

Oral Administration of Acidic Xylooligosaccharides Prevents the Development of Atopic Dermatitis-Like Skin Lesions in NC/Nga Mice

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Summary We examined whether two types of xylooligosaccharides (neutral or acidic xylooligosaccharides) derived from hardwood kraft pulp ameliorate the development of atopic dermatitis (AD)-like skin lesions induced by repeated application of picryl chloride (PiCl) in NC/Nga mice. Oral administration of acidic xylooligosaccharides at a daily dose of 100 mg/kg significantly prevented the development of AD-like skin lesions. Serum histamine level was significantly suppressed, but serum total IgE level was not significantly suppressed. Moreover, the secretion of inflammatory cytokine IL-12 from splenic lymphocytes was significantly suppressed. On the other hand, neutral xylooligosaccharides showed no significant preventive effect on the development of AD-like symptoms. These results suggest that oral administration of acidic xylooligosaccharides may be effective in preventing the development of AD-like skin disease and one of the mechanisms is the suppressive effect on IL-12.

Key Words acidic xylooligosaccharides, prebiotics, atopic dermatitis, NC/Nga mouse, interleukin-12

Atopic dermatitis (AD) is a chronic eczematous skin disorder accompanied by severe itching and frequently repeated episodes (1). Histopathologic findings of AD exhibit an inflammation with thickened epidermis, and infiltration of a lymphocyte, eosinophil and mast cell to the corium. Blood biochemistry findings of AD frequently show an increase of immunoglobulin E (IgE) antibodies against many kinds of allergens and histamine levels (2, 3). However, the relationship between IgE and clinical disease is not always exclusive. There are three primarily therapeutic approaches to AD in the AD treatment guideline. First, the drug therapies with agents like corticosteroids, antiallergic drugs, and antibiotics. Second, the skin care therapy with bathing, using skin moisturizer. Third, the control of environmental and food factors in individual patients (4). From the viewpoint of the adjunctive treatment, attention has recently been paid to supplements containing prebiotics, probiotics, highly-unsaturated fatty acid, etc. Clinical evidences of some of these supplements have been reported (5–8).

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, or upon treatment with repeated challenge with 2,4,6-trinitrochlorobenzene (picryl chloride) under specific pathogen free (SPF)

conditions (9–11). The induced AD-like symptoms were accompanied by increased expression of Th2 cytokines, eosinophil accumulation in the lesions and frequent scratching behavior (9).

Xylooligosaccharides are indigestible oligosaccharides obtained by degrading xylans, a common component of plant cell walls, and have the structure of β -1,4-linked D-xylose. Among previously reported indigestible oligosaccharides, xylooligosaccharides have the distinctive feature of modifying gastrointestinal conditions at a low dose of 0.4 g (12), and many foods specified for health, claiming “to modify gastrointestinal conditions” and containing xylooligosaccharides have been approved by the Ministry of Health, Labour and Welfare in Japan. Xylooligosaccharides are currently produced primarily by the treatment of corncob-derived xylans with xylanase (13). Acidic xylooligosaccharides, in which glucuronic acid is linked to xylooligosaccharide by α -1,2 bonds, have been reported to have an antibacterial effect (14), inhibitory effects on stress-induced gastric inflammation (15), preventive effects on contact hypersensitivity (16), and inhibitory effects on binding and uptake of *Salmonella enteritidis* to macrophages (17).

We focused on glucuronoxylan adsorbed on the surface of hardwood kraft pulp and established a method for producing two types of xylooligosaccharides, neutral xylooligosaccharides (XOS; a homo-oligomer of xylose) and acidic xylooligosaccharides (U-XOS; a hetero-oligomer of xylose with uronic acid), by treating hardwood

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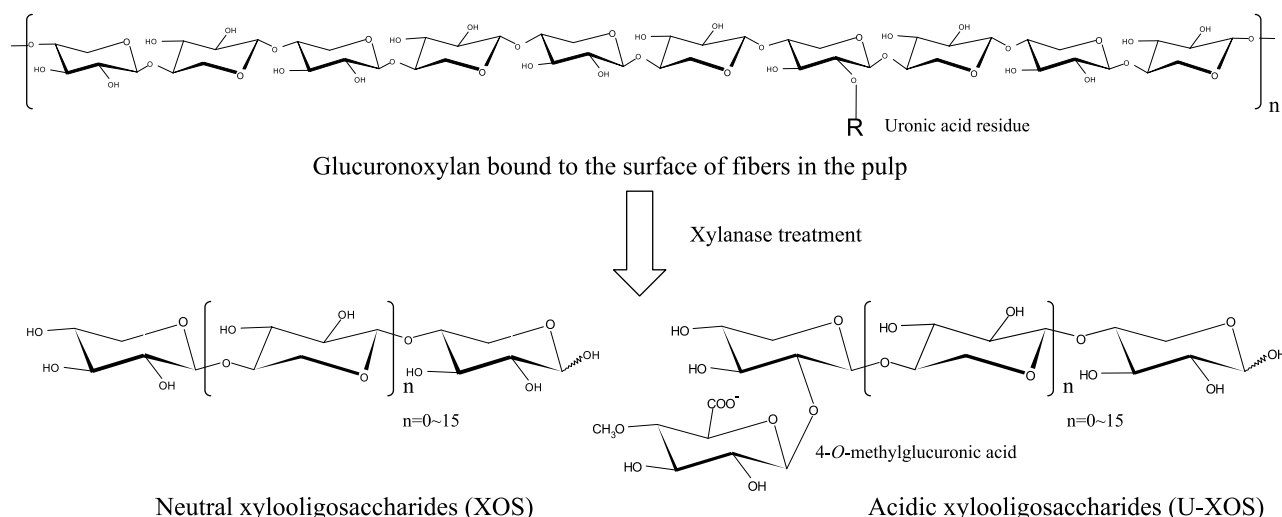


Fig. 1. Production of xylooligosaccharides by xylanase treatment. XOS is a xylooligomer in the D.P. range of dimer to approximately 17 mer containing approximately 12% xylobiose. The other was an acidic xylooligosaccharide (U-XOS), a xylooligomer in the D.P. range of dimer to approximately 17 mer with a major side chain of 4-O-methyl-glucuronic acid and other side chains of glucuronic acid and 2-O-galactopyranosyl-4-O-methyl-glucurono acid.

kraft pulp with xylanase (18, 19). Moreover, we previously reported that both xylooligosaccharides exhibited growth-promoting effect on bifidobacteria (20).

In this study, we investigated the effects of oral administration of XOS and U-XOS on the development of AD-like symptoms in NC/Nga mice, and the effect on the serum total IgE and histamine levels, and splenocyte cytokine production.

MATERIALS AND METHODS

Preparation of XOS and U-XOS. XOS (neutral xylooligosaccharide) and U-XOS (acidic xylooligosaccharide) were prepared from hardwood kraft pulp according to the method described by Izumi et al. (18, 19). The pH of 10% hardwood kraft pulp slurry (1,500 L) was adjusted to 6.0 with sulfuric acid, and then *Trichoderma reesei*-derived xylanase available as a food additive (Xylanase Conc., Specialty Enzymes and Biochemicals Co.) was added to the pulp slurry at a final concentration of 5,000 U/L. After incubation with stirring at 50°C for 45 min, the pulp was removed by solid-liquid separation to obtain 1,000 L of 0.2% saccharide solution. A reverse osmosis membrane was used to concentrate the solution 50-fold, and the pH was adjusted to 3.0 with sulfuric acid. Subsequently, lignin was associated and precipitated by heating at 120°C and 1 atmospheric pressure for 100 min. Then, the saccharide solution was decolorized with activated carbon and applied to the following resins: strong cation-exchange resin (200CT, Organo Corporation), and then weak anion-exchange resin (IRA96SB, Organo Corporation). The flow-through fraction of ion exchange resins was concentrated and spray-dried, resulting in 1.1 kg of XOS. A fraction obtained by applying 50 mM NaCl solution to the weak anion-exchange resin was similarly concentrated and spray-dried, yielding 0.7 kg of U-XOS. Figure 1 shows the structure of XOS and U-XOS.

Animals. Male 5-wk-old NC/Nga mice were pur-

chased from Charles River Japan (Yokohama, Japan) and housed individually in plastic cages in a room controlled for temperature ($23 \pm 2^\circ\text{C}$), humidity ($55 \pm 5\%$), and light (lights on: 7:00–19:00 h). The mice were kept in specific pathogen free (SPF) conditions. The mice were fed an MF diet (Oriental Yeast Co., Ltd., Tokyo) and water ad libitum. Experiments were performed in accordance with the Guideline for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animal Science.

In vivo experiments. The abdomen, dorsal skin and scalp of NC/Nga mice aged 7 wk were shaved before sensitization and challenge. These mice were sensitized by the transdermal administration of 150 μL of 0.8% picryl chloride (PiCl) in acetone/ethanol (1 : 4) to the abdomen on day 0. The mice were challenged by the application of 100 μL of 5% PiCl in olive oil to the dorsal skin and scalp once a week for 10 wk (Days 7 to 77). Twenty-one mice were divided into three groups ($n=7$ /group), and the administration of test samples was started on day 29. XOS and U-XOS, dissolved in distilled water, were orally administered to each group at a daily dose of 100 mg/kg. The test samples were administered daily via gastric tubes. Distilled water was administered to the control group. Dermatitis symptoms were evaluated by a clinical severity score at 0, 1, 2, 3, 4, 5, 6 and 7 wk after the first administration of test samples. The clinical severity score was evaluated by the method of Hirasawa et al. (21) with some modifications. The score was defined as the sum of the individual scores graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for each of five symptoms (itching, edema, hemorrhage, excoriation/erosion, and scaling/dryness, total score=15). The itch score was evaluated by observing scratching and biting behaviors. The mice were individually put into a clear plastic cage. After a 10 min adaptation, the time of a series of scratching and biting behaviors was measured as a bout of events for 5 min.

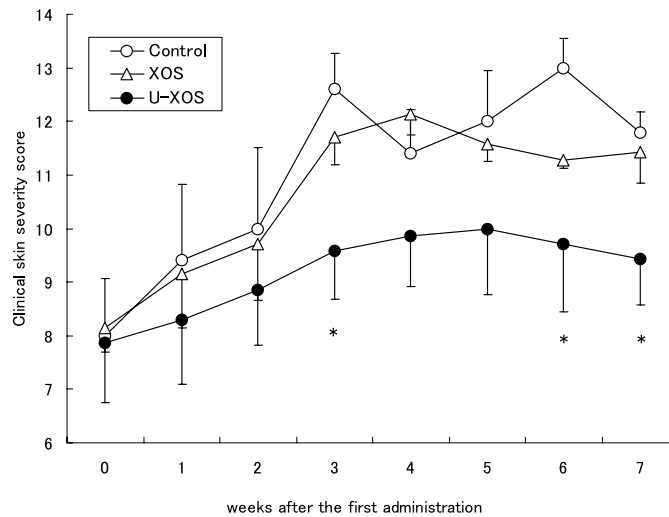


Fig. 2. Effect of administration of XOS or U-XOS on changes in the clinical skin severity score of NC/Nga mice. Each value represents the mean $\pm$ SE, $n=7$. *Significantly different from the corresponding control group at $p<0.05$.

Table 1. Score of the five symptoms of NC/Nga mice in week 7.

	Itching	Edema	Hemorrhage	Excoriation/Erosion	Scaling/Dryness	Total
Control	2.4 $\pm$ 0.2	2.2 $\pm$ 0.2	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0	1.2 $\pm$ 0.2	11.8 $\pm$ 0.4
XOS	2.0 $\pm$ 0.4	1.9 $\pm$ 0.3	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0	1.6 $\pm$ 0.2	11.4 $\pm$ 0.6
U-XOS	1.4 $\pm$ 0.3*	1.6 $\pm$ 0.4*	2.7 $\pm$ 0.2	2.6 $\pm$ 0.4	1.1 $\pm$ 0.1	9.4 $\pm$ 0.9*

Each value represents the mean $\pm$ SE, $n=7$.

*Significantly different from the corresponding control group at $p<0.05$.

The itch score was defined as accumulated time of scratching and biting behaviors graded as 0 (no continuous behavior observed), 1 (~1 min), 2 (1–2 min), and 3 (>2 min). On day 77, the mice were sacrificed by cervical dislocation with etherization and whole blood was taken from the abdominal aorta, and the spleen was dissected. Serum samples prepared by centrifugation (1,700 $\times g$, 10 min) were stored at -80°C until assay (Total IgE and histamine). Splenocytes were obtained by passing the pieces of spleen through a mesh cellstrainer (Becton Dickinson, USA) and treatment with a polysucrose solution (Lympholyte-M, Cederlane Laboratories, Canada) to remove erythrocytes and dead cells. After centrifugation (1,200 $\times g$, 20 min), the splenocytes were suspended at the density of 1.0×10^6 cells/well on a 48-well culture plate and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Valley Biochemical, USA), 100 U/mL of penicillin and 100 $\mu\text{g/mL}$ of streptomycin (MP Biomedicals, USA). These cells were cultured for 72 h at 37°C at a 5% CO_2 concentration, and the supernatants were collected to measure cytokines (IL-4, IL-6, IL-12 and IFN- γ).

Enzyme-linked immunosorbent assay (ELISA). The concentration of IL-4, IL-6, IL-12 and IFN- γ in the culture supernatant, and total IgE and histamine in serum was measured using sandwich ELISA kits (IgE, Yamasa, Japan; histamine, Oxford Biochemical Research, USA; IL-4, IFN- γ and IL-12, BioLegend, USA; IL-6, BD Pharmingen, CA) according to manufacturers' instruc-

tions.

Statistical analysis. All values were expressed as mean $\pm$ SE (standard error of the mean). The clinical severity score was analyzed using Mann-Whitney's *U* test. The concentrations of the serum total IgE, histamine and cytokines were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. A probability value of less than 0.05 was considered to be statistically significant.

RESULTS

Effect of oral administration of XOS or U-XOS on the development of dermatitis

Figure 2 shows the changes in the clinical skin severity score of NC/Nga mice. The clinical skin severity score increased in the control group, whereas the increase in the score was suppressed in the U-XOS group in week 3, 6 and 7. The XOS group showed no significant inhibitory effect on the increase in the score. In the U-XOS group, the progression of itching and edema symptom was suppressed markedly compared with the control group (Table 1). The development of dermatitis in NC/Nga was suppressed by the oral administration of U-XOS.

Effect of oral administration of XOS or U-XOS on the serum total IgE and histamine

The clinical severity of dermatitis in NC/Nga mice is reported to be associated with elevated serum IgE and histamine levels. The serum total IgE and histamine lev-

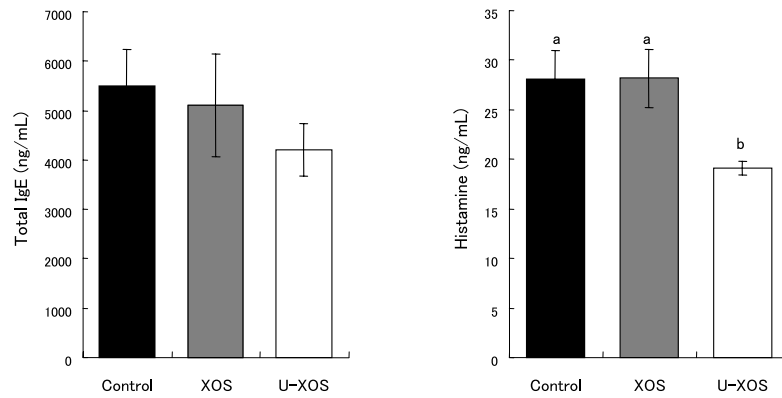


Fig. 3. Effect of administration of XOS or U-XOS on serum total IgE and histamine levels of NC/Nga mice on day 77. Each value represents the mean $\pm$ SE, $n=7$. Means not sharing the same letter are significantly different from each other, $p<0.05$.

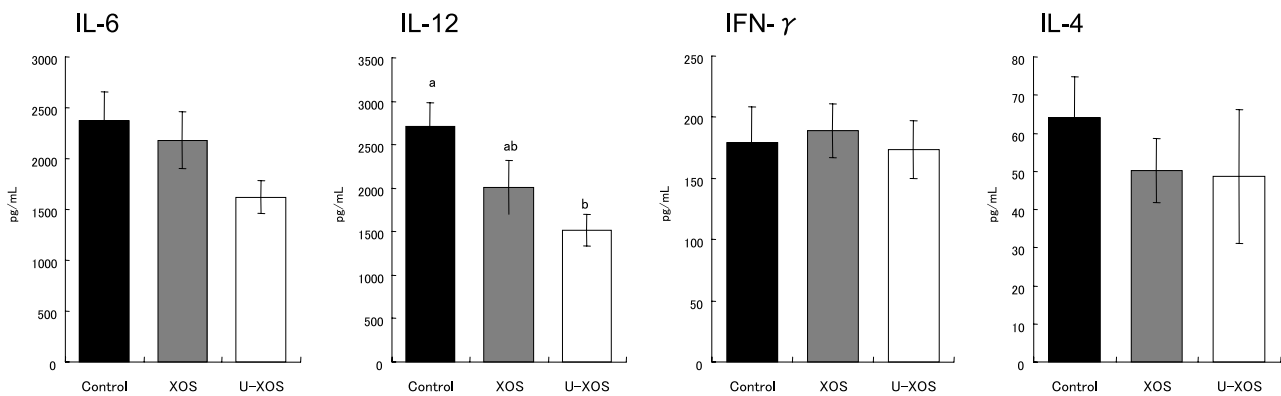


Fig. 4. Cytokine production by splenocyte cells prepared from NC/Nga mice on day 77. Each value represents the mean $\pm$ SE, $n=7$. Means not sharing the same letter are significantly different from each other, $p<0.05$.

els of each group were measured on day 77. In the serum total IgE levels, there were no significant differences among three groups. The serum histamine level of the U-XOS group was significantly lower than that of control and XOS groups. XOS administration did not significantly affect the serum total IgE or histamine levels (Fig. 3).

Effect of oral administration of XOS or U-XOS on cytokine production from splenocyte cells of NC/Nga mice

To clarify the mechanism of the preventive effect of U-XOS on AD-like dermatitis in NC/Nga mice, spontaneous cytokine production from splenocyte cells was measured. The spontaneous IL-6 production showed no difference among the three groups. The spontaneous IL-12 production of the U-XOS group was significantly lower than that of the control group, but XOS administration did not significantly affect the IL-12 production. XOS or U-XOS administration did not significantly affect the IFN- γ or IL-4 production (Fig. 4).

DISCUSSION

In this study, we examined whether XOS or U-XOS, two types of xylooligosaccharides derived from hardwood kraft pulp, ameliorates the development of AD-like skin lesions, and whether these oligosaccharides alter serum total IgE and histamine levels, and spleno-

cyte cytokine status in NC/Nga mice. The administration of U-XOS prevented the development of dermatitis symptoms, especially itching behavior and edema symptoms, with the alteration of histamine levels.

Th2 phenotype is one of the aspects of human AD (22). IL-4 secreted from Th2 cells is an important factor for immunoglobulin class switching to IgE in B cells. On the other hand, IFN- γ strongly suppresses IgE production in B cells via suppression of CD40L mRNA expression in Th2 cells. Our results indicate that oral administration of U-XOS did not influence the serum total IgE level, IL-4 or IFN- γ secretion from cultured splenocyte. Previously we examined whether oral administration of XOS or U-XOS suppresses OVA (ovalbumin) specific immunoglobulin production in OVA immunized mice. As a result, XOS and U-XOS did not show any effects on the alteration of OVA specific immunoglobulin (unpublished data). These results suggest that xylooligosaccharides might not be involved in the alteration of Th2 phenotype.

Delayed-type allergic reaction and Th1 immune response are also likely to be involved in pathogenesis in AD. IL-12, one of the Th1 cytokines, is a key cytokine modulating differentiation of Th0 cells to Th1 cells (23). Hamid et al. reported that IL-12 may participate in the chronicity of skin inflammation, in agreement with the

finding that the IL-12 positive cells increase in chronic skin lesions compared with acute lesions and uninvolved skin from AD patients (24). Tomimori et al. reported that intravenous administration of anti IL-12 antibody strongly prevented the prolongation of AD-like dermatitis in NC/Nga mice (25). From these results, it is considered that U-XOS may prevent the development of dermatitis symptoms via suppression of IL-12 accentuation. Moreover, as the preventive effects were only observed in U-XOS administered mice, it is conceivable that these beneficial effects are due to the presence of uronic acid residues.

There are a few reports regarding the immunomodulating effects of oligosaccharides. Hosono et al. reported that oral administration of fructooligosaccharides (FOS), one of the major indigestible oligosaccharides used for many functional foods and supplements, up-regulated IgA secretion in the intestinal mucosa and suppressed serum IgG1. They predict that the immunopotentiating effect could be exerted via the effect of FOS on the increase in the intestinal population of beneficial gram positive bacteria such as *Lactobacillus* and *Bifidobacterium*. Cell wall components of these bacteria such as peptideglycans and polysaccharides stimulate intestinal immune tissues (26).

On the other hand, Sonoyama et al. reported either oral or intraperitoneal treatment with α -linked galactooligosaccharide (GOS), an oligosaccharide mixture consisting largely of α -1,6-galactobiose, exhibited a suppressive effect on allergic airway eosinophilia in allergen-sensitized Brown Norway rats. Moreover, oral treatment with GOS in cecectomized rats administered neomycin showed a suppressive effect on eosinophilia. They predict that these suppressive effects involve post-absorptive action of GOS rather than prebiotic action, although they didn't rule out the prebiotic action (27).

We previously reported that the low molecular weight fraction of U-XOS was absorbed intact in the gastrointestinal tract of rats (28). In addition, U-XOS was not well utilized by *Lactobacillus* (20), the dominant bacterial species rather than *Bifidobacterium* among commensal bacteria in the intestine of mice (29), but was utilized by *Bifidobacterium*. These results suggest that U-XOS might take effect on the immune system by postabsorptive action rather than prebiotic action, although further investigation is needed.

U-XOS is a mixture of xylooligosaccharides with various degrees of polymerization (D.P.) and uronic acid residues which is linked to arbitrary xylose. Therefore, investigation into the relationship between the physiological activity and the structure of U-XOS (the D.P. of xylooligosaccharide and the location of uronic acids, etc.) is considered to be an interesting approach.

In conclusion, the results of this study demonstrate that oral administration of U-XOS prevents the development of AD-like symptoms in NC/Nga mice and modulates the synthesis of Th1 cytokine such as IL-12. Therefore, it is expected that U-XOS could be used with usual AD therapies.

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