Construction and immunogenicity of recombinant fowlpox vaccines coexpressing HA of AIV H5N1 and chicken IL18

Ma Mingxiao a,b,1, Jin Ningyi a,∗, Wang Zhenguo a, Wang Ruilin a,1, Fei Dongliang a, Zheng Min a, Yin Gefen a, Li Chang a, Jia Leili a, Jin Kuoshia, Zhang Yingjiuc

a Genetic Engineering Laboratory, Academy of Military Medical Sciences, Changchun 130062, PR China
b College of Animal Science and Veterinary Medicine, Jin Zhou Medical University, Jinfen 121001, PR China
c Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, Jilin University, Changchun 130023, PR China

Received 10 November 2005; received in revised form 17 February 2006; accepted 2 March 2006
Available online 29 March 2006

Abstract
cDNAs of the HA genes of subtype H5N1 AIV were fused to form a single open reading frame, designated H5HA-H7HA. The H5HA-H7HA cDNA and chicken Interleukin-18 (IL18) were inserted into the fowlpox virus (FPV) expression vector pUTA-16-LacZ to produce pUTAL-H5-H7-IL18. cDNA of H5N1 AIV HA was inserted into the FPV expression vector pUTA2 to create the recombinant expression plasmid pUTA2-H5. Plasmids were then co-transected into CEF cells. The two recombinant fowlpox viruses (rFPV) were produced by three cycles with the BrdU and verified by RT-PCR, IFA and Western blotting. One-day-old specific pathogen free (SPF) chickens and 7-day-old commercial Leghorn egg-laying chickens were inoculated with 10^6 PFU recombinant or parental fowlpox vaccine viruses by wing-web puncture. Hemagglutination inhibition (HI) antibody titer and nonspecific cellular immunity level were assessed after 1–3 weeks post-immunization. We found that all rFPV-vaccinated groups produced HI-specific antibodies, and the level of cellular immunity induced by the rFPV-H5-H7-IL18 strain was significantly higher than that induced by rFPV-H5HA. At 3 weeks post-inoculation, immunized SPF and Leghorn chickens were challenged with H5N1 HP AIV. The rFPV-H5-H7-IL18 vaccine strains were able to induce complete (10/10) protection, while the rFPV-H5HA vaccine strain induced (9/10) protection. Cloacal swabbing samples were collected from immunized Leghorn chickens during the first week post-challenge; no shedding was found in the rFPV-H5-H7-IL18 vaccinated group. The rFPV-H5-H7-IL18 vaccinated group displayed significantly increased weight gain relative to the rFPV-H5HA group. This study reports a significant step in the further development of new AIV vaccines.

© 2006 Elsevier Ltd. All rights reserved.
Keywords: AIV; rFPV vaccine; Immunogenicity; Hemagglutinin, IL18

1. Introduction
Avian influenza (AI) is an infectious disease caused by avian influenza virus (AIV), which is divided into 15 hemagglutinin (HA) subtypes and 9 neuraminidase (NA) subtypes on the basis of antigenicity of the HA and NA surface glycoproteins. Highly pathogenic avian influenza (HPAI) is caused by particular AIV subtypes such as H5 or H7 and is classified as a disease by the Office Internationale des Epizooties [1].

Outbreaks of severe disease caused by influenza viruses have been reported frequently in recent years, leading to substantial economic loss. In 1997, a highly pathogenic H5N1 AIV strain was transmitted directly from birds to humans in Hong Kong [2,3].

Since then H7 and H9 AIV have been identified from humans and other mammals. These facts have made clear that the avian influenza virus can directly cross the species barrier and replicate in mammals, causing severe disease, which were proved by the event AIV conveying form birds to humans in Hong Kong [4,5]. Presently, the H5 and H7 HPAIV remain active in Southeast Asia and other parts of the world [6,7], necessitating the development of an efficacious...
vaccine that can protect chickens from multiple subtypes of lethal AIV and block spread of the virus.

It is believed that inactivated whole AIV virus vaccines show sufficient efficacy against HPAIV, but they also induce immune responses to the group-specific influenza A nucleoprotein (NP) antigen, which interferes with epidemiological surveillance by prohibiting direct serological distinction between vaccinated and field-exposed birds [8]. The basis of protective humoral immunity is the development of neutralizing antibody against HA and a variety of vaccines derived from HA gene of AIV, including recombinant virus vaccines [4,9,10], subunit hemagglutinin protein, and DNA vaccines [11] that could induce subtype-specific immunity and have shown efficacy against challenge with homologous virus are under development.

FPV have a large double stranded DNA genome and a host range limited to avian species [12] and have been used to successfully express protective immunogen genes from several viruses, including the Newcastle disease virus, Marek’s disease virus, foot and mouth disease virus, HIV and others. Fowlpox virus vectors for poultry, it has a great deal of advantages, but its toxico-side effect cannot be neglected: its inhibitory action for the increase of chicken's weight and immune function [5]. It has been confirmed that some cytokines can relieve its toxico-side effect. Some of the cytokines have been proven to be effective immunomodulator in animal model or clinical test in many reports, For example, IL-1, IL-8, IL-12, IFN, colony-stimulating factor and TNF, etc. as the immunomodulator, it has been demonstrated that the cytokine adjuvant can promote the vaccines of bacterium, virus, parasite to induce protective immune response, and enhance the vaccines effect of immunoprotection [13], so the cytokine adjuvant is widely used lately. In 2000, the cDNA encoding IL18 of chicken was cloned for the first time by Schneider et al. [14], and expressed in the Bacterium coli, it indicated that the recombinant IL18 of chicken has the biologic activity of inducing IFN-γ production. In 2003, Thomas et al. [15] reported that the IL18 of chicken has a great many biologic activities, as the Th cell effective activator, that it can induce CD4+ T cell to secrete IFN-γ and the proliferation of T cell, in addition, it has the positive accommodation effect for the composition of MHC Class-I Ag. In this study, we have constructed three recombinant fowlpox viruses that coexpressing the HA genes of H5 and H7 subtype AIV and the chicken IL18 gene, which was included to overcome the FPV vector-induced inhibition and increase the efficacy of immunization.

2. Materials and methods

2.1. AIV isolated (H5N1) strain inactivated vaccine and objective gene

Inactivated whole AIV (H5 subtype) vaccine was purchased from HaErBin, 282E4 FPV was purchased from the Department of Virology of the China Institute of Veterinary Drug Control (TCID50 = 1 × 10^5.0 ml). AIV (A/Chicken/JiLin/9/2004) strain (ELD50 = 1 × 10^{-7.0} ml, LD50 = 1 × 10^{-6.0} ml) from chickens was isolated by Genetic Engineering Laboratory of Academy of Military Medical Sciences. cDNAs encoding the whole HA genes of H5N1 subtype AIV were previously cloned and sequenced [16]. H5N1 subtype AIV whole HA cDNA and chicken IL18 were previously cloned and sequenced [17]. A cDNA copy of HA gene of H5N1 subtype AIV was previously manually synthesized from Biotechs Co. Ltd. (Dalian, China). Goad anti-chicken IgG antibodies labeled by the alkaline phosphatase, NBT/BCIP and Non-Radioactive Cytotoxicity Assay Kit were the products of Promega (USA).

2.2. Animals

One-day-old chickens were provided by the Changchun Academy of Agricultural Sciences and 9–10-day-old specific-pathogen free (SPF) chickens were purchased from Harbin Veterinary Research Institute. All experiments commenced in the under pressure isolator after the chickens had adapted to the environmental chamber for at least 1 week. All animal experiments were approved by the Harbin Veterinary Research Institute.

2.3. Plasmids, vectors and chicken embryo fibroblasts (CEF)

The fowlpox virus vector plasmid pUTA1L, which was composed of the combined promoter ATI-P7.5 (ATI promoter of cowpox virus and 20 tandemly repeated mutant P7.5 early promoters of vaccinia virus), with LacZ gene controlled by the single promoter (16 tandemly repeated mutant early p7.5 promoters of vaccinia virus) as a reporter gene. 282E4 strain of fowlpox virus (FPV282E4) is an attenuated vaccine produced by Animal Pharmaceutical Factory of Nanjing (Nanjing, China). The FPV expression vectors, pUTA2-LacZ and pUTA2 [18,19], and recombinant plasmids encoding chicken IL18 and HA cDNA clones of H5 and H7 AIV were used [16,17]. The CEF was produced using method referenced Cell A Laboratory Manual (translated by Pei Tang Huang et al., Science Publishing Company, p. 71-8).

2.4. The homologous recombination and screening of the recombinant virus

cDNAs encoding the whole HA genes of H5N1 subtype AIV was cloned into vector pUTA2 under the control of ATI-P7.5 × 20 promoter to produce the plasmid, pUTA2-H5. To create the plasmids, pUTA1L-H5-H7-IL18, the HA cDNAs of subtype H5N1 and H7N1 were ligated together to form a single opening read frame (ORF), then subcloned along with chicken IL18 into the expression vector, pUTA16-LacZ under the respective control of the ATI-P7.5 × 20 and...
P.5 × 16 promoters. In the same way, the plasmid pUTAL-HS-IL18 was constructed. The restriction enzyme digestion, ligation, transformation were carried out according to the method of Sambrook et al. [20].

The recombinant fowlpox virus was prepared as described previously [19,21,22]. Briefly, three recombinant plasmids described above and fowlpox virus of 282E4 strain were cotransfected to 80% confluent CEF cells via liposome. The viruses were collected after a cytopathic effect (CPE) appeared, and the viruses were screened under the presence of 40 μg/ml BUdR for three times, and then cultured in MEM medium without BUdR. The individual virus phage was picked out, amplified and purified when CPE appeared. Then the recombinant FPV were identified by RT-PCR, Western blotting analysis was carried out as described previously [20].

2.5. PCR analysis of the recombinant virus

The genomic DNA of recombinant virus, extracted by the method of SDS-Protease K-Phenol, was used as PCR templates and amplifications were performed with TaKara LA Taq polymerase applying 30 cycles of 94°C for 1 min, 55°C for 30 s, and 72°C for 1 min. The HA and IL18 specific primers: H5 (sense: 5′-ATGCCTTTTG-3′, antisense: 5′-ATGGTGCA-3′), IL18 (sense: 5′-ACCCGGCA-TCACTTGCAGTTCCAAT-3′), H7 (sense: 5′-AAAAATGAAACACCTCAAAAT-3′, antisense: 5′-AACCTTATATACAA-ATGGTGGCA-3′), IL18 (sense: 5′-GAAGATGGAAATGGC-ATGCTTTTGG-3′, antisense: 5′-TGGATATCGAGATGG-AATGCGAAGCTTTTG-3′).

2.6. Western blotting analysis

Western blotting analysis was carried out as described previously [20]. Briefly, CEF cell layer was infected by PCR positive recombinant virus at a MOI (multiplicity of infection) of 10, and the infected cells were collected when 90% cytopathogenic effects appeared. After the cell suspension was centrifuged for 10 min at 2000 × g, the cell pellet was suspended in PBS and sonicated for 4 × 15 s, then the suspension was centrifuged for 10 min at 2000 × g, and the supernatant was collected for measurement of virus PFU. PFU of FPV and rFPV inoculated Plaque forming units of rPFV-HSHA, rPFV-HSHA-IL18, rPFV-HSHA-HSHA-IL18, and 282E4 FPV were calculated by inoculation of 10-fold serial dilutions into CEF cells.

2.8. Immunization and challenge infection

Vaccine trials and challenge experiments in specific pathogen free (SPF) chickens and commercial egg-laying Leghorn chickens were conducted in BL3+ facility. All immunizations were done by wing-web puncture with a double needle. Approximately 50 μl inoculum containing 10⁶ PFU of FPV was administered to each chicken according to State Standard of the People’s Republic of China. All challenge experiments were done 3 weeks after the administration of vaccine. The challenge was homologous (virus subtype identical to that of immunizing gene).

2.8.1. Experiment 1

Fifty 1-day-old SPF chickens randomly assigned into five groups of 10 chickens were inoculated as described above. These groups of chickens were vaccinated with rPFV-HSHA, wt-FPV, rPFV-HSHA-IL18, rPFV-HSHA-HSHA-IL18, inactivated vaccine respectively. Another 1-day-old SPF-chickens (n = 20) remained untreated. At 3 weeks post-vaccination, each group was challenged with 0.1 ml of 10⁶ ELD₅₀ of H5 subtype HPAI virus (A/Chicken/Jilin/9/2004) by intramuscular injection into the pectoral muscle. Control chickens were divided into two subgroups and a group was challenged. The chickens were monitored daily for 2 weeks for survival and clinical signs of infection, which include swollen face, malaise, loss of appetite, diarrhoea, cyanosis of the comb or wattles, and paralysis and death.

2.8.2. Experiment 2

Seventy 7-day-old commercial egg-laying Leghorn chickens were randomly assigned into seven groups for vaccination and challenge. Vaccination and challenge was performed according to the method described above. Group 7 was used as control which was not immunized and challenged. Mortality and survival was monitored daily to calculate the protection ratio as described before [23]. Seven days after challenge, cloacal swabs were collected for virus isolation from each group.

2.9. Serology

HA and hemagglutinin inhibition (HI) assays were performed with 0.5 chicken red blood cells as previously described [24]. Sera from chickens were tested individually after treatment with receptor-destroying enzyme. HI titers were determined as the reciprocal of the highest serum dilution that completely inhibited hemagglutination. (Diagnostic

2.10. Comparison of spleen lymphocytes proliferation and the effect of rFPV on the body weight

Fifty 1-day-old commercial Leghorn chickens were randomly assigned into five groups for comparison of spleen lymphocyte proliferation. Seven days after breeding, chickens were immunized as above with rFPV-H5HA, rFPV-HSHA-IL18, wt-FPV, rFPV-HSHA-H7HA-IL18 or PBS as a control.

To investigate the effect of rFPV on body weight, 100 one-day-old SPF chickens were immunized in the same manner. At 1 and 2 weeks after immunization, all SPF chickens were weighed.

2.11. Statistical analysis

Differences between groups were analyzed by Student’s t-test. Results were expressed as the mean ± standard deviation (S.D.). P values less than 0.05 were considered statistically significant in all cases.

3. Results

3.1. Construction and identification of the recombinant plasmids

To prepare recombinant fowlpox viruses expressing H5N1, coexpressing H5N1 and IL18, coexpressing H5N1, H7N1 and IL18, and three recombinant plasmids were constructed (Fig. 1). The fowlpox virus expression vector pUTA2 was used to construct the plasmid pUTA2-H5. The fowlpox virus expression vector pUTAIL was flanked with TK gene and contained two kinds of promoters whose directions were opposite and reporter gene LacZ. The reporter gene LacZ, which was located on the downstream of the single promoter P7.5, was replaced with IL18 gene, and HA genes were inserted into the downstream of the combined promoter ATI-P7.5. The restriction endonuclease digestion pattern showed that the two genes were inserted correctly into the vector (data not shown).

3.2. Screening of recombinant virus by PCR analysis

To detect whether the interest genes were inserted successfully into the genome of fowlpox virus via homologous recombination, the genome of the recombinant fowlpox virus was extracted as the template and PCR was performed with three pairs of specific primers. Three specific DNA bands of 1.7 kb were amplified for positive recombinant virus, whereas there was no specific DNA band was detected for negative virus (data not shown).

3.3. Biological characteristics of HA protein expressed by rFPV-H5, rFPV-H5-IL18 and rFPV-H5-H7-IL18 identified by Western blot

AIV HA proteins expressed by rFPV-H5, rFPV-H5-IL18 and rFPV-H5-H7-IL18 were revealed by Western blot analysis (Fig. 2). The PCR positive recombinant virus was inoculated into CEF cell, and the infected cells were collected and
Fig. 2. Detection of expressed protein by Western blot assay: (1) prestained protein marker (broad range); (2) H5HA expressed by rFPV-H5HA-IL18; (3) H5HA expressed by rFPV-H5HA; (4) H7H9HA expressed by rFPV-H5HA-H7HA-IL18.

the cellular lysates were prepared after CPE appeared. The lysates were sampled into SDS-PAGE and transferred into nitrocellulose membrane. The proteins were probed by anti-H5N1 antibodies and goat anti-chicken antibody conjugated with alkaline phosphatase sequentially and visualized with NBT/BCIP. The results showed that the recombinant virus expressed the foreign proteins in infected CEF cells. Two bands with molecular weights of 56 kDa and one band with molecular weights of 112 kDa were detected, demonstrating that HA from AIV H5 and H7 subtype were expressed in CEF cells (Fig. 3).

3.4. Protection induced by immunization with rFPV-H5HA-H7HA-IL18, rFPV-H5HA-IL18 and rFPV-H5HA

Chickens immunized with rFPV-H5HA-H7HA-IL18, rFPV-H5HA-IL18 and rFPV-H5HA were challenged with 0.1 ml isolated H5N1 AIV. Morbidity, mortality, and reduced viral shedding were detected 2 days after infection with an otherwise lethal dose of virus. SPF chickens immunized with 10^6 PFU rFPV-H5HA-H7HA-IL18 and rFPV-H5HA-IL18 were completely protected (10/10). None of these chickens presented clinical signs of AIV infection or died after challenge with H5 subtype HPAIV; whereas, 9 out of 10 of the SPF chickens immunized with the inactivated whole AIV (H5 subtype) vaccine or rFPV-H5HA were protected. Similarly, commercial Leghorn egg laying chickens immunized with 10^6 PFU rFPV-H5HA-IL18 and rFPV-H5HA-H7HA-IL18 were also 100% (10/10) protected, while were 9/10 were protected when immunized with inactivated whole AIV (H5 subtype) vaccine and rFPV-H5HA. In contrast to the vaccine groups, control chickens injected with wt-FPV or PBS did show clinical signs by the third day of infection and died within a week after challenge. Furthermore, we were unable to isolate virus from chickens immunized with rFPV-H5HA-H7HA-IL18 and rFPV-H5HA-IL18 1 week post-challenge, while virus was discovered in one chicken immunized with rFPV-H5HA. All surviving chickens of the control groups were found to shed challenge viruses in the cloaca. These results demonstrate that immunization with rFPV-H5HA-H7HA-IL18, rFPV-H5HA-IL18 and rFPV-H5HA is able to protect chickens from lethal challenge with H5 subtype of HPAI viruses.

Seven days following challenge, AIV was isolated and propagated in the allantoic cavities of 11-day-old embryonated chicken eggs. The isolated viruses were characterized

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose of inoculation</th>
<th>Vaccination age (days)</th>
<th>Ratio of chicken shedding virus 7 days post-challenging</th>
</tr>
</thead>
<tbody>
<tr>
<td>rFPV-H5HA-IL18</td>
<td>10^6 PFU</td>
<td>7/28</td>
<td>0/10</td>
</tr>
<tr>
<td>rFPV-H5HA-H7HA-IL18</td>
<td>10^6 PFU</td>
<td>7/28</td>
<td>0/10</td>
</tr>
<tr>
<td>rFPV-H5HA</td>
<td>10^6 PFU</td>
<td>7/28</td>
<td>1/10</td>
</tr>
<tr>
<td>Inactivated vaccine</td>
<td>0.2 ml</td>
<td>7/28</td>
<td>1/9</td>
</tr>
<tr>
<td>wt-FPV</td>
<td>10^6 PFU</td>
<td>7/28</td>
<td>–</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.2 ml PBS</td>
<td>7/28</td>
<td>–</td>
</tr>
</tbody>
</table>

Negative control all died.

Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose of inoculation</th>
<th>Vaccination age (days)</th>
<th>Ratio of chicken shedding virus 7 days post-challenging</th>
</tr>
</thead>
<tbody>
<tr>
<td>rFPV-H5HA-IL18</td>
<td>10^6 PFU</td>
<td>7/28</td>
<td>0/10</td>
</tr>
<tr>
<td>rFPV-H5HA-H7HA-IL18</td>
<td>10^6 PFU</td>
<td>7/28</td>
<td>0/10</td>
</tr>
<tr>
<td>rFPV-H5HA</td>
<td>10^6 PFU</td>
<td>7/28</td>
<td>1/10</td>
</tr>
<tr>
<td>Inactivated vaccine</td>
<td>0.2 ml</td>
<td>7/28</td>
<td>1/9</td>
</tr>
<tr>
<td>wt-FPV</td>
<td>10^6 PFU</td>
<td>7/28</td>
<td>–</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.2 ml PBS</td>
<td>7/28</td>
<td>–</td>
</tr>
</tbody>
</table>

Negative control all died.

Fig. 3. Splenocyte lymphocytes proliferation stimulated by Con A.
by hemagglutination inhibition assays with subtype-specific antisera against HA (Table 1). These results demonstrated that the rFPV described here, especially rFPV-HSHA-H7HA-IL18 and rFPV-HSHA-IL18, which was more effective than rFPV-HSHA, can remarkably reduce the release of AIV.

### 3.5. Antibody responses of chicken’s immunized with rFPV

We examined the relative capacities of HA-rFPV vaccines to induce serum antibodies. Serum samples from chickens of all groups were collected for analysis of the anti-HA antibody levels by HI (Tables 2 and 3). Although SPF chickens immunized with rFPV-HSHA-H7HA-IL18, rFPV-HSHA-IL18 and rFPV-HSHA were positive for HA antibody after 1 week, the level was very lower. However, the HI antibody titers increased by 2–3 weeks after immunization. A similar change was observed in immunized commercial Leghorn chicken.

### 3.6. Comparison of spleen lymphocyte proliferation and the effect of rFPV on body weight

Two weeks after vaccination, splenocytes were collected from commercial Leghorn chickens and stimulated with concanavalin A (Con A) free of pathogenic microorganisms. As shown in Fig. 3, proliferation of lymphocytes from the rFPV-HSHA-H7HA-IL18 and rFPV-HSHA IL18 immunized chickens were significantly higher than that of chickens immunized with rFPV-HSHA or wt-FPV (P < 0.05). We suggest that expression of IL18 by rFPV-HSHA-H7HA-IL18 and rFPV-HSHA-IL18 is responsible for this effect and may enhance immunization with FPV.

Immunized SPF chickens were weighed every week after immunization (Table 4). Chickens vaccinated with rFPV-HSHA-H7HA-IL18, rFPV-HSHA-IL18 or unvaccinated controls had higher body weights than chickens vaccinated with rFPV-HSHA or wt-FPV, suggesting that IL18 can reduce the effects of the FPV vector on chicken body weight.

### 4. Discussion

When some H5 and H7 AIV detected in infected poultry, control measures are frequently instituted to prevent the spread of virus due to the potential for a virulence shift resulting in a serious disease outbreak [25]. Conventional inactivated vaccines have been considered to be effective for the control and prevention of avian influenza outbreaks, but the host response, antibodies against the vaccine NP antibody, prevents distinction between vaccinated and field-virus exposed birds. A more advanced approach may be to use a recombinant vaccine that avoids the above caveats. Fowlpox viruses are a member of the avipoxvirus genus of the family poxviridae and possess many of the desirable characteristics of vaccinia virus [26]. Fowlpox viruses undergo abortive replication in nonavian cells and cannot be adapted to produce infective progeny virus in mammalian tissue. They readily infect mammalian cells in vitro and induce synthesis of foreign genes products, which are displayed on the cell membrane in the case of viral surface glycoproteins [27,28]. Despite their inability to replicate in these hosts, recombinant fowlpox virus vaccines have induced protective immune responses to inserted foreign genes products in several mammalian species. The lower level of antigen production from fowlpox virus vaccines compared to vaccinia virus may preferentially stimulate T-cell rather than antibody responses. Furthermore, antigenic competition from immune responses to vector antigens is likely to be lower for the weakly replicating fowlpox virus than for vaccinia virus. And fowlpox viruses only propagate strictly in the cytoplasm, which avoids the possibility of the integration of FPV genome into the host’s chromosome, they are safer as vaccine vectors. Therefore, fowlpox viruses are used not only as a live vector vaccine for avian disease, but also as an ideal vector for mammals and humans [29].

HA is the major protective antigen of AIV and HA antibodies against it constitute the primary defense against virus infection. Thus it is an appropriate gene to produce genetically engineered vaccine. The protective effect of various
kinds of vaccines targeting HA protein has been established in previous studies, but this immunity is limited to the specific homologous HA subtype [30]. When compared to conventional vaccines, these recombinant vaccines have the advantage of not interfering with epidemiologic surveillance studies, which detect antibodies against NP, to monitor natural infection. In addition, immunization with recombinant NP, or NP expressed by pox vectors induces only partial protection against challenge infection with homologous influenza virus [31,32]. In the current study, chickens inoculated with the rFPV expressing fused HA genes of H5 and H7 AIV and chicken IL18 were protected from intramuscular challenge with H5 AIV. Neither viral replication nor clinical signs of infection were observed in these chickens. Meanwhile, chickens inoculated with rFPV expressing only HA from H5 AIV were also protected from H5 AIV challenge, but some chickens did become infected and local viral replication was observed. Furthermore, rFPV-H5-H7-IL18 and rFPV-H5HA-IL18 vaccinated groups displayed significant body weight gain compared to groups vaccinated with rFPV-H5HA. At 3 weeks post-inoculation, immunized SPF and Leghorn chickens were challenged with H5N1 HPAIV. rFPV-H5-H7-IL18 and rFPV-H5-IL18 vaccine strains were able to induce complete (100%) protection against an otherwise lethal challenge with HPAIV, while the rFPV-H5HA vaccine strain induced 90% (9/10) protection.

We found that all rFPV-vaccinated groups produced HI-specific antibodies, and the level of cellular immunity induced by the rFPV-H5-H7-IL18 and rFPV-H5-H7-IL18 strain was significantly higher than that induced by rFPV-H5HA. The rFPV-H5-H7-IL18 and rFPV-H5-IL18 vaccinated group displayed significantly increased weight gain relative to the rFPV-H5HA group. Through comparison of spleen lymphocyte proliferation and the effect of rFPV on body weight, it showed indirectly that the IL18 can increase the immunogenicity of vaccine just as other study showing [33,34]. Although the H7 challenge experiment was not conducted, the HI-specific antibodies from H7 HA viruses were significant which was shown in Table 3. The protective efficacy exhibited by rFPV-H5-H7-IL18 suggests that it can be a valuable candidate vaccine for the control of H7 AIV and may be for the control of H7 AIV. The effectiveness of this vaccine in actual field application remains to be determined in the future.

Acknowledgements

This work was supported by a grant from the China Department of Science and Technology (CDST) and Jinhua Provincial Department of Science and Technology (JSTD).

References


