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Rapid communication

Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of β-glucan

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Abstract

Mice were orally administered with β-glucan, isolated from baker's yeast, daily for one week (25 mg/day/mouse) and several immunoparameters in the digestive tract were examined. The most prominent change was an increase in the number of intraepithelial lymphocytes (IEL) in the intestine, although the number of lymphocytes in the liver remained unchanged. The absolute number of both αβT cells and γδT cells expressing CD8 antigens increased among IEL in the intestine. Primarily, liver lymphocytes showed a spontaneous production of Type 0 cytokine (simultaneous production of IFNγ and IL-4) while IEL did not produce any cytokines without stimulation. However, mice administered with β-glucan produced Type 1 cytokine, namely, production of IFNγ alone. These results suggest that β-glucan may be an important potentiator for mucosal immunity in the digestive tract.

1. Introduction

It is widely known that oats, mushrooms, and yeasts (including baker's yeast and beer yeast) are good for the health because they stimulate intestinal movement [1–12]. Thus, they can be used to ameliorate constipation and treat other bowel disorders. The major component of oats, mushrooms, and yeast is β-glucan, which is an undigestive sugar in humans. In light of these facts, it is speculated that β-glucan may also modulate mucosal immunity in the digestive tract. Thus, intestinal functions and the immune system are mutually regulated by parasympathetic nerves [13].

Cumulative evidence for mucosal immunity in the digestive tract has been raised during the past decade. Both the intestine [14–16] and the liver [17,18] contain unique lymphocyte subsets of extrathymic origin. This is because the liver originated from the intestine in phylogeny as an excretion organ of bile [19]. However, the intestine and the liver developed in somewhat different ways as immune organs in subsequent phylogeny [20]. In this regard, we investigated how β-glucan differently modulates the immune system of the intestine and that of the liver. β-glucan was found to be an important potentiator for mucosal immunity in the intestine, but not in the liver.

2. Materials and methods

2.1. Mice

C57BL/6 mice, maintained in the animal facility of Niigata University, were used in this study. All mice were fed under specific pathogen-free conditions.

2.2. Oral administration of β-glucan

β-glucan (Norwegian Beta 1, 3/1, 6 Glucan, Nakajima Suisan, Tokyo, Japan) was orally administered to mice (25 mg/day/mouse) for one week. Control mice were given PBS (pH 7.4).

2.3. Cell preparation

Hepatic lymphocytes were isolated by a previously described method [21]. Briefly, the liver was removed,

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pressed through 200-gauge stainless steel mesh, and suspended in Eagle's MEM medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 5 mM Hepes and 2% heat-inactivated newborn calf serum. After being washed once with the medium, the cells were fractionated by centrifugation in 15 ml of 35% Percoll solution (Amersham Biosciences, Piscataway, NJ) for 15 min at 2000 rpm. The pellet was resuspended in erythrocyte lysing solution (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA-Na, and 170 mM Tris, pH 7.3).

Intraepithelial lymphocytes (IEL)[1] were collected from the intestine according to a previously described method [22]. Briefly, the small intestine was removed and flushed with PBS to eliminate luminal contents. The mesentery and Peyer's patches were then resected. The intestine was opened longitudinally and cut into 1–2 cm fragments. These fragments were incubated for 15 min in 20 ml Ca^{2+}- and Mg^{2+}-free Dulbecco's PBS containing 5 mM EDTA, in a 37 °C shaking-water bath. The supernatant was then collected. The cell suspensions were collected and centrifuged in a discontinuous 40/80% Percoll gradient at 2800 rpm for 25 min. Cells from the 40/80% interface were collected. Splenocytes were obtained by pressing the spleen through 200-gause stainless steel mesh. Splenocytes were used after erythrocyte lysing.

2.4. Immunofluorescence assay

For flow cytometric analysis [22], anti-CD4 (RM4-5), anti-CD8α (53–6.7), anti-TCRαβ (H57-597), anti-TCRγδ (GL3), anti-CD8β (53–5.8), and anti-CD45 (30-F11) mAb were purchased from PharMingen (San Diego, CA). All mAb were used in a FITC-, PE-, or Cychrome-conjugated form. Cells were analyzed by FACScan (Becton Dickinson, Mountain View, CA). To prevent nonspecific binding of mAb, CD32/16 (2.4G2) was added before staining with labeled mAb. Dead cells were excluded by forward scatter, side scatter gating. To analyze lymphocytes, $CD45^+$ cells were gated.

2.5. Reverse transcription (RT)-PCR for IFNγ and IL-4

Total RNA was extracted from mononuclear cells (MNC). To detected mRNAs of IFNγ and IL-4, RNA was reverse-transcript (RT) using the primers of these genes and such cDNA was further amplified by PCR method. Briefly, total RNA was prepared from MNC in various organs by using RN-easy Mini kit (Qiagen, Valencia, CA). cDNA was synthesized using 5 μg RNA with Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences, Piscataway, NJ) and Oligo(dT) 15 Primer (Promega, Madison, WI). PCR amplification of synthesized cDNA was conducted as previously described [23]. The primers of IL-4, IFNγ, and G3PDH for PCR amplification were as follows: IL-4 (5′-CCA GCT AGT TGT CAT CCT GC-3′ and 5′-GTG ATG TGG ACT TGG ACT CA-3′); IFNγ (5′-AAT GAA CGC TAC ACA CTG CA-3′ and 5′-TGA AGA AGG TAG TAA TCA GG-3′); and G3PDH (5′-ACC ACA GTC CAT GAA ATC AC-3′ and 5′-TCC ACC ACC CTG TTG CTG TA-3′). PCR products as well as markers were estimated by staining with ethidium bromide. Primers for G3PDH were used to assess the integrity of the RNA preparation.

2.6. Statistical analysis

The difference between the values was determined by Student's *t*-test.

3. Results

3.1. Increase in the number of IEL in the intestine by oral administration of β-glucan

Mice were orally administered with or without β-glucan for a week and the numbers of lymphocytes yielded by the intestine and liver were compared (Fig. 1). The number of IEL in the intestine was elevated up to fourfold by the administration of β-glucan in comparison with control mice. However, the number of lymphocytes in the liver remained unchanged by β-glucan.

In a preliminary study, we determined the dose of β-glucan in this study. Lower doses (e.g., 10 mg/day/

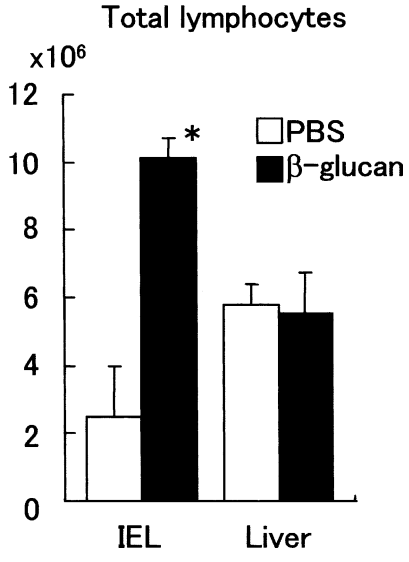

Fig. 1. The number of lymphocytes yielded by the intestine and liver of mice administered with or without β-glucan. IEL were obtained from the small intestine. The mean and one SD were produced from 4 mice. $^*P < 0.05$.

[1] *Abbreviations used:* IEL, intraepithelial lymphocytes; RT, reverse transcription.

mouse) of β-glucan than 25 mg/day/mouse decreased the change in the number of IEL in the intestine. However, the maximum change was reached by 25 mg/day/mouse. The change induced by 50 mg/day/mouse was almost the same as 25 mg/day/mouse. When we continued the administration of β-glucan up to one month, a little more prominent change than one week was produced.

3.2. Phenotypic characterization of lymphocytes in mice administered with β-glucan

To identify the phenotype of cells, two-color stainings with various combinations of mAbs were conducted in the intestine and liver (Fig. 2). Two-color staining for TCRαβ and TCRγδ revealed that the proportion of γδT cells prominently increased in the intestine. This change was not seen in the liver. Two-color staining for CD4 and CD8 showed that the proportion of both $CD4^+$ and $CD8^+$ cells increased in the intestine. In the case of the intestine, a unique population of $CD4^+8^+$ double-positive cells is present. This population also tended to increase in this organ. $CD4^+8^+$ double-positive cells were all $\alpha\beta TCR^+$ (data not shown). Among $CD8^+$T cells in the intestine, the $CD8\alpha\alpha^+$ subset and the $CD8\alpha\beta^+$ subset are present. Two-color staining for CD8α and CD8β revealed that both subsets increased proportionally.

By repeated experiments ($n = 4$), the absolute number of various lymphocyte subsets were calculated in the intestine and liver (Fig. 3). Although the most prominent change was seen in the number of γδT cells of the intestine, the number of αβT cells was also elevated in this organ. These changes were mainly seen in the number of $CD8^+$ cells. The number of $CD4^+$ cells also increased but the absolute number of $CD4^+$ cells among IEL was primarily small. $CD8^+$ cells in the intestine

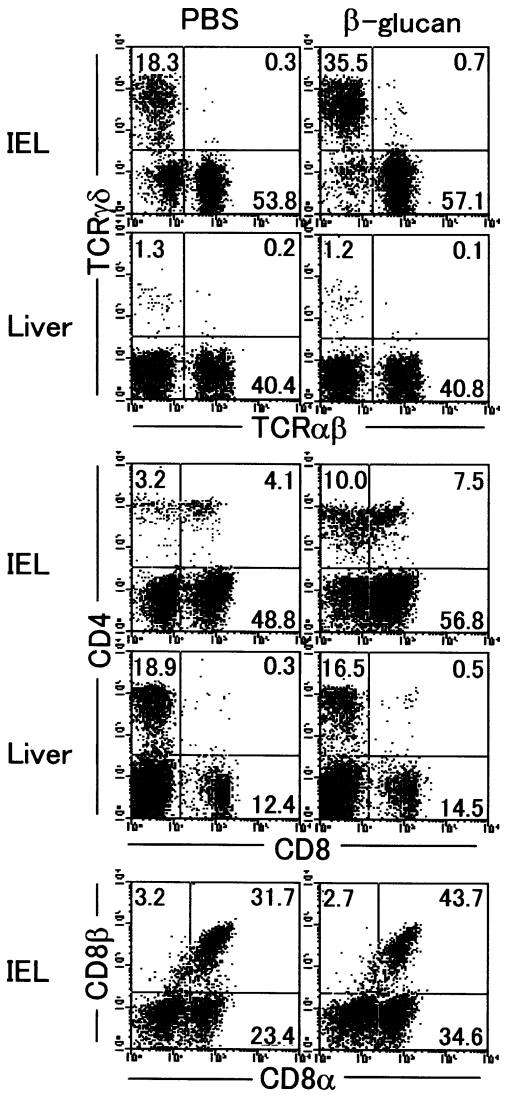

Fig. 2. Phenotypic characterization of lymphocytes in the intestine and liver. Two-color staining for TCRαβ and TCRγδ, that for CD4 and CD8, and that for CD8α and CD8β were conducted. Numbers in the figure represent the percentages of fluorescence-positive cells in corresponding areas. The data shown here are representative of three experiments.

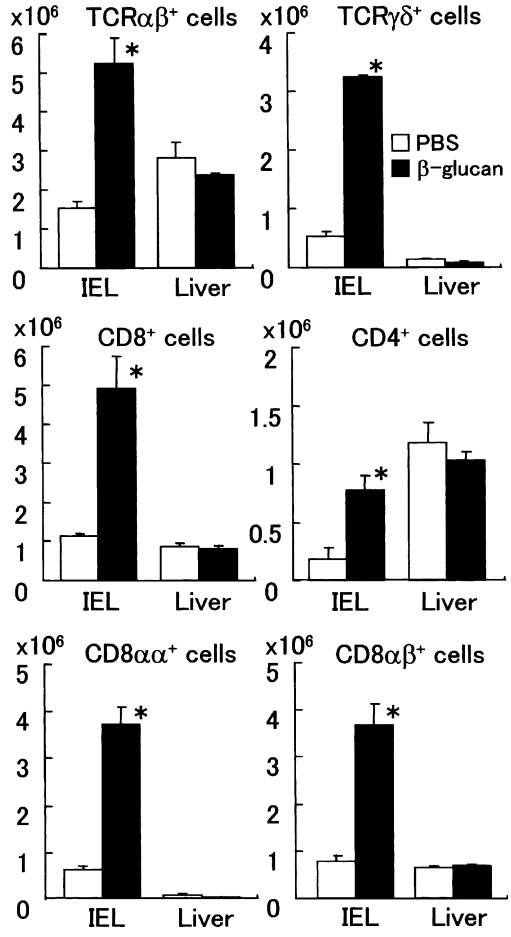

Fig. 3. Expansion of various lymphocyte subsets in the intestine and liver of mice administered with β-glucan. Repeated experiments of the phenotypic study were conduced. The mean and one SD were produced from independent experiments ($n = 4$). *$P < 0.05$.

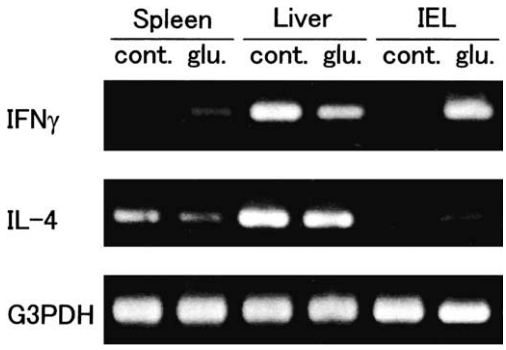

Fig. 4. Production of IFNγ and IL-4 by the administration of β-glucan. Signs of mRNAs of IFNγ and IL-4 were identified by the PCR method. The data shown here are representative of three experiments.

were the half-and-half mixture of CD8αα$^+$ cells and CD8αβ$^+$ cells. The number of both CD8αα$^+$ and CD8αβ$^+$ cells increased at almost the same level. Oral administration of β-glucan did not influence the number of lymphocyte subsets in the liver.

3.3. Cytokine profile after the administration of β-glucan

In a final portion of these experiments, the cytokine profile was examined in the spleen, liver, and intestine by the PCR method (Fig. 4). Without the administration of β-glucan, spleen lymphocytes expressed the sign of IL-4, but liver lymphocytes expressed those of both IFNγ and IL-4. In contrast, no signs of IFNγ and IL-4 were detected in the intestine. When β-glucan was administered, this pattern of profile did not significantly change in the spleen and liver. However, it was found that the sign of IFNγ appeared prominently in the intestine. In addition to the liver, a weak sign of IFNγ also appeared in the spleen.

4. Discussion

In the present study, we demonstrated that β-glucan isolated from baker's yeast increased the number and function of IEL in the intestine of mice when administered daily for a week. The number of lymphocytes yielded by the intestine increased more than fourfold in comparison with that of control mice. The most prominent change among IEL was seen in γδT cells expressing CD8 antigens. Spontaneous production of IFNγ (i.e., Type 1 cytokine profile) was also found in IEL of the intestine. These results suggested that β-glucan may potentiate mucosal immunity in the digestive tract.

In contrast to other immune organs, γδT cells are extremely abundant in the intestine [14–16]. The most prominent increase in the proportion of lymphocyte subsets by β-glucan was seen in this population. It is known that these γδT cells among IEL are a mixture of CD8αα$^+$ and CD8αβ$^+$. The proportion of both CD8αα$^+$ and CD8αβ$^+$ cells was found to be increased by β-glucan. In addition to γδT cells, the absolute number of αβT cells among IEL in the intestine was increased by β-glucan.

It is widely known that many undigestive sugars, including β-glucan, stimulate the movement of intestine and result in the amelioration of constipation and other intestinal disorders [1–12]. One speculation is that this effect might be related to the stimulation of parasympathetic nerves. Since lymphocytes carry cholinergic receptors on the surface [13], it is conceivable that such stimulation of parasympathetic nerves then activates the mucosal immune system in the intestine. The mechanism underlying the present phenomenon remains to be further investigated.

We previously reported that not only the intestine but also the liver contain unique lymphocytes of extrathymic origin [17,18]. This is due to the fact that the liver originated from the intestine in phylogenic development [19]. However, the present study demonstrated that the effect of β-glucan on immunopotentiation was not seen at all in the liver. This result might be associated with the properties of β-glucan as an undigestive sugar and its nonadsorption from the portal vein. Since a weak sign of IFNγ appeared in the spleen after the administration of β-glucan, we could not deny completely that some fragments of β-glucan were absorbed into the body. We have also to consider mitogenic effects for β-glucan in nature. At this time, some lectin-like molecules for sugar moiety on lymphocytes might be working.

We think that increase in the number of IEL in the intestine was due to their in situ generation, although this subject needs further experiments. A spontaneous sign of IFNγ in the intestine after the administration of β-glucan was of interest. If this is the case, a switching from the resting state to the Type 1 cytokine was induced. This might be related to the potentiation of cytotoxicity mediated by CD8$^+$T cells. Further investigations are required according to the present results from β-glucan. In any case, β-glucan was found to be an important potentiator for mucosal immunity in the digestive tract.

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