Activation of Immune Responses in Mice by an Oral Administration of Bunching Onion (*Allium fistulosum*) Mucus

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Bunching onion (*Allium fistulosum* L. (Liliaceae)) secretes the mucus in the cavities of its green leaves. The effects of the mucus, which is consumed as food, were examined. The mucus augmented the production of tumor necrosis factor (TNF-α) and monocyte chemotactic protein (MCP)-1 from RAW 264 cells and of interleukin (IL)-12 from J774.1 cells; however, extracts from green leaves and white sheaths did not. An oral administration of this mucus to mice augmented the immune functions of peritoneal cells by increasing TNF-α and IL-12 production and phagocytosis. It also augmented interferon (IFN)-γ production from spleen cells and natural killer (NK) activity. These results suggest that an oral administration of the *A. fistulosum* mucus can enhance natural immunity.

Key words: bunching onion; mucus; immune activation; cytokine; natural killer

*A. fistulosum* L. (Liliaceae) is a perennial herb that is widely cultivated throughout the world, from tropical Asia to Siberia, and especially in Japan, Korea, and China. One of its common names, “Welsh onion” is derived from the German *welschhe*, meaning foreign. Other local names in English-speaking countries include Japanese bunching onion, Spanish onion, two-bladed onion, spring onion, green bunching onion, scallion, green trail, and Chinese small onion. In Japan, Korea, and China, it is respectively called *negi*, *pa*, and *cong*. *A. fistulosum* is considered to have its origin in northwestern China, and both the white sheaths and green leaves are edible.

*A. fistulosum* has also been used as a herbal medicine for many diseases. In Japan, it is considered to activate immunity and prevent colds. In addition, in China, *A. fistulosum* is used for treating febrile disease, headache, abdominal pain, diarrhea, eye-related disorders, and habitual abortion. It has been reported to inhibit platelet aggregation, modulate the aortic vascular tone, and lower blood pressure and hyperglycemia. The aqueous extract from its green leaves has reportedly attenuated lipoprotein oxidation and excessive nitric oxide and prostaglandin generation by macrophages to protect against oxidative damage from reactive oxygen and nitrogen species, and to increase high-density lipoprotein receptor expression in macrophages.

Other members of the Allium family (*e.g.*, garlic and onion) have also been used as antihypertensives, anti-atherosclerotics, anti-thrombotics, anti-hyperlipidemics, and antioxidants. Many studies have suggested that these beneficial effects were primarily due to organosulfur compounds (*e.g.*, S-allylcysteine and diallyl disulfide).

However, there have been few studies on the immunomodulatory effects of the bunching onion. It has recently been reported that a hot-water extract from bunching onions interfered with influenza A virus infection through activation of the host-immune system, with fructan as one of its active principles. We report here that the bunching onion possessed certain immunostimulatory effects because of the mucus in the inner cavities of the green leaves.

Materials and Methods

*Plant material and sample preparation.* The shimonita cultivar of *A. fistulosum* L. was cultivated at the National Institute of Vegetable and Tea Science in Mie Prefecture, Japan. The plants were weighed and divided into green leaves and white sheaths. The green leaves were dipped into distilled water, and the mucus from the inner cavities of the leaves was dissolved and collected by repeated stroking. The remaining green leaves and white roots were homogenized in a blender (Panasonic, Osaka, Japan) and filtered through nylon mesh sheets of 1-mm pore size (Sanplatec, Osaka, Japan). The filtrate and mucus were lyophilized in a tray lyophilizer (Tokyo Rika, Tokyo, Japan). The yield of lyophilized residue obtained was 0.63% (mucus), 0.06% (green leaves extract), and 0.11% (white roots extract) of the original materials.

*Animals.* Male ICR mice (5 weeks or 14 months old) were purchased from Nihon SLC (Tokyo, Japan). The mice were housed under normal laboratory conditions (25°C temperature, 40–60% relative humidity, with a 12-h light/dark cycle) and were fed with standard rodent food and water *ad libitum*. All experiments were performed according to the guidelines for animal experimentation (no. 105) and notification (no. 6) implemented by the government of Japan and approved by the Animal Experiment Committee of the National Institute of Vegetable and Tea Science.

*Cells and medium.* RAW 264 cells (mouse leukemic monocytes), J774.1 cells (mouse macrophage-like cells) and YAC-1 cells (mouse lymphocyte-like cells) were obtained from RIKEN Bio Resource Center (Tsukuba, Japan). The cells were grown in Eagle’s minimum essential medium (Mediatech, Manassas, VA, USA) containing 2 mM L-glutamine (Mediatech) and supplemented with 10% fetal bovine serum (Invitrogen Canada, ON, Canada), 100 U/mL of penicillin G

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**Abbreviations:** ELISA, enzyme-linked immunosorbent assay; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MCP, monocyte chemotactic protein; NK, natural killer; TNF, tumor necrosis factor
Results and Discussion

We harvested 11 bunching onions (shimonita cultivar, 5.50 kg) and cut into pieces the green leaves (3.15 kg), white sheaths (2.31 kg), and bottoms with roots (0.04 kg). Since the green leaves were pipe-shaped and clear mucus was secreted in the cavity, the leaves were cut, dipped into distilled water, and stroked by hand to obtain the mucus. The yield was 3.6 L with 2 L of distilled water, suggesting that the mucus absorbed water and swelled. The mucus was lyophilized and yielded 34.8 g of fine white powder. In contrast, the green leaves, with the mucus extracted, and the white sheaths respectively yielded 3.3 g and 68.2 g of lyophilized powder; this was distinct from the dried mucus powder which was coarse and rough. The bottoms and insoluble fibers obtained as residues from the green leaves and white sheaths were not used.

We first investigated the in vitro effects of the plant extracts on several inflammatory cytokines produced by macrophage-like cell lines that are regarded as markers of immunomodulation. We reported that the oral administration to mice of a hot-water extract of A. fistulosum yielded 34.8 g of fine white powder obtained as residues from the green leaves and white sheaths that had some immune stimulating activity.
Oral administration of the mucus can activate other immune cells. A difference was apparent in the responsiveness of the spleen cells to LPS in mice that had been administered with the oral mucus sample. The spleen cells obtained from these mice produced significantly more IFN-γ than those of the control group after LPS stimulation (Table 1). However, no IFN-γ could be detected in unstimulated spleen cells. IFN-γ is primarily produced from spleen T cells, so it may have a role in defending against bacterial and viral infection. These results also suggest that the spleen cells had already been stimulated in vivo with the cytokines produced by macrophages.

An oral administration of the mucus to the older mice activated NK cells (Fig. 4). The cellular toxicity against YAC-1 cells by spleen cells obtained from the mice that had been orally administered with the mucus was augmented in a dose-dependent manner. These effects were also detected in the younger adult mice (data not shown), but were more apparent in the older mice because of their decreased NK activity. The mechanism underlying the enhanced NK activity resulting from oral administration of the mucus is unclear. NK cells are large granular lymphocytes derived from the bone marrow, and they act as cytolytic effector cells of the innate immune system. Since NK cells can distinguish normal cells from tumor cells, they are likely to provide the first line of defense against tumors and viral infections and contribute to antitumor immunosurveillance.

We report here that the A. fistulosum mucus significantly activated splenic NK cells and macrophages. It has been reported that the early production of IL-12 by macrophages contributes to the maturation of both NK and CD8+ T cells, leading to a T helper type of 1-biased response. Those with low NK cell activity have been found to have increased cancer risk in an 11-year follow-up study of the general population. It has also been reported that the endogenous production of both TNF-α
and IFN-γ was required to restrict primary bacterial infections. It may be possible that the *A. fistulosum* mucus could stimulate the antitumor and infection-preventive functions of NK cells and macrophages.

It has been suggested that some components of the *A. fistulosum* mucus stimulate the host immune system. However, we initially doubted the effects of contaminated LPS derived from soil bacteria. Although LPS was detected in the *A. fistulosum* extracts by the limulus test, the value obtained was different between samples and did not correlate with the activities (data not shown). The quantity of LPS in the extracts of green leaves and white sheaths was higher than that of the mucus, but this was not affected in vitro. The *A. fistulosum* mucus could activate peritoneal macrophages derived from LPS hyporesponsive C3H/He J mice (data not shown). The *A. fistulosum* mucus could stimulate natural immunity in vitro as well as orally in vivo. It has been reported that orally administered LPS was barely absorbed from the intestinal tract and detoxified in the liver. It could therefore be suggested that the in vitro data shown in Fig. 1 may not completely inhibit the involvement of contaminated LPS, and that other oral effects were due to another molecule.

![Fig. 3. Phagocytic Activity of Peritoneal Cells from Mice Orally Administered with the *A. fistulosum* Mucus.](image)

The *A. fistulosum* mucus (10 mg/400 μL/mouse) was orally administered to mice 3 h and 27 h before isolating the immune cells. The control group was administered with 400 μL of distilled water (DDW). Peritoneal cells were collected and seeded in a 96-well culture plate (1 x 10^5 cells/200 μL/well). Peritoneal macrophages were incubated at 37°C for 2 h with prelabeled zymosan, and phagocytosed zymosan was determined by its absorbance intensity. Data are presented as the mean ± SD (n = 6 mice). *p < 0.05 by Student’s t-test.

![Fig. 4. Effect on the NK Activity of an Oral Administration of the *A. fistulosum* Mucus to Mice.](image)

The *A. fistulosum* mucus (2.5–10 mg/400 μL/mouse) was orally administered to the older mice 3 h and 27 h before isolating the spleen cells. Spleen cells (5 x 10^5 cells/mL) and YAC-1 cells (5 x 10^5 cells/mL) were plated in 96-well plates and co-cultured for 18 h. The WST-1 reagent was added and the absorbance level at 450 nm measured after 2 h of culture. Data are presented as the mean ± SD (n = 4 mice). The test and control groups were significantly different at p < 0.01 by Tukey’s test.

It has recently been reported that one of the active molecules present in an *A. fistulosum* hot-water extract with anti-influenza effects may be fructan. Fructan from an aged garlic extract has also been reported to possess a delayed immunoadjuvant response to the ovalbumin antigen in BALB/c mice. We therefore examined the effects of such known ingredients as allicin, which is characteristic of the Allium species, and vitamin C, fructan and fructose, but were unable to explain the reported effects (data not shown). Further examination is necessary to validate these activities, and further studies will be required to address the effects of lower doses. The mucus administered to the mice was equivalent to 20 g in humans of 60 kg body weight, and it was obtained from 5.5 kg of *A. fistulosum*. Since bunching onions are usually cooked and their volume condensed, it is not difficult for humans to eat them. We found that even autoclaving or boiling the *A. fistulosum* mucus for 60 min did not deactivate it (data not shown). The same effects may therefore be expected when humans eat cooked bunching onion.

In conclusion, oral administration of the *A. fistulosum* mucus to mice enhanced natural immunity. This was demonstrated by the significantly increased cytokine release, the phagocytic activity of macrophages and the NK cell activity. Further work will be focused on human studies based on this evidence.

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**References**