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Evaluation of a multi-epitope subunit vaccine against avian leukosis virus subgroup J in chickens

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ABSTRACT

The intricate sequence and antigenic variability of avian leukosis virus subgroup J (ALV-J) have led to unprecedented difficulties in the development of vaccines. Much experimental evidence demonstrates that ALV-J mutants have caused immune evasion and pose a challenge for traditional efforts to develop effective vaccines. To investigate the potential of a multi-epitope vaccination strategy to prevent chickens against ALV-J infections, a recombinant chimeric multi-epitope protein X (rCMEPX) containing both immunodominant B and T epitope concentrated domains selected from the major structural protein of ALV-J using bioinformatics approach was expressed in *Escherichia coli* Rosetta (DE3). Its immunogenicity and protective efficacy was studied in chickens. The results showed that rCMEPX could elicit neutralizing antibodies and cellular responses, and antibodies induced by rCMEPX could specifically recognize host cell naturally expressed ALV-J proteins, which indicated that the rCMEPX is a good immunogen. Challenge experiments showed 80% chickens that received rCMEPX were well protected against ALV-J challenge. This is the first report of a chimeric multi-epitope protein as a potential immunogen against ALV-J.

1. Introduction

Avian leukosis virus subgroup J (ALV-J) was first isolated from meat-type chickens in 1989 and different from ALV subgroups A, B, C, D and E with greater pathogenicity and transmission ability (Bai et al., 1995a,b; Payne et al., 1991, 1992). ALV-J was emerged via a recombination event between an exogenous ALV and an endogenous counterpart of the ev/J family (Bai et al., 1995a,b; Benson et al., 1998). Significant antigenic variations of ALV-J strains have been observed in recent years (Pandiri et al., 2010; Zavala et al., 2007), which contribute to the development of novel variants with new antigenicity, host range or pathopoiesis (Venugopal et al., 1998; Xu et al., 2004). Diseases associated with ALV-J have caused severe economic losses in poultry industry worldwide (Fadly and Smith, 1999).

The proviral DNA of ALV contains three major structural genes *gag*, *pol* and *env*, which encode proteins of virion group-specific antigens (GSA), the reverse transcriptase (RT) and integrase (IN) and envelope glycoproteins, respectively (Payne, 1992). Like other retroviruses, ALV inserts its proviral DNA into the genome of infected cells where it could persist for years, increasing the possibility of morbidity in chickens. Although some large breeding companies have succeeded in the eradication of ALV-J from breeding flocks in some countries (Payne and Nair, 2012), it still has a long way to achieve this purpose in China due to the high cost, the strain diversities of ALV-J, and complexities of infections and transmissions (Witter et al., 2000; Witter and Fadly, 2001). Vaccination is a cost-effective and feasible way to control the avian leukemia. Avian leukemia is a disease mainly caused by vertical transmission. It has been reported that it is easy for ALV to induce immune tolerance of host but hard to induce specific antibodies, which makes the development of vaccines difficult (Payne and Nair, 2012). There is no commercial avian leukosis vaccine available so far. Recently, subunit vaccines using gp85 protein of ALV-J either adjuvanted with CpG-ODN (Dou et al., 2013) or entrapped by liposomes (Zhang et al., 2014) were reported to provide partial immune protections against ALV-J infections in chickens. It is believed that vaccination with one antigen usually leads to less immunogenicity and limited protection (Gil et al., 2013). A novel epitope-driven vaccine prepared with multiple epitopes of protective antigens of pathogens can break through these weaknesses.

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Multi-epitope-based vaccines containing epitopes of different target genes could increase immunogenicity and enhance immune responses (Berzofsky et al., 2001; Conceição et al., 2006; Grassmann et al., 2012). Although there has been much progress in research of epitope-based vaccines against viral, bacterial and parasitic infections (Ding et al., 2000; Sette and Fikes, 2003; Sette et al., 2001; Wei et al., 2010), multi-epitope based vaccine has never been tried in protection against ALV-J infections. We hypothesized that a multi-epitope based subunit vaccine may elicit immunity against ALV-J infections and would be an alternative approach for ALV-J vaccine development. In the present study, we expressed a chimeric multi-epitope-based protein X (rCMEPX) containing both immunodominant B and T epitope concentrated domains selected from the whole structural protein of ALV-J using bioinformatics approach (Xu et al., 2015), and used it as a multi-epitope-based subunit vaccine. Its protection efficacy against ALV-J infection in chickens was evaluated.

2. Materials and methods

2.1. Cells and viruses

DF-1 cells were grown and maintained in the Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Beijing, China) complemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, 37 °C incubator. ALV-J strain NX0101 was kindly provided by Dr. Zhizhong Cui (Shandong Agricultural University, China) and propagated on monolayer of DF-1 cells.

2.2. Design, construction and expression of the immunogen

Twenty ALV-J full length genome sequences from the NCBI GenBank database were analyzed and the highly conserved fragments from the main structure proteins (Gag, Pol, Env) were selected for epitope prediction. The corresponding protein sequences of NX0101 strain (DQ115805.1) were analyzed by bioinformatics software to predict epitopes. B cell epitopes were predicted by a DNAstar software and online prediction websites (http://imed.med.ucm.es/Tools/antigenic.pl and ProtScale: http://us.expasy.org/) according to the algorithms concerning the hydrophilicity, secondary structure, flexibility, antigenic value and surface probability. T-cell epitopes were analyzed using T-cell epitope online prediction servers (ProPred-I: http://www.imtech.res.in/raghava/propred1/, ProPred MHC Class-II: http://www.imtech.res.in/raghava/propred/ and Rankpep tool: http://bio.dfci.harvard.edu/Tools/rankpep.html). Manual optimizations of results were performed by inspections of amino acid sequences and comparisons of the predicted results of each software. Four T and B-cell linear epitopes concentrated segments from Gag (278–376aa), Pol (784–855aa) and Env (Gp85: 145–156aa and Gp37: 412–538aa) proteins were selected (Table 1) and clustered as a single chimeric multi-epitope gene X by using polymerase chain reaction and ligation reactions. The segments were separated from one another with GAGS flexible peptides to enhance appropriate epitope processing. The chimeric gene X was cloned into pET-30a(+) vector (Novagen, Beijing, China) and expressed in *Escherichia coli* Rosetta (DE3). The expressed recombinant chimeric multi-epitope protein X (rCMEPX) in inclusion bodies was purified using urea purification assay, quantified using a Bio-Rad protein assay kit (Bio-Rad, Beijing, China), and verified using western blot analysis with ALV-J positive serum (Xu et al., 2015). The purified rCMEPX was used as antigen for immunization experiments.

Table 1
Amino acid sequences and positions of epitopes in rCMEPX.

Origin	Epitope type and position (aa)	Amino acid sequences
Gag (278–376aa)	B (281–292)	ALMSSPLLPHDV
	B (294–307)	NLMRVILGPAPYAL
	B (312–321)	WGVQLQTVIA
	B (358–371)	GQAALLRPGELVAI
	Tc (287–295)	LLPHDVTNL
	Tc (298–306)	VILGPAPYA
	Tc (306–314)	ALWMDAWGV
	Tc (314–322)	VQLQTVIAA
	Tc (361–369)	ALLRPGELV
	Tc (368–376)	LVAITASAL
	Th (296–304)	MRVILGPAP
	Th (314–322)	VQLQTVIAA
	Th (368–376)	LVAITASAL
Pol (784–855aa)	B (787–798)	PTVLTEGPPVKI
	B (809–815)	WNVLVWG
	B (828–839)	KVIWVPSRKVKP
	Tc (788–796)	TVLTEGPPV
	Tc (803–811)	GEWEKGWNV
	Tc (812–820)	LVWGRGYAA
	Tc (829–837)	VIWVPSRKV
	Th (812–820)	LVWGRGYAA
Gp85 (145–156aa)	B (145–156)	LPWDPQELDILG
Gp37 (412–538aa)	B (415–422)	LACWSVKQ
	B (424–432)	NLTSLILNA
	B (440–447)	IRHAVLQN
	B (450–473)	AIDFLLLAQGHGCQDVEGMCCFNL
	B (475–484)	DHSESIHKAL
	B (516–533)	LAKGVKTLLFALLVIVCL
	Tc (421–429)	KQANLTSLI
	Tc (432–440)	AILEDTNSI
	Tc (465–473)	VEGMCCFNL
	Tc (472–480)	NLSDHSESI
	Tc (515–523)	WLAKGVKTL
	Tc (525–533)	FALLVIVCL
	Th (412–420)	IERLACWSV
	Th (454–462)	LLLAQGHGC
	Th (465–473)	VEGMCCFNL
	Th (509–517)	FGGLGGWLA
	Th (528–536)	LVIVCLLAI

2.3. Immunization

Fifty one-day-old specific-pathogen-free (SPF) chickens were purchased (Shandong Institute of Poultry Science, PR China) and housed in a SPF environment at the Laboratory Animal and Resources Facility, Shandong Agricultural University. The study protocol and all animal studies were approved by the Shandong Agricultural University Animal Care and Use Committee (SACUC Permission number: AVM120301-10). The chickens were randomly divided into two groups (25 chickens each) and immunized on 1, 3 and 5 weeks old respectively. The chickens in group I were injected subcutaneously with 200 μg purified rCMEPX and that in group II with PBS as negative control. Pre-immune sera were collected from each chicken before the immunization. For the primary immunization, the rCMEPX was mixed with an equal volume of complete Freund's adjuvant (Sigma, Beijing, China). Chickens were boosted twice at a 2-week interval with same immunogen emulsified in equal volume of incomplete Freund's adjuvant (Sigma, Beijing, China). Sera from five chickens per group were collected randomly 1 week after each immunization for anti-rCMEPX specific antibodies detections.

2.4. Evaluation of the immune effect of rCMEPX

2.4.1. Determination of antibody titers by indirect ELISA

Flat-bottomed 96-well plates were coated with 100 μL purified rCMEPX per well at the concentration of 0.125 μg/mL in 50 mM