

***Phellinus linteus* grown on germinated brown rice suppresses IgE production by the modulation of Th1/Th2 balance in murine mesenteric lymph node lymphocytes**

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Abstract

We compared the immunomodulating effects of *Phellinus linteus* (PL), germinated brown rice (BR) and *P. linteus* grown on germinated brown rice (PB) on IgE production in murine mesenteric lymph node (MLN) lymphocytes. All extracts decreased IgE concentrations by 43–65% compared to control mice in both serum and MLN lymphocytes. In addition, PL and PB increased the proportion of CD4⁺ T cells by 9% and 12% in MLN lymphocytes. IFN- γ concentration, Th1 cytokine, was significantly increased by 44–67%, whereas IL-4 and IL-10 concentrations, Th2 cytokine, significantly decreased by 30–60% in the three treated groups compared to control group. These results suggest that PB suppresses IgE production through the modulation of Th1/Th2 balance to down-regulate Th2 response in MLN lymphocytes, even though a synergistic effect of PB was not found.

Introduction

Phellinus linteus (PL) in the family of Hymenochaetaceae has been used for the immunochemo-therapy of various cancers in oriental countries. The fungus has antitumor and immunostimulatory properties (Kim *et al.* 1996, Han *et al.* 1999). The immunological activities of *P. linteus* have been investigated extensively (Oh *et al.* 1992, Kim *et al.* 1996, Lee *et al.* 1996); It positively stimulates the immune function of T-lymphocytes, natural killer (NK) cells, macrophages and B-lymphocytes. However, *P. linteus* is not easily available for food in large amounts because of its high production cost and difficult growing techniques. From this point of view, we grew *P. linteus* on germinated brown rice

which is known to have rich nutrients (Oh & Choi 2000) and arabinoxylan, which augments interferon (IFN) γ secretion and activates NK cells (Ghoneum 1998, Ghoneum & Jewett 2000).

Allergic responses are associated with immunoglobulin (Ig) E for antigens in the air and food. The production of IgE is regulated by Th lymphocytes which have been classified into Th1 and Th2 subtypes. Th2 cells secrete both interleukin (IL)-4, which is the major inducer of class-switching to IgE production in B lymphocytes, and IL-10, which down-regulates cytokine synthesis in Th1 cells (Mosmann & Coffman 1989, Mosmann & Moore 1991). On the other hand, IFN- γ produced by Th1 cells suppresses the development of Th2 cells (Coffman & Carty 1986). As enhancement of Th2-mediated immu-

nity causes IgE-dependent allergic diseases, modulation of the Th1/Th2 balance is considered to be a rational strategy for the prevention and therapy of the diseases.

P. linteus stimulates systemic immunity (i.e., the spleen). However, the immuno-inhibitory properties of the fungus on the intestinal immune system have not previously been investigated with respect to allergic responses. Therefore, we compared the immunomodulating effects of *Phellinus linteus* germinated brown rice (BR) and *Phellinus linteus* grown on germinated brown rice (PB) on IgE production and Th1/Th2 balance in murine mesenteric lymph node (MLN) lymphocytes.

Materials and methods

Materials

Propylene glycol and concanavalin A (Con A) were from Sigma. RPMI 1640 medium and fetal bovine serum (FBS) were from Invitrogen Corporation (Carlsbad, NM). CD4-FITC and CD8-PE monoclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies used to detect IgE were purchased from Biosource International (Comarillo, CA). Monoclonal antibodies used to measure cytokines were purchased from ID Labs (Ontario, Canada).

Preparation of *Phellinus linteus* grown on germinated brown rice extracts

Phellinus linteus (PL), germinated brown rice (BR) and *Phellinus linteus* grown on germinated brown rice (PB) were provided by Cell Activation Research Institute (CARI, Korea). PB preparations were made according to the original method, as described previously (Jeon *et al.* 2003). PB (3 kg) was extracted with ethanol (36 l) at room temperature for 1 day. After filtration, the residue was extracted with ethyl acetate (24 and 30 l) at room temperature for 2 days and then filtered again. The combined filtrates were concentrated in vacuum at 40 °C. The extract (60 g) was stored at -20°C until used. PL and BR were also extracted by the same procedure. All extracts were dissolved in propylene glycol and used for the animal experiments.

Animals

Four-week-old male ICR mice were obtained from Samtako Bio Korea (Osan, Korea). All animal care was performed within the guidelines approved by the institutional animal care and use committee. All mice were kept in stainless steel bottom cages in a room controlled at 23 ± 1 °C and $55 \pm 5\%$ humidity under a 12-h dark/light cycle. Mice were fed a commercial diet (Purina chow) and given free access to water *ad libitum*. After adaptation for a week, mice were randomized into four groups of ten mice each. Control group mice received the vehicle (propylene glycol) only. The PL, BR and PB group mice were perorally administered the respective extract (2 mg/mouse). After 4 weeks of experimental feeding, mice were anesthetized, and peripheral blood was collected by retroorbital bleeding for the analysis of IgE production.

Lymphocyte culture

The MLN lymphocytes isolated from each group of mice were cultured in 10% FBS/RPMI 1640 medium. MLN lymphocytes were adjusted to 2×10^6 cells ml^{-1} in 24-well microtiter plates. Cells were incubated at 37 °C for 48 h in the absence or presence of 25 μg Con A ml^{-1} . The concentrations of IgE were measured by sandwich ELISA methods in supernatant, as described previously (Lim *et al.* 1997). The concentrations of cytokines (IFN- γ , IL-2, IL-4 and IL-10) were also measured by ELISA using cytokine-specific capture and detection monoclonal antibodies as described previously (Lim *et al.* 2000).

T-cell population analysis

Either CD4-FITC or CD8-PE monoclonal antibodies (5 μl) were added to MLN lymphocytes suspended at 1×10^6 cells ml^{-1} and incubated at 4 °C for 30 min. The lymphocytes were rinsed three times with PBS containing 10% (v/v) FBS and centrifuged at $300 \times g$ for 5 min. The stained lymphocytes were fixed with 2% paraformaldehyde and counted by EPICS Altra flow cytometry (Beckman Coulter, Cupertino, CA). Each analysis, including the negative control samples, was based on at least 104 events after the dead cells and residual erythrocytes were eliminated by gating on the basis of the forward angle light scatter.

Statistics

The data are presented as mean $\pm$ SEM. Differences between the means of the individual groups were assessed by one-way ANOVA with Duncan's multiple range tests. Differences were considered significant at $p < 0.05$. The statistical software package SPSS 10.0 (SPSS institute, Chicago, IL) was used for these analyses.

Results and discussion

In the current study, we demonstrated that PB modulates the pattern of Th1/Th2 cytokine production, and suppresses IgE production in both serum and murine MLN lymphocytes. The MLN plays a pivotal role in the intestinal immunity against foreign antigens such as food and microbes. IgE is the primary mediator in type I hypersensitivity reactions to food allergens (Sampson & Metclafe 1992). Here, we showed that all extracts (PL, BR and PB) drastically decreased IgE concentrations in both serum and MLN lymphocytes (Figure 1). In addition, PL and PB increased the ratio of CD4⁺/CD8⁺ T cells in MLN lymphocytes (Table 1). CD8⁺ T cells may regulate the development of CD4⁺ helper T cells by producing IFN- γ or other regulatory cytokines which suppress the development of Th2 cells and favor Th1 cell growth (Cher & Mosmann 1987). All extracts significantly increased IFN- γ concentrations in MLN lymphocytes (Figure 2). A greater proportion of CD4⁺ T cells and greater IFN- γ concentrations were observed in the PL and PB groups compared to the control group. Therefore, T cell differentiation to Th1 cells may be increased by the increased concentration of Th1 cytokines induced by PL and PB administration.

In this study, all extracts significantly increased Th1 cytokine production, IFN- γ , and decreased Th2 cytokine, IL-4 and IL-10 concentrations (Figure 2). The Th1/Th2 balance is considered to be important in maintaining homeostasis in the host. Once this balance is disturbed, Th1 cells mediate delayed-type hypersensitivity and autoimmune disease, and abnormal Th2 responses are implicated in allergic disease (Neurath *et al.* 2002). The cytokines themselves play the most critical role in helper T cell differentiation. IFN- γ and IL-12

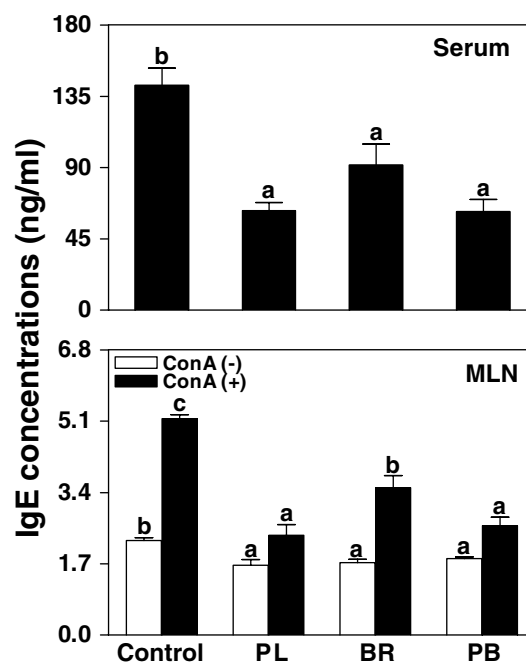


Fig. 1. IgE concentrations in serum (top panel) and mesenteric lymph node (MLN) lymphocytes (bottom panel). The lymphocytes were adjusted to 2×10^6 cells/ml in 24-well microtiter plates and incubated at 37 °C for 48 h in the absence or presence of 25 μ g Con A/ml. Each bar represents mean $\pm$ SEM of ten mice. Mean values with different superscripts are significantly different ($p < 0.05$). PL, *Phellinus linteus*; BR, germinated brown rice; PB, *Phellinus linteus* grown on germinated brown rice.

Table 1. T-cell population of MLN lymphocytes in mice given extracts (Unit: %).

Groups	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺
Control	55.1 $\pm$ 1.0 ^a	15.2 $\pm$ 0.4 ^b	3.6 $\pm$ 0.1 ^a
PL	61.1 $\pm$ 0.3 ^b	14.4 $\pm$ 0.1 ^a	4.3 $\pm$ 0.1 ^b
BR	54.7 $\pm$ 0.6 ^a	14.8 $\pm$ 0.2 ^{ab}	3.7 $\pm$ 0.1 ^a
PB	63.2 $\pm$ 0.5 ^c	15.5 $\pm$ 0.2 ^b	4.1 $\pm$ 0.1 ^b

Values represent the means $\pm$ SEM of ten mice. Values with different superscripts are significantly different ($p < 0.05$). Either CD4-FITC or CD8-PE monoclonal antibodies (5 μ l) were added to MLN lymphocytes suspended at 1×10^6 cells/ml and incubated at 4 °C for 30 min. The stained lymphocytes were fixed with 2% (w/v) paraformaldehyde and counted.

induce differentiation to Th1 from Th0, whereas IL-4 controls Th2 differentiation. These cytokines induce the generation of their own T-helper subset, and simultaneously inhibit the production of the opposing subset. Therefore, it is believed that an increase in IFN- γ and IL-4 shifts the Th1/Th2

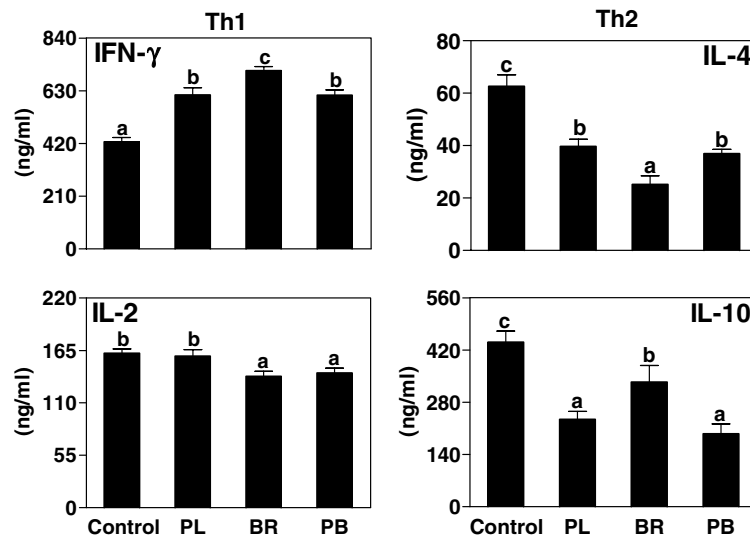


Fig. 2. Con A-induced Th1 and Th2 cytokine secretion in MLN lymphocytes. MLN lymphocytes were adjusted to 2×10^6 cells/ml in 24-well microtiter plates and incubated at 37 °C for 48 h in the absence or presence of 25 µg Con A/ml. The concentrations of all cytokines were measured by sandwich ELISA methods in supernatant, as described in Materials and methods. Each bar represents mean $\pm$ SEM of ten mice. Mean values with different superscripts are significantly different ($p < 0.05$).

balance to predominantly Th1 and Th2, respectively (McKnight *et al.* 1994, Finkelman *et al.* 1988, Glimcher & Murphy 2000) Th2 cells are immunologically the dominant cell type associated with several notable allergic reactions (Romagnani 2000). Indeed, OVA/alum-sensitized allergic mice harbor a systemic Th2 response characterized by elevated production of Th2 cytokines and antigen-specific IgE synthesis (Brewer *et al.* 1996).

In summary, we demonstrate that PB led to significant decrease in the Th2 response with concomitant increase in Th1 response, indicating modulation of intestinal Th1/Th2 balance. Moreover, the down-regulation of Th2 response was reflected in the suppression of IgE production in both serum and MLN lymphocytes. By these immunomodulatory activities, PB may be useful for prevention or therapy of allergic disease.

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