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Absorption and Tissue Distribution of an Immunomodulatory α-D-Glucan after Oral Administration of *Tricholoma matsutake*

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α-D-Glucan (MPG-1) separated from *Tricholoma matsutake* (CM6271) has been reported to show immunomodulatory activities. In this study, the plasma concentration and tissue distribution of MPG-1 after CM6271 oral administration were investigated as part of the action mechanism analysis. When CM6271 was orally administered in a single dose to mice, MPG-1 was absorbed via the intestinal tract, appeared in plasma after 16 h, was gradually excreted from the blood, and fell to background level after 48 h. The time course analysis of MPG-1 in plasma showed the following pharmacokinetic parameters of MPG-1: t_{max} = 24 h; C_{max} = 161.1 ng/mL; $AUC_{(0-\infty)}$ = 2559.7 ng·h/mL. Moreover, MPG-1 was confirmed to localize in Peyer's patches, mesenteric lymph nodes (MLN), and the spleen and to promote IL-12 p70 production and NK cell activity. These results suggest that MPG-1 stimulated the intestinal immune system through Peyer's patches; moreover, it was taken into the blood and stimulated the systemic immune system.

KEYWORDS: Glucan; polysaccharide; *Tricholoma matsutake*; mycelium; ELISA

INTRODUCTION

From the results of many epidemiological and animal studies, scientific evidence has been accumulated that the oral intake of foods containing certain polysaccharides and protein−polysaccharide complexes shows beneficial effects, such as reduced levels of blood cholesterol and reduced rates of cancer (*1*–*4*). To understand some of the functions of these foods panoptically, it is important to identify not only the active ingredient and the action mechanism but also the gastrointestinal absorption and distribution of the active components in target tissues and cells after intake. However, although the pharmacokinetics of the intravenous injection of purified polysaccharides and protein−polysaccharide complexes have been analyzed in detail (*5*–*8*), there have been few evaluations after the oral administration of such high molecular weight substances (*9*–*13*).

In the current search for edible mushrooms and herbs that modulate immune response by oral administration, a mycelial preparation of *Tricholoma matsutake* strain BP-7304 (CM6271), a member of the Tricholomataceae family of basidiomycetes, was found to significantly enhance the recovery of natural killer cell activity that had been reduced by the loading of restraint stress (*14, 15*). This effect possibly occurred through the induction of immunoenhancing cytokine IL-12 and not through the direct action on stress-induced immunosuppressive hormones such as corticosterone and ACTH (Ishihara et al., unpublished results). Furthermore, a sodium hydroxide extract of mycelia was purified by a combination of ion exchange chromatography and gel filtration to identify the components involved in the expression of the activity, and a single-peak fraction (MPG-1) was obtained by reversed-phase chromatography. Its relative molecular mass was 360 000 (glucan/protein = 94.3:5.7), and the sugar chain structure, which accounts for 94.3% of the molecular weight, is α-(1→4)-D-glucans with α-(1→2)- and α-(1→6)-D-linkages. The amino acid composition of the protein moiety was rich in 10 amino acids, namely, glutamine, alanine, asparagine, leucine, glycine, valine, serine, threonine, isoleucine, and proline (*16*). The purpose of this study was to investigate the pharmacokinetics of MPG-1 after CM6271 oral administration to mice.

MATERIALS AND METHODS

CM6271 and MPG-1. CM6271, cultured mycelia of *T. matsutake* strain BP-7304, was supplied by Kureha Corp. (Tokyo, Japan). The preparation was a white powder containing 73% carbohydrates, 16% proteins, 5% lipids, and 5% ash, and its endotoxin content was 1.5 ng/mg, as determined colorimetrically using a commercially available

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kit (Seikagaku Kogyo, Tokyo, Japan). CM6271 was sealed and kept in the dark until use. Fixed amounts of CM6271 were suspended in a 0.5% aqueous solution of carboxymethylcellulose and homogenized. The resulting suspension was administered in a single dose to mice orally at 0.1 mL per 10 g of body weight using a dosing needle. MPG-1 was prepared according to the method of Hoshi et al. (*16*). Briefly, an equal volume of 1:1 (v/v) methanol/chloroform solvent mixture was added to an alkaline extract of CM6271, and the aqueous phase was recovered. Subsequently, this aqueous phase was sequentially fractionated by DEAE ion exchange chromatography and gel filtration chromatography to recover MPG-1. MPG-1 showed a single peak by reversed-phase HPLC. The endotoxin content of the MPG-1 preparation was below 2.5 ng/mg, and MPG-1 content was 764 μg in 1 g of CM6271.

Biotin-Labeled Anti-MPG-1 Polyclonal Antibody. Biotin-labeled anti-MPG-1 polyclonal antibody (pAb) was prepared according to the method of Hoshi et al. (*17*). Briefly, 500 μg of MPG-1 was emulsified with an equal volume of Freund's complete adjuvant (Sigma-Aldrich Japan, Tokyo, Japan) and injected subcutaneously into female New Zealand white rabbits (Charles River Laboratories Japan, Inc., Kanagawa, Japan). After 2 weeks, an emulsion of MPG-1 with Freund's incomplete adjuvant (Sigma-Aldrich Japan) was injected subcutaneously four times at 2 week intervals, followed by a mixture of MPG-1 with TiterMax gold adjuvant (Interchim, Montluçon, France) four times at 2 week intervals. Seven days after the final immunization, antiserum was separated and stored at −80 °C until use. Antiserum was purified by protein A Sepharose (GE Healthcare U.K. Ltd., Buckinghamshire, U.K.), and purified pAb was labeled using a Sulfo-OSu biotinylation kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. Earlier studies have shown that this antibody detects the glucan moiety of MPG-1 (*17*).

Transforming Growth Factor β1 (TGF-β1) Binding Activity. TGF-β1 binding activity was evaluated according to the method of Matsunaga et al. (*18*). Briefly, to avoid nonspecific protein adsorption, ^{125}I-rhTGF-β1 (PerkinElmer Japan Co., Ltd., Kanagawa, Japan) was dissolved in 50 mM Tris-HCl buffer at pH 7.5 containing 2% bovine plasma albumin and 0.2 M sodium chloride and adjusted to 10 ng/mL. A mixture of 100 μL of ^{125}I-rh TGF-β1 (10 ng/mL) and 100 μL of MPG-1 (100 μg/mL) was incubated at 37 °C for 3 h. To assess the effect of the anti-MPG-1 antibody on MPG-1-TGF-β1 binding, MPG-1 was preincubated with 10 or 50 μg/mL anti-MPG-1 antibody at 37 °C for 3 h, and then the mixture was incubated with ^{125}I-rhTGF-β1 at 37 °C for another 3 h. Then, the reaction mixture was applied to a Sephacryl S-100HR column (10 × 500 mm; GE Healthcare U.K. Ltd.), and the level of radioactivity was measured using a COBRA γ-counter (PerkinElmer Japan Co., Ltd.). Radioactivity between the elution fraction of the complex (MPG-1 and ^{125}I-rhTGF-β1) and the noncomplex (^{125}I-rhTGF-β1 alone) was compared.

Animals. Specific-pathogen-free male C57BL/6 and female BALB/c mice purchased from Charles River Laboratories Japan, Inc., were acclimatized and then used in the experiments at the age of 8 weeks. The mice were allowed free access to food CE-2 (Oriental Yeast, Tokyo, Japan) and sterilized tap water. The mice were bred at 25 ± 2 °C, humidity of 55 ± 7%, laminary air flow, and 12 h light/12 h dark cycle at 150−300 lx. To maintain a uniform environment, noise was carefully avoided, and only keepers and experimenters were allowed into the rearing room. CM6271 was suspended in 0.5% aqueous solution of carboxymethylcellulose and was orally administered to mice using a dosing needle. As a rule, each experimental group consisted of six mice, and the same experiment was repeated at least twice. The experimental design was reviewed by the Committee of Ethics on Animal Experiments of the Biomedical Research Laboratories of Kureha Corp., and the experiments were carried out in accordance with the guidelines of this committee.

Plasma and Tissue Sampling. Blood was transferred to a refrigerated centrifuge to separate the plasma, and the collected plasma was stored at −80 °C until determinations were made. The intestinal tract, mesenteric lymph nodes (MLN), spleen, and liver were fixed in 10% buffered formalin and embedded in paraffin.

Quantitation of Plasma MPG-1 by ELISA. ELISA was performed by the following method. Wells of a 96-well microtiter plate (Corning Inc., Corning, NY) were coated with 5 μg/mL of anti-MPG-1 antibody and kept at 4 °C overnight. The plate was washed with phosphate-buffered saline (PBS) and blocked with 1% BSA at room temperature (RT) for 30 min. Subsequently, the sample or MPG-1 solution was added to each well and incubated at RT for 2 h. After each well had been washed with PBS containing 0.05% Tween-20 (PBS-T), 1 μg/mL of biotinylated Ab was added to the wells and incubated at RT for 2 h. After further washing with PBS-T, 0.1 μg/mL of horseradish peroxidase (HRP)-conjugated streptavidin (Zymed Laboratories, Inc., San Francisco, CA) was added to the well and incubated at RT for 1 h. After further washing, glycine−citric acid buffer containing 2,2′-azinodi[3-ethylbenzthiazoline sulfonate] and hydrogen peroxide (ABTS Substrate Solution, Kirkegaard & Perry Laboratories, Inc., Washington, DC) was added to each well. Color development was stopped by the addition of 5% sodium dodecyl sulfate solution (ABTS Peroxidase Stop Solution, Kirkegaard & Perry Laboratories, Inc.), and the optical density of each well was measured at 405 nm (reference wavelength = 630 nm). The concentration of MPG-1 in each sample was estimated by extrapolating the optical density to a calibration curve. In this system, the detection limit was 10 ng/mL. In addition, this ELISA procedure was insensitive to typical α- and β-glucans, such as glycogen, starch, yeast glucan, barley glucan, etc., and was considered to predominantly recognize MPG-1 (*17*).

Immunostaining of Tissues. Paraffin-embedded sections of 4 μm thickness were deparaffinized with xylene and ethyl alcohol, treated with a 3% hydrogen peroxide solution for 20 min to inactivate endogenous peroxidases, and blocked with 2% porcine plasma for 15 min. Then, the anti-MPG-1 antibody (2 μg/mL) was added, and the sections were incubated at RT for 2 h and washed with PBS-T three times. HRP-labeled anti-rabbit IgG antibody solution (Kirkegaard & Perry Laboratories, Inc.; 0.2 μg/mL) was added as the secondary antibody, and the sections were incubated at 25 °C for 1 h and washed with PBS-T three times. Then, the sections were reacted with Liquid DAB Chromogen (DAKO Japan Co. Ltd., Kyoto, Japan) and examined using a microscope.

Natural Killer (NK) Cell Activity. Immediately after the mice were sacrificed by cervical dislocation, their MLN and spleens were removed and suspended in RPMI 1640 medium with 10% heat-inactivated fetal calf serum (FCS) so that a single cell suspension was obtained. NK cell activity was determined by the 51Cr release assay using YAC-1, a mouse leukemia cell line, as a target cell, as reported previously (*14*).

Quantification of Total IL-12. Cells separated from the MLN or spleen were dispensed at 2 × 10^6 /mL in RPMI 1640 medium containing 10% heat-inactivated FCS and 2 mM glutamine into each well of a 96-well tissue culture plate. Then, 5 μg/mL of rat antimouse CD40 monoclonal antibody (mAb) derived from clone 3/23 (BD Pharmingen, Franklin Lakes, NJ) and 10 ng/mL recombinant mouse (rm) IFN-γ (BD Pharmingen) were dispensed into the wells and incubated for 72 h at 37 °C in a 5% CO_2 atmosphere. After culture, the supernatant was recovered by centrifugation, and the IL-12 p70 concentration in the supernatant was determined using an ELISA kit (R&D Systems, Minneapolis, MN).

MPG-1 Binding Assay. Peyer's patches (PPs) were obtained by surgical excision from the small intestines of 8−12-week-old female BALB/c mice (Charles River Laboratories Japan, Inc.) and allowed to incubate for 30 min at 37 °C in calcium/magnesium-free Hank's balanced salt solution (HBSS; Sigma-Aldrich Japan) containing 10% heat-inactivated fetal bovine serum (ICN Pharmaceuticals, Costa Mesa, CA), 25 mM HEPES (Invitrogen Corp., Tokyo, Japan), 1 mM dithiothreitol (Sigma-Aldrich Japan), 5 mM EDTA (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 50 μg/mL gentamicin (Sigma-Aldrich Japan) to remove intestinal epithelial cells. After vigorous washing with HBSS, PPs were minced and digested with collagenase (400 units/mL; Wako Pure Chemical Industries, Ltd.) for 1 h at 37 °C in RPMI 1640 (Sigma-Aldrich Japan) containing 10% heat-inactivated fetal bovine serum, 25 mM HEPES, 2 mM L-glutamine, and 50 μg/mL gentamicin (complete medium). The digested tissue was filtered through a 100 μm nylon mesh (Sigma-Aldrich Japan) to yield a single cell suspension, which was layered over Lympholyte-M

Table 1. Effect of Anti-MPG-1 Antibody on the TGF-β1-Binding Activity of MPG-1[a]

^{125}I rhTGF-β1 (ng/mL)	MPG-1 (μg/mL)	anti-MPG-1 antibody (μg/mL)	radioactivity (cpm) MPG-1−^{125}I rhTGF-β1 complex	radioactivity (cpm) unbound ^{125}I rhTGF-β1
10	100	0	35000	14250
10	100	10	35716	17640
10	100	50	23921	34644
10	0	0	245	78950

[a] MPG-1 (100 μg/mL) and anti-MPG-1 antibody were mixed in a test tube and pre-incubated at 37 °C for 3 h. The mixture was incubated with 10 ng/mL of ^{125}I-rhTGF-β1 at 37 °C for another 3 h. Then the reaction products were subjected to gel filtration, and the radioactivity of the eluate was measured using a γ-counter. Elution of the volume between 60 and 70 mL was designated the high molecular weight fraction (MPG-1−^{125}I rhTGF-β1 complex) and that between 155 and 170 mL as the low molecular weight fraction (unbound ^{125}I rhTGF-β1). cpm, counts per minute. Data shown are representative of three independent experiments.

(Cedarlane Laboratories, Hornby, ON, Canada) and centrifuged for 15 min at 1000*g* at RT. Cells at the interface were carefully collected and washed with complete medium.

Statistical Analysis. Data are expressed as the mean ± standard deviation (SD) of six mice. Statistical differences were tested using Student's *t* test. *P* values of <.05 were considered to be significant. All experiments were repeated at least twice.

RESULTS

Effect of Anti-MPG-1 Polyclonal Antibody on MPG-1-TGF-β1 Binding. Anti-MPG-1 antibody was prepared by immunizing rabbits with MPG-1. Then, whole blood was collected and sera were separated according to a standard method. Antibody was purified by HiTrap rProtein A FF Column (GE Healthcare U.K. Ltd.) and used for the following experiments. TGF-β1 is a biomolecule known to inhibit the growth and proliferation of immunocompetent cells, and its overproduction causes immunosuppression. Moreover, the intracellular signal pathway of TGF-β1 is affected by glucocorticoids produced in stress-loaded individuals (*9*). In a previous study, it was confirmed that MPG-1 binds directly to TGF-β1 and inhibits its activity (*16*); therefore, it was examined whether the anti-MPG-1 antibody could combine with MPG-1 and block the interaction between MPG-1 and TGF-β1. Representative findings are shown in **Table 1**. The reaction mixture of MPG-1 and ^{125}I-rh TGF-β1 was subjected to gel filtration on a Sephacryl S-100HR column to separate it into an ^{125}I-rhTGF-β1−MPG-1 complex and unbound ^{125}I-rhTGF-β1. Accordingly, the radioactivity levels of the ^{125}I-rhTGF-β1−MPG-1 complex and unbound ^{125}I-rhTGF-β1 were 35000 and 14250 cpm, respectively. The radioactivity of the high molecular weight fraction showed an obvious increase compared to when ^{125}I-rhTGF-β1 alone was applied. Subsequently, MPG-1 was pre-incubated with anti-MPG-1 antibody, and then the mixture was incubated with ^{125}I-rhTGF-β1. In this case, the radioactivity levels of the ^{125}I-rhTGF-β1−MPG-1 complex and unbound ^{125}I-rhTGF-β1 were 35716 and 17640 cpm (10 μg/mL of anti-MPG-1 antibody) or 23921 and 34644 cpm (50 μg/mL of anti-MPG-1 antibody), respectively. The ^{125}I-rhTGF-β1−MPG-1 complex formation was suppressed by antibody treatment dose-dependently. In addition, even if nonimmune IgG was used instead of the anti-MPG-1 antibody, it did not affect the reactivity of MPG-1 and ^{125}I-rhTGF-β1 (data not shown). These results suggest that the anti-MPG-1 antibody inhibits the direct binding of MPG-1 to rhTGF-β1 and possibly neutralizes MPG-1 activity.

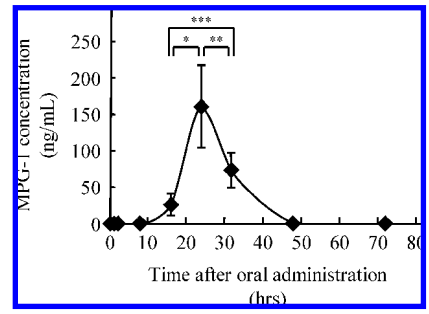

Figure 1. Plasma MPG-1 concentrations at various times after oral administration of CM6271. The MPG-1 concentration was determined in blood serially collected after a single oral administration of *T. matsutake* CM6271 to mice at 450 mg/kg by ELISA. Each value represents the mean of six mice, and the vertical bar indicates SD. ∗, $P = 0.001$; ∗∗, $P = 0.012$; ∗∗∗, $P = 0.017$.

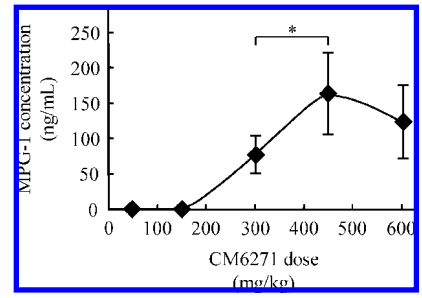

Figure 2. Dose dependency of plasma MPG-1 concentrations after oral administration of CM6271. The MPG-1 concentration was determined by ELISA in blood collected at 24 h after a single oral administration of *T. matsutake* CM6271 to mice at 0, 50, 150, 300, 450, and 600 mg/kg. Each value represents the mean of six mice, and the vertical bar indicates SD. ∗, $P = 0.018$.

Pharmacokinetics of MPG-1 after Oral Administration of CM6271. As described under Materials and Methods, this ELISA system was able to detect an MPG-1 concentration of 10 ng/mL or more. Then, to determine its pharmacokinetics, *T. matsutake* CM6271 was given in a single dose by oral administration to C57BL/6 mice at 450 mg/kg. Blood was collected over time (0, 1, 4, 8, 16, 24, 32, 48, and 72 h), and the MPG-1 level in plasma was measured. As shown in **Figure 1**, the levels of plasma MPG-1 at 1, 4, and 8 h after administration were equivalent to those in untreated mice and below the detection limit. After 16, 24, and 32 h, the levels were 31.4 ± 15.1, 161.1 ± 57.2, and 71.0 ± 28.9 ng/mL, respectively, and the MPG-1 level declined to the background level after 48 h. Furthermore, as shown in **Figure 2**, MPG-1 levels in the blood depended on the amount of CM6271, and at 24 h after oral administration of 300, 450, and 600 mg/kg they were 78.4 ± 36.0, 161.1 ± 57.2, and 121.8 ± 62.8 ng/mL, respectively. From these results, the pharmacokinetics parameters were calculated for MPG-1 using WinNonlin Noncompartmental Analysis version 5.0.1. The maximum drug concentration in plasma (C_{max}) was 161.1 ng/mL, the maximum drug concentration time in plasma (t_{max}) was 24 h, and the area under the plasma concentration time curve ($AUC_{0-\infty}$) was 2559.7 ng·h/mL. The terminal elimination half-life ($t_{1/2}$) was about 28 h. This evaluation result using ELISA showed that MPG-1 gradually entered the blood circulation after 16 h following the oral administration of CM6271, reaching a peak at 24 h, and then MPG-1 was excreted from plasma and declined to a level comparable to the background after 48 h. In addition, the plasma levels showed dose-proportionality between 300 and 450 mg/kg and reached a plateau at a 450 mg/kg dose and over.

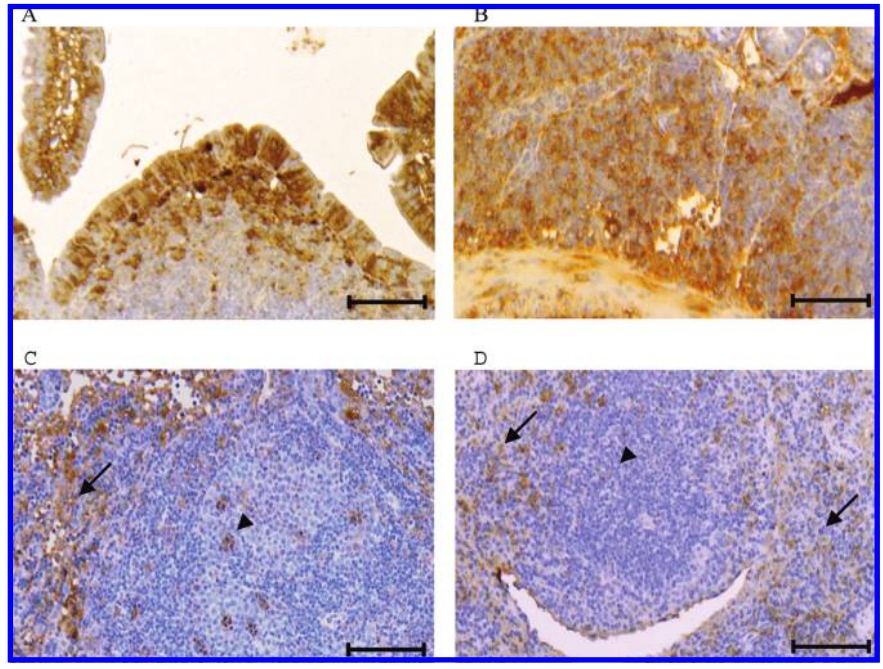

Figure 3. Immunostaining of immune tissues after oral administration of CM6271. *T. matsutake* CM6271 was administered orally in a single dose to mice at 450 mg/kg, and Peyer's patches and MLN, which are intestinal immune tissues, and the spleen were removed. These samples were fixed with formalin, and paraffin sections were prepared, which were then stained with an anti-MPG-1 antibody: (**A**) M cell region of Peyer's patch at 4 h after administration.; (**B**) parafollicular region of Peyer's patch at 8 h after administration; (**C**) MLN at 24 h after administration (arrow shows reticulum cell region, and the arrowhead shows a germinal center); (**D**) spleen at 24 h after administration (arrows show red pulp, and the arrowhead shows a white pulp). Bar = 100 μm. All experiments were repeated at least twice, and representative immunohistochemical profiles are shown.

Tissue Distribution of MPG-1 after Oral Administration of CM6271. Furthermore, whether MPG-1 contacted with the intestinal immune system while maintaining its bioactive structures after oral administration of *T. matsutake* CM6271 was investigated by an immunostaining method. Mice were orally administered CM6271 at 450 mg/kg and sacrificed at set time points. Histological specimens were prepared by a standard procedure and stained immunologically using the anti-MPG-1 antibody. MPG-1 localization was detected in the Microfold cell (M cell) region of Peyer's patches at 4 h after administration and, 8 h afterward, also in the parafollicular cell region. MPG-1 was detected in Peyer's patches up to 32 h and disappeared after 72 h. In addition, at 8−32 h after administration, MPG-1 reached both reticulum cell regions and macrophages of the germinal center in the MLN with mild enlargement of the lymphatic sinus and mild hyperplasia of reticulum cells. MPG-1 was also distributed in reticulum cells of splenic red pulp and macrophages of splenic white pulp at 24 h after administration, although it was not observed in periarteriolar lymphoid sheath (PALS), marginal zone, or corona (**Figure 3**). Conversely, MPG-1 was hardly accumulated in the liver. Summary data are presented in **Table 2**. These results suggest that MPG-1 remains a bioactive structure in contact with immune tissues.

Acceleration of NK Cell Activity and IL-12 p70 Production in MLN Cells and Spleen Cells of CM6271-Administered Mice. MLN cells and spleen cells were separated from mice administered CM6271 orally for 10 consecutive days at 450 mg/kg/day, and the NK cell activity was measured by co-incubation with YAC-1 cells for 4 h in vitro. Moreover, these NK cells were cultured in the presence of anti-CD40 mAb and rm IFN-γ, and each culture supernatant was collected and examined for IL-12 p70 by ELISA. As a result, in both cells, NK cell activity and IL-12 p70 product in the CM6271-administered group were significantly higher than in the control group (**Figure 4**). These results suggest that NK cell activity and IL-12 p70 production were accelerated in tissue cells in which MPG-1 was localized.

Table 2. Distribution of MPG-1 in Immune Tissues after Oral Administration of CM6271[a]

	distribution of MPG-1					
tissue	0 h	4 h	8 h	24 h	32 h	72 h
mucosal layer	−	+	+	+	+	−
Peyer's patches	−	+	+	+	+	−
mesenteric lymph nodes	−	−	+	+	+	−
spleen	−	−	−	+	−	−
liver	−	−	−	−	−	−

[a] Tissues of mice orally administered *T. matsutake* CM6271 at 450 mg/kg were investigated as to whether MPG-1 contacted with the intestinal immune system as described under Materials and Methods: +, detectable; −, undetectable.

DISCUSSION

Surveying the literature on the body distribution of polysaccharides after oral administration, it has been reported that the distribution is affected by various factors, such as molecular weight, particle size, fine structure, charge, association, and susceptibility to enzymatic hydrolysis (*9–12*). Rice et al. (*10*) showed that when rats were orally administered three kinds of fluorescently labeled soluble polysaccharides with different properties, such as molecular weight and polydiversity, the blood kinetics of these polysaccharides differed. The bioavailabilities of seaweed-derived laminarin and fungus-derived scleroglucan, which are neutral β-(1→3)(1→6)-D-glucans, were 4.9 and 4.0%, respectively. β-(1→3)-D-Glucan phosphate, which is a polyelectrolyte, showed a bioavailability of 0.5%. Furthermore, it has been reported that water-insoluble particulate glucan was not detected in blood following oral administration (*10*). Triplex β-D-glucan is difficult to absorb, as it forms an aggregate through intermolecular hydrogen bonds and becomes a macromolecule.

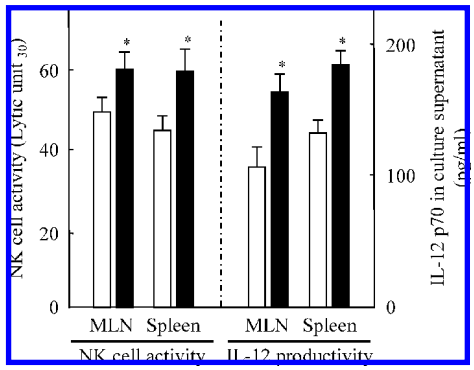

Figure 4. NK cell activity and IL-12 production in immune tissues. MLN and spleens after the oral administration of *T. matsutake* CM6271 were removed, and NK cell activity was determined by the 51Cr release assay using YAC-1 as a target cell. White bars represent the control group, and the black bars represent the CM6271-administered group. Each bar represents the mean of six mice, and the vertical bars indicate SD. An asterisk (∗) indicates statistical significance at $P < 0.05$ (vs control group).

As a result, it was considered that its bioavailability was negligible (*11*). On the other hand, α-D-glucan of dextran sulfate and dextran (MW about 8000) was taken up into endothelial cells at the early stage after oral administration, so its plasma levels were negligible (*11, 12*). Therefore, the findings of the pharmacokinetic analysis of MGP-1, which differ from the above glucans in terms of its properties, are novel results.

Protein or microorganism transport systems in the digestive tract are mainly based on the intracellular transcytosis pathway across M cells of Peyer's patches, tight junctions, and intestinal epithelial cells (*20, 21*). This active transport vesicular system consists of endosome formation and fusion and recycling of membrane vesicles. For this mechanism to operate, G proteins and the polarized cytoskeleton of intestinal epithelial cells are needed. In this study, the MPG-1 structure was detected in the mucosal layer and the M cell region of Peyer's patches at 4 h after administration, and 4 h later it was observed also in the parafollicular cell region. At 16 h, migration to MLN and the spleen was also identified, and it was thought that CM6271 orally administered was digested in the gastrointestinal tract, MPG-1 (and its degradation products) reached the intestinal immune system, and parts of these were taken into the blood and reached the spleen and so on. The level of plasma MPG-1 at 16−32 h after administration was 31−161 ng/mL and peaked after 24 h, but then declined to an undetectable level at 48 h. The MPG-1 level in the blood after 24 h depended on the amount of CM6271 administered, which peaked at an oral dose of 450 mg/kg and plateaued at a 600 mg/kg dose (**Figure 2**). This is the first study to analyze the pharmacokinetics of α-D-glucans. In a previous study it was reported that when ^{14}C-labeled CM6271 prepared by biosynthesis was orally administered to healthy rats, radioactivity was rapidly absorbed from the intestinal tract into the blood and became distributed throughout almost the entire body, including the liver and intestinal tissue, and about 90% was excreted from the body in 168 h (*22*). Whereas the PK parameters of CM6271 at that time were $t_{max} = 2.3$ h, $C_{max} = 53.5$ ng/mL, and $AUC_{(0-\infty)} = 1760.0$ μg·h/mL, the PK parameters of MPG-1 in this study were $t_{max} = 24$ h, $C_{max} = 161.1$ ng/mL, and $AUC_{(0-\infty)} = 2559.7$ ng·h/mL; the two studies clearly gave different results. Moreover, we analyzed radioactivity in the plasma from ^{14}C-CM6271 orally administered rats by gel filtration chromatography with a Superose 12 column. At 1 h after administration, a low molecular weight substance was detected, and at 8 h, a high molecular weight substance was also detected; radioactivity peaked at 16−24 h but disappeared at 168 h (data not shown). In this study, MPG-1 was detected specifically by an anti-MPG-1 antibody. On the other hand, in the previous study, which administered ^{14}C-CM6271, radioactivity (^{14}C) was detected, so this finding is not specific to MPG-1, and other polysaccharides, proteins, etc., were detected simultaneously. The high molecular weight substance that was absorbed into the blood at 16−24 h after oral administration of ^{14}C-CM6271 may be MPG-1.

The mechanism of the effect of β-D-glucan after oral administration is characterized by modulating the host immune system via contact with macrophages and dendritic cells present in the intestinal mucosa, and β-D-glucan is barely absorbed into the bloodstream. Hong et al. (*9*) proposed that fluorescently labeled β-(1→3)-D-glucan of yeast origin is taken up by intestinal macrophages that transported it to the spleen, lymph nodes, and bone marrow. Macrophages degrade the large glucan into smaller soluble active fragments that are taken up by marginated granulocytes. As a result, these granulocytes are activated and show tumor growth inhibition. In addition, Rice et al. (*10*) reported that soluble β-(1→3)-D-glucan phosphate showed anti-infective activity by elevating the systemic level of IL-12 after it bound to certain immune cells and was internalized. In contrast, the present study shows that MPG-1 transported by the intestinal tract circulates throughout the body in the bloodstream, contacts immunocytes in the MLN and spleen, and stimulates the host immune system.

Mechanism-based studies of bioactive α-D-glucans have rarely been reported as compared to β-D-glucans. Cell-surface receptors of β-D-glucans, such as C-type lectin-like receptor, dectin-1, and toll-like receptor have been studied extensively, but those of α-D-glucans have not yet been elucidated (*23*). The affinity of fluorescently labeled MPG-1 to MLN cells was unaffected by β-D-glucan pretreatment, and even if MPG-1 was used to pretreat MLN cells, the affinity between these cells and fluorescently labeled β-D-glucan was not affected. This result shows that both glucans bind to a different cell-surface receptor. Moreover, MPG-1 has high affinity for immune cells, particularly in dendritic cells (DC) positive for CD11c or CD11b-positive macrophages (Hoshi et al., unpublished results). Here, it was demonstrated that MPG-1 reaches immunocytes, directly or indirectly affects interferon or interleukin production, and enhances antitumor or anti-infectious activities by an action mechanism different from that of β-D-glucan. Additional elucidation of the mechanism at the molecular level is now in progress.

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