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Astragalus polysaccharides enhance the immune response to avian infectious bronchitis virus vaccination in chickens

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ABSTRACT

Astragalus polysaccharides (APS) are biological macromolecules extracted from *Astragalus* species that have strong immunoregulatory properties. In this study, APS were employed as an adjuvant for an avian infectious bronchitis virus (IBV) vaccine, and its effects on the cellular immune and humoral immune responses to vaccination in chicken were investigated. One hundred and fifty chicken were randomly divided into five groups (n = 30, each group). The chickens in all groups, except for the unvaccinated control group, were vaccinated with an IBV DNA vaccine. Three of the four vaccinated groups were administered different doses of APS (APSL, 10 mg/kg; APSM, 50 mg/kg; and APSH, 100 mg/kg) after the first vaccination, and the remaining vaccinated group served as a control, without any additional treatment. At 14, 28, and 42 days after the first vaccination, serum anti-IBV antibody titers; peripheral lymphocyte proliferation; and the mRNA expression of *IL-1β*, *IL-2*, *IL-8*, and *TNF-α* in the spleen were assessed by enzyme-linked immunosorbent assay (ELISA), the cell counting kit-8 (CCK-8), and real time quantitative RT-PCR (qRT-PCR), respectively. At most time points, the titer of IBV-specific antibodies, lymphocyte proliferation, and *IL-1β*, *IL-2*, *IL-8*, and *TNF-α* mRNA expression levels were higher in three APS groups than in the vaccine control group, and these increases were dose-dependent. These data suggest that APS could be used as an adjuvant for IBV vaccination to provide better protection against IBV infection.

1. Introduction

Infectious bronchitis (IB) is one of the most relevant infectious diseases of poultry, and it is caused by the infectious bronchitis virus (IBV) [1]. IBV infects chickens of all ages and causes respiratory disease, diarrhea, reduced feeding and weight gain, and decreases egg production and quality, leading to severe economic losses [2]. There are no effective drugs for the treatment of IB [3]; thus, vaccination is the most promising measure for preventing this disease. However, the extensive diversity of IBV strains worldwide hinders the use of vaccination due to poor cross-protection. Therefore, an effective, safe vaccine is greatly needed [4]. Most commercially available IB vaccines are inactivated whole-virus preparations that do not induce long-term protection [5]. This short fall may be overcome by administration of an effective immune adjuvant that generates a strong enough immune response to provide long-term protection against infection [6].

Chinese herbal medicinal ingredients have been used as safe and effective adjuvant [7,8]. They can be administered along with a vaccine to elicit a stronger, more rapid immune response, and they exert strong immunomodulatory effects, by increasing cytokine expression [9–11], enhancing $CD4^+$ and $CD8^+$ T-cell activation, and increasing NK cell activity, etc. [12]. Astragalus polysaccharides (APS), which are active ingredients extracted from the Chinese medicinal herb *Astragalus*, were shown to have multiple biological activities, including immunomodulatory [13], antioxidant [14], anti-tumor [15], anti-diabetes [16], anti-inflammatory [17], and antiviral effects [18]. APS have been widely used in veterinary clinics and have been shown to enhance the immune response to several vaccines against viruses, such as H9N2 avian influenza virus [19], foot-and-mouth disease virus [20], Newcastle disease virus

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[21], and infectious bursal disease virus [22]. However, whether APS can enhance the immunity of an IBV vaccine is unknown. Therefore, the aim of this study was to evaluate the effects of APS as an adjuvant for an IBV inactivated vaccine in chickens.

2. Results

2.1. Effect of APS on the humoral immune response

To investigate whether APS have adjuvant effects on the humoral immune response in chickens, serum antibody titers were determined by ELISA. As shown in Table 1, on day 14 after the first IBV vaccination, the antibody titers in the high (APSH) and medium (APSM) dose APS groups were significantly higher than those in the vaccine only control group ($p < 0.05$). On day 28 and 42, the antibody titers in all APS groups (APSH, APSM, and low [APSL]) were significantly higher than those in the vaccinated group ($p < 0.05$), and the titers were also significantly higher in the APSH and APSM groups than in the low APS group (APSL, $p < 0.05$; Table 1). During the experimental period, compared to the unvaccinated controls, the vaccinated group showed significantly higher antibody titers ($p < 0.05$; Table 1). These results suggest that APS can improve the humoral immune response to an IBV DNA vaccine in a dose-dependent manner.

2.2. The effect of APS on lymphocyte proliferation

To determine whether APS affect cell-mediated immunity, the changes in lymphocyte proliferation were determined by the cell counting kit-8 (CCK-8) assay. The OD_{450} values are shown in Table 2. On day 14 after the first vaccination, the optical density at 450 nm (OD_{450}) values in the APSH and APSM groups were significantly higher than the value in the vaccinated group ($p < 0.05$; Table 2). On days 28 and 42, the OD_{450} values in all APS groups were significantly higher than those in the vaccine group ($p < 0.05$; Table 2), and the values in the APSH and APSM groups were significantly higher than those in the APSL group ($p < 0.05$; Table 2). Compared to the unvaccinated control group, the vaccinated group showed higher OD_{450} values throughout the experimental period. These results clearly demonstrate that the OD_{450} value was significantly and dose-dependently higher in the APS treatment groups than in the untreated vaccinated group, suggesting that APS can improve the cell-mediated immune response to IBV DNA vaccination.

2.3. The effect of APS on cytokine expression

2.3.1. The effect of APS on IL-1β

To determine whether APS influenced cytokine secretion after IBV vaccination, serum *IL-1β*, *IL-2*, *IL-8*, and *TNF-α* mRNA expression levels were determined by qRT-PCR. As shown in Fig. 1, on days 14, 28, and 42, the *IL-1β* mRNA expression levels in all APS groups were significantly higher than those in the vaccinated group ($p < 0.05$).

Table 1
The changes of antibody titer in different groups (OD_{650} value).

Groups	14 days	28 days	42 days
Control	0.28 ± 0.03^{a}	0.35 ± 0.04^{a}	0.32 ± 0.05^{a}
Vaccine	0.41 ± 0.04^{b}	0.48 ± 0.06^{b}	0.52 ± 0.07^{b}
APSL	0.43 ± 0.05^{b}	0.68 ± 0.06^{c}	0.74 ± 0.08^{c}
APSM	0.56 ± 0.05^{bc}	0.88 ± 0.09^{cd}	0.98 ± 0.09^{cd}
APSH	0.58 ± 0.06^{c}	0.95 ± 0.09^{d}	1.02 ± 0.11^{d}

[a–d]Data within a column without the same superscripts differ significantly ($p < 0.05$). APS, Astragalus polysaccharides; L, Low dose, M, Medium dose; H, High dose.

Table 2
The changes of lymphocyte proliferation in different groups (OD_{450} value).

Groups	14 days	28 days	42 days
Control	0.21 ± 0.02^{a}	0.24 ± 0.03^{a}	0.28 ± 0.03^{a}
Vaccine	0.35 ± 0.04^{b}	0.38 ± 0.04^{b}	0.44 ± 0.05^{b}
APSL	0.37 ± 0.04^{b}	0.59 ± 0.06^{c}	0.63 ± 0.07^{c}
APSM	0.50 ± 0.05^{bc}	0.77 ± 0.07^{cd}	0.82 ± 0.08^{cd}
APSH	0.54 ± 0.05^{c}	0.84 ± 0.08^{d}	0.88 ± 0.09^{d}

[a–d]Data within a column without the same superscripts differ significantly ($p < 0.05$). APS, Astragalus polysaccharides; L, Low dose, M, Medium dose; H, High dose.

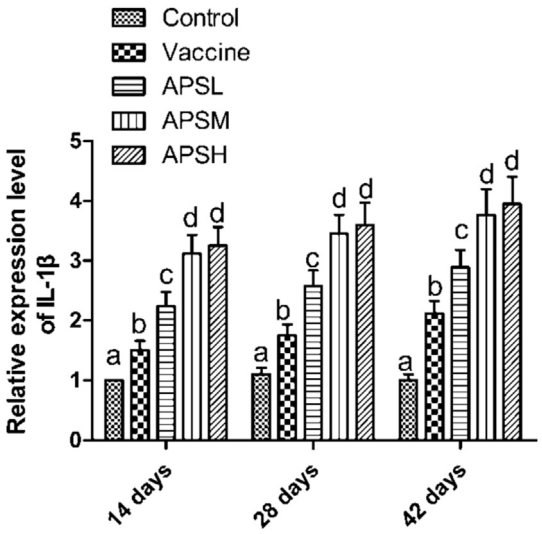

Fig. 1. Effect of APS on the mRNA expression of *L-1β*. Superscripts with different letters (a–d) differ significantly ($p < 0.05$).

Moreover, the expression levels in the APSH and APSM groups were significantly higher than those in the APSL group ($p < 0.05$). During the entire experimental period, the vaccinated group showed higher *IL-1β* expression than the unvaccinated control group ($p < 0.05$).

2.3.2. The effect of APS on IL-2 expression

The qRT-PCR results showed that *IL-2* mRNA expression levels were significantly higher in all APS groups than in the vaccinated group on days 14, 28, and 42 ($p < 0.05$; Fig. 2). In addition, on days 14 and 28, *IL-2* mRNA expression levels in the APSM and APSH groups were significantly higher than those in the APSL group (Fig. 2). There were no significant differences in *IL-2* mRNA expression among the APSL, APSM, and APSH groups at 42 days. During the entire experimental period, *IL-2* mRNA expression was higher in the vaccinated group than in the unvaccinated control group ($p < 0.05$).

2.3.3. The effect of APS on IL-8 expression

The dynamic changes in *IL-8* mRNA expression levels were similar to those observed for *IL-2* mRNA expression. As shown in Fig. 3, the mRNA expression levels of *IL-8* in all APS groups were significantly higher than those in the vaccinated group at all time points ($p < 0.05$). The APSM and APSH groups showed significantly higher *IL-8* mRNA expression levels than the APSL group at days 14 and 28 (Fig. 3). On day 42, there were no significant differences in *IL-8* mRNA expression levels among all APS groups ($p > 0.05$; Fig. 3). During the entire experimental period, *IL-8* mRNA expression was higher in the vaccinated group than in the unvaccinated control