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Astragalus polysaccharide and sulfated epimedium polysaccharide synergistically resist the immunosuppression

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ABSTRACT

The immunoenhancement of compound polysaccharides, APS–sEPS composed with astragalus polysaccharide (APS) and sulfated epimedium polysaccharide (sEPS), was observed in immunosuppressed model chicken induced by cyclophosphamide (Cy). 11-day-old chickens were injected with Cy once a day for three successive days except vaccine control group. At day-14-old, all chickens were vaccinated with ND vaccine, and in experimental groups simultaneously administrated with APS–sEPS at three dosages, APS and sEPS once a day for three successive days. On days 7, 14, 21 and 28 after the administration, the peripheral T-lymphocyte proliferation, serum antibody titers, IFN-γ, IL-2, IgG and IgM were determined. The results displayed that APS–sEPS could overcome Cy-induced immunosuppression, significantly promote T-lymphocyte proliferation and raised serum antibody titers, IFN-γ, IL-2, IgG and IgM levels, its high and medium doses were superior to single APS or sEPS. This demonstrated that APS and sEPS could synergistically resist the immunosuppression and APS–sEPS was an effective immunopotentiator.

1. Introduction

Animal immunosuppression is common and generally caused by infection, stress, abusing of antibiotics and chemicals, and so on. Some immunosuppressive diseases in poultry are very pervasive, which usually can be ignored for their subclinical signs (Islam et al., 2002; Markowski-Grimsrud & Schat, 2003; Sharma, Kim, Rautenschlein, & Yeh, 2000). Immunosuppressed animals may have an increasing incidence of secondary infections, immunodeficiency, which could reduce immune response to commonly used vaccines (Sharma et al., 2000). Immunosuppression resulted in a great deal of economic losses to poultry industry. So, it is necessary to find a useful immunopotentiator for enhancing the immunity and improving productivity of animal farms.

In recent years, polysaccharides isolated from natural plants have been regarded as an important class of biological response modifiers (Leunga, Liu, Koon, & Fung, 2006). They have attracted a great deal of attention in the biomedical area owing to their broad spectrum of therapeutic properties and the relatively low toxicity. The polysaccharides from natural plants have been reported to possess activity in promoting lymphocyte proliferation and improving the expression of cytokines so as to enhance the immunity (Kodama, Murata, & Nanba, 2004; Lim, Na, Choi, Chung, & Hwang, 2004; Noriko et al., 2005; Wang, Li, & Chen, 2009). They can correct immune imbalance and make the immune function recover to normal level. Many studies have demonstrated that biological activities of polysaccharides are obviously increased by chemical modification (Xing et al., 2005), above all, sulfated polysaccharides exerted obvious potent biological properties in comparison with non-sulfated polysaccharides (Liu et al., 2009). Therefore, sulfated modification could be considered as the effective way to enhance the biological activities of polysaccharides.

Radix astragali and *Herba epimedii* are two plants commonly used as traditional Chinese medicine or veterinary medicine to improve immune functions in humans and animals (Cao et al., 2003; Luo, Shao, Gu, & Li, 2009). APS and EPS could markedly promote lymphocyte proliferation of chicken and mice in vivo and in vitro, and positively improve the immune system of the immunesuppressed animals (Huang et al., 2008; Kim et al., 2001; Mckenna,

Abbreviations: cPS, compound polysaccharides; APS, astragalus polysaccharide; sEPS, sulfated epimedium polysaccharide; APS–sEPS, compound polysaccharides composed with astragalus polysaccharide and sulfated epimedium polysaccharide; Cy, cyclophosphamide; HI, hemagglutination inhibition; ND, Newcastle disease; VC, vaccine control group.

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Hughes, & Jones, 2002; Qiu, Cheng, Xu, & Zhang, 2010; Shao et al., 2004). Our previous studies have proved that the sulfated polysaccharides exerted better potent biological properties in comparison with non-sulfated polysaccharides, such as anti-virus and immunoenhancing activities of APS and EPS (Huang et al., 2008; Lu, Wang, Hu, Huang, & Wang, 2008; Lu et al., 2009). Consecutive research confirmed that APS and sEPS could synergistically enhance the immune effect of Newcastle disease vaccine and avian influenza vaccine, and APS–sEPS at medium dose possessed the best efficacy (Guo et al., 2012). It has been applied for registration of national new veterinary drug.

In order to investigate the immunomodulatory actions of APS–sEPS, the immunosuppression model chicken was prepared with cyclophosphamide (Cy). The effects of APS–sEPS were evaluated from peripheral lymphocyte proliferation, serum antibody titer, cytokine production and Ig levels in the chicken. It may provide a basis for the use of APS–sEPS as an efficacious immunopotentiator.

2. Materials and methods

2.1. Preparation of polysaccharides

APS and sEPS were provided as previously described (Huang et al., 2008; Lu et al., 2008). APS–sEPS was prepared according to a certain proportion and diluted with water for injection, then sterilized and detected for endotoxin by pyrogen tests. When the endotoxin amount was up to the standard of Chinese Veterinary Pharmacopoeia (less than 0.5 EU mL^{-1}) (Veterinary Pharmacopoeia Commission of the People's Republic of China, 2000), they were stored at 4 °C until for use.

2.2. Vaccine and reagents

ND vaccine (La Sota strain) was provided by Nanjing Tianbang Biotechnology Co. Ltd.

Cyclophosphamide, purchased from Shanghai Kayon Biological Technology Co. Ltd., was dissolved with PBS and filtered through a 0.22 μm filter. RPMI-1640 medium (Gibco) supplemented with benzylpenicillin 100 IU mL^{-1}, streptomycin 100 IU mL^{-1} and 10% fetal bovine serum, was used for washing, re-suspending and culturing cells. Phytohemagglutinin (PHA, Sigma), as the T-cell mitogen, was dissolved with RPMI-1640 medium. Hank's solution was used for diluting blood. 3-(4,5-Dimethyithiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) was dissolved into 5 mg mL^{-1} with calcium and magnesium-free phosphate-buffered saline. These reagents were filtered through a 0.22 μm filter. RPMI-1640 medium and Hank's solution were stored at 4 °C, and MTT solution was stored at 4 °C in dark. Lymphocytes Separation Medium (ρ: 1.077 ± 0.002, No. 110812) was purchased from Tianjin Haoyang Biological manufacture Co. Ltd. DMSO was provided by Chemical Agent Company of Chinese Medicine Groups.

2.3. Animals

One-day-old White Roman chickens, purchased from Tangquan Poultry Farm, were housed in wire cages (100 cm × 60 cm × 40 cm) in air-conditioned room at 37 °C and lighted for 24 h per day at the beginning of pretrial period. The temperature was gradually declined to the room temperature and the light time to 12 h per day, which were kept constant in the following days. The chickens were fed with the commercial starter diet provided by the Feed Factory of Jiangsu Academy of Agricultural Science. All the procedures were performed in strict accordance with internationally accepted principles and the P.R. China legislation on the use and care of laboratory animals.

2.4. Protocols for immunosuppression induction and treatment

Three hundred and fifty 11-day-old chickens were randomly assigned into 7 groups (n = 50). One group of healthy chickens was used as vaccine controls (VC group). From day 11 to 13, the other 6 groups of chickens were subjected to immunosuppression by administration of Cy (80 mg/kg/d) intramuscularly, and one group of those Cy-treated chickens was used as a model group (Cy group). At day-14-old the chickens were vaccinated with ND vaccine in all groups, and then administered as follows: VC group and Cy group with normal saline; three APS–sEPS groups respectively with 150, 100 and 50 mg/kg of APS–sEPS; APS group with 60 mg/kg of APS; sEPS group with 40 mg/kg of sEPS. All administrations were once a day for 3 successive days by intramuscular injection (0.5 ml) (Table 1). Booster vaccination was administered on day 28. On days 7, 14, 21 and 28 after the administration, the blood was sampled for determination of lymphocyte proliferation by MTT method, serum hemagglutination inhibition (HI) antibody titer by micro-method and the concentrations of IFN-γ, IL-2, total IgG, total IgM by enzyme-linked immunosorbent assay (ELISA).

2.5. Lymphocyte proliferation assay

Blood samples (2 mL per chicken) from heart were collected and transferred immediately into aseptic capped tubes with sodium heparin, then diluted with equal volume of Hanks' solution and carefully layered on the surface of lymphocyte separation medium. After centrifugation at 800 × g for 20 min, a white cloud-like lymphocytes' band was collected and washed twice with RPMI1640 media without fetal bovine serum. The resulting pellet was re-suspended to 2.5×10^6 mL^{-1} with RPMI 1640 media and incubated in 96-well culture plates with 80 μL per well, then another 20 μL of PHA was added into each well, each sample seeded four wells. The cells were incubated at 38.5 °C for 48 h in a humid atmosphere of 5% CO_2. Cell proliferation was measured by MTT assay 48 h after culture. After 44 h incubation, 30 μL of MTT (5 mg mL^{-1}) was added into each well, and the plates were reincubated for 4 h. 100 μL of DMSO was added into each well. The plates were shaken for 5 min to dissolve the Formazan crystals completely. The absorbance at 570 nm (A_{570} value) was measured by microliter enzyme-linked immunosorbent assay reader (Model DG-3022, East China Vacuum Tube Manufacturer) as the index of lymphocytes proliferation (Wang et al., 2005).

2.6. Serum HI antibody assay

Blood samples (0.5 mL per chick) from brachial vein were drawn into Eppendorf tubes and allowed to clot at 37 °C for 2 h. Serum was separated by centrifugation for determination of HI antibody. The serum was inactivated at 56 °C for 30 min, twofold serial dilution were made in a 96-well V-shaped bottom microtiterplate containing 50 μl of CMF-PBS in each well, then 50 μL of NDV antigen (4 HA units) was added into all the wells except for the last row as the controls. Serum dilutions ranged from 1:2 to 1:2048. The plate was incubated at 37 °C for 20 min, then 50 μL of 1% rooster erythrocytes suspension was added to each well and continued to incubate for 30 min. A positive serum, a negative serum, erythrocytes and antigens were also included as controls. The highest dilution of caused complete inhibition was considered as the endpoint. The geometric mean titer was expressed as reciprocal log 2 values of the highest dilution that displayed HI (Thekisoe, Mbati, & Bisschop, 2004).

2.7. Serum IFN-γ, IL-2, IgG and IgM assay

Blood samples (2.0 mL per chick) from brachial vein were drawn into Eppendorf tube and allowed to clot at 37 °C for 2 h. Serum

Table 1
The protocols for immunosuppression induction and treatment.

Group	Cy-induced (mg/kg/d)	ND vaccine	Drug and dosage (mg/kg) Once a day for 3 successive days (0.5 ml)
APS–sEPS$_H$	80	+	APS–sEPS 150
APS–sEPS$_M$	80	+	APS–sEPS 100
APS–sEPS$_L$	80	+	APS–sEPS 50
APS	80	+	APS 60
sEPS	80	+	sEPS 40
Cy	80	+	Normal saline
VC	–	+	Normal saline

–, groups not administration of cyclophosphamide (Cy). +, groups vaccinated with ND vaccine. APS–sEPS, the compound polysaccharides (cPS) composed with astragalus polysaccharides (APS) and sulfated epimedium polysaccharides (sEPS). H, high dose of 150 mg/kg; M, medium dose of 100 mg/kg; L, low dose of 50 mg/kg.

Table 2
The changes of lymphocyte proliferation in every group (A_{570} value).

Group	Days after administration			
	7	14	21	28
APS–sEPS$_H$	0.209 ± 0.004	0.220 ± 0.027^{b}	0.279 ± 0.027^{a}	0.276 ± 0.005^{a}
APS–sEPS$_M$	0.206 ± 0.011^{b}	0.222 ± 0.011^{ab}	0.258 ± 0.046^{a}	0.276 ± 0.008^{a}
APS–sEPS$_L$	0.196 ± 0.002^{b}	0.204 ± 0.004^{c}	0.189 ± 0.005^{b}	0.190 ± 0.014^{cd}
APS	0.160 ± 0.045^{d}	0.149 ± 0.011^{d}	0.137 ± 0.007^{c}	0.200 ± 0.003^{bc}
sEPS	0.178 ± 0.011^{c}	0.195 ± 0.002^{c}	0.188 ± 0.005^{b}	0.213 ± 0.010^{b}
Cy	0.144 ± 0.020^{e}	0.127 ± 0.005^{e}	0.106 ± 0.002^{d}	0.179 ± 0.003^{d}
VC	0.233 ± 0.002^{a}	0.232 ± 0.002^{a}	0.266 ± 0.028^{a}	0.215 ± 0.003^{b}

(a–e) Data within a column with different letters differ significantly ($P < 0.05$). APS–sEPS, the compound polysaccharides (cPS) composed with astragalus polysaccharides (APS) and sulfated epimedium polysaccharides (sEPS). H, high dose of 150 mg/kg; M, medium dose of 100 mg/kg; L, low dose of 50 mg/kg. Cy, only cyclophosphamide treated without vaccine and polysaccharides. VC, only vaccination control.

was separated by centrifugation for determination of the concentrations of IFN-γ, IL-2, total IgG and total IgM.

2.8. Statistical analysis

Data were expressed as the mean ± S.D. Duncan multiple range test was used to analyze the difference among groups with the software SPSS 16.0. The statistical significance ($P < 0.05$) was evaluated by one-way ANOVA with simultaneous multiple comparisons among different groups.

3. Results

3.1. The changes of peripheral lymphocyte proliferation

The changes of A_{570} values are listed in Table 2. After the administration, the A_{570} values in VC group at all time points were significantly higher than those in Cy group ($P < 0.05$). On day 7, the A_{570} values in three doses of APS–sEPS groups were significantly higher than that in APS and sEPS groups ($P < 0.05$). On day 14–28, the A_{570} values in APS–sEPS$_H$ and APS–sEPS$_M$ groups were significantly higher than those in APS, sEPS and Cy groups ($P < 0.05$). On day 7–21, the A_{570} values in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$) and in sEPS group was significantly higher than that in APS group ($P < 0.05$). On day 28, the A_{570} values in APS–sEPS$_H$ and APS–sEPS$_M$ groups were significantly higher than that in VC group ($P < 0.05$).

3.2. The changes of serum antibody titer

The changes of antibody titers were listed in Fig. 1. After the administration, the antibody titers in VC group at all time points were significantly higher than those in Cy group ($P < 0.05$). On day 14, the antibody titers in three doses of APS–sEPS groups were significantly higher than those in APS group ($P < 0.05$). On day 21–28, the antibody titers in APS–sEPS$_H$ and APS–sEPS$_M$ groups were significantly higher than those in APS, sEPS and VC groups ($P < 0.05$). On day 7–28, the antibody titers in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$) and in three doses of APS–sEPS groups were higher or significantly higher than that in APS group ($P < 0.05$). On day 21–28, the antibody titers in APS–sEPS$_H$ and APS–sEPS$_M$ groups were significantly higher than those in VC group ($P < 0.05$).

3.3. The changes of IFN-γ concentration

The changes of the IFN-γ concentration were listed in Table 3. After the administration, the IFN-γ concentration in VC group at all time points were significantly higher than those in Cy group ($P < 0.05$). On day 7, the IFN-γ concentration in APS–sEPS$_M$ group was significantly higher than that in APS group ($P < 0.05$) and in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$). On day 14, the IFN-γ concentrations in APS–sEPS$_H$, APS–sEPS$_M$ and sEPS groups were significantly higher than that in Cy group ($P < 0.05$). On day 21, the

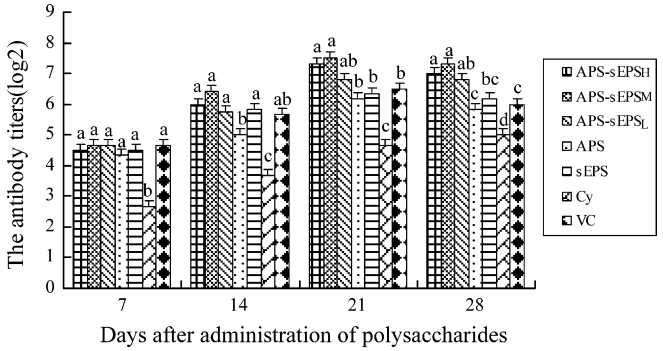

Fig. 1. The changes of antibody titer in every group (log 2). (a–d) Data in bar with different letters differ significantly ($P < 0.05$). APS–sEPS, the compound polysaccharides (cPS) composed with astragalus polysaccharides (APS) and sulfated epimedium polysaccharides (sEPS). H, high dose of 150 mg/kg; M, medium dose of 100 mg/kg; L, low dose of 50 mg/kg. Cy, only cyclophosphamide treated without vaccine and polysaccharides. VC, only vaccination control.

Table 3
The changes of IFN-γ concentration (ng/L).

Group	Days after administration			
	7	14	21	28
APS–sEPS$_H$	149.63 ± 22.53^{bc}	129.72 ± 6.42^{bc}	141.91 ± 20.09^{ab}	133.33 ± 27.76^{a}
APS–sEPS$_M$	162.09 ± 4.45^{ab}	140.86 ± 18.89^{ab}	162.65 ± 15.74^{a}	130.09 ± 5.89^{a}
APS–sEPS$_L$	138.95 ± 2.68^{c}	115.49 ± 11.85^{cd}	117.40 ± 17.81^{b}	93.83 ± 9.15^{b}
APS	138.27 ± 3.34^{c}	121.42 ± 4.17^{bcd}	123.24 ± 3.27^{b}	87.69 ± 4.06^{bc}
sEPS	142.46 ± 11.^{50bc}	126.11 ± 10.46^{bc}	143.70 ± 20.16^{ab}	88.61 ± 9.82^{bc}
Cy	113.21 ± 16.26^{d}	99.69 ± 5.60^{d}	79.57 ± 5.09^{c}	63.06 ± 3.01^{c}
VC	176.11 ± 6.55^{a}	158.51 ± 11.79^{a}	130.74 ± 5.76^{ab}	106.75 ± 8.77^{ab}

(a–d) Data within a column with different letters differ significantly ($P < 0.05$). APS–sEPS, the compound polysaccharides (cPS) composed with astragalus polysaccharides (APS) and sulfated epimedium polysaccharides (sEPS). H, high dose of 150 mg/kg; M, medium dose of 100 mg/kg; L, low dose of 50 mg/kg. Cy, only cyclophosphamide treated without vaccine and polysaccharides. VC, only vaccination control.

IFN-γ concentration in APS–sEPS$_M$ group was significantly higher than those in APS and Cy groups ($P < 0.05$) and in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$). On day 28, the IFN-γ concentrations in APS–sEPS$_H$ and APS–sEPS$_M$ groups were significantly higher than those in APS–sEPS$_L$, APS, sEPS and Cy groups ($P < 0.05$), and in APS–sEPS$_L$ group was significantly higher than that in Cy group ($P < 0.05$). On day 21–28, the IFN-γ concentration in APS–sEPS$_M$ group was higher than that in VC group ($P > 0.05$).

3.4. The changes of IL-2 concentration

The changes of the IL-2 concentration were listed in Table 4. After the administration, the IL-2 concentration in VC group at all time points were significantly higher than those in Cy group ($P < 0.05$). On day 7, the IL-2 concentration in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$) and in APS–sEPS$_H$ and APS–sEPS$_M$ groups were higher than that in APS group. On day 14, the IL-2 concentration in APS–sEPS$_M$ group was significantly higher than those in APS and sEPS groups ($P < 0.05$) and in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$). On day 21, the IL-2 concentration in APS–sEPS$_M$ was significantly higher than those in other 6 groups ($P < 0.05$), in APS–sEPS$_H$ and APS–sEPS$_L$ groups were significantly higher than those in APS, sEPS and Cy groups ($P < 0.05$) and in APS group was significantly higher than that in Cy group ($P < 0.05$). On day 28, the IL-2 concentration in APS–sEPS$_M$ was significantly higher than those in other 6 groups ($P < 0.05$), and in APS–sEPS$_H$ group was significantly higher than those in APS, sEPS and Cy groups ($P < 0.05$). On day 14–28, the IL-2 concentration in APS group was higher than that in sEPS group. On day 21–28, the IL-2 concentrations in APS–sEPS$_M$ group was significantly higher than those in VC group ($P < 0.05$).

3.5. The changes of total IgG concentration

The changes of the total IgG concentration were listed in Fig. 2. After the administration, the IgG concentration in VC group at all time points were significantly higher than those in Cy group ($P < 0.05$). On day 7, the IgG concentration in three doses of APS–sEPS groups were significantly higher than those in APS and sEPS groups ($P < 0.05$) and in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$). On day 14, the IgG concentration in APS–sEPS$_M$ group was significantly higher than those in other 6 groups ($P < 0.05$), and in APS–sEPS$_H$, APS–sEPS$_L$ and sEPS groups were significantly higher than those in Cy group ($P < 0.05$). On day 21, the IgG concentration in three doses of APS–sEPS group were significantly higher than those in APS, sEPS and Cy groups ($P < 0.05$). On day 28, the IgG concentration in APS–sEPS$_M$ group was significantly higher than those in APS, sEPS and Cy groups ($P < 0.05$). On day 7–14 and 28, the IgG concentration in sEPS group was higher or significantly higher than that in APS group ($P < 0.05$). On day 14, the IgG concentration in APS–sEPS$_M$ group was significantly higher than that in VC group ($P < 0.05$).

3.6. The changes of total IgM concentration

The changes of the total IgM concentration were listed in Fig. 3. After the administration, the IgM concentration in VC group at all time points were significantly higher than those in Cy group

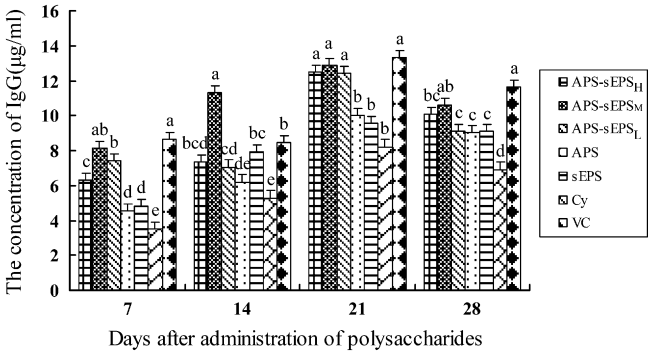

Fig. 2. The changes of IgG concentration (μg/ml). (a–e) Data in bar with different letters differ significantly ($P < 0.05$). APS–sEPS, the compound polysaccharides (cPS) composed with astragalus polysaccharides (APS) and sulfated epimedium polysaccharides (sEPS). H, high dose of 150 mg/kg; M, medium dose of 100 mg/kg; L, low dose of 50 mg/kg. Cy, only cyclophosphamide treated without vaccine and polysaccharides group. VC, only vaccination control.

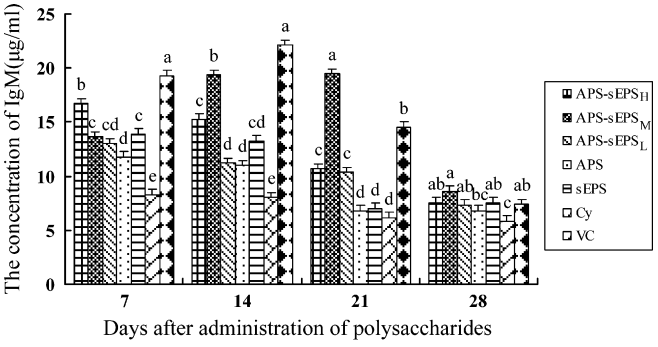

Fig. 3. The changes of IgM concentration (μg/ml). (a–e) Data within a column with different letters differ significantly ($P < 0.05$). APS–sEPS, the compound polysaccharides (cPS) composed with astragalus polysaccharides (APS) and sulfated epimedium polysaccharides (sEPS). H, high dose of 150 mg/kg; M, medium dose of 100 mg/kg; L, low dose of 50 mg/kg. Cy, only cyclophosphamide treated without vaccine and polysaccharides group. VC, only vaccination control.

Table 4
The changes of IL-2 concentration (ng/L).

Group	Days after administration of polysaccharides			
	7	14	21	28
APS–sEPS$_H$	4.28 ± 0.20^a	3.35 ± 0.71^{ab}	3.02 ± 0.15^b	2.41 ± 0.18^b
APS–sEPS$_M$	4.18 ± 0.10^a	3.88 ± 0.25^a	3.88 ± 0.42^a	3.03 ± 0.60^a
APS–sEPS$_L$	3.68 ± 0.18^b	3.03 ± 0.34^b	2.89 ± 0.42^b	2.27 ± 0.25^{bc}
APS	3.71 ± 0.09^b	3.02 ± 0.20^b	2.15 ± 0.21^c	1.79 ± 0.28^{cd}
sEPS	3.89 ± 0.19^{ab}	2.80 ± 0.26^b	1.90 ± 0.20^{cd}	1.64 ± 0.18^d
Cy	3.05 ± 0.16^c	1.72 ± 0.25^c	1.54 ± 0.14^d	1.28 ± 0.11^d
VC	4.32 ± 0.43^a	3.32 ± 0.21^{ab}	3.10 ± 0.39^b	2.42 ± 0.30^b

(a–d) Data within a column with different letters differ significantly ($P < 0.05$). APS–sEPS, the compound polysaccharides (cPS) composed with astragalus polysaccharides (APS) and sulfated epimedium polysaccharides (sEPS). H, high dose of 150 mg/kg; M, medium dose of 100 mg/kg; L, low dose of 50 mg/kg. Cy, only cyclophosphamide treated without vaccine and polysaccharides. VC, only vaccination control.

($P < 0.05$). On day 7, the IgM concentration in APS–sEPS$_H$ and APS–sEPS$_M$ groups were significantly higher than that in APS group ($P < 0.05$), in APS–sEPS$_H$ group was significantly higher than that in sEPS group ($P < 0.05$) and in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$). On day 14, the IgM concentrations in APS–sEPS$_H$ and APS–sEPS$_M$ groups were significantly higher than that in APS group ($P < 0.05$), in APS–sEPS$_M$ group was significantly higher than that in sEPS groups ($P < 0.05$), and in three doses of APS–sEPS, APS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$). On day 21, the IgM concentrations in three doses of APS–sEPS groups were significantly higher than those in APS, sEPS and Cy groups ($P < 0.05$), and in APS–sEPS$_M$ group was the highest. On day 28, the IgM concentration in APS–sEPS$_M$ group was significantly higher than that in APS group ($P < 0.05$), and in three doses of APS–sEPS and sEPS groups were significantly higher than that in Cy group ($P < 0.05$). On day 7–28, the IgM concentration in sEPS group was higher or significantly higher than that in APS group ($P < 0.05$). On day 21, the IgM concentration in APS–sEPS$_M$ group was significantly higher than that in VC group ($P < 0.05$).

4. Discussion

Cyclophosphamide (Cy) is an immunosuppressive agent and primarily used to treat several types of cancer as a cytotoxic drug. It can inhibit both humoral and cellular immunity, and is referred to be as well known immunosuppressive in case of mammals and birds. Up to now, experimental infections have been carried out on immunosuppressed animals mainly treated with Cy (Chamorro et al., 2007; Ojha, Hayes, Turner, & Maclnnes, 2007). It has been reported that injection of Cy for newborn chickens primarily induced selective B cell damage resulting in irreversible humoral immunosuppression (He, Yang, & Guo, 2007; Hirota & Bito, 1978; Moshira et al., 2004; Reynolds & Maraqa, 1999). Therefore immunosuppressive model was prepared with chicken by injection of Cy in this research. The results of model group showed that Cy could reduce the lymphocytes proliferation, serum antibody titers, the level of serum IFN-γ, IL-2, IgG and IgM and the model could be used to evaluated the effect of immunopotentiator.

The effects of APS–sEPS on cellular and humoral immune responses in immunosuppressive model chicken were observed. The results showed that the peripheral T lymphocyte proliferations in APS–sEPS at dosages of 100 and 150 mg/kg groups were numberly or significantly increased in comparison with those in Cy, APS, sEPS and VC groups (Table 2), the antibody titers in these two groups were numberly or significantly higher than those in Cy, APS, sEPS and VC groups (Fig. 1). T lymphocyte proliferation is the indicator reflecting the cellular immunity state and antibody titers accurately reflect the humoral immunity state of animal (Hadden, 2003; Marciani et al., 2000). The results confirmed that APS–sEPS at suitable dosage could resist the immunosuppression induced by cyclophosphamide and enhance the immune response from cellular and humoral immune aspect. It was worth noticing that in 100 and 150 mg/kg of APS–sEPS groups, the A_{570} values on day 7–28 and the antibody titers on day 21–28 were significantly higher than those in APS and sEPS groups, and the A_{570} values on day 28 and the antibody titers on day 21–28 were significantly higher than that in VC group. This indicated that APS and sEPS could synergistically resist the immunosuppression and enhance the immune response.

Many studies have proved that a lot of polysaccharides can stimulate the production of cytokines (Schepetkin & Quinn, 2006; Xie et al., 2008). The changes of serum IFN-γ and IL-2 were detected in this experiment. The results displayed that the administration of APS–sEPS could prohibit the Cy-induced decline of IFN-γ and IL-2 level. Especially in APS–sEPS at 100 and 150 mg/kg groups, the effects were superior to those of APS and sEPS group, and in APS–sEPS at 100 mg/kg group on day 21–28, the effect was superior to VC group. This indicated that APS and sEPS could synergistically resist the immunosuppression and enhance the secretion of some cytokines. IFN-γ and IL-2 are the main cytokines that improve cell immunity. IL-2 can induce proliferation of B and NK cells, IFN-γ can activate macrophages and NK cells and promote the differentiation from TH0 cells into TH1 cells (Basinski et al., 2009; Decker, Müller, & Stockinger, 2005; Zimmermann et al., 2011). Thus, it is likely that APS–sEPS was effective on Th1 cells.

Some reports have pointed out that some polysaccharides can enhance humoral immune response by promoting the production of specific IgA, IgM and IgG (Yang et al., 2009). The results in this study showed that the level of serum IgG and IgM could be increased by administration of APS, sEPS and APS–sEPS at three doses in comparison with Cy group. This indicated that APS, sEPS and APS–sEPS could resist the immunosuppression induced by cyclophosphamide. At all time points after administration, the IgG concentrations in APS–sEPS groups were maintained at higher level than those in APS and sEPS groups, and the IgM concentrations in APS–sEPS at 100 mg/kg and 150 mg/kg groups were higher than those in APS and sEPS groups, especially in APS–sEPS at 100 mg/kg group, the IgG concentration on day 14 and the IgM concentration on day 21 were significantly higher than those in VC group. These results indicated that APS and sEPS had synergistical effect and APS–sEPS could promote the production of serum IgG and IgM in dose-dependent relationship. IgG and IgM are the major immunoglobulins which are central to humoral immune responses and involved in the complement activation, opsonization, neutralization of toxins, etc. (Miller, Ludke, Peacock, & Tomar, 1991). Therefore, the conclusion could be obtained that APS–sEPS could improve the humoral immune state by increase of IgG and IgM levels for immunosuppressed chickens.

5. Conclusion

APS and sEPS could synergistically resist the immunosuppression induced by cyclophosphamide. APS–sEPS possessed significant immunoenhancement by promoting lymphocyte proliferation and up-regulating the levels of antibody titer, IFN-γ, IL-2, total IgG and total IgM, and was an effective immunopotentiator.

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