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Immunogenicity and protective efficacy of virus-like particles and recombinant fiber proteins in broiler-breeder vaccination against fowl adenovirus (FAdV)-8b

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ABSTRACT

Inclusion body hepatitis (IBH) is an economically important diseases in broiler chicken industry. Several serotypes of fowl adenovirus (FAdV) can cause IBH, among them, serotype FAdV-8b is associated with the majority of the IBH cases in Canada. Here, we evaluated FAdV-8b virus-like particles (VLPs) and recombinant FAdV-8b fiber proteins (expressed in *E. coli*) as potential broiler-breeder vaccines against IBH. For assessing the immunogenicity of vaccines, we investigated both humoral and cellular immunity. The humoral immune response was evaluated by determining total IgY and virus-neutralizing antibody in serum at 14, 28, 35 and 60 days post-immunization (dpi). We examined cellular immunity using flow cytometry by determining CD4:CD8 ratio change in peripheral blood after the booster vaccination. The protective effect of vaccines was tested by challenging 14 day-old progeny (n = 30/group) carrying maternal antibodies (MtAb) by challenging with virulent FAdV-8b virus (1×10^7 $TCID_{50}$, FAdV-8b-SK). Although total IgY levels were comparable in all groups, the neutralizing antibody response in broiler-breeders at 35 and 60 dpi was significantly ($p < 0.05$) higher those vaccinated with FAdV-8b VLPs followed by FAdV-8b fiber compared to fiber-knob. Moreover, vaccines comprised of FAdV-8b VLPs and FAdV-8b fiber rather than FAdV-8b fiber-knob efficiently elicited the cell-mediated immune response as evidenced by a statistically significant ($p < 0.05$) $CD8^+$ T-cell proliferative response in broiler-breeders four days after the booster vaccination. Unlike FAdV-8b fiber-knob, FAdV-8b VLPs, and FAdV-8b fiber vaccinated broiler-breeders were able to transfer a substantial amount (28.4 ± 9%) of MtAb to their progeny. Challenge revealed that MtAb provided 100% and 82.7% protection in progeny hatched from FAdV-8b VLPs, and FAdV-8b fiber vaccinated broiler-breeders, respectively. Collectively, our data suggest that FAdV-8b subunit vaccine-induced MtAb efficiently protected progeny against clinical IBH and broiler-breeder vaccination with subunit vaccines is a potential approach to protect against IBH.

1. Introduction

Fowl adenoviruses (FAdVs) are non-enveloped, double-stranded (ds) DNA viruses of the genus *Aviadenovirus* in the family *Adenoviridae* [1]. They are classified into five species (A-E) [2] and twelve serotypes (FAdV 1–7, 8a, 8b, 9–11). FAdV 1 and FAdV 4 are associated with gizzard erosion and ulceration (GEU) and hydropericardium hepatitis syndrome (HHS), respectively [3]. Whereas, serotypes FAdV 2, 3, 8a, 8b, 9 and 11 cause inclusion

Abbreviations: CTL, cytotoxic T-cell-mediated immunity; $TCID_{50}$, tissue culture infective dose 50; PBMC, peripheral blood mononuclear cells; H&E, hematoxylin and eosin; FAdV, fowl adenovirus; MtAb, maternal antibodies; NAb, neutralizing antibodies; VLPs, virus-like particles; dpi, days post immunization; IBH, inclusion body hepatitis.

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body hepatitis (IBH) [4–6], a worldwide problem [4,7,8]. FAdVs infect 2- to 5-week-old neonatal chicks [3]. The majority of cases of IBH in Canada, Australia, Hungary and South Korea have been associated with FAdV 8a [7,9–11]. The disease is characterized by liver necrosis, hemorrhage and basophilic intranuclear inclusion bodies in the hepatocytes [12,13]. Mortality associated with the disease ranges from 10% to 30% [5,6]. In the broiler industry, broiler-breeders are vaccinated with autogenous vaccines to control IBH in broiler chicks [4,14,15] through maternal antibody transfer (MtAb) [16]. Despite the use of autogenous vaccines, sporadic outbreaks of IBH occur in the vaccinated chicks [15,17] incurring considerable economic losses [12,18]. Hence, the development of effective and safe IBH vaccine is highly desired.

Among the various vaccine types, subunit or virus-like particle (VLP) based vaccines are potentially good alternatives to autogenous vaccines due to their safety, effectiveness and easy of production on large scale. A subunit vaccine is a recombinant small immunogenic component of a microorganism which elicits a protective immune response in combination with an adjuvant [19]. A VLP is a type of subunit vaccine, which mimics the natural virus conformation, but lacks a complete genome [20,21]. VLPs for Newcastle disease virus have been developed [22]; though, these vaccines are only in the preliminary stages of animal testing. Several fowl adenoviral proteins, like hexon, penton, and fiber capsid, have been identified as potential subunit vaccine antigens [10,23–26]. Among these, FAdV fiber protein is the most suitable immunogen for a subunit vaccine due to its ability to induce neutralizing antibody responses [25,26] as well preventing clinical disease (HHS) in chicks upon exposure to a lethal virus dose [10]. Previous studies on subunit vaccines against FAdVs examined protective immunity (humoral and cellular) by directly challenging vaccinated chicks. However, it remains to be seen if subunit vaccines can be used in broiler-breeder vaccination programs, wherein, progeny acquire only humoral immunity via maternal antibody (MtAb) transfer.

Therefore, the present study was designed to investigate the suitability of various subunit vaccines as a broiler-breeder vaccine against IBH. Here, we compared the immunogenicity of FAdV-8b VLPs, recombinant fiber, and fiber-knob in broiler-breeders. The protection efficacy against lethal virus was evaluated through progeny challenge. Results suggest that subunit vaccines may provide a promise for use in broiler-breeder vaccination programs against IBH.

2. Material and methods

2.1. Virus, cell line, and antibodies

A fowl adenovirus (FAdV-8b-SK) isolated from a clinical case of IBH in the broiler chicken industry in Saskatchewan was used in this study. Based on the loop-1 hexon protein sequence, it had 93.9% amino acid similarity to FAdV-8b [27,28] and FAdV-8b strain 764 [29]. Leghorn male hepatoma (LMH) cell line was (ATCC #CRL-2117) [30] used to propagate FAdVs. Anti-6x-His epitope tagged mouse monoclonal antibody and AP conjugated goat-anti-mouse IgG (H + L) polyclonal antibody were purchased from ThermoFisher Scientific (Waltham, MA, USA).

2.2. Purification of FAdV-8b-SK VLPs

The empty capsids of FAdV-8b, lacking genome, hereafter, referred to a VLPs, were conventionally purified using Cesium Chloride (CsCl) density gradient method. Briefly, supernatant containing VLPs was loaded onto CsCl (1.25/1.34 g/ml) cushion and spun at 35,000 rpm for 24 h at 10 °C. The capsid (upper band) and virus (lower band) was aspirated separately and dialyzed in 0.1 M Tris-HCl (pH-8) three times for 8 h each. The capsid protein concentration was measured by Bradford assay in 96-well plate as per manufacturer's instructions. The optical density (OD) was read in SoftmaxPro5 data acquisition and analysis software (Molecular Devices, LLC, Sunnyvale, CA, USA) at 590 nm. Generation of VLPs was confirmed by transmission electron microscopy (TEM). Samples for negative staining and TEM were processed using standard techniques as described before [31].

2.3. Cloning and expression of fiber and fiber-knob of FAdV-8b-SK

Full-length fiber gene (1575 bp DNA, ~62 kDa protein) was amplified using forward primer (5′AA*GCATGC*ATGGCGAC CTCIACTCCTCA-3′) and reverse primer (5′- ATT*AAGCTT*TTACG GAGCGTTGGCTGTGCTTAGGG-3′). Whereas, the fiber-knob of FAdV-8b-SK (453 bp DNA, ~15 kDa protein) was amplified using forward primer (5′-CG*GGATCC*TATTTCACGTTCTGGGTAGG-3′) and reverse primer (5′-ATT*AAGCTT*TTACGGAGCGTTGGCTGTGCTTAG GG-3′). The purified PCR products of fiber or fiber knob were cloned between *SphI* and *Hind III* or *Bam HI* and *Hind III* sites of the linearized pQE30 plasmid vector (Addgene, Cambridge, MA, USA) in frame with the 6X His gene, respectively. The products were named as pQE30-F8b-SK or pQE30-K8b-SK, respectively.

2.4. Protein expression and purification

pQE30-F8b-SK or pQE30-K8b-SK plasmids were transformed into *M17 E. coli* cells and proteins were purified by affinity column chromatography using Profinity™ IMAC Nickel Charged Resins (Biorad Laboratories, Inc) as per the manufacturer's protocol.

2.5. Broiler-breeder vaccination

Day-old broiler-breeders were obtained from Aviagen (Alabama, USA) and were reared in the animal care facility of the Western College of Veterinary Medicine as per Aviagen guidelines. The animal experiments were conducted following the approved protocols from Animal Research Ethics Board (AREB) of the University of Saskatchewan. At the age of 30 weeks, the birds were randomly divided into four groups (n = 20 birds/group) and day 0 blood samples were collected. Each of the three groups received FAdV-8b VLPs, FAdV-8b fiber or FAdV-8b fiber-knob (50 μg/bird) with 20% Emulsigen-D (MVP technologies, Omaha, NE, USA) were injected intramuscularly in the pectoral muscle. The fourth group was kept as an unvaccinated control. Twenty-one days after the primary vaccination, immunized groups were boosted with the respective vaccine (50 μg/bird). Sera (n = 10) were collected at 14, 21, 28, 35 and 60 days post-immunization (dpi) to determine serum neutralization. Also, blood was collected at 21 days after primary and four days following booster (25th day) vaccination to measure $CD4^+$ and $CD8^+$ T-cells in PBMC.

2.6. Progeny challenge

Three weeks after the booster vaccination, eggs were collected from the immunized and unimmunized broiler-breeders to produce chicks (n = 30/group) for the progeny challenge. Sera samples were collected at day-13 post-hatch (dph) to test for maternal antibodies against FAdVs. Progeny were challenged with FAdV-8b-SK ($1x10^7$ $TCID_{50}$/bird) at 14 dph. Cloacal swabs (n = 5) were collected from all the groups before FAdV challenge (day-0) and at days 3, 7 and 10 post-challenge (dpc) to determine fecal shedding of FAdV. Briefly, cloacal swabs were collected with dry cotton swabs (Satrplex) and weighted before placing in 1 ml Tryptose broth (TB) (Sigma) supplemented with an antibiotic-antimycotic solution