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Full Length Research Paper

Effects of the three Chinese herbal crude polysaccharides on immunoglobulin A secreting cells and serum antibody titers in immunization chickens

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This study was conducted to evaluate the effects of three Chinese herbal crude polysaccharides on immunoglobulin A secreting cells and serum antibody titers in vaccinated chickens. A total of 450 14-day-old chickens were randomly assigned to nine equal groups and all chickens were vaccinated. Concurrent with the first vaccination, chickens in groups 1 to 8 were intramuscularly injected with four crude polysaccharides among which the astragalus polysaccharide (APS) was selected as positive control at high and low doses, and group 9 (control group) with saline once a day for three successive days. The numbers of positive immunoglobulin A (IgA) secreting cells and the serum specific immunoglobulin G (IgG) antibody were determined by immunohistochemistry and micro method. The results showed that the individual administration of any of the three crude polysaccharides could significantly increase the number of IgA secreting cells, and the maximum numbers of increased IgA secreting cells in the cecum tonsil and duodenum in the three polysaccharides groups were 37.7 and 33.5 when compared with the controls, and those of the APS groups were 33.9 and 32.7. These three crude polysaccharides at appropriate doses also significantly enhance anti-Newcastle disease virus antibody titers, and the maximum antibody titer increase in the three polysaccharides groups was 1.6 $\log_2$ when compared with the control group, and those of the APS groups was 1.7 $\log_2$. These findings indicated that the appropriate doses of the three crude polysaccharides possess significant immune enhancing properties of mucosa and humoral immune response s, which have similar effect with astragalus polysaccharide, and may be useful as a new type of immune potentiator during vaccination.

Key words: Chinese herbal crude polysaccharides, vaccine, immunity, chickens.

INTRODUCTION

In recent years, many unknown and latent forms of infections have emerged in addition to the prevailing diseases. Among the various emerging diseases, viral diseases in general and immunosuppressive viruses in particular are suspected as the etiological agents for a variety of clinical conditions in poultry. Newcastle disease (ND) virus is one of the most important avian infection agents, because of high mortality rates in acute infections caused by subclinical infections. Many researchers in Africa, Asia and Australia have identified ND as the major cause of mortality in chicken production. To protect chickens against ND virus, both live and inactivated vaccines have been used. The goal of vaccination is to generate a strong immune response providing long term protection against infection. However, it is difficult to provide full protection of chickens against virus infection. Thus, it is necessary to study and develop safer and more efficacious vaccine immune potentiators that are safe for

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Abbreviations: APS, Astragalus polysaccharide; **IRPS,** isatis root polysaccharide; **ARPS,** achyranthes root polysaccharide; **CYPS,** Chinese yam polysaccharide; **ND,** Newcastle disease; **IB,** infectious bronchitis; **HI,** hemagglutination inhibition; **IgG,** immunoglobulin G; **IgA,** immunoglobulin A; **CMF,** calcium and magnesium -free; **PBS,** phosphate-buffered saline; **DAB,** diaminobenzidine.

chickens and have no risk of producing antigenic and pathogenic variants. The simultaneous application of a vaccine and an immune potentiator could improve the efficacy of vaccination.

Many Chinese herbal medicines and their ingredients have been reported to enhance immune responses (Hu, 1997; Xie, 2000; Ma et al., 2012), and thus have great potential in practical applications. Polysaccharides, one of the main classes of bioactive substances from Chinese herbal medicine, have been indicated to have wide pharmacological activities, especially broad immunomodulatory and antitumour effects. Thus, polysaccharides are regarded as biological response modifiers and attract attention, because of their natural origin, low toxicity in humans and animals, and long - standing use as folk medicines (Lu et al., 2003; Yon et al., 2006). It has been reported that astragalus, isatis root, achyranthes root and Chinese yam are common traditional Chinese medicinal plant widely used to enhance the body's natural defense mechanisms and the immune responses, and polysaccharides are the main effective components of immunological enhancement from these Chinese herbal medicines. Especially, the immune enhancement of astragalus polysaccharide has been reported by many researchers, and has been successfully used in li vestock breeding industry (Cho et al., 2007; Cui et al., 2011; Sun and Xie, 2011; Zhao et al., 2008). In this study, we investigated the immunoenhancing effects of three kinds of crude polysaccharides with respect to mucosal and humoral immune responses following vaccination in chickens, astragalus polysaccharide as positive control. The aim of this study is to determine the potential of these three polysaccharides as a new type immune potentiator during vaccination.

MATERIALS AND M ET HODS

Preparation of Chinese herbal crude polysaccharides

The four crude polysaccharides w ere extracted using w ater-decoction-ethanol-precipitation method as prev iously described (Xue, 1985). Total polysaccharide w as measured by Vitriol-anthracene ketone, us ing glucose w ithout H_2O as a standard control (Liu et al., 1994). The contents (%) of total astragalus polysaccharide (APS), isatis root polysaccharide (IRPS), achyranthes root polysaccharide (A RPS) and Chinese yam polysaccharide (CY PS) (comparable w ith those of glucose) w ere 65.1, 56.4, 54.3 and 72.8%, respectively. Based on our previous studies and on the polysaccharide content of the extracts prepared here, the four crude polysaccharides w ere diluted w ith deionized water into 2 concentrations as follow s: 4 and 2 mg/ml for APS, 3 and 1.5 mg/ml for IRPS, 6 and 3 mg/ml for ARPS and CY PS, respectively. The diluted preparations w ere sterilized by pasteurization and tested for endotox in by pyrogen tests (Veterinary Pharmacopoeia Commiss ion of the People's Republic of China, 2000). Follow ing confirmation that all polysaccharide crude extracts met the acceptable standard of Chinese Veterinary Phar macopoeia (less than 0.5 EU/ml), the preparations w ere stored at 4°C until use.

Reagents

Mouse anti-chicken IgA antibody (dilution: 1:100), biotinylated rabbit anti- mouse immunoglobulin (IgG) antibody, horseradish peroxidase-labeled chain ovalbumin (all from Shenzhen Jingmei Biotechnology Co. Ltd., Shenzhen, China), normal goat serum (Henan Tumor Hospital Pathology Laboratories, Zhengzhou, China), developer diaminobenzidine (DAB, Sigma) purchased from Beijing Zhongshan Biotechnology Co. Ltd., Beijing, China; hydrogen peroxide, citric acid, sodium citrate are produced by Zhejiang Mitaka chemical reagent Co., Ltd., Lanx i, China; methanol, formaldehyde are produced by Hubei Xinda Li Biochemical Co., Ltd., Wuhan, China; sodium dihydrogen phosphate, disodium hydrogen phosphate are produced by Bei Jing Kang Pu Hui Wei Technology Co., Ltd., Beijing, China.

Vaccine

ND (Lasota strain)-IB (H_{120} strain) live virus vaccine (No. 315) and ND- IB oil adjuvant vaccine (No. 551) w ere provided by the Institute of Veterinary Medicine, Animal Husbandry Bureau of Henan Pr ovince, Zhengzhou, China.

Birds and housing

One-day-old white Roman male chickens (layer type), purchased from Zhengzhou Ruixiang Co. Ltd., were housed in wire cages (60 × 60 × 100 cm) in climate controlled rooms at 36±1°C and kept under 24 h light at the beginning of the pretrial period, with ten chickens per w ire cage. The temperature was gradually reduced to room temperature in spring and the light time w as kept constant to 12 h per day. Chickens were fed with a commercial starter diet, provided by Feed Factory of Animal Husbandry Bureau of Henan province.

Experimental design

At 14 days of age, 450 chickens w ere vaccinated w ith ND- IB live virus vaccine by intr anasal and intraocular administrations, and then were randomly divided into nine treatment groups of 50 chic kens each, 5 cages per treatment. Their mean titer of maternal antibody against ND virus w as 4.5 $\log_2$ and the average body w eight w as 97.6 g. Each chicken in groups 1 to 8 w as injected subcutaneously w ith 0.5 ml of one of the four crude polysaccharides at one of tw o concentrations, once a day for three successive days. In group 9, as the control, each chicken w as injected w ith 0.5 ml saline at the same times as treatment groups. At 28 days of age, all chic kens were vaccinated for the second time w ith ND- IB oil adjuvant vaccine by subcutaneous injection in the dorsal region of the cervix. On days 10, 20, 30, 40, 50, and 60 after the first vaccination, eight chickens w ere sampled randomly from each group to determine changes in the number of IgA secreting cells in the duodenum and cecum tonsil mucosa by immunohistochemistry (Yang et al., 2002), and to determine temporal changes of serum ND antibody titers by micro method (Thekisoe et al., 2004).

Immunohistochemical examination for IgA secreting cells

After sacrifice, a fragment of the duodenum from the same region and cecum tonsil from the same side of each chicken w ere excised, fixed in 10% neutral formalin solution and embedded in paraffin. Immunohistochemistry was performed on 0.6 mm thick paraffin sections. After deparaffinization and dehydration, endogenous peroxidase was inactivated by incubation w ith 0.3% hydrogen peroxide in methanol, and w as washed in phosphate-buffered saline

(PBS, 0.01 M phosphate, 0.13 M NaCl, pH 7.4) for 10 min, then demas ked by microw ave oven treatment and c itrate buffer. After washing in PBS, the sections w ere treated w ith 5% normal goat serum in PBS in a humid chamber for 30 min at room temperature to block non-spec ific binding. The sections w ere rinsed three times w ith PBS for 5 min and then stained separately w ith monoclonal mouse anti-chic ken IgA antibody and the preparations w ere incubated at 4°C overnight. Tissues w ere rinsed in PBS for 5 min and then incubated for 30 min w ith biotiny lated secondary antibody diluted (1:50) in PBS. After rinsing w ith PBS, sections w ere incubated w ith horseradish peroxidase-labeled chain ovalbumin for 30 min, w ashed w ith PBS and the reactions w ere made visible w ith DAB substrate. Sections w ere then counterstained w ith hematoxylin, rinsed w ith distilled w ater and cleared w ith xylene. All incubations were performed in a moist chamber. Control staining w as carried out simultaneously, in w hich the primary antibody w as replaced w ith PBS.

Hemagglutination inhibition examination for serum specific IgG antibody

Blood samples (1.0 ml per chicken) w ere draw n into Eppendorf tubes from the main brachial vein of the chicken and allow ed to clot at 37°C for 2 h prior to collecting serum. Serum w as separated by centrifugation and stored at -20°C for use. Briefly, after inactivation of serum at 56°C for 30 min, tw o-fold serial dilution of serum in a 96-w ell, V-shaped bottom microtiter plate containing 50 µl PBS w as performed, and 50 µl of ND v irus antigen (4 hemagglutinin (HA) units) w as added to all the w ells except for the last row , as controls. Serum dilutions ranged from 1:2 to 1:2048. The antigen serum mixture w as incubated for 10 min at 37°C. Then 50 µl of a 1% rooster erythrocyte suspension w as added to each w ell and re-incubated for 30 min. Pos itive serum, negative serum, erythrocytes, and antigens w ere included as controls. The highest dilution of serum causing complete inhibition of erythrocyte agglutination w as considered the endpoint. The geometric mean titer w as expressed as reciprocal log $_2$ values of the highest dilution that displayed anti-ND virus hemagglutination inhibition.

Statistical analysis

The sections w ere observed using an LEICA microscope (Model DM2000, Germany, purchased from Leica Microsystems Trading Ltd. Shangha, China) and analyzed by Qw in image analysis system of LEICA image w orkstations (CD 2000, Germany, purchased from Leica Microsystems Trading Ltd., Shangha, China). Tw enty different fields of view w ere chosen per section, w ith 8 sections per group and analysis of positive IgA secreting cells w hich appear as brow n (Figure 1) w as calculated by number. The number of IgA secreting cells in regional units w as used for the statistical analysis of the data.

Data are expressed as mean ± standard deviation for analysis; single factor analysis of variance (A NOVA) test w as performed using Statistical Package for Social Sciences (SPSS) to determine the difference among herbal polysaccharides and control groups. $P < 0.05$ w as considered significant for all analyses.

RESULTS

Increased numbers of IgA secreting cells in duodenum of treated chickens

On day 20 after the first vaccination, the numbers of IgA secreting cells in the duodenum in the APS_L group were significantly elevated when compared with controls ($P < 0.05$). On day 30, the numbers of IgA secreting cells in APS_H, APS_L, $IRPS_L$, $ARPS_H$ and $CYPS_H$ groups were significantly elevated when compared with the controls ($P< 0.05$), and the largest mean number of IgA secreting cells was 139.1 in $IRPS_L$ group, and 133.2 in APS_H, which in control group was 101.4. On days 40 and 50, the numbers of IgA secreting cells in APS_L, $IRPS_L$, $ARPS_H$ and $CYPS_H$ groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells in $CYPS_H$ and $IRPS_L$ groups was 128. 9 and 120.9, respectively, and was 131. 4 and 118.6 in APS_L, which in control group was 97.5 and 88.1. On day 60, the numbers of IgA secreting cells in $IRPS_L$ and $CYPS_H$ groups were significantly elevated when compared with controls ($P < 0.05$), and the largest mean number of IgA secreting cells in $IRPS_L$ group was 99.3, and 97. 6 in APS_L, which in control group was 75. 7 (Table 1).

Increased numbers of IgA secreting cells in cecum tonsils of treated chickens

On day 20 after the first vaccination, the numbers of IgA secreting cells in the cecum tonsil in the treatment groups were elevated when compared with the controls, and there was no significant difference ($P > 0.05$). On day 30, the numbers of IgA secreting cells in APS_L, $IRPS_H$, $IRPS_L$, $ARPS_H$ and $CYPS_H$ groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells in $IRPS_L$ group was 138.3, and was 141.4 in APS_L, which in the control group was 108.7. On days 40 and 50, the numbers of IgA secreting cells in APS_L, $IRPS_L$, $ARPS_H$ and $CYPS_H$ groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells in $IRPS_L$ group was 128.9 and 110.2, and was 122.5 and 108.8 in APS_L group, which in the control group was 95.4 and 85.5. On day 60, the numbers of IgA secreting cells in APS_L, $IRPS_L$ and $CYPS_H$ groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells in $IRPS_L$ group was 105.3, and for APS_L group was 102.2, which in control group was 77.1 (Table 2).

Dynamic changes in serum ND virus-specific IgG antibody titers

On day 10 after the first vaccination, ND virus-specific IgG antibody titers among the 9 groups showed no significant difference ($P > 0.05$). For APS, on days 20, 30, 40, and 50, ND virus-specific IgG antibody titers in the APS_L group were higher when compared with the controls significantly ($P < 0.05$). And on day 60, the titers in APS_H and APS_L groups were higher when compared with the controls significantly ($P < 0.05$); the antibody titer in APS_L group was 8.9 $\log_2$, which in control group was 7. 2 $\log_2$; the antibody titer increased in the APS_L group was the

Table 1. Dynamic changes in the number of IgA secreting cells in the duodenum of vaccinated chickens.

Group	D10	D20	D30	D 40	D50	D 60
IRPSH	72. 3±1 1. 2^{a}	99. 1±1 4. 3^{b}	119. 6 ±16. 1^{b}	119. 2±17. 7^{b}	97. 8±1 3. 6^{b}	90. 2±1 2. 9^{b}
IRPSL	77. 8± 14. 7^{a}	105. 5 ±15. 2^{b}	139. 1 ±19. 5^{a}	12 7. 1± 18. 2^{a}	120. 9 ±15. 5^{a}	99. 3±1 2. 6^{a}
ARPSH	75. 9± 10. 9^{a}	108. 9 ±14. 5^{b}	135. 5 ±18. 3^{a}	12 5. 5± 17. 5^{a}	114. 7 ±14. 5^{a}	95. 9±1 4. 4^{ab}
ARPSL	73. 6±1 1. 6^{a}	97. 1±1 3. 9^{b}	118. 8 ±15. 4^{b}	114. 6±17. 9^{b}	95. 7±1 3. 2^{b}	87. 6±1 3. 8^{b}
CYPSH	71. 4± 13. 1^{a}	110. 6±15. 9^{ab}	136. 4 ±19. 8^{a}	12 8. 9± 17. 3^{a}	115. 2 ±14. 9^{a}	98. 4±1 4. 1^{a}
CYPSL	75. 1± 12. 8^{a}	98. 7±1 3. 5^{b}	114. 2 ±15. 6^{b}	110. 8±16. 8^{b}	101. 3 ±15. 8^{b}	84. 7±1 3. 2^{b}
APSH	79. 2± 14. 2^{a}	96. 9±1 3. 3^{b}	133. 2 ±21. 9^{a}	12 2. 3± 18. 4^{b}	105. 1 ±15. 1^{b}	85. 9±1 5. 2^{b}
APSL	74. 7± 12. 4^{a}	117. 3 ±17. 4^{a}	129. 7 ±13. 4^{a}	13 1. 4± 18. 9^{a}	118. 6 ±14. 8^{a}	97. 6±1 4. 6^{ab}
Control group	74. 9± 13. 7^{a}	95. 3±1 4. 8^{b}	101. 4 ±17. 7^{b}	97. 5±1 5. 1^{b}	88. 1±1 4. 3^{b}	75. 7±1 2. 9^{b}

Column data marked w ithout the same superscripts differ significantly (P<0.05). D, day; APS, astragalus polysaccharide which is the positive control; IRPS, isatis root polysaccharide; ARPS, achyranthes root polysaccharide; CYPS, Chinese yam polysaccharide.

Table 2. Dynamic changes in the number of IgA secreting cells from cecal tonsils of vaccinated chickens.

Group	D 10	D 20	D 30	D 40	D 50	D60
IRPSH	69. 7±1 2. 9^{a}	93. 6± 14. 6^{a}	12 8. 7± 17. 9^{a}	10 6. 3± 16. 9^{b}	99. 1± 15. 4^{b}	92. 6±1 3. 2^{b}
IRPSL	68. 4±1 1. 8^{a}	102. 8 ±15. 2^{a}	13 8. 3± 19. 3^{a}	12 8. 9± 16. 2^{a}	110. 2 ±17. 3^{a}	105. 3 ±16. 1^{a}
ARPSH	70. 3±1 2. 7^{a}	99. 2± 14. 3^{a}	12 7. 8± 14. 8^{a}	119. 8 ±15. 7^{a}	107. 9 ±16. 6^{a}	97. 5±1 5. 2^{ab}
ARPSL	60. 8±1 1. 1^{a}	95. 3± 13. 7^{a}	117. 9±15. 6^{b}	113. 4±17. 1^{ab}	96. 3±15. 8^{ab}	83. 8±1 3. 3^{b}
CYPSH	65. 5±1 1. 5^{a}	98. 4± 14. 1^{a}	12 9. 6± 16. 3^{a}	12 0. 7± 16. 4^{a}	106. 5 ±15. 1^{a}	99. 9±1 2. 4^{a}
CYPSL	67. 9±1 2. 2^{a}	91. 6± 13. 9^{a}	112. 3±16. 8^{b}	10 1. 5± 16. 1^{b}	92. 4± 14. 2^{b}	85. 9±1 3. 5^{b}
APSH	62. 2±1 0. 8^{a}	100. 7 ±14. 9^{a}	120. 1± 16. 5^{ab}	10 3. 2± 15. 9^{b}	97. 6± 14. 5^{b}	88. 5±1 3. 1^{b}
APSL	64. 9±1 1. 3^{a}	94. 5± 13. 8^{a}	14 1. 4± 18. 7^{a}	12 2. 5± 15. 5^{a}	108. 8 ±16. 7^{a}	102. 2 ±14. 3^{a}
Control group	65. 3±1 1. 9^{a}	89. 9± 13. 6^{a}	10 8. 7± 17. 7^{b}	95. 4±1 5. 3^{b}	85. 5± 14. 7^{b}	77. 1±1 2. 8^{b}

Column data marked w ithout the same superscripts differ significantly (P<0.05). D, day; APS, astragalus polysaccharide which is the positive control; IRPS, isatis root polysaccharide; ARPS, achyranthes root polysaccharide; CYPS, Chinese yam polysaccharide.

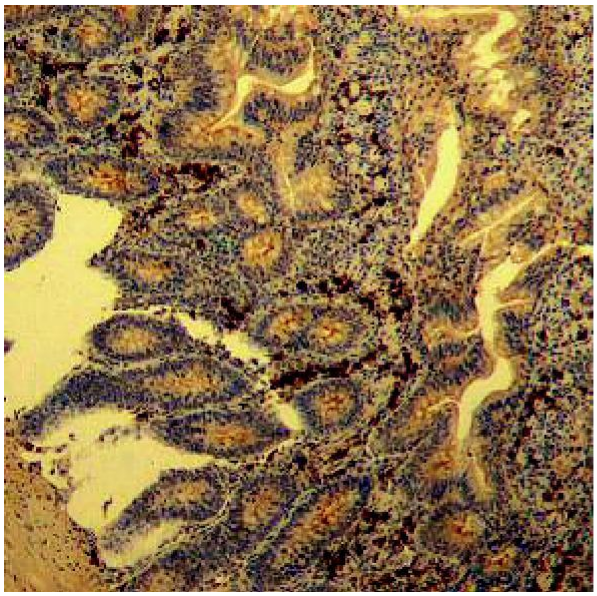

Figure 1. The section by Immunohistochemical staining which w ere observed using optical microscope. Distr ibution of SIgA positive cells w hich appears brow n in the chicken duodenum and the section w as observed at 400× magnification.

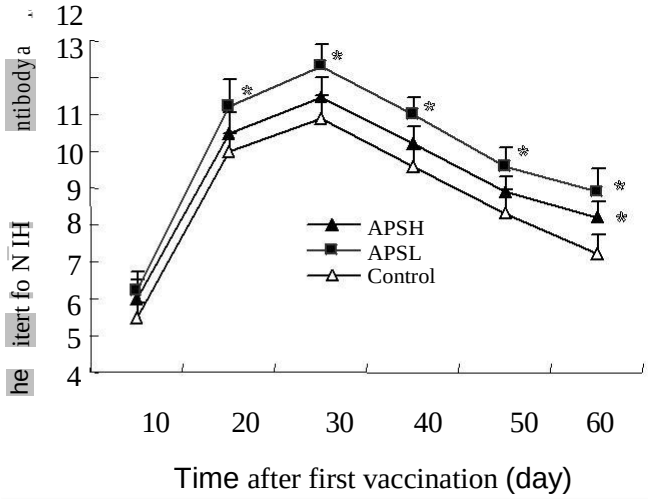

Figure 2. Dynamic changes of serum specific IgG antibody titers (Log_2) in A PS (pos itive control) and control groups. Data represent the mean ± SD. *P<0.05 compared w ith controls. ND, New castle disease; A PS, astragalus polysaccharide; A PS_H, high dose astragalus polysaccharide; A PS $_L$, low dose astragalus polysaccharide.

maximum (1. 7 log_2) when compared with the controls (Figure 2). For IRPS, on days 20, 40, and 50, the titers in

$IRPS_L$ group were higher when compared with the controls significantly ($P < 0.05$). On days 30 and 60, the titers in $IRPS_H$ and $IRPS_L$ groups were higher when compared with controls significantly ($P < 0.05$). And on days 60, the antibody titer in $IRPS_L$ group was 8.8 log_2, which in control group was 7.2 log_2; the antibody titer increased in $IRPS_L$ group was the maximum (1.6 log_2) when compared with the controls (Figure 3). For ARPS, on days 20, 30, 40, 50, and 60, the titers in $ARPS_H$ group were higher when compared with the controls ($P < 0.05$). And on days 60, the antibody titer in $ARPS_H$ group was 8.5 log_2, which in control group was 7.2 log_2; the antibody titer increased in the $ARPS_H$ group was the maximum (1.3 log_2) when compared with the controls (Figure 4). For CYPS, on days 20, 30, 40, 50, and 60, the titers in the $CYPS_H$ group were higher when compared with controls significantly ($P < 0.05$). And on days 60, the antibody titer in $CYPS_H$ group was 8.4 log_2, which in control group was 7.2 log_2; the antibody titer increased in CYPSH group was the maximum (1.2 log_2) when compared with controls (Figure 5). It showed that IRPS is the most similar to APS in raising ND virus-specific IgG antibody titers.

DISCUSSION

The mucosal immune system is equipped with unique innate and acquired defense mechanisms, which provide a first line of protection against ingested infectious agents (Mick and Karin, 2006). Secretory IgA is the major antibody isotype present in mucosal secretions and has many functions, both direct and indirect, that prevent infective agents such as bacteria and viruses from breaching the mucosal barrier (Egmond et al., 2001; Russell and Sibley, 1999). Therefore, IgA secreting cells are important for the protection of mucosal surfaces. Changes in the numbers of IgA secreting cells are one of the standards used to estimate mucosal immunity. In this study, the presence of positive IgA secreting cells was detected from duodenum and cecum tonsils of chickens by immunohistochemistry. We found that the numbers of positive IgA secreting cells per unit area from tissues of treatment groups were much higher than those from control groups at most time points, especially in the APS_L, $IRPS_L$, $ARPS_H$ and $CYPS_H$ treatment groups. This suggests that APS, IRPS, ARPS and CYPS might promote the differentiation and proliferation of IgA secreting cells in the intestinal mucosa of chickens. This demonstrated that Chinese herbal polysaccharides could effectively stimulate mucosal immune responses to resist external microbial invasion. Interestingly, we found that the effects of APS and IRPS at low doses were better than ARPS and CYPS at high doses. This phenomenon concurs in part with a study by Zhang et al. (2007), where two compound adjuvants (cMIA I and cMIA II) promoted IgA secreting cells and intestinal intraepithelial lymphocytes in chickens vaccinated with attenuated Newcastle-disease vaccine.

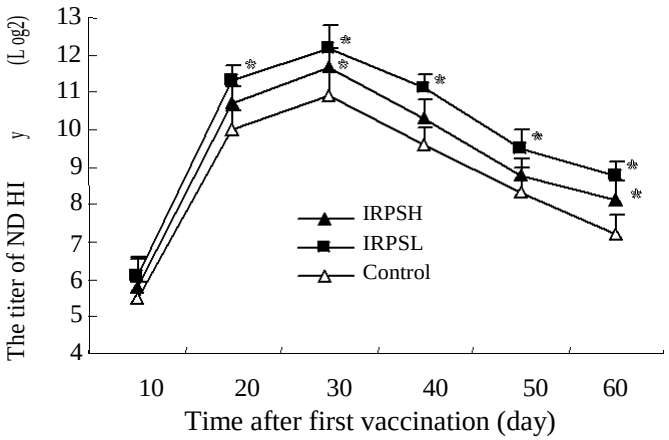

Figure 3. Dynamic changes of serum spec ific IgG antibody titers (Log_2) in IRPS and control groups. Data represent the mean ± SD. *P<0.05 compared w ith controls. ND, New castle disease; IRPS, isatis root polysaccharide; $IRPS_H$, high dose isatis root polysaccharide; $IRPS_L$, low dose isatis root polysaccharide.

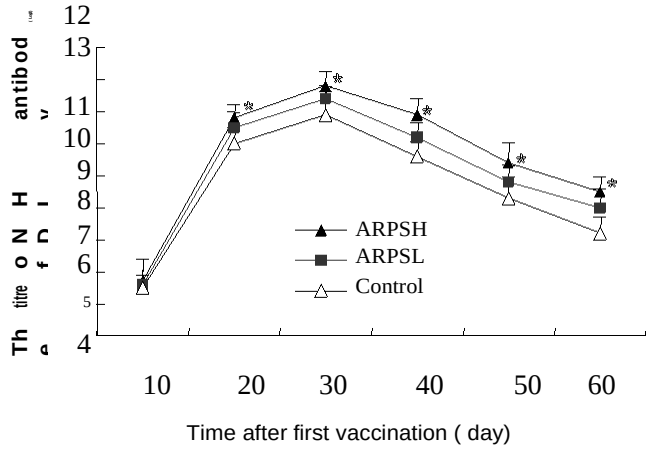

Figure 4. Dynamic changes of serum spec ific IgG antibody titers (Log_2) in A RPS and control groups. Data represent the mean ± SD. *P<0.05 compared w ith controls. ND, New castle disease; A RPS, achyranthes root polysaccharide; A RPS_H, high dose achyranthes root polysaccharide; A RPS_L, low dose achyranthes root polysaccharide.

Merz et al. (1981) reported that humoral immune responses played important roles in the host's defense against ND virus infection. The specific antibodies could neutralize or inactivate the free virus by binding to virus surface glycoproteins, thus inhibiting the attachment of virus to cells, and blocking viral spread. The dynamic changes of specific serum IgG antibody titers reflect the state of humoral immunity in the animals. Our results showed that the antibody titers in most treatment groups at many time points were significantly higher than in the control group.

Titers in the APS_L, $IRPS_L$, $ARPS_H$ and $CYPS_H$ groups at five time points were significantly higher than in controls, indicating that low dose IRPS and high

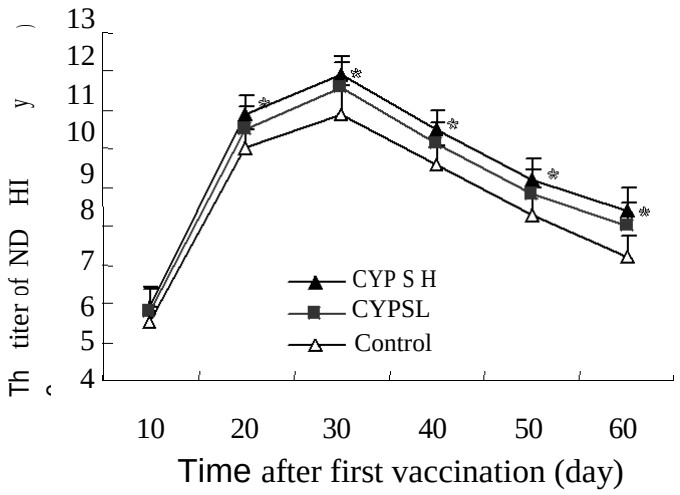

Figure 5. The dynamic changes of serum specific IgG antibody titers (Log 2) in CY PS and control groups. Data represent the mean ± SD. *P<0.05 compared w ith controls. ND, New castle disease; CY PS, Chinese yam polysaccharide; CY PS_H, high dose Chinese yam polysaccharide; CY PS_L, low dose Chinese yam polysaccharide.

dose ARPS and similar to APS,CYPS had the best effect on enhancing humoral immunity. Antibody titers in low dose APS and IRPS and high dose ARPS and CYPS chickens up to 60 days old were still higher than 8.4 Log2, while the titer in control group was 7. 2 Log2. This indicated that APS, IRPS, ARPS and CYPS at suitable doses could maintain higher antibody titers, because of a slower decline of antibody titer. Gu et al. (2005) reported that Chinese herbal medicine compound polysaccharides could promote the development of immune organs in chickens. The main immune organ for specific antibody production is the thymus and bursa of Fabricius (Alam et al. 1997). Thus, these three Chinese herbal crude polysaccharides could strengthen humoral immunity in vaccinated chickens through promoting development of immune organs.

These results showed that immune-enhancing effects of the three different crude polysaccharides are very similar to APS. In our previous preliminary test, a variety of traditional Chinese medicine were selected and extracted to observe the immunomodulatory effects on mice, and then these crude polysaccharides which had the better effect were selected to observe its immune - enhancing effect in chickens, when compared with APS. This may be the reasons why the experimental results are very similar, but it indicated that the suitable doses of these crude polysaccharides were not the same, and the effect of IRPS which was most similar to APS at low doses were slightly better than ARPS and CYPS at high doses. Similar research also verified the remarkable potential benefits of crude polysaccharides derived from Chinese medicines (Chen et al., 1997; Sun et al., 2005).

Conclusion

This study confirmed that low dose IRPS and high dose ARPS and CYPS could significantly promote the differentiation and proliferation of IgA secreting cells in the intestinal mucosa and increase serum ND virus-specific IgG antibody titers, and thus, enhance mucosal and humoral immune responses. It takes longer time to inhibit the multiplication of virus when compared with antiviral drug, but Chinese herbal crude polysaccharides has the advantages of natural, safe, less toxic or side effect at suitable dose, because the antiviral efficacy of these Chinese herbal crude polysaccharides is achieved through enhancing the body's immune system. Therefore, Chinese herb polysaccharides should be used for the prevention of viral diseases, rather than treatment. These three crude polysaccharides may form the basis for a new immune potentiating drug in the domestic animal and poultry industry. The dosage used is an important factor and must be considered in the development of a Chinese herbal medicinal immune potentiating drug. Further study on the mechanism of protective vaccination effects of the three Chinese herbal polysaccharides is underway.

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