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Preparation and immunoprotection of subgroup B avian leukosis virus inactivated vaccine

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ABSTRACT

Objectives: To develop an inactivated vaccine against subgroup B avian leukosis virus (ALV-B) and determine if vaccination of chicken breeders could protect young chicks from ALV-B horizontal infection at early stage and accelerate eradication progress.
Methods: Chicken embryo fibroblast (CEF) cells were inoculated with SDAU09C2 strain of ALV-B and ALV-CEF was inactivated for preparation of oil-adjuvant vaccine. Eggs were collected from un-vaccinated and 9 vaccinated great parent female chickens for incubation. 1-day-old chicks were bled for testing their maternal antibodies to ALV-A/B and then inoculated with ALV-B. Viremia and cloaca p27 detection dynamics were tested and compared between chick groups with or without maternal antibody to ALV.
Results: In 3 weeks after 3 vaccination with the inactivated vaccine, all 9 vaccinated breeders developed high antibody titers against ALV-A/B with ELISA read values of 1.69–1.89 (the positive base line was 0.4) and kept at the high titers for at least another 4 weeks. Maternal antibody was detected in 70% (12/17) of chicks from breeders with high antibody titers to ALV-A/B. Only 4 of 12 chickens with maternal antibodies developed temporary viremia and no viremia was detected in the left 8 maternal antibody positive chickens during the whole 14 week after inoculation of ALV-B at 1 day of age. But the persistent viremia was detected in 2–8 weeks in all 9 maternal antibody negative chickens and the viremia persisted in the whole tested period of 14 weeks after inoculation of ALV-B.
Conclusions: The inactivated ALV-B vaccine could induce high titer antibody reaction to ALV-B, it could provide maternal antibodies to 1-day-old chickens and protect chickens from early infection of ALV-B.

1. Introduction

Avian leukosis is one of the main diseases of causing poultry tumor, whose pathogen is avian leukosis virus (ALV). According to the viral envelope glycoprotein antigenic structure, host range and mutual interference between different strains cultured in cells, ALV can be divided into 10 subgroups A–J, and subgroups A, B, C, D, E and J exist in chickens [1]. Subgroups A, B and J are the main exogenous ALV that cause chicken tumors, but it is rare to hear tumors caused by subgroups C, D. Subgroup E is endogenous leukosis virus, which has low or no pathogenicity to chickens. In the last century before the 80s, avian leukosis virus subgroups A, B caused chicken lymphoid leukemia and various tumors, but in the middle of the 80s, international large breeder companies have basically eliminated the infection of ALV-A and ALV-B. But in 1989, Payne et al. discovered a new virus caused myelocytomas [2,3], that is avian leukosis virus subgroup J. From the last century, the 90s, avian leukosis subgroup J has been widely infected among the meat type broiler chickens, which caused great economic loss to the poultry industry. After decades of control and eradication measures, most of the international large breeder companies have eradicated the infection of ALV-J. In the past decade, there have been a large number of reports about ALV-J epidemic situation in China, the virus initially appeared in introduction of meat type broiler chickens [4–7], then we found that ALV-J also widely existed in egg-type chicken,"yellow" chicken and Chinese local strains [8–11].

In recent decades, China has never carried out any nationwide eradication measures against ALV, exogenous ALV infection exist in chicken flocks in China, especially in the local strains. In recent years, Zhu et al. [12] isolated a strain of ALV-A from Shandong local strains, Zhao et al. [13] isolated a subgroup B avian leukosis virus

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from chickens of Chinese native breed luhua. It shows that chicken has infection of A, B subgroup avian leukosis virus in China.

In foreign countries eradication programs such as antibody testing, antigen testing, and virus isolation were merely mentioned during the process of avian leukemia purifying, pathways of vaccine immune were never mentioned, that is to say that the world has no available avian leukemia vaccine. This research first successfully prepared ALV-B inactivated vaccine in the world, and successfully caused all 9 vaccinated breeders developed high antibody titers against ALV-A/B, and chicks of immunized chickens have maternal antibodies, which can significantly slow down the positive appearance time of viremia and cloaca p27, as well as reduce the positive duration time of viremia and cloaca p27, and significantly reduce the S/P value of viremia and cloaca p27.

2. Materials and methods

2.1. The exploration of virus cell culture conditions

ALV-B SDAU09C2 was isolated from chickens of Chinese native breed luhua by Zhao et al. in 2009 [13]. SDAU09C2 was inoculated into the logarithmic growth phase DF1cells in tissue culture plate 12 well, after 2 h incubation, changing the maintenance fluid of different serum concentrations (0.5%, 1%, 2%, 3%), 150 μl supernatant was collected in centrifugal tube daily, which was used for p27 detection, so as to determine the optimal concentration of serum (newborn calf serum, Gibco). The logarithmic growth phase cells of DF1 in tissue culture plate 24 well were inoculated into SDAU09C2, after 2 h incubation, changing the optimal serum concentration maintenance fluid of different pH value (6.6–7.7), 150 μl supernatant was collected in centrifugal tube daily, which was used for ALV-p27 detection, thereby determining the optimum pH. The logarithmic growth phase cells DF1 were inoculated into SDAU09C2, after 2 h incubation, changing DMEM culture medium (pH = 6.8, containing 0.5% of newborn bovine serum) for 10 days, 150 μl supernatant was collected in two 1.5 ml centrifugal tubes daily, (one tube is used for p27antigen detection, another tube used for $TCID_{50}$ determination). From the result of the above test one can identify the optimized virus cell culture conditions, providing the guarantee for the antigen amount of inactivated vaccine. SDAU09C2 was inoculated into 20 cell culture flask (Corning, NY, USA) of new growth monolayer CEF and passed through 3 passages (the last passage to tissue culture Plate 6 well), maintained under optimal conditions for 5 days, the cell supernatant was taken to test ALV-p27 antigen using ALV-p27 antigen test kit (IDEXX, Westbrook Maine, USA), the residual supernatants were stored in −70 °C for the virus content detection and injection. Chicken embryo fibroblast (CEF) was used to prepare vaccine.

2.2. Indirect fluorescent antibody test (IFA)

The CEF on cover glass was fixed for 5 min using a fixative (acetone:alcohol = 3:2), 1:500 diluted ALV-A/B single factor [14] mouse serum was added and incubated at 37 °C for 45 min; after washing 3 times in PBS, 1:200 diluted sheep anti mouse antibody labeling FITC (Sigma company) was added, effected at 37 °C for 45 min, and washed 3 times in PBS; a drop of 50% glycerol was added on the cover glass, and the experimental results were observed under fluorescence microscopy.

2.3. Virus quantitation

100 μl cells supernatant was taken to make continuous 10-fold dilution, each dilution of the virus was inoculated to one column of a 96 well cell plate. After 9 days under the condition of 37 °C, 5% CO_2, it was tested whether there is ALV-p27 in the supernatant or not, TCID50 was calculated according to the Reed–Muench method.

2.4. The preparation of the cells inactivated vaccine

ALV-CEF cells (10 million ALV-CEF cells per 1 ml) were taken at 37 °C, effected for 24 h with 0.2% volume ratio of formaldehyde and fully emulsified ALV-CEF and MONTANIDE ISA 775 VG (adjuvant) of volume ratio of 2.6:7.4, 30 ml ALV-B inactivated vaccine was prepared.

2.5. Experimental animals and their subgroups, immunization, protective immunity

Multiple inoculations of 1 ml (contain 10 millions ALV-CEF) inactivated vaccine were made into the 35 week grandfather chickens (chest inoculations of 0.5 ml ALV-B inactivated vaccine, thigh inoculations of 0.5 ml ALV-B inactivated vaccine). The eggs were collected (including control group without antibodies) at the highest antibody levels (the second and third week after the third immunization) of Grandfather chickens for incubation. Muscle multi-point injection of SDAU09C2 1 ml (TCID50 of $10^{4.6}$/0.1 ml), was administered to 4 immunized chickens and 4 control group chickens, anticoagulant blood and non-anticoagulant blood and cloacal swabs were collected weekly. 33 chicks were hatched, in which 17 chicks hatched from the eggs of chickens having antibodies and 16 chicks hatched from chickens not having antibodies. Chicks were divided into four groups, in the first group, 13 chicks hatched from chickens having antibodies, abdominal injection of ALV-B(TCID50 of $10^{4.6}$/0.1 ml) was given at the age of 1 day, the second group, 4 chicks hatched with maternal antibodies, abdominal injection of PBS was given (the purpose is to test maternal antibodies dynamics), the third group had 13 chicks hatching from the eggs of chickens not having antibodies, abdominal injection of ALV-B was given at the age of 1 day, the fourth group had 3 chicks hatching from chickens not having antibodies, abdominal injection of PBS was given, as control group. Collected anticoagulant blood and non-anticoagulant blood and cloacal swabs were collected every week.

2.6. Adult chicken ALV-A/B specific antibody test

5 grandparent chickens were immunized 4 times and 0.5 ml non-anticoagulant sanguis was collected weekly, to 4 immunized grandparent chickens at the appearance of high antibodies level, abdominal injection of ALV-B (TCID50 of $10^{4.6}$/0.1 ml) and to 4 grandparent chickens abdominal injection of ALV-B (TCID50 of $10^{4.6}$/0.1 ml) was given, 0.5 ml non-anticoagulant sanguis was collected weekly, serum was separated and tested in Avian Leukosis Virus Antibody (ALV-A/B) Test Kit of IDEXX Company.

2.7. Adult chicken viremia and cloacal swab specificity p27 antigen test

0.5 ml anticoagulant blood was collected before injection of ALV-B for viremia test, cloacal swabs were taken for p27 antigen test. After injection of ALV-B, cloacal swabs p27 antigen and cell supernatant of viremia were collected weekly and tested in Avian Leukosis Virus Antigen Test Kit of IDEXX Company.

2.8. Chick ALV-A/B specific antibody test

0.3 ml non-anticoagulant blood was collected at the age of 1 day, serum was separated, and maternal antibodies were tested. After injection of ALV-B, 0.3 ml non-anticoagulant blood was collected