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Interleukin 8 enhances the immune response of ducks to avian influenza vaccine

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Summary. – Interleukins are reported to be valuable immunostimulants in enhancing the immune efficiency of conventional vaccines. In this study, the effect of expression of interleukin 8 (IL-8) on the immune response of ducks to avian influenza vaccine was investigated. The results showed that the serum antibody titer, lymphocyte transformation efficiency and serum interferon gamma (IFN-γ) level of ducks injected with avian influenza vaccine along with a plasmid expressing duck IL-8 were higher than those of ducks injected with conventional immunostimulant Astragalus polysaccharide (APS) or empty plasmid. Therefore, the duck IL-8 may be used as a good immunostimulant to enhance the immune efficiency of avian influenza vaccine in ducks.

Keywords: interleukin 8; avian influenza virus; vaccine; Astragalus polasaccharide; duck; immune response

Introduction

In recent years, highly pathogenic avian influenza H5 virus has caused considerable losses to poultry industry around the world and has caused a great threat to public health in several countries since the first appearance in 1996 (Capua and Alexander, 2007; Giese *et al*., 2008; He *et al*., 2013). Many studies revealed that domestic ducks are one of the primary natural reservoirs of avian influenza viruses (AIVs) of different subtypes, and they have been always thought to be the interface between the natural gene pool of wild aquatic birds and land-based poultry in the ecology of influenza viruses (Webster *et al*., 1992; Ellis *et al*., 2004). The conventional animal production pattern makes it possible for domestic ducks to contact with wild waterfowls and terrestrial poultry simultaneously, providing the opportunities to transmit viruses asymptomatically from the former to the latter. Therefore, it is important to prevent the potential transmission of AIVs from ducks to other susceptible animals or humans.

Several studies have suggested that the immune efficiency of AIV vaccines in ducks was lower than that in chickens (Webster and Hulse, 2005). To improve the immune response of AIV vaccines in ducks, immunostimulants were applied to enhance the production of IFNs and regulate immune function in immunized ducks (Yao *et al*., 2010; Garçon *et al*., 2012). In this study, we evaluated duck IL-8 and Astragalus polysaccharide (APS) as immunostimulants in enhancing the immune response of ducks to an AIV vaccine.

Materials and Methods

Ducks and vaccine. Five-day-old ducks (clean animals) were purchased from Sansui Duck Breeding Farm, Sansui, Guizhou, China. AIV vaccine (Recombinant avian influenza inactivated vaccine (H5N1 subtype, Re-6 strain) was purchased from Qingdao Yibang Biological Engineering Co., Qingdao, China.

Plasmid construction. The ORF of duck IL-8 gene (GenBank Acc. No. AB236335) was synthesized and inserted into eukaryotic

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Abbreviations: APS = Astragalus polysaccharide; AIV(s) = avian influenza virus(es); IFN-γ = interferon gamma; IL-8 = interleukin 8; p.i. = post immunization

expression plasmid pcDNA3.1 (+) (Invitrogen, USA) at the *Xho*I and *Bam*HI sites to generate pcDNA3.1-dIL-8. The obtained clones were verified by DNA sequencing.

Animal experiment. Ducks were injected with the AIV vaccine and then randomly divided into three experimental groups (IL-8, empty plasmid and APS). Ten and 24 days post immunization (p.i.) with 0.5 ml of avian influenza vaccine, the IL-8 and the empty plasmid groups were injected with 200 μg pcDNA3.1-dIL-8 and pcDNA3.1, respectively, whereas the APS group was injected with 0.01 g APS. The schedules and doses of vaccination as well as injection of immunostimulants were performed according to the instructions of AIV vaccine manufacturer: the first immunization and the second immunization were performed at 10- and 24-day-old, respectively, with the injection dose of avian influenza vaccine 0.5 ml per animal. The schedules of immunostimulant injection were carried out as described previously (Yu *et al*., 2012). Unvaccinated and plasmid-uninjected ducks were used as blank control.

The weight of ducks was recorded and the blood was collected from the wing vein on days 10, 24, 38, 52, and 66 p.i. Sera were used for determination of lymphocyte transformation efficiency by MTT assay (Wu *et al*., 2007), serum antibody titer by hemagglutination-inhibition test and serum IFN-γ level by ELISA as previously described (Yun *et al*., 2000).

Results and Discussion

In this study, the effect of expression of duck IL-8 in ducks and injection of conventional immunostimulant APS on the immune response of ducks to an AIV vaccine was investigated. The results showed that the antibody titer was undetectable in all vaccinated groups on day 10, but increased from day 24 to day 52 p.i. (Fig. 1a). The highest antibody titer levels were exhibited by the IL-8 group, followed by the APS group and the empty plasmid group. The corresponding differences on days 38, 52 and 66 were significant ($P < 0.01$).

As for the T lymphocyte transformation efficiency, it was very low on day 10 in all the four groups, but then it increased continuously on days 24 and 38 to maximum values, which were reached in the IL-8 group, whereas the APS group showed significantly lower values ($P < 0.01$) (Fig. 1b).

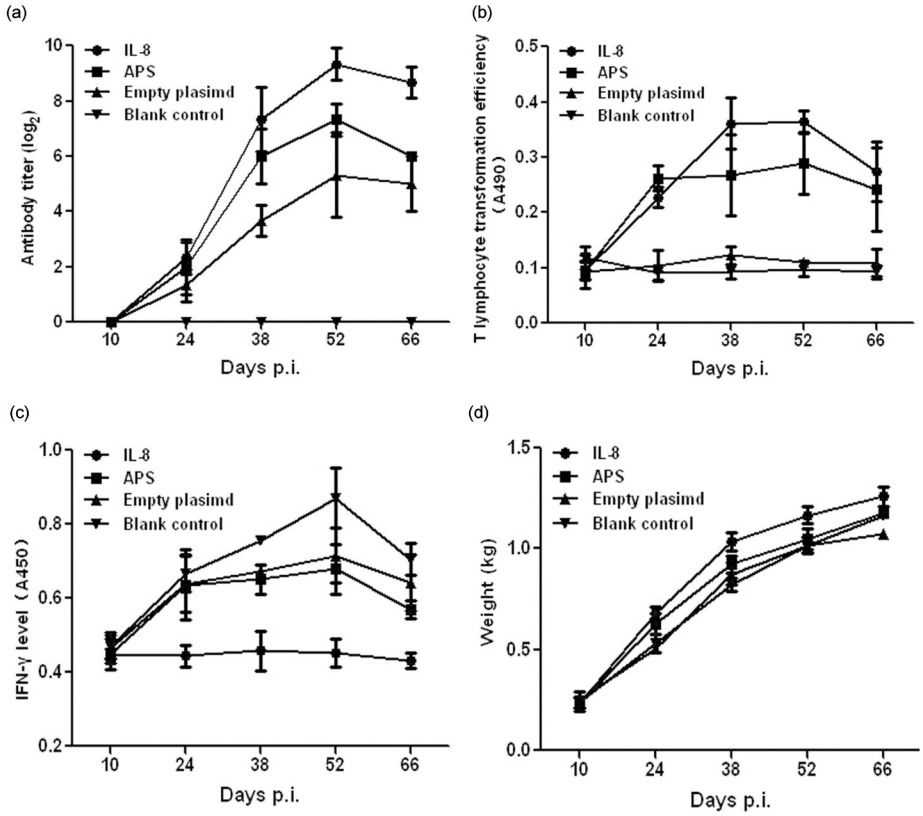

Fig. 1
Effects of IL-8 on the immune response of ducks to AIV vaccine
The serum antibody titer (log_2) (a), lymphocyte transformation efficiency (A_{490}) (b), serum IFN-γ level (A_{450}) (c), and weight of ducks on days 10–66 p.i. (kg) (d) in duck IL-8 group, APS group, empty plasmid group and blank control group were investigated.

Assays of serum IFN-γ levels showed that they increased continuously from day 10 to 52 in all three vaccinated groups to levels descending in the order IL-8, APS and empty plasmid group (Fig. 1c).

Eventually, the recording of weight of ducks showed a picture similar to those from previous assays, namely the highest weight increase in the IL-8 group, followed closely by the APS and the empty plasmid group (Fig. 1d), indicating that the use of IL-8 did not affect the weight growth of ducks.

Taken together, our study demonstrated that the effect of duck IL-8 on the immune response of ducks to AIV vaccine was superior to that of conventional immunostimulant APS, providing a candidate immunostimulant in enhancing the immune efficiency of AIV vaccine in ducks.

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