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Immune Recovery Effects of Immunopotentiator from *Pantoea agglomerans* 1 (IP-PA1) on Low Antibody Productions in Response to *Salmonella* Enteritidis Vaccine and Sheep Red Blood Cells in Dexamethasone-Treated Stressed Chicken Models

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ABSTRACT. Considering the usefulness of the immunopotentiator from *Pantoea agglomerans* 1 (IP-PA1), which is a purified lipopolysaccharide (LPS) derived from symbiotic gram-negative bacteria of food crops, in controlling immunosuppression in poultry husbandry, in this study, we examined its immune-recovery effects in dexamethasone-treated stressed chicken models. Three-week-old chickens daily administered 10 μg/kg of dexamethasone for 35 days to induce stress showed more whole body weight loss; relative thymic, bursal, and splenic weight losses; and decrease in the number of peripheral blood lymphocytes, as compared with the control chickens on day 35; the IP-PA1-pretreated, dexamethasone-treated chickens showed reduced weight losses. Five- to eight-week-old chickens administered 5 mg/kg of dexamethasone showed excessive apoptosis of thymic and bursal lymphocytes 24 hr after a single dexamethasone treatment; apoptosis was inhibited in the IP-PA1-pretreated, dexamethasone-treated chickens. Chickens daily administered 10 μg/kg of dexamethasone for 35 days and injected with commercial *Salmonella* Enteritidis (SE) vaccine or sheep red blood cells (SRBC) on days 7 and 21 showed about 8- or 2-fold lower antibody production in response to SE or SRBC, respectively, as compared with the control chickens on day 35; the antibody production in response to SE or SRBC was increased in the IP-PA1-pretreated, dexamethasone-treated chickens. These results indicate that IP-PA1 exerts inhibitory effects on dexamethasone-induced immunosuppression and that it may be useful in controlling immunosuppression in poultry husbandry.

KEY WORDS: chicken, immunomodulator, immunosuppression, IP-PA1, stress.

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Immunosuppression is often induced by various stresses such as overcrowded feeding and exposure to excessively high or low temperature. A typical phenomenon of stress-induced immunosuppression observed in chickens is low antibody production to antigens [15, 31, 34], which can cause low vaccine responses or low resistance against infections. Therefore, control of immunosuppression is critical for maintaining animal health; moreover, there has been an earnest demand of practically useful immunomodulators [4].

Under various stress conditions, the hypothalamus-pituitary-adrenal axis is activated and glucocorticoids are secreted by the adrenal gland. Glucocorticoids, often regarded as a hallmark of stress, play a critical role in affecting physiological changes, such as anemia, body weight loss, and hyperlipidemia in stressed animals [25]. Glucocorticoids considerably contribute to stress-induced immunosuppression through their role in affecting physiological changes, including direct suppression of immune cells [9]. Thus, animals administered dexamethasone, a typical synthetic glucocorticoid, has been used as stress models in many studies [10, 23, 29].

IP-PA1 is a low-molecular-weight (5 kDa) LPS derived from the cell walls of symbiotic gram-negative bacteria commonly found in various food crops, such as cereals, fruits, and vegetables [18, 19, 22, 28]. It has been shown that IP-PA1 has immune-enhancing effects such as macrophage-activation and protection against bacterial and parasitic infections in laboratory animals [19, 28].

However, thus far, the immunological effect of IP-PA1 has not been analyzed in domestic animals, including chickens. Therefore, in this study, considering the usefulness of IP-PA1 in controlling immunosuppression manifested as low antibody production in poultry husbandry, its immune-recovery effects were investigated in dexamethasone-treated immunosuppressed (stressed) chicken models. First, the effects of long-term dexamethasone treatment on whole body weight and relative lymphoid organ weights were examined. The inhibitory effects of IP-PA1 on dexamethasone-induced losses in the weights of the thymus and bursa

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of Fabricius and excessive apoptosis of lymphocytes in these tissues were then investigated. Finally, the effects of IP-PA1 pretreatment on dexamethasone-induced low antibody productions in response to SE vaccine or SRBC were examined.

MATERIALS AND METHODS

Reagents: IP-PA1, an LPS derived from the cell walls of *Pantoea agglomerans*, symbiotic gram-negative bacteria commonly found in food crops, was purified to >99% according to previously described methods [32]. Dexamethasone 21-phosphate disodium salt, a water-soluble dexamethasone analog, was purchased for treating the animal models (Sigma-Aldrich, St. Louis, MO, U.S.A.). A complete culture medium, Roswell Park Memorial Institute (RPMI) 1640 (Gibco; Invitrogen, Carlsbad, CA, U.S.A.) supplemented with L-glutamine, 10% fetal bovine serum (FBS; HyClone; Thermo Scientific, South Logan, UT, U.S.A.), 100 μg/m*l* of penicillin and streptomycin (Nakalai Tesque, Kyoto, Japan), 0.05 mM mercaptoethanol (Wako Pure Chemical Industries Ltd., Osaka, Japan) and 10 mM HEPES (Wako Pure Chemical Industries Ltd.) was used for cell culture. Calcium- and magnesium-free Hank's balanced salt solution (Sigma-Aldrich) supplemented with 10% fetal bovine serum, 100 μg/m*l* of penicillin and streptomycin and 10 mM HEPES was used as the washing medium in the preparation of lymphocytes [36].

Chickens: Three- to eight-week-old male and female inbred White Leghorn chickens of the major histocompatibility complex (MHC) haplotype B^2B^2 [6], maintained at the Tokyo University of Agriculture and Technology, Tokyo, Japan, were used in this study. All the chickens were kept in an air-conditioned room and fed standard laboratory food and water *ad libitum*. All experiments with animals were approved by the University Animal Care and Use Committee of the Tokyo University of Agriculture and Technology.

Long-term dexamethasone treatment: Three-week-old chickens were intramuscularly administered a daily dose of 10 μg/kg of dexamethasone for 35 days. The whole body weight of each chicken was measured daily. In order to calculate the peripheral white blood cell (WBC) count, a blood sample was collected from each chicken via the ulnar vein, and blood smears were prepared on day 35 after the initiation of the dexamethasone treatment. Differential WBC counts were determined by microscopy (× 400 magnification), counting 200 intact WBCs after Wright-Giemsa staining. After blood sample collection, the chickens were sacrificed by treating them with an overdose of diethyl ether, and the thymic, bursal and splenic weights were measured. For the control group, the same number of chickens as above were injected with PBS and treated in the same way as described above. The thymic, bursal and splenic weights of each chicken were divided by the whole body weight and expressed as relative organ weights (mg/100 mg body weight).

Vaccination: Three-week-old chickens were orally administered different doses of IP-PA1 daily 2 hr prior to the daily intramuscular injections of 10 μg/kg of dexamethasone for 35 days. On days 7 and 21 after initiation of the IP-PA1 and dexamethasone treatments, each chicken was subcutaneously injected with 500 μ*l* of a commercial inactivated SE vaccine (Layermune SE; CAF Laboratories, Hiroshima, Japan) according to the manufacture's instructions. Another group of chickens treated in the same way as above was intravenously injected with a PBS-diluted suspension of 5×10^8 SRBC [11], following the same schedule as that for SE vaccination. On days 11, 14, 18, 21, 25, 28, 32, and 35, serum samples were collected, and specific antibody titers against SE or SRBC were determined by an indirect agglutination test. On day 35, the chickens were sacrificed by treating them with an overdose of diethyl ether, and the thymic, bursal, and splenic weights were measured. The relative organ weights of the dexamethasone-treated chickens were divided by those of the dexamethasone-untreated chickens and expressed as a ratio of organ weight of the treated chickens to that of the control group.

Indirect agglutination test: Specific antibody titers against SE and SRBC were determined by performing an indirect agglutination test [12] with slight modifications. In brief, 50 μ*l* of serum samples serially diluted with PBS in 2-fold steps was placed into round-bottomed microtest plates, and 50 μ*l* of 100-fold diluted formalin-fixed whole SE clone #40 (10^8 cfu/m*l* [2]) or 2.5% SRBC was added to each well. The plates were incubated for 2 hr at 37°C and additionally for 20 min at 4°C. Subsequently, 50 μ*l* of the total volume of 100 μ*l* was transferred to a new conical-bottomed microtest plate, and 100 μ*l* of 2,000-fold diluted goat anti-chicken IgG (heavy chain- and light chain-specific; Kirkegaard and Perry Laboratories, Gaithersburg, MD, U.S.A.) was added to each well. The agglutination titer was read 4 hr after the addition of anti-IgG and was expressed as the $\log_2$ of the reciprocal of the highest dilution showing agglutination.

Preparation of lymphocytes: Five- to eight-week-old chickens were orally administered IP-PA1 2 hr prior to intramuscular injections of 5 mg/kg of dexamethasone. Twenty-four hours after a single dexamethasone treatment, thymic and bursal lymphocytes were isolated employing the method described by Zhu *et al.* [36], with slight modifications. In brief, the thymus and bursa of Fabricius of each chicken were removed and homogenized in the washing medium. The cell suspension was placed on ice for 20 min to allow the cell clumps to settle. After washing once, lymphocytes were isolated from the cell suspension by a density-gradient centrifugation, layering 10 m*l* of the cell suspension over 3 m*l* of Lymphoprep (specific gravity 1.077; Nycomed Pharma AS, Oslo, Norway) and centrifuging at 800 *g* for 30 min at room temperature. The lymphocytes were washed thrice with the culture medium, and the number of viable cells was counted by the Trypan blue dye exclusion test.

Apoptosis of lymphocytes: The percentage of apoptotic cells in the isolated lymphocytes was determined using a

TACS annexin-V kit (Trevigen Inc., Gaithersburg, MD, U.S.A.) according to the manufacturer's instruction. In brief, 10^6 cells were washed in ice-cold PBS and incubated with annexin-V for 15 min at room temperature. Apoptotic cells (annexin-V-positive cells) were distinguished using a flow cytometer (Epics Elite; Coulter, Tokyo, Japan).

Statistical analysis: All data are expressed as mean ± SEM. One-way ANOVA with post hoc analysis with the Bonferroni's test was used for multiple comparisons, and Student's *t*-test was used for comparing the means of 2 groups.

RESULTS

Long-term dexamethasone treatment: The whole body weight gains of the dexamethasone-treated chickens were observed to decrease from day 7 after the initiation of the treatment until the end of the experiment (day 35; Fig. 1A). On day 35, the whole body weight gains were reduced from 623.0 ± 16.8 to 385.0 ± 14.8 g, and the average daily weight gains decreased from 12.8 ± 0.8 to 6.2 ± 0.4 g/d after the dexamethasone treatment (Fig. 1A and 1B). In dexamethasone-treated chickens, muscular atrophy was generally observed. General health abnormalities such as anorexia and diarrhea were not observed. The relative thymic, bursal,

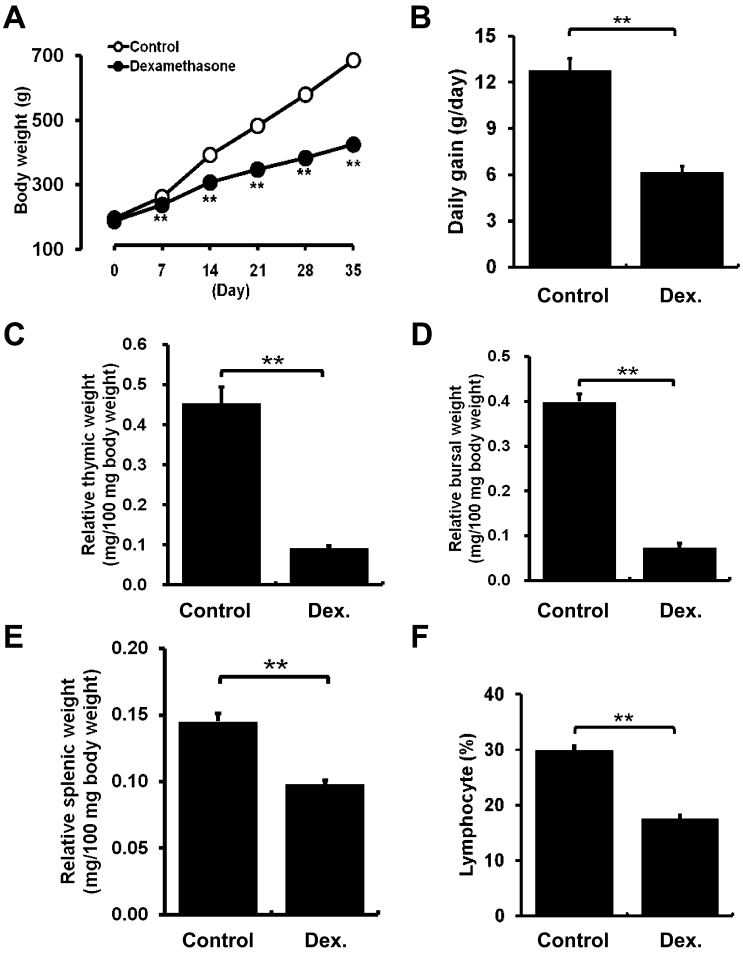

Fig. 1. Whole body weights, relative immunological organ weight losses and decreases in the numbers of peripheral blood lymphocytes in dexamethasone-treated chickens. Three-week-old chickens were intramuscularly administered a daily dose of 10 μg/kg of dexamethasone for 35 days. Whole body weights (A) and average daily weight gains (B) on the indicated days (A) or on day 35 (B) in the control chickens and dexamethasone-treated chickens are shown. Relative thymic (C), bursal (D) and splenic (E) weights on day 35 are shown as a ratio of the organ weight to the whole body weight of each chicken. The percentage of lymphocytes in peripheral WBCs are shown in F. The data represent the means ± SEM of values obtained for 5 chickens. Significant differences in the means of the 2 indicated groups are indicated as ** P<0.01.

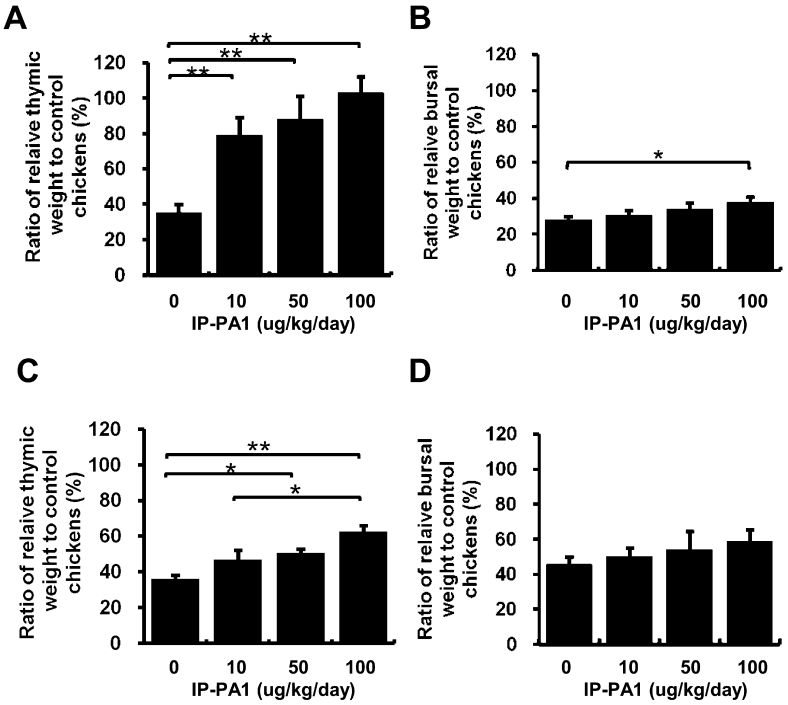

Fig. 2. Reductions in thymic and bursal weight losses in chickens pretreated with IP-PA1. Three-week-old chickens administered different daily doses of IP-PA1 and a daily dose of 10 μg/kg of dexamethasone for 35 days were subcutaneously injected with 500 μl of a commercial SE vaccine (A and B) or intravenously injected with 5×10^8 of SRBC (C and D) on days 7 and 21. Twenty-four hours after the last dexamethasone treatment, chickens were sacrificed, and the weights of thymus (A and C) and bursa of Fabricius (B and D) were measured. Data are expressed as ratios of the relative thymic or bursal weights of the dexamethasone-treated chickens to those of the control chickens. The data represent the means ± SEM of values obtained for at least 5 chickens. Significant differences in the means of the 2 indicated groups are indicated as * $P<0.05$ and ** $P<0.01$.

and splenic weights of the dexamethasone-treated chickens were decreased by 79.3, 81.5, and 32.4%, respectively, compared to those of the control chickens (Fig. 1C–E, respectively). In dexamethasone-treated chickens, parts of thymus or bursa of Fabricius replaced adipose tissues; and some leaves of thymus presented the string-shaped appearance. The number of peripheral blood lymphocytes, determined microscopically, decreased from 29.9 ± 0.8 to 17.5 ± 0.6% after the dexamethasone treatment (Fig. 1F).

Inhibitory effects of IP-PA1 on thymic and bursal weight losses: SE- or SRBC-injected chickens were sacrificed, and the thymic and bursal weights were measured on day 35 after initiation of the IP-PA1 and dexamethasone treatments. Both the relative thymic and bursal weights were observed to decrease in the dexamethasone-treated chickens on day 35 (65.50 ± 4.54 and 71.69 ± 1.76% weight loss, respectively, in SE-injected chickens (Fig. 2A and 2C) and 64.00 ± 2.21 and 54.22 ± 4.16% weight loss, respectively, in SRBC-injected chickens (Fig. 2B and 2D). With regard to the SE-injected chickens, thymic weight loss was reduced in the dexamethasone-treated chickens pretreated with a daily dose of 10 μg/kg of IP-PA1, compared to the IP-PA1-untreated, dexamethasone-treated chickens; the weight loss was inhibited almost completely by pretreatment with a daily dose of 50 or 100 μg/kg of IP-PA1 (Fig. 2A). With regard to the SRBC-injected chickens, thymic weight loss was reduced in the dexamethasone-treated chickens pretreated with a daily dose of 50 or 100 μg/kg of IP-PA1 (Fig. 2C). Bursal weight loss was reduced in the SE-injected (Fig. 2B) and only showed a tendency to reduce in the SRBC-injected (Fig. 2D) dexamethasone-treated chickens pretreated with a daily dose of 100 μg/kg of IP-PA1. Abnormality of shape of thymus and bursa of Fabricius mentioned above were reduced by pretreatment of IP-PA1; no difference was observed in the whole body weights and relative splenic weight loss between the IP-PA1-untreated chickens and IP-PA1-pretreated chickens in both the experiments (data not shown).

Inhibitory effects of IP-PA1 on apoptosis of thymic and bursal lymphocytes: Chickens were orally administered IP-PA1 2 hr prior to the intramuscular injections of 5 mg/kg of dexamethasone. Twenty-four hours after a single dexam-

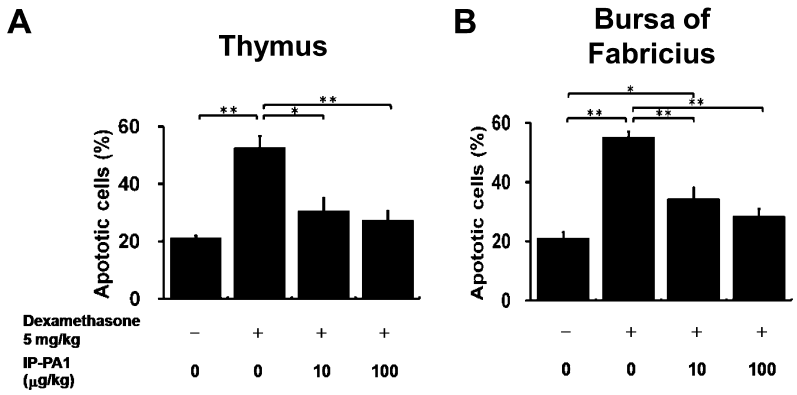

Fig. 3. Inhibition of dexamethasone-induced excessive apoptosis of thymic and bursal lymphocytes in chickens pretreated with IP-PA1. Five- to eight-week-old chickens were orally administered IP-PA1 2 hr prior to intramuscular injections of 5 mg/kg of dexamethasone, and apoptosis of thymic (A) and bursal (B) lymphocytes isolated 24 hr after dexamethasone treatment was analyzed. Annexin V-positive apoptotic cells were counted by flow cytometric analysis. The data represent the means ± SEM of values obtained from 3 independent experiments. Significant differences in the means of the 2 indicated groups are indicated as * $P<0.05$ and ** $P<0.01$.

ethasone treatment, thymic and bursal lymphocytes were isolated, and the percentage of apoptotic cells in them was determined by flow cytometric analysis. Dexamethasone treatment caused about 2.5-fold increase in the percentage of apoptotic cells in both thymic (from 21.13 ± 1.93 to 52.67 ± 4.12%) and bursal (from 21.13 ± 1.93 to 54.88 ± 1.98%) lymphocytes. Pretreatment with IP-PA1 inhibited this increase in the percentage of apoptotic cells in thymic (Fig. 3A) and bursal (Fig. 3B) lymphocytes.

Response to SE vaccine: Chickens were daily administered IP-PA1 and dexamethasone for 35 days and injected with inactivated SE vaccine on days 7 and 21 after the initiation of the IP-PA1 and dexamethasone treatments. Specific antibody titers against SE in the dexamethasone-treated chickens, as determined by the indirect agglutination test, were lower than those in the control chickens; pretreatment with a daily dose of 100 μg/kg of IP-PA1 before the dexamethasone treatment increased the antibody production in response to the SE vaccine at the examined time points (Fig. 4A). On day 35, the dexamethasone-treated chickens showed more than 8-fold lower antibody production in response to the SE vaccine than the control chickens (Fig. 4B). Dexamethasone-treated chickens pretreated with a daily dose of more than 10 μg/kg of IP-PA1 showed increased serum levels of SE-specific antibody as compared to the IP-PA1-untreated, dexamethasone-treated chickens (Fig. 4B). The serum levels of SE-specific antibody were similar in the dexamethasone-treated chickens pretreated with daily dose of 10, 50, and 100 μg/kg of IP-PA1 (Fig. 4B).

Response to SRBC: Chickens with daily administered IP-PA1 and dexamethasone were injected with SRBC following the same schedule as that followed for SE vaccination, and antibody productions in response to SRBC was determined by an indirect agglutination test. Antibody production in response to SRBC was almost 2-fold lower in the dexamethasone-treated chickens than in the control chickens at most of the examined time points; this dexamethasone-induced hyporesponsiveness to SRBC was not observed clearly in dexamethasone-treated chickens pretreated with a daily dose of 100 μg/kg of IP-PA1 (Fig. 4C). On day 35, dexamethasone-treated chickens pretreated with a daily dose of more than 10 μg/kg of IP-PA1 showed a tendency for a dose-dependent increase in the serum levels of SRBC-specific antibody compared with the IP-PA1-untreated, dexamethasone-treated chickens; however, the increase was not significant (Fig. 4D).

DISCUSSION

It has been reported that stressed chickens produce high levels of glucocorticoids, which induce immunosuppression manifested as low antibody responses to antigens [16, 31, 34]. Glucocorticoid-mediated immunosuppression is characterized by functional impairment or anatomical defects in immunobiological organs [15, 20], peripheral blood lymphopenia and excessive apoptosis of thymic and bursal lymphocytes [17, 20, 27]. In order to evaluate the usefulness of IP-PA1 in controlling glucocorticoid-mediated immunosuppression in domestic chickens, we investigated the inhibitory effects of IP-PA1 on immunosuppression in dexamethasone-treated stressed chicken models in this study.

In previous reports, daily administration of 0.2–5 mg/kg of dexamethasone for 30 days induced low antibody productions and physical abnormality, but did not affect survival and general health status in chickens or mallards [10, 13]. The dexamethasone dose in the long-term studies performed in the present research (10 μg/kg, daily) was determined by pilot studies as the minimum dose that induces immunosup-

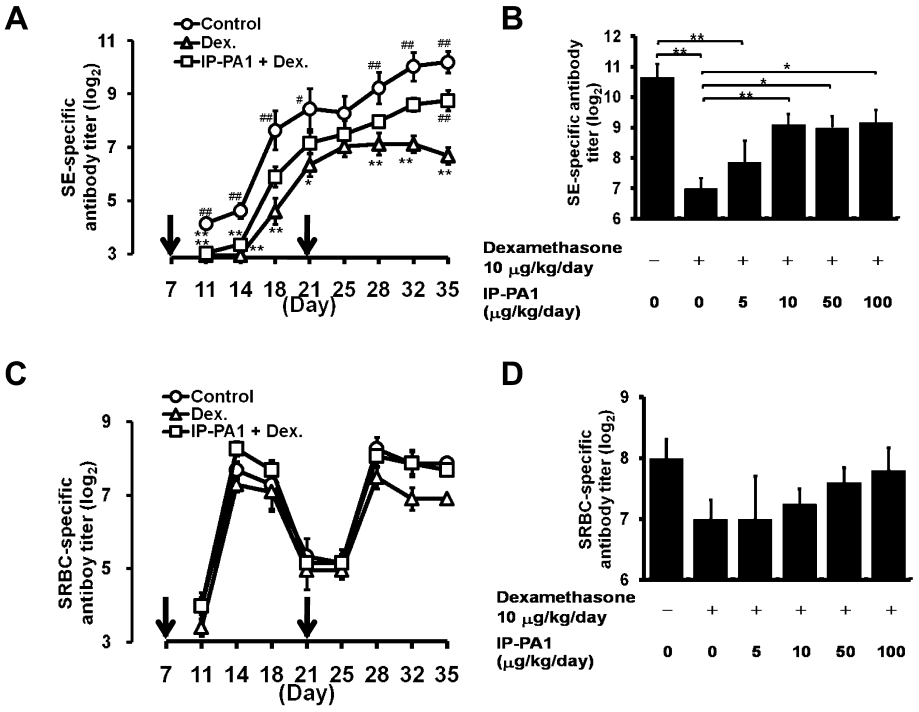

Fig. 4. Modulation of dexamethasone-induced low antibody production in response to SE vaccine and SRBC in chickens pretreated with IP-PA1. Three-week-old chickens were orally administered different daily doses of IP-PA1 2 hr prior to daily intramuscular injections of 10 μg/kg of dexamethasone for 35 days. On days 7 and 21, chickens were subcutaneously injected with 500 μl of a commercial SE vaccine (A and B) or intravenously injected with 5×10^8 SRBC (C and D). Specific antibody titers against SE (A and B) or SRBC (C and D) were determined by an indirect agglutination test. The titers on the indicated days (A and C) or on day 35 (B and D) are shown. Arrows (↓) in A and C indicate vaccination days. The data represent the means ± SEM of values obtained for at least 5 chickens. In A and C, significant differences compared with the control chickens are indicated as * P<0.05 and ** P<0.01; significant differences compared with the IP-PA1-untreated, dexamethasone treated chickens are indicated as $^{\#\#}$ P<0.01. In B and D, significant differences in the means of the 2 indicated groups are indicated as * P<0.05 and ** P<0.01.

pression at a level similar to that induced by stresses [16]. Although it would be important and interesting to examine how IP-PA1 affects the lymphocyte apoptosis induced by long-term treatment with dexamethasone, physiological responses could also alter the course of immunosuppression. Thus, in the present study, the dexamethasone dose that induced lymphocyte apoptosis clearly and directly in a previous study (5 mg/kg) [23] was chosen for evaluation of the inhibitory effects of IP-PA1 on lymphocyte apoptosis.

Oral administration of IP-PA1 reduced dexamethasone-induced thymic weight loss in both the SE-injected (Fig. 2A) and SRBC-injected (Fig. 2C) chickens; this finding suggests that IP-PA1 exerts inhibitory effects on dexamethasone-induced thymic atrophy. Inhibitory effects of IP-PA1 on dexamethasone-induced excessive apoptosis of thymic lymphocytes (Fig. 3A) and peripheral blood lymphopenia (data not shown) were also observed. Inhibition of T-cell apoptosis is one of the possible reasons for the prevention of thymic weight loss (Fig. 2A and 2C) and increase in antibody responses to antigens (Fig. 4) after IP-PA1 treatment. The increase in the relative thymic weights observed in the SE- and SRBC-injected chickens were considerably more than those observed in the unvaccinated chickens (data not shown); this suggested that IP-PA1-induced thymic weight gain was enhanced by T-cell proliferation due to vaccination.

IP-PA1 seemed to inhibit dexamethasone-induced lymphocyte apoptosis (Fig. 3) by an indirect mechanism since pretreatment with IP-PA1 did not protect cultured bursal and splenic lymphocytes isolated from naïve chickens from dexamethasone-induced cell death (data not shown). LPS activates macrophages and dendritic cells (DC) mainly via Toll-like receptor (TLR)-4 [1], and expression of functional TLR-4 has been reported in chicken macrophages [8]. Activated macrophages and DC affect other immune cells through several signals including cytokines. Oral administration of IP-PA1 to mice increases the serum levels of interleukin (IL)-12 and interferon (IFN)-γ (T. Hebishima, unpublished data), typical cytokines produced by activated macrophages and DC, suggesting that oral administration of

IP-PA1 activates macrophages and DC in the gut mucosal tissue or other tissues. Several cytokines produced by activated macrophages and DC stimulate lymphocyte proliferation and prevent their apoptosis. Both IL-12 and IFN-γ enhance memory T-cell expansion and differentiation [36]. Apoptosis of human immature thymocytes is inhibited by IL-1 [26]. Immature T-cell survival and development require IL-2, IL-7, and IL-15 [5]. Thus, cytokines produced by activated macrophages and DC by IP-PA1 treatment are one of the possible causes of the inhibition of apoptosis of thymic lymphocytes and may also induce lymphocyte proliferation.

IP-PA1 inhibited thymus weight loss more strongly than bursal weight loss (Fig. 2), although its inhibitory effects on dexamethasone-induced apoptosis of lymphocytes were similar in the thymus and bursa of Fabricius (Fig. 3); this suggests that IP-PA1 stimulated T-cell proliferation due to vaccination more strongly than B cell proliferation. Macrophages and DC are known as antigen-presenting cells (APC), which stimulate antigen-specific T-cell clonal expansion via direct interaction between MHC molecules and T cell receptor (TCR). Two steps of antigen presentation by APC are enhanced by LPS, phagocytosis of antigens [3] and surface expression of MHC molecules [14]. Thus, it is possible that oral administration of IP-PA1 enhances antigen-specific T cell clonal expansion via activation of APC, resulting in increase in thymic weight.

IP-PA1 was observed to exert similar inhibitory effects on the apoptosis of B cells and T cells (Fig. 3), which suggests that it enhanced the production of B cell-activating cytokines such as B cell-activating factor belonging to the tumor necrosis factor family (BAFF [24]), which prevents immature B cell apoptosis by macrophages and DC. IP-PA1 reduced bursal weight loss; however, the reduction was marginal in the SE-injected chickens (Fig. 2B) and not significant in the SRBC-injected chickens (Fig. 2D); this indicates that B cell proliferation due to vaccination was considerably weaker than T cell proliferation. It is possible that B cell activation by IP-PA1 directly or indirectly in the vaccinated chickens was insufficient for their proliferation.

Control of immunosuppression is important not only for maintaining animal health but also for public hygiene. Excessive SE infection of the chicken gastrointestinal tract is considered a major cause of foodborne salmonellosis, and the serum levels of SE-specific antibody have been found to be correlated with cecal bacterial load [21]. In the present study, low antibody production in response to the SE vaccine in the dexamethasone-treated stressed chicken models suggests a high risk of foodborne salmonellosis in stressed chickens, and the immune-recovery effects of IP-PA1 on the low antibody production suggest the usefulness of IP-PA1 in preventing foodborne salmonellosis.

In spite of its strong immune-enhancing effects *in vitro*, clinical application of LPS from pathological gram-negative bacteria such as *Escherichia coli* has been limited because they cause severe septic shock when large amounts enter the blood stream directly [17]. However, in contrast to injection, no toxicities of LPS have been reported when administered orally [22]. IP-PA1 derived from symbiotic gram-negative bacteria found in crops is considered safe because it has been ingested by humans and animals for a long time [22]. In addition, no adverse reaction of IP-PA1 injection has been found in some toxicity tests [19, 33]. Furthermore, IP-PA1 provides more effective protection from gastric ulcer and parasitic infection by oral administration than LPS from *E. coli* or other conventional bacteria [28, 35]. Thus, there is great hope for IP-PA1 as an edible immunomodulator. In the present study, we have shown the immune-recovery effects of oral administration of IP-PA1, such as increase in specific antibody production in response to the SE vaccine or SRBC, in dexamethasone-treated stressed chicken models. This is the first report on the immune-enhancing effects of IP-PA1 in domestic animals. Because immune responses, especially antibody production to antigens, vary among chickens with different MHC (B) haplotypes [30], further evaluation of the application of IP-PA1 in poultry husbandry with strains other than B^2B^2 White Leghorn is required. However, comparable immune enhancement by IP-PA1 is expected because IP-PA1 stimulates macrophages and DC, via TLR-4, which is conserved in all chicken strains. Furthermore, IP-PA1 will enhance innate immunity through macrophage activation in treated chickens, which also supports maintenance of the animal's health. Although the effects of IP-PA1 on healthy chickens should be evaluated for field application, the immune-recovery effects of IP-PA1 shown in the dexamethasone-treated stressed chicken models strongly suggest the usefulness of IP-PA1 in controlling immunosuppression in poultry husbandry.

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