (MAbs) for the hatched chickens against early infection, the gp85 gene was amplified from the proviral cDNA of ALV-A-SDAU09C1 strain using PCR and the recombinant plasmid containing target gene was constructed and expressed in *Escherichia Coli*. The expressed product was confirmed using SDS–PAGE and western blot that it is about 46 KD of recombinant protein. The purified recombinant proteins combining with CpG-ODN adjuvant or Freund's adjuvant were inoculated into the breeder hens, the ALV-A antibodies in serum and in egg-yolk were detected; the fertilized eggs from the vaccinated hens with different titers of egg-yolk antibody were hatched and then challenged with $10^{4.2}$/0.1 mL $TCID_{50}$ of ALV-A-SDAU09C1 strain, all the hatched chickens were weekly detected for the viremias and the cloacal swab P27 antigen and pathological lesions; the neutralizing test of antisera in vitro was conducted. The results showed that the recombinant gp85 proteins combining with CpG-ODN adjuvant could induce the breeder hens to produce better antibody responses than gp85 protein with Freund's adjuvant or without adjuvant; the MAbs with higher titers induced by CpG-ODN + gp85 proteins could obviously decrease the ratios of viremias (13% *vs* 33%), cloacal detoxification (20% *vs* 67%) and death (0% *vs* 22%) caused by ALV-A infection than those by gp85 protein without adjuvant. The results of the neutralizing test indicated that the antisera from the hatched chickens could neutralize the ALV-A-SDAU09C1 strain in vitro, but which depends on the antibody titers. The results of IFA confirmed that the serum antibody could combine with the ALV in DF1 cells. It can be concluded that the prepared ALV-A gp85 subunit vaccine combining with CpG-ODN adjuvant could induce the breeder hens to produce better neutralizing antibody responses and protect 80% of their offspring chickens against early infection.

1. Introduction

Avian leukosis virus (ALV) is the causal agent of the most common naturally occurring avian retroviral infections causing neoplastic diseases and other production problems in the poultry industry worldwide (Gao and Wang, 2012). There are six well-characterized chicken subgroups of ALV (A to E and J). The chicken lymphoid leukosis and sarcoma are mainly induced by subgroup A or B ALV, which were widely prevalent in the 1970s–1980s (Burstein et al., 1984). Although in some countries, large poultry breeding companies took several years to eradicate ALV-A and reduced the economic loss it caused, several studies still have examined the prevalence of ALV-A strains and its association with diseases in poultry in recent years (Fenton et al., 2005; Relova et al., 2013; Zhang et al., 2010; Liu et al., 2011a; Li et al., 2013a).

Because ALV (including ALV-A/B/C/D/J) mainly depends on vertical and early horizontal infection (parents-eggs or chickens), some measures can be taken to block the infected pathways for controlling it in bird flocks. Li et al. reported that the inactivated vaccine against ALV-B could induce high titer antibody reaction and it could provide maternal antibodies to protect chickens against early infection (Li et al., 2013b), Recently we also reported that the subunit vaccine containing ALV-J gp85 protein combining with CpG-ODN adjuvant can induce breeding hens to produce

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maternal antibody and protection for hatched chickens against ALV-J early infection (Dou et al., 2013; Zhang et al., 2014), but there are no reports on ALV-A vaccines till now. In this study, the subunit vaccine containing gp85 protein of ALV-A was prepared using *Escherichia coli* and combining CpG-ODNs adjuvant was inoculated into breeding hens for 3 times, and the results indicated that the gp85 protein plus CpG-ODN adjuvant could produce better antibody responses than single gp85 protein and protect 80% of the offspring chickens against ALV-A early infection.

2. Materials and methods

2.1. Materials and animals

The ALV-A-SDAU09C1 strain was isolated from the imported meat-type grand-parent chickens in 2009 in our laboratory (Zhang et al., 2010). The pMD18-T simple vector was purchased from Dalian Takara Biotechnology Co., Ltd. and was used for plasmid-cloning experiments. The pET32a expression vector was obtained commercially (Invitrogen, Shanghai, China) and was used for gp85 gene expression. The CpG-ODN containing sequence TCGTCGTTTTGTCGTTTTGTCGTT was synthesized using a phosphorothioate backbone at Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. and was diluted appropriately and emulsified as adjuvant for subunit vaccine. The Freund's adjuvant used in this study included complete Freund's adjuvant and incomplete Freund's adjuvant, both purchased from the Sigma Company.

The local "Nongda 3#" layer breeder hens were purchased from Beijing Beinongda breeding poultry Co. Ltd. and housed in a clean and comfortable room. Before the start of the experiment, ALV-A/B-antibody and ALV P27 antigen assays were performed to confirm that each hen was negative for ALV antibody and virus. The Animal Ethics Committee at the Shangdong Province Animal Protection and Welfare Institute approved the animal experiments.

2.2. The preparation of ALV-A subunit vaccine

A forward primer (5'-CGC*GGATCC*CACTTACTCGAGCAGCC-3') and a reverse primer (5'-CCC*AAGCTT*TCACCCTACCGGACGACTG-3') were used to amplify the gp85 gene with proviral cDNA (PMD-18T-env) as the template. Using BamH I and Hind III restriction enzyme sites in the forward and reverse primers, respectively, the primers were designed by referring to the ALV-A-SDAU09C1 gp85 gene sequence published in GenBank (No. HM452339). The PCR product was gel-purified (Gel/PCR Extraction Kit, Biomiga) and transformed into the PET28a vector to generate the recombinant clone vector PET28a-gp85, which was confirmed by DNA sequencing and then amplified in *E. coli* (DH5α).

The recombinant ALV-A gp85 gene was expressed in BL21 (DE3) cells using 1.0 mM IPTG at 37 °C. The soluble fraction was harvested and applied to a high-affinity Ni–NTA column. The eluted proteins were further purified by running the eluate through an SD200 gel filtration column twice, with and without 1% nadeoxycholate, to remove the endotoxins. The purity of the proteins was evaluated by SDS–PAGE in 12% polyacrylamide gels and was analyzed using western bolt mediated by the monoclonal antibody (McAb) against His-tagged proteins. The protein concentration was determined by performing thin-layer chromatography scanning and Bradford's total protein content assay using a Bio-Rad protein assay kit (Bio-Rad); here, bovine serum albumin was used as the standard.

The purified recombinant protein was mixed with CpG-ODN adjuvant or Freund's adjuvant into emulsions as subunit vaccines for the vaccinated breeder hens.

2.3. Immunization and sample collection

32 layer breeder hens (aged 60 weeks) were randomly divided into four groups: control group, gp85 group, F + gp85 group, and CpG + gp85 group. Each group contained 8 hens, and each group was maintained in a clean and comfortable room. Hens were intramuscularly inoculated with 1.0 mL emulsions containing 500 μg purified recombinant gp85 protein, 1.0 mL emulsions containing 500 μg recombinant gp85 protein plus Freund's adjuvant and 1.0 mL emulsions containing 500 μg recombinant gp85 protein plus 300 μg CpG-ODN adjuvant for the gp85 group, F + gp85 group and CpG + gp85 group, respectively. The hens in the control group were intramuscularly inoculated with 1.0 mL PBS served as a negative control. All experimental hens were boosted at the 3rd and 6th week post first inoculation (wpfi). From the day of the first inoculation, the serum samples were collected from each hen at weekly intervals up to 10 weeks. One egg was collected from each hen at the 4rd week after the second booster vaccination to be used to detect egg yolk antibodies as maternal antibodies (MAbs) and the hens in control group, gp85 group, and CpG + gp85 group were fertilized artificially and the fertilized eggs from the corresponding hens were hatched in a dedicated chicken incubator. The serum samples were also collected from the hatched chicken at 1-day-age and were used to detect the MAbs.

2.4. Challenge and evaluation on protection in the hatched chickens

After being hatched, each chicken in three groups was challenged intraperitoneally with $10^{4.2}$/0.1 mL $TCID_{50}$ of ALV-A-SDAU09C1 strain at 1-day-age, and was monitored weekly for viremias and cloacal swab P27 antigen of ALV-A and pathological lesions, death of the challenged chickens.

2.5. Serological and egg-yolk antibody assay

The collected serum samples from the vaccinated hens and the hatched chickens were detected using commercial ALV-A/B antibody test kits (IDEXX USA Inc., Beijing, China). The egg-yolk antibodies were also detected using the above test kits according to the published method (Liu et al., 2013). The relative antibody titers in the serum or in the egg-yolks was determined by calculating the sample to positive (*S*/*P*) ratio [(mean of sample optical density) − (mean of negative control optical density)]/[(mean of positive control optical density) − (mean of negative control optical density)]. The sera from each group were tested in triplicate, and the serum samples with a sample to positive (*S*/*P*) ratio higher than 0.4 were considered ALV-A/B antibody positive.

2.6. Detection of chicken viremia and cloacal swab specificity ALV P27 antigen

0.5 mL anticoagulant blood and cloacal swabs were weekly collected from each chicken to be used for viremias test and for P27 antigen test, respectively. The viremias were detected using the method in the literature reference (Dou et al., 2013). Briefly described as follows: the plasma samples were inoculated into DF1 cells in logarithmic growth, following incubation at 38.5 °C, 5% CO_2 for 5 days, and the supernatant of DF1 cells of each sample was checked for the presence of the virus using ALV P27 antigen ELISA test kits (IDEXX USA Inc., Beijing, China).

The cloacal swab specificity ALV P27 antigen were detected using ALV P27 antigen ELISA test kits in accordance with the manufacturer's protocol. The relative antigen titer level was determined by calculating the sample to positive (*S*/*P*) ratio using the formula shown in Section 2.5. Each sample was tested in triplicate