Review

Recovery from Immunosuppression-related Disorders in Humans and Animals by IP-PA1, An Edible Lipopolysaccharide

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Abstract. *Immunopotentiator from Pantoea agglomerans 1 (IP-PA1), an edible lipopolysaccharide (LPS) derived from symbiotic bacteria in crops, is a promising immunomodulator. It activates macrophages and protects from chemotherapeutic agent-induced growth inhibition in macrophages in vitro. We showed the immune-recovery effects of IP-PA1 in a chicken model of dexamethasone-induced stress in which IP-PA1 inhibited thymic and bursal atrophy and improved antibody production in response to vaccination. Furthermore, we showed IP-PA1 improved survival of melanoma-bearing, doxorubicin-treated mice, although not directly affecting the proliferation of melanoma cells, dominantly through the improvement of host antitumor immunity. These results suggest that IP-PA1 could have other possible applications in the treatment of various immunosuppression-related disorders in humans and animals.*

Immunopotentiator from *Pantoea agglomerans*

An immunopotentiator from *Pantoea agglomerans*, IP-PA1, is a low molecular weight (5 kDa) lipopolysaccharide (LPS) derived from the cell wall of symbiotic gram-negative bacteria on various crops such as cereals, fruits, and vegetables (1-4). LPS activates macrophages and dendritic cells (DC) *via* toll-like receptor (TLR)-4, a specific receptor of LPS (5). IP-PA1 induces the activation of macrophages to produce tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) by activating the transcription factor nuclear factor (NF)-κB, a target of TLR-4 (6).

IP-PA1 is a particularly appealing therapeutic because it can be orally administered. It has immunoenhancing effects that protect experimental animals from bacterial and parasitic infections (2, 3). For example, in naive BALB/c mice, orally administered IP-PA1 increases the serum levels of immunostimulatory cytokines such as TNF-α, interferon (IFN)-γ, and interleukin (IL)-12 within 12 hours after ingestion. Furthermore, orally administered IP-PA1 provides greater protection from parasitic infections and a more recovery from indomethacin-induced gastric ulcer than does LPS from *Escherichia coli* or other common bacteria (3, 7). Therefore, IP-PA1 is a promising edible immunomodulator.

Immune Recovery by IP-PA1 in a Chicken Model of Dexamethasone-induced Stress

In domestic animals, immunosuppression is often induced by stresses such as overcrowding during feeding and exposure to excessively high or low temperature. In chickens, stress-induced immunosuppression decreases antibody production in response to antigens (8-10), which can reduce vaccine efficacy and increase susceptibility to infections. Since

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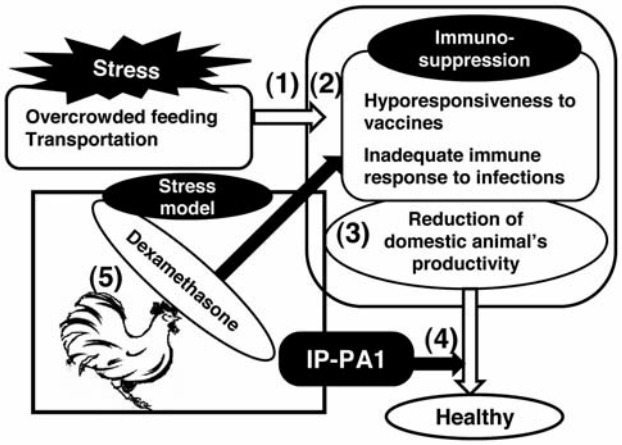

Figure 1. *Summary of experimental objective. Stress induced by overcrowding during feeding and transportation (1) often causes immunosuppression manifested by decreased antibody production and reduced responses to vaccines and infections (2). Thus, stress-induced immunosuppression can reduce the productivity of husbandry (3). To develop a novel immunomodulator, the recovery effects of IP-PA1 on stress-induced immunosuppression were examined (4) by using a chicken model of dexamethasone-induced stress (5).*

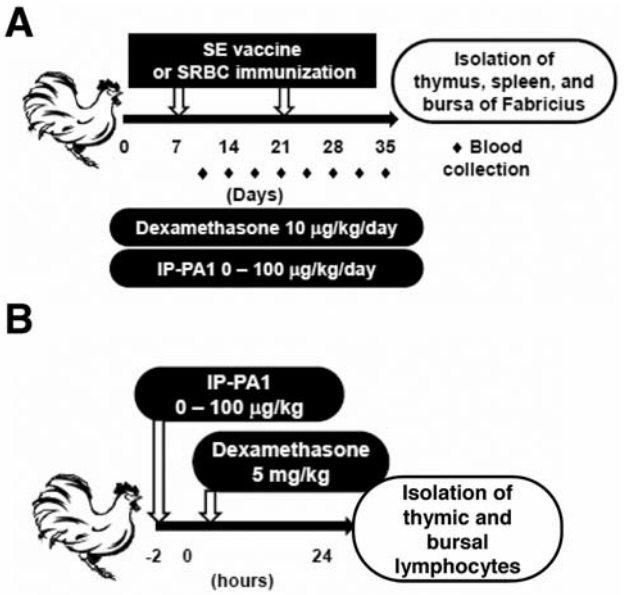

Figure 2. *Summary of experimental procedures. A: Three-week-old chickens that were administered different daily doses of IP-PA1 and a daily dose of 10 μg/kg of dexamethasone for 35 days were intravenously injected with 500 μl of SE vaccine or 5×10^8 SRBC on days 7 and 21. Serum samples were collected for antibody titration on day 35. Chickens were sacrificed and their thymus, spleen, and bursa of Fabricius were removed and weighed. B: Five- to 8-week-old chickens were orally administered IP-PA1 2 hours prior to intramuscular injections of 5 mg/kg dexamethasone. Twenty-four hours after dexamethasone treatment, thymic and bursal lymphocytes were isolated and analyzed for lymphocyte apoptosis.*

controlling immunosuppression is important for maintaining health in domestic animal populations, the development of a practical immunomodulator would be beneficial (11). As a result, we examined the immune recovery effects of IP-PA1 in a stress model of dexamethasone-treated chickens to evaluate the practicality of using IP-PA1 in immunologically suppressed animals (12) (Figure 1).

Dexamethasone is a synthetic glucocorticoid used to induce stress in animal models; it can be used for stress induction without excessive pain (13-15). Two experiments in a chicken model of dexamethasone-induced stress were conducted. In one experiment, White Leghorn chickens were fed IP-PA1 2 hours prior to intramuscular injections of 10 μg/kg dexamethasone. The chickens were treated in this manner every day for 35 days. On days 7 and 21, they also were subcutaneously injected with commercial *Salmonella enteritidis* (SE) inactivated vaccine or sheep red blood cells (SRBC). Antibody titers against SE and SRBC were measured by an indirect agglutination test twice a week after day 11. Finally, on day 35, the thymuses and bursas were weighed (Figure 2A). In the second experiment, 5- to 9-week-old chickens were fed IP-PA1 2 hours prior to the intramuscular injection of 5 mg/kg of dexamethasone. Twenty-four hours later, recovery from dexamethasone-induced excessive apoptosis in thymic and bursal lymphocytes by IP-PA1 was examined by using annexin V staining and flow cytometry (Figure 2B).

Dexamethasone-induced immunosuppression in chickens is characterized by both anatomical defects in and functional impairment of immune organs (15, 16), as well as increased apoptosis of thymic and bursal lymphocytes (17, 18). These characteristics were clearly observed in our experiments (Table I). Dexamethasone reduced the production of anti-SE and anti-SRBC-specific antibodies by approximately 8- and 2-fold, respectively, in response to the vaccination. However, daily ingestion of 10-100 μg/kg of IP-PA1 inhibited these immunosuppressive changes (Tables II and III). The treatment of 5 mg/kg dexamethasone induced excessive apoptosis in thymic and bursal lymphocytes; however, ingestion of IP-PA1 effectively abrogated these increases (Table IV).

Chronic Stress and Immunosuppression

The acute stress response is important for wound healing and protection against infections and is highly conserved in animals. This response enhances immune responses by increasing cytotoxic T lymphocytes (CTL) (19) and redistributing leukocytes from the blood to the skin (20, 21). In addition, the hypothalamus–pituitary–adrenal axis is activated and the adrenal gland secretes glucocorticoids that rapidly induce physiological changes such as leukocyte redistribution from the blood to the skin (22).

Unlike acute stress, chronic stress suppresses immune responses (23, 24). These immunosuppressive effects include

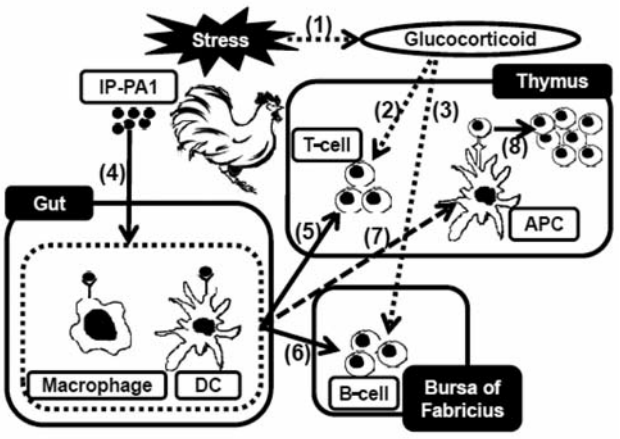

Figure 3. *A model of possible mechanisms of immune recovery effects of IP-PA1 on stress-induced immunosuppression. Increased glucocorticoid levels in chickens responding to various stressors (1) increase T-cell (2) and B-cell (3) apoptosis and thymic and bursal atrophy. IP-PA1 stimulates macrophages and dendritic cells (DCs) via toll-like-receptor (TLR)-4 in gut mucosal tissue (4). Cytokines produced by activated macrophages and DCs inhibit apoptosis of thymic T-cells (5) and bursal B-cells (6), and enhance major histocompatibility complex expression or activation of antigen-presenting cells (APCs) (7). This increases APC T-cell interactions and promotes T-cell clonal expansion (8).*

Table I. *Dexamethasone-induced losses of whole-body weight and relative organ weights.*

Treatment	Number	Body weight (g)	Relative organ weight (mg/g)		
			Thymus	Spleen	Bursa of Fabricius
PBS	5	623.0±16.8	4.54±0.41	1.45±0.06	3.99±0.17
Dexamethasone	5	385.0±14.7*	0.92±0.06*	0.98±0.03*	0.74±0.09*

*$p<0.01$ Compared to phosphate-buffered saline (PBS)-treated control chickens.

Table II. *Recovery from dexamethasone-induced relative thymic and bursal weight losses.*

IP-PA1 (μg/kg)	Number	Ratio of relative organ weight to that of control chickens (%)	
		Thymus	Bursa of Fabricius
0	7	35.50±4.54	28.21±1.76
10	6	79.25±9.69**	30.83±2.44
50	5	88.33±12.64**	34.45±2.97
100	6	103.17±8.91**	37.96±2.93*

*$p<0.05$ and ** $p<0.01$ Compared to control chickens not treated with IP-PA1.

a shift in the cytokine balance from type 1 to type 2 cytokine-driven responses (25) and a reduction in the quantity of (26) and function of (27) protective immune cells. Immunosuppression is the result of increased regulatory T-cells (28) and glucocorticoid resistance caused by frequent activation of the glucocorticoid receptor (29).

Consequently, chronic stress may help cause or exacerbate many conditions such as infection and cancer, and slow wound healing. For example, chronic stress in mice increases their susceptibility to infection by the influenza virus and suppresses their production of antibodies and inflammatory cytokines such as IL-2 (30, 31). Chronic stress also increases susceptibility to UV-induced squamous cell carcinoma in mice due to decreased type 1 cytokine and increased regulatory T-cells (28). Similarly, in humans, caregivers for dementia patients frequently chronically stressed and have poorer immune function than controls as indicated by their greater susceptibility to infection and shorter life span (32, 33). Specifically, in these caregivers, immunosuppression inhibits the proliferation of immune cells in response to mitogens and cytolytic activity of NK cells in response to recombinant IFN-γ and IL-2 (34, 35).

Possible Mechanisms of the Immunoenhancing Effects of IP-PA1

In chickens with dexamethasone-induced stress, IP-PA1 resulted in improved antibody production in response to both SE and SRBC vaccination. The observation that IP-PA1 inhibited dexamethasone-induced apoptosis of thymic and bursal lymphocytes may help explain how IP-PA1 prevents thymic and bursal weight loss and increases antibody responses. However, IP-PA1 must indirectly inhibit dexamethasone-induced lymphocytic apoptosis because pre-treatment with IP-PA1 did not protect cultured bursal and splenic lymphocytes from dexamethasone-induced cell death in our previous study (13).

One possible mechanism of this inhibition is activation of macrophages and DC by IP-PA1 *via* TLR-4 in gut mucosal tissue (4), followed by production of cytokines which can affect other immune cells such as those in the thymus, bursa, or spleen. For example, IL-1, IL-2, IL-7, and IL-15 are essential for the survival and development of immature T-cells (36, 37). Similarly, B-cell-activating cytokines such as B-cell-activating factor belonging to the tumor necrosis factor family (BAFF) (38) prevent immature B cell apoptosis. The involvement of cytokines in this mechanism also may explain the observation that the inhibitory effect of IP-PA1 on dexamethasone-induced apoptosis of lymphocytes was more pronounced in the thymus than in the bursa. Specifically, IP-PA1 may stimulate T-cell proliferation more strongly than B-cell proliferation through differential effects of cytokines in the thymus and bursa, respectively.

Table III. *Recovery from dexamethasone-induced low antibody production in response to Salmonella enteritidis vaccine.*

IP-PA1 (μg/kg)	Dexamethasone 10 μg/kg	Number	Specific antibody titer to *Salmonella enteritidis* on day (log_2)			
			14	21	28	35
0	–	6	4.83±0.31	8.83±0.79	9.67±0.61	10.67±0.42
0	+	11	3.09±0.09*	6.64±0.45	7.45±0.43*	7.00±0.33*
100	+	12	3.50±0.22	7.50±0.43	8.33±0.21	9.17±0.40

*$p<0.01$ Compared to control chickens not treated with dexamethasone.

Alternatively, IP-PA1 may stimulate antigen-specific T-cell clonal expansion by activating antigen-presenting cells (APCs), such as macrophages and DCs. LPS enhances two aspects of antigen presentation by APCs, namely, phagocytosis of antigens (39) and surface expression of major histocompatibility complex (MHC) molecules (40). Since these processes help mediate T-cell clonal expansion, it is possible that IP-PA1 could increase thymic weight by this mechanism.

We have shown that oral administration of IP-PA1 promotes immune recovery, specifically by increasing antibody production in response to the SE vaccine and SRBC, in a chicken model of dexamethasone-induced stress (Figure 3). Our results strongly support the usefulness of IP-PA1 for recovery from stress-induced immunosuppression.

Table IV. *Recovery from dexamethasone-induced excessive apoptosis of thymic and bursal lymphocytes.*

IP-PA1 (mg/kg)	Dexa-methasone 5 mg/kg	Apoptotic lymphocytes (%)	
		Thymus	Bursa of Fabricius
0	–	21.32±0.66	21.13±1.93
0	+	52.67±4.12**	55.16±1.98**
50	+	30.58±4.58	34.38±3.65*
100	+	27.47±3.07	28.56±2.50

*$p<0.05$ and **$p<0.01$ Compared to control chickens not treated with dexamathasone.

Immunosuppression and Cancer Outcome

One of the prognostic factors for cancer patients is the function of antitumor immunity, *i.e.* the defense mechanisms against the occurrence and progression of cancer (41, 42). The tumor lytic function of tumor-infiltrating lymphocytes is associated with the clinical response in melanoma patients (43). Macrophages located in all peritoneal tissues recognize and kill tumor cells directly (44, 45); take in apoptotic tumor cells by phagocytosis (46); and process and present tumor-specific antigens to $CD8^+$ cytotoxic T lymphocytes (CTLs), thus activating them (47). These functions are essential not only for preventing the occurrence and development of tumors, but also for the elimination of tumor cells that are damaged or apoptotic because of chemotherapeutic agents; these cells can potentially suppress antitumor immunity (48).

The majority of tumor cells can potentially inhibit host immunity through various mechanisms such as the production of immunosuppressive IL-10 (49); in addition, conventional chemotherapy (50), radiotherapy (51), and surgery (52, 53) frequently suppress host immunity; therefore, cancer patients are generally immunologically suppressed.

Therefore, considering the usefulness of IP-PA1 as a supportive drug in melanoma therapy, we evaluated the effects of the oral administration of IP-PA1 in a melanoma-inoculated mouse model. Five- to 6-week-old female C57BL/6 mice, intraperitoneally injected with 2.0×10^5 B16 melanoma cells, were administered 0-1 mg/kg IP-PA1 orally every day and doxorubicin intraperitoneally on alternate days starting 1 day after the melanoma inoculation. The mean survival period of melanoma-bearing, doxorubicin-treated mice was prolonged from 31.4±7.1 days to 35.3±8.4, 51.1±5.4, and 45.0±8.4 days by combinatory treatment of IP-PA1 at the daily doses of 0.1, 0.5, and 1 mg/kg, respectively, with doxorubicin.

The enhancement of antibody production in a chicken model of dexamethasone-induced stress was thought to be an indirect result of macrophage activation induced by IP-PA1. Activated macrophages may produce cytokines that stimulate several kinds of immune cells other than lymphocytes. For example, IFN-γ is a strong stimulator of NK cells (54) which have a pivotal role in the surveillance and rejection of tumor cells (55). As IP-PA1 did not affect the proliferation of B16 cells directly *in vitro*, it is suggested that the significant improvement of survival induced by IP-PA1 treatment in melanoma-bearing mice was predominantly due to the enhancement of antitumor immunity.

A Wide Range of Applications for IP-PA1

In spite of its strong immunoenhancing effects *in vitro*, clinical application of LPS has been limited due to the severe septic shock that results when large amounts are injected into the bloodstream (56). In contrast, no adverse reactions to LPS have been reported when it is administered orally (4). Ingesting IP-PA1 is very safe. Rats that consumed 1000 mg/kg of IP-PA1 daily, much more than the dose required for immunoenhancing effects, did not have any toxic effects (2, 57). Moreover, the bacterial source of IP-PA1 is common in crops that have been safely eaten by humans and animals for thousands of years (4).

The immunoenhancing effects of immunomodulators such as IP-PA1 are found not only in mammals and birds, but also in fishes and crustaceans (2, 3). The conservation of these functions may be due to the strong conservation of molecules in the TLR-4–NF-κB signaling pathway in many organisms (5). As a result, IP-PA1 is expected to have applications in the prevention and treatment of a wide range of disorders either caused or exacerbated by stress-induced immunosuppression in many animals, including humans.

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