Oral Administration of the Extract from Hatakeshimeji (Lyophyllum decastes Sing.) Mushroom Inhibits the Development of Atopic Dermatitis-Like Skin Lesions in NC/Nga Mice

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Note

Summary We examined whether the extract from Hatakeshimeji (Lyophyllum decastes, LD) mushrooms suppresses the development of atopic dermatitis (AD)-like skin lesions induced by repeated application of picryl chloride (PicCl) in NC/Nga mice. Oral administration of LD extract to NC/Nga mice inhibited the development of AD-like skin lesions based on lower total skin severity scores and serum immunoglobulin E (IgE) levels. Splenic lymphocytes were stimulated with the T cell mitogen concanavalin A, and secretion of a Th1 cytokine (IFN-γ) and a Th2 cytokine (IL-4) was determined by ELISA. IFN-γ production was not inhibited by treatment with LD extract. On the other hand, IL-4 production was significantly decreased by treatment with LD extract. These results suggest that LD extract exerts anti-allergic actions by suppressing the serum IgE and Th2-type immune responses.

Key Words Hatakeshimeji (Lyophyllum decastes Sing.), atopic dermatitis, NC/Nga mouse, interleukin-4, interferon-γ

Atopic dermatitis (AD) is a complex eczematous skin disease accompanied by severe itching and frequently repeated episodes (1). Both genetic and environmental factors are involved in AD. Acute lesions of AD have significantly increased serum immunoglobulin (IgE) levels and an increased number of cells expressing T helper 2 (Th2) cytokines, such as interleukin (IL)-4.

NC/Nga mice, established as an inbred strain from Japanese fancy mice in 1957, spontaneously develop AD-like dermatitis with IgE hyperproduction under conventional housing conditions. NC/Nga mice raised in specific-pathogen free (SPF) conditions, however, do not develop skin lesions (2). Th2-specific chemokines and their receptors are highly expressed in the lesions of the NC mice (3).

Hatakeshimeji (Lyophyllum decastes, LD) belongs to the same genus as Honshimeji (Lyophyllum shimeji Hongo), which is well known as the most delicious mushroom in Japan. Technology for the artificial cultivation of Hatakeshimeji in pots was recently developed (4) and cultivated mushrooms are now available to consumers.

We previously reported the antitumor activities of 11 polysaccharides that were isolated from hot-water extracts of LD fruiting bodies by ion-exchange and gel permeation chromatographies (5). Among them, (1→3)-β-D-glucan and (1→6)-β-D-glucan have marked antitumor activities against Sarcoma 180. On the other hand, cold-water, hot-water, and ethanol extracts of LD all have significant angiotensin I converting enzyme inhibitory activity (6). Furthermore, the hypocholesterolemic action of LD mushrooms has been reported (7).

In the present study, we examined the effect of LD extract on serum IgE levels, total skin severity scores, and spleen cytokine production in NC/Nga mice.

Materials and Methods

LD extract preparation. The fresh fruiting body of LD (500 kg) was treated with 50 kL of ion-exchanged water at 93°C for 3 h to prepare the hot-water extract. After centrifugation at 14,000 × g for 30 min, the supernatant was spray-dried. The yield of the hot-water extract from 500 kg of LD fresh fruiting body was about 20 kg. Nutritional ingredients of LD extract of 100 g is carbohydrate of 51.8 g, protein of 17.4 g, a dietary fiber of 16.8 g, lipid of 0.5 g, and the main sugar composition is glucose.

Animals. Male 5 wk-old NC/Nga mice were purchased from Charles River Japan (Yokohama, Japan) and housed individually in plastic cages in a room controlled for temperature (23 ± 2°C), humidity (55 ± 5%), and light (lights on: 7:00–19:00 h). The mice were fed an MF diet (Oriental Yeast Co., Ltd., Tokyo) and water ad libitum. Experiments were performed in accordance with the Guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animal Science.

Atopic dermatitis mice. The dorsal skin and scalp were shaved from NC/Nga mice aged 7 wk before initiating sensitization. These animals were sensitized by the
application of 150 μL of 0.8% picryl chloride (PiCl) in acetone/ethanol (1:4) to the shaved skin on day 0, and challenged by the application of 100 μL of 5% PiCl in olive oil once a week for 9 wk (days 7 to 70). LD extract (100 mg/kg body weight) was orally administered every day for 6 wk (days 29 to 70). The dermatitis score was defined as the sum of the individual scores graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for each of the five symptoms (itching, edema, hemorrhage, excoriation/erosion, and scaling/dryness, total score = 15).

Quantification of serum IgE. Blood was collected from a tail vein once every 2 wk using a capillary glass tube. Serum samples were obtained by centrifugation (1,700 × g, 10 min) and stored at −80°C until assayed. Total serum IgE levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Bethyl Laboratories, Montgomery, TX). Serum (100 μL of 10 to 100-fold diluted serum) was applied to each well. The total IgE concentration in each well was estimated using a standard sample from the manufacturer’s kit.

Quantification of serum histamine. On day 70, whole blood was withdrawn from the abdominal aorta and the spleen was collected under ether anesthesia. Serum samples were obtained by centrifugation (1,700 × g, 10 min) and stored at −80°C until assayed. Total serum histamine levels were measured using an ELISA kit (Biochemical Research, Oxford, MI). Serum (50 μL of 0 to 5-fold diluted serum) was applied to each well. The total histamine concentration in each well was estimated using a standard sample from the manufacturer’s kit.

Preparation of mouse splenocytes. The spleen was collected as described above. Splenocytes were obtained by passing pieces of spleen through a mesh cellstrainer (Becton Dickinson, USA) and then treatment with a polysucrose solution (Lympholyte-M, Cedarlane Laboratories, Canada) to remove erythrocytes and dead cells. After centrifugation (1,200 × g, 20 min), splenic lymphocytes were suspended and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum in 5% CO2.

Quantification of cytokine production. Splenic lymphocytes (1 × 10^5 cells/well) suspended in 500 μL of RPMI-1640 medium were cultured in 24-well U-bottom culture plates with or without concanavalin A (5 μg/mL) for 72 h at 37°C. After centrifugation, the supernatant was collected from each well and stored at −80°C until assayed. Interleukin (IL)-4 and interferon (IFN)-γ levels in the culture supernatants were determined using an ELISA kit (BD Pharmingen, San Diego, CA) according to the manufacturer’s instructions.

Statistical analysis. All results were expressed as mean ± SE and all data were analyzed statistically using Student’s t-test. A p value of less than 0.05 was considered statistically significant.

Results and Discussion

The NC/Nga mouse strain develops AD-like skin lesions following repeated application of PiCl under conventional housing conditions (8). Consistent with this report, the clinical skin severity in the control NC/Nga mice gradually increased depending on the number of PiCl challenges. Oral administration of LD extract to NC/Nga mice inhibited the development of AD-like skin lesions based on the total skin severity scores (Fig. 1).

In approximately 80% of patients with AD, serum IgE levels and blood eosinophil counts are elevated (9, 10). It is well established that Th2 cytokines have an important role in the onset and development of AD. CD4+ helper T lymphocytes are divided into two groups, Th1 and Th2, based on the cytokines they secrete (11, 12). The T lymphocytes responsible for delayed-type skin reactions that secrete IL-2 and IFN-γ belong to the Th1 subset, and those that are responsible for IgE production and eosinophil activation and secrete IL-4 and IL-5, respectively, comprise the Th2 subset. The activation and function of both subsets of T lymphocytes are mutually regulated through the cytokines they secrete, and the balance of both subsets is considered to be skewed to Th2 in atopic diseases, including AD (13, 14).

Plasma IgE levels gradually increased with repeated PiCl challenges. The increase in the IgE level was significantly attenuated by treatment of the group administered LD extract (Fig. 2). In addition, serum histamine levels were significantly decreased in the control group compared with those of LD-administered group (Fig. 3).

Because IgE production is an indicator of the Th2 response, our findings suggest that the oral administration LD extract downregulates the Th2 response. To clarify the effect of LD on the Th1/Th2 cytokine balance, we demonstrated that splenic lymphocytes stimulated with LD extract improved or inhibited the production of Th1 and Th2 cytokines. IFN-γ production was not inhibited by treatment with LD extract, although IL-4 production was significantly decreased (Fig. 4A...
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Fig. 2. Effects of LD on plasma IgE levels in NC/Nga mice. LD (100 mg/kg) was administered orally to the NC/Nga mice sensitized and challenged with PiCl once a week for 10 wk. Plasma samples were obtained by centrifugation and stored at −80°C until use. Total IgE levels were determined by ELISA. Values are expressed as means±SE of 8 animals per group. *p<0.05, significantly different from the control group.

Fig. 3. Effects of LD on plasma histamine levels in NC/Nga mice. LD (100 mg/kg) was administered orally to the NC/Nga mice sensitized and challenged with PiCl once a week for 10 wk. Plasma samples were obtained by centrifugation and stored at −80°C until use. Serum histamine levels were determined by ELISA. Values are expressed as means±SE of 8 animals per group. *p<0.05, significantly different from the control group.

Fig. 4. Effects of LD on the production of IFN-γ (A) and IL-4 (B) in immunized splenic lymphocytes. LD (100 mg/kg) was administered orally to the NC/Nga mice sensitized and challenged with PiCl once a week for 10 wk. Mice were sacrificed on day 70 and splenic lymphocytes (1×10^5/well) were cultured with ConA (5 μg/mL) for 72 h. The supernatants were collected and amounts of IFN-γ and IL-4 were measured by ELISA. Values are expressed as means±SE of 8 animals per group. *p<0.05, significantly different from the control group.

and B). These results suggested that LD extract suppressed the production of the Th2-type cytokine IL-4 and improved the balance of Th1/Th2 in a model of AD.

In summary, LD extract exerts an anti-allergic action through suppression of serum IgE and Th2-type immune responses. Thus, LD extract might be an effective dietary supplement for the prevention of AD. Further study on the production of other cytokines by lymphocytes and antigen-presenting cells will enhance our understanding of the anti-allergic activity of LD extract. Further studies are also required to clarify the inhibition of histamine release and Th2 responses in vivo, especially in some pathogenic animal models.

REFERENCES

