学位論文

Immunomodulatory effects of D-allose on cytokine production by plasmacytoid dendritic cells

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Immunomodulatory effects of D-allose on cytokine production by plasmacytoid dendritic cells

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ABSTRACT

D-Allose is classified as a 'rare sugar,' i.e., part of the group of monosaccharides that are present in low quantities in the natural world. D-Allose has been demonstrated to exert many physiological functions. The effects of the rare sugars on immune responses are largely unexplored. Here, we investigated the physiological effects of D-allose on murine dendritic cells' cytokine production. When plasmacytoid dendritic cells (pDCs) were stimulated with a Toll-like receptor 7 (TLR7) ligand, a single-stranded RNA (ssRNA), or a TLR9 ligand, CpG DNA, in the medium containing D-allose, the productions of both interferon-alpha (IFN-α) and interleukin (IL)-12p40 were severely decreased. In contrast, a normal production of these cytokines was observed when pDCs were stimulated with other TLR7 ligands, an imidazoquinoline, or a guanosine analog. In contrast to the pDCs, conventional dendritic cells (cDCs) produced IL-12p40 and tumor necrosis factor-alpha (TNF-α) in response to an imidazoquinoline or CpG DNA even though D-allose was present in the medium. D-Allose did not induce pDC death, and not inhibit the endocytic uptake of fluorophore-labeled CpG DNA into pDCs. These results suggested that D-allose exerts its inhibitory effects after CpG DNA is internalized. We analyzed the TLR7/9 signal-induced activation of downstream signaling molecules in pDCs and observed that when pDCs were stimulated with a ssRNA or CpG DNA, the phosphorylation status of the MAPK family, which includes Erk1/2, JNK/SAPK, and p38 MAPK, was attenuated in the presence of D-allose compared to D-glucose controls. The stimulation of pDCs with an imidazoquinoline induced a strong phosphorylation of these MAPK family members even in the presence of D-allose. These findings reveal that D-allose can inhibit the cytokine production by pDCs stimulated with ssRNA or CpG DNA via an attenuation of the phosphorylation of MAPK family members.

1. Introduction

D-Allose, a C-3 epimer of D-glucose, is one of the monosaccharides, and it is classified in the group of 'rare sugars' that consists of monosaccharides that are present in low quantities in the natural world (Suppl. Fig. S1) [1,2]. It was reported that D-allose has an inhibitory effect on the production of reactive oxygen species (ROS) from neutrophils [4], and that D-allose suppresses the development of salt-induced hypertension [5], protects against inflammatory and oxidative ischemia/reperfusion (I/R) in a few organs [6], enhances the antitumor effects of chemoradiotherapy while sparing normal tissues [7], and ameliorates hepatic inflammation [8].

Dendritic cells (DCs) are activated by sensing the pathogen-associated molecules with pattern recognition receptors including Toll-like receptors (TLRs), and this activation gives rise to the production of various cytokines and/or antigen presentation. DCs play important roles in linking innate immunity and acquired immunity.

Abbreviations: BCG, Bacillus Calmette-Guerin; BM, bone marrow; cDC, conventional dendritic cell; D-All, D-allose; DC, dendritic cell; D-Glc, D-glucose; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; IFN, interferon; IL, interleukin; pDC, plasmacytoid DC; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ssRNA, single-stranded RNA; TLR, Toll-like receptor; TNF, tumor necrosis factor.

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They are classified into two subsets: plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs). pDCs are capable of producing cytokines including interleukin (IL)-12 and high levels of type I interferon (IFN) through the activation of TLR7 or TLR9 signaling [9–11].

The effects of the rare sugars on immune responses of the DCs are poorly understood. It has been reported that D-allose decreases the endocytosis of Bacillus Calmette-Guerin (BCG)-*anti*-BCG IgG immune complexes into cDCs [12]. We conducted the present study to investigate the effects of rare sugars on cytokine production by DCs.

2. Materials and methods

2.1. Mice

C57BL/6 N mice (8–10-week-old males) were purchased from CLEA Japan (Tokyo). This study was approved by the Animal Care and Use Committee of Kagawa University and was carried out according to the Animal Experiment guidelines of Kagawa University.

2.2. Reagents

RPMI 1640 medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 μM 2-mercaptoethanol, 50 units/ml penicillin (Meiji Seika Pharma, Tokyo), and 50 μg/ml streptomycin sulfate (Meiji Seika Pharma) was used for the cell cultures. RPMI 1640 medium (no D-glucose) (Nacalai Tesque) was supplemented with the above-described reagents and the test monosaccharide, and used for cell stimulation. The following reagents were used for cell stimulation: polyuridylic acid (polyU) (Sigma-Aldrich, St. Louis, MO, USA), R848 (Enzo Life Sciences, New York, NY), Gardiquimod (InvivoGen, San Diego, CA), Loxoribine (InvivoGen), and ODN1668 and D19 (Eurofins Genomics, Ebersberg, Germany).

Cy5-D19, which was labeled at 5′-end of D19 with a Cy5, was purchased from Integrated DNA Technologies (Coraville, IA). Human Flt3 ligand (Flt3L) was purchased from Thermo Fisher Scientific (Waltham, MA). Crystalline forms of D-glucose and D-allose (100% pure monosaccharides) were supplied by the Rare Sugar Research Center, Kagawa University, Kagawa, Japan [1,3].

2.3. Flow cytometric analysis

Cells were incubated with *anti*-mouse CD16/32 antibody (2.4G2) to block Fc receptors. The following monoclonal antibodies (mAbs) were used for staining: FITC-*anti*-mouse CD11c antibody (N418; Biolegend, San Diego, CA), PE-CD45R (B220) antibody (RA3-6B2; Biolegend), APC-*anti*-mouse CD317 antibody (BST2, PDCA-1, 927; Biolegend), and Alexa Fluor 647 *anti*-mouse Siglec-H antibody (551; Biolegend). Cell proportions were analyzed with a FACSCalibur flow cytometer (BD Biosciences, Mountain View, CA) and FlowJo software (BD Biosciences).

2.4. Preparation of DCs

For the isolation of Flt3L-induced pDCs and cDCs, we collected bone marrow (BM) cells from the femur and tibiae of male C57BL/6 N mice and incubated the BM cells in the culture medium supplemented with 100 ng/ml human Flt3L. On day 8, loosely adherent cells were harvested and used as Flt3L-induced bone marrow dendritic cells (Flt3L-BMDCs). The Flt3L-BMDCs were first incubated with *anti*-CD45R (B220) microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). $CD45R^+$ cells and $CD45R^-$ cells were then sorted using the MACS system (Miltenyi Biotec) and used as Flt3L-pDCs and Flt3L-cDCs, respectively. Flt3L-pDCs ($CD11c^+CD45R^+CD317^+$ cells) and Flt3L-cDCs ($CD11c^+CD45R^-CD317^-$ cells) were ≥98% and ≥95% pure, respectively.

For BM-pDC enrichment, BM cells were collected as described above and incubated with *anti*-mouse CD16/32 antibody. The BM cells were then stained with APC-*anti*-mouse CD317 antibody. $CD317^+$ cells were labeled with MojoSort™ mouse *anti*-APC nanobeads (Biolegend) and purified with the MACS system. Enriched cells contained >40% $CD11c^+CD45R^+CD317^+$ cells.

For spleen pDC enrichment, murine spleen cells were incubated with *anti*-mouse CD16/32 antibody. Spleen pDCs were stained with APC-*anti*-mouse CD317 antibody and then labeled with MojoSort™ mouse *anti*-APC nanobeads and purified with the MACS system. Enriched cells contained >40% $CD11c^+CD45R^+CD317^+$ cells. For spleen cDC enrichment, $CD317^-$ cells obtained from the above pDC purification step were incubated with Pan DC microbeads (Miltenyi Biotec). Spleen cDCs were purified with the MACS system. The enriched cells contained >80% $CD11c^+CD45R^-CD317^-$ cells.

2.5. Measurement of cytokine production

Cells were plated on 96-well flat-bottomed plates at 1×10^5 cells/well/100 μl in RPMI 1640 medium (no D-glucose) supplemented with 0.2% (w/v) D-glucose or D-allose. The RPMI 1640 medium, which was used for *in vitro* DC culture, was supplemented with 0.2% (w/v) D-glucose by the manufacturer. In order to avoid changes in osmotic pressure between two culture media used for cell culture and cell stimulation, the concentration of monosaccharide added to the medium was fitted to 0.2% (w/v). The cells were then stimulated with the TLR7/9 ligands for 24 h. PolyU was complexed with Lipofectamine® 2000 reagent (Thermo Fisher Scientific) and used. DCs were stimulated at the concentrations of TLR ligands that most strongly induced cytokine production by DCs.

Culture supernatants were collected and analyzed for cytokine production. Cytokine concentrations in the supernatants were measured with an enzyme-linked immunosorbent assay (ELISA). ELISA kits for mouse IL-12p40 and mouse tumor necrosis factor-alpha (TNF-α) were purchased from Biolegend, and the ELISA kit for mouse IFN-α was obtained from InvivoGen.

2.6. CpG DNA uptake assays

Flt3L-pDCs were incubated with 1.0 μM Cy5-D19 for 30 min at 37 °C or on ice and extensively washed with ice-cold fluorescence-activated cell sorting (FACS) staining buffer before analysis on a flow cytometer. For the detection of internalized Cy5-D19, the cell surface-bound Cy5-D19 signal was quenched by adding 0.1% (w/v) trypan blue to samples just before the flow cytometric measurement [13,14].

2.7. Western blotting

Flt3L-pDCs were stimulated with TLR7/9 ligands for 0, 30, or 60 min in the presence of 0.2% (w/v) D-glucose or D-allose. The cells were harvested and lysed in lysis buffer (50 mM Tris-HCl pH8.0, 150 mM NaCl, 1.0% (v/v) NP-40, 10 mM NaF, 10 mM β-glycerophosphate, and 1 mM Na_3VO_4) containing protease inhibitor cocktail (Nacalai Tesque). Cell lysates were centrifuged at 15,000 rpm for 15 min at 4 °C, and the supernatants was collected. Equal amounts of protein in the lysates were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto a polyvinylidene difluoride (PVDF) membrane. The activations of ERK, JNK, p38, and NF-κBp65 were evaluated by an immune blot analysis.

The following primary antibodies were obtained from Cell Signaling Technology (Beverly, MA): phospho-p44/42 MAPK (Erk1/

2) (Thr202/Tyr204) (D13.14.4E) XP rabbit mAb (#4370), p44/42 MAPK (Erk1/2) (137F5) rabbit mAb (#4695), phospho-SAPK/JNK (Thr183/Tyr185) (81E11) rabbit mAb (#4668), SAPK/JNK polyclonal antibody (#9252), phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP rabbit mAb (#4511), p38 MAPK (D13E1) XP rabbit mAb (#8690), phospho-NF-κB p65 (Ser536) (93H1) rabbit mAb (#3011), and NF-κB p65 (D14E12) XP rabbit mAb (#8242). *Anti*-rabbit horseradish peroxidase-conjugated secondary antibodies (Cytiva, Tokyo) was used to detect primary antibodies. Protein bands were visualized using Clarity Western ECL Substrate (Bio-Rad, Hercules, CA).

3. Results

3.1. Effect of D-allose on cytokine production by Flt3L-pDCs or cDCs

We first evaluated the effect of D-allose on the cytokine production by Flt3L-BMDCs. Productions of both IFN-α and IL-12p40 by Flt3L-BMDCs stimulated with TLR7 ligand (single-stranded RNA [ssRNA]; polyU) or TLR9 ligands (CpG DNA; D19 or ODN1668) was decreased in a D-allose dose-dependent manner (Suppl. Figs. S2 and S3). Flt3L-BMDCs consist of two major subsets, pDCs and cDCs. When Flt3L-BMDCs are stimulated with TLR7/9 ligand, IFN-α is produced solely by pDCs, but IL-12p40 is produced by both pDCs and cDCs. For this reason, we next analyzed the effect of D-allose on the production of cytokines by TLR7/9 signal-stimulated Flt3L-pDCs and -cDCs separately. When Flt3L-pDCs were stimulated with polyU or CpG DNA (D19 or ODN1668) in the medium containing D-allose, the productions of both IFN-α and IL-12p40 were severely decreased (Fig. 1A). In contrast, when two different types of TLR7 ligands, i.e., imidazoquinolines (R848 or Gardiquimod) or a guanosine analog (Loxoribine), were used to stimulate Flt3L-pDCs, both IFN-α and IL-12p40 were produced in

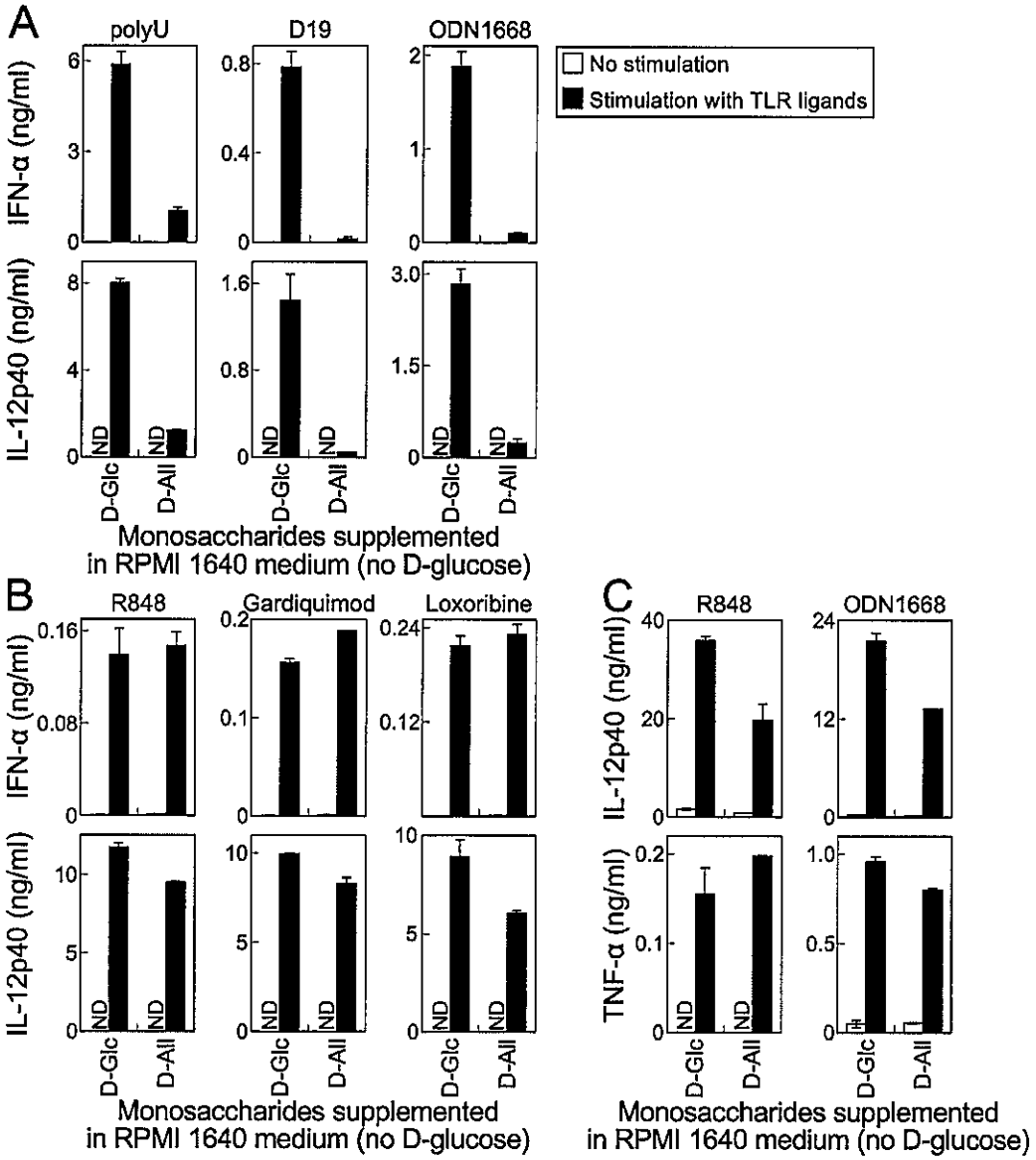

Fig. 1. The effect of D-allose on the production of cytokines by Flt3L-induced pDCs or cDCs. All DCs were cultured in media containing D-glucose (D-Glc) or D-allose (D-All). **(A)** Flt3L-pDCs were stimulated with 2.5 μg/ml polyU, 3.0 μM D19, or 0.03 μM ODN1668. **(B)** Flt3L-pDCs were stimulated with 100 nM R848, 3.0 μM Gardiquimod, or 1.0 mM Loxoribine. **(C)** Flt3L-cDCs were stimulated with 100 nM R848 or 1.0 μM ODN1668. The concentrations of IFN-α, IL-12p40, and TNF-α in the culture supernatants obtained after 24 h of stimulation were measured by ELISA. Data are representative of three independent experiments (mean ± SD). ND: below the detectable limit.

the medium containing D-allose, as was observed in the medium containing D-glucose (Fig. 1B). When Flt3L-cDCs were stimulated with R848 or ODN1668 in the medium containing D-allose, although the IL-12p40 production was slightly decreased, the TNF-α production was comparable to that of the D-glucose-containing medium (Fig. 1C). These results indicated that D-allose has an ability to inhibit cytokine production by only Flt3L-pDCs stimulated with polyU or CpG DNA. Other monosaccharides (including D-allulose, L-allulose, and D-fructose) did not inhibit TLR7/9 signal-induced cytokine production by Flt3L-pDCs or cDCs. We analyzed the viability of Flt3L-BMDCs stimulated with polyU by flow cytometry (Suppl. Fig. S4). The viability of unstimulated Flt3L-BMDCs was slightly decreased in the medium containing D-allose as compared with D-glucose control. In contrast, the viability of polyU-stimulated Flt3L-BMDCs was not markedly decreased in the medium containing D-allose. The percentages of Flt3L-pDCs ($CD11c^+$ $Siglec\text{-}H^+$ cells) stimulated with polyU were also comparable between the media containing D-allose and D-glucose.

3.2. Effects of D-allose on cytokine production by DCs purified from BM or spleen

We also analyzed the TLR7/9 signal-induced cytokine production by pDCs and/or cDCs isolated from mouse BM or spleen. The stimulation of BM-pDCs or spleen pDCs with polyU, D19, or ODN1668 in the medium containing D-allose resulted in severely decreased productions of IFN-α and IL-12p40 (Fig. 2A and B). In contrast, R848-stimulated BM-pDCs and R848-stimulated spleen pDCs produced almost the same amounts of IFN-α and IL-12 in D-allose-containing medium as the amounts observed in D-glucose-containing medium. Spleen cDCs stimulated with R848 or ODN1668 produced almost equal amounts of IL-12p40 even in the presence of D-allose (Fig. 2C). These results were consistent with

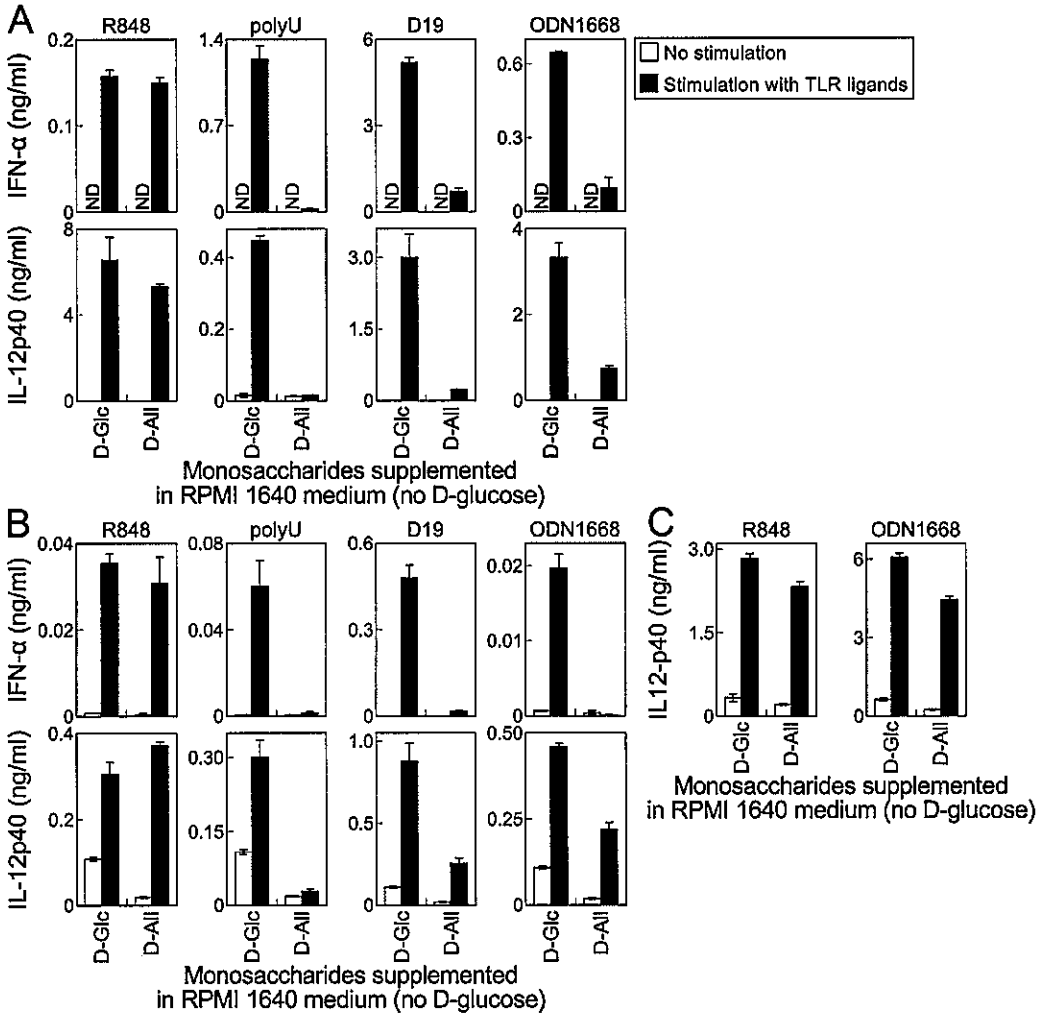

Fig. 2. The effect of D-allose on the production of cytokines by DCs purified from mouse bone marrow (BM) or spleen. MACS-purified BM-pDCs **(A)** or spleen pDCs **(B)** were stimulated with 100 nM R848, 2.5 μg/ml polyU, 3.0 μM D19, and 0.03 μM ODN1668. **(C)** MACS-purified spleen cDCs were stimulated with 100 nM R848 or 1.0 μM ODN1668. The concentrations of IFN-α and IL-12p40 in the culture supernatants obtained after 24 h of stimulation were measured by ELISA. Data are representative of three independent experiments (mean ± SD). ND: below the detectable limit.

our observations obtained using Flt3L-pDCs and -cDCs. We thus decided to use Flt3L-pDCs for the subsequent analyses.

3.3. Uptake of CpG DNA into Flt3L-pDCs or -cDCs

We investigated whether the incorporation of CpG DNA into Flt3L-pDCs was inhibited in the medium containing D-allose. We synthesized fluorophore-labeled D19 and investigated whether it could be used as a tracer for D19. In the medium containing D-glucose, Flt3L-pDCs stimulated with Cy5-D19 produced the same levels of IFN-α and IL-12p40 as those observed when Flt3L-pDCs were stimulated with unlabeled D19 (Fig. 3A). In addition, the amounts of these cytokines produced by Cy5-D19-stimulated Flt3L-pDCs were markedly decreased in the medium containing D-allose (Fig. 3A). We thus decided to use Cy5-labeled D19 as a tracer for visualizing CpG DNA.

Flt3L-pDCs were incubated with Cy5-D19 at 37 °C or on ice. These pDCs were then analyzed by flow cytometry for the incorporation of Cy5-D19. The frequency of pDCs incorporating Cy5-D19 was not decreased in the medium containing D-allose compared with that in the medium containing D-glucose (Fig. 3B). Similar results were observed when cDCs were analyzed for the incorporation of Cy5-D19. These data indicate that D-allose does not inhibit the endocytic uptake of CpG DNA into Flt3L-pDCs, and they suggest that D-allose exerts its inhibitory effects after CpG DNA is internalized.

3.4. The effect of D-allose on the phosphorylation of intracellular signaling molecules in Flt3L-pDCs stimulated with TLR7/9 ligands

We next investigated whether the activation of intracellular signaling molecules could be inhibited in the presence of D-allose. We conducted Western blot analyses to evaluate the phosphorylation status of Erk1/2, SAPK/JNK, p38 MAPK, and NF-κB p65 in Flt3L-pDCs stimulated with TLR7/9 ligands. The stimulation of Flt3L-pDCs with D19 induced the phosphorylation of Erk1/2, SAPK/JNK, p38 MAPK, and NF-κB p65 in the medium containing D-glucose. However, when D19 stimulation was carried out in the presence of D-allose, the band intensities indicating the phosphorylation status of Erk1/2, SAPK/JNK, and p38 MAPK became weaker compared to those of the D-glucose control (Fig. 4).

In contrast, the phosphorylation status of NF-κB p65 was not

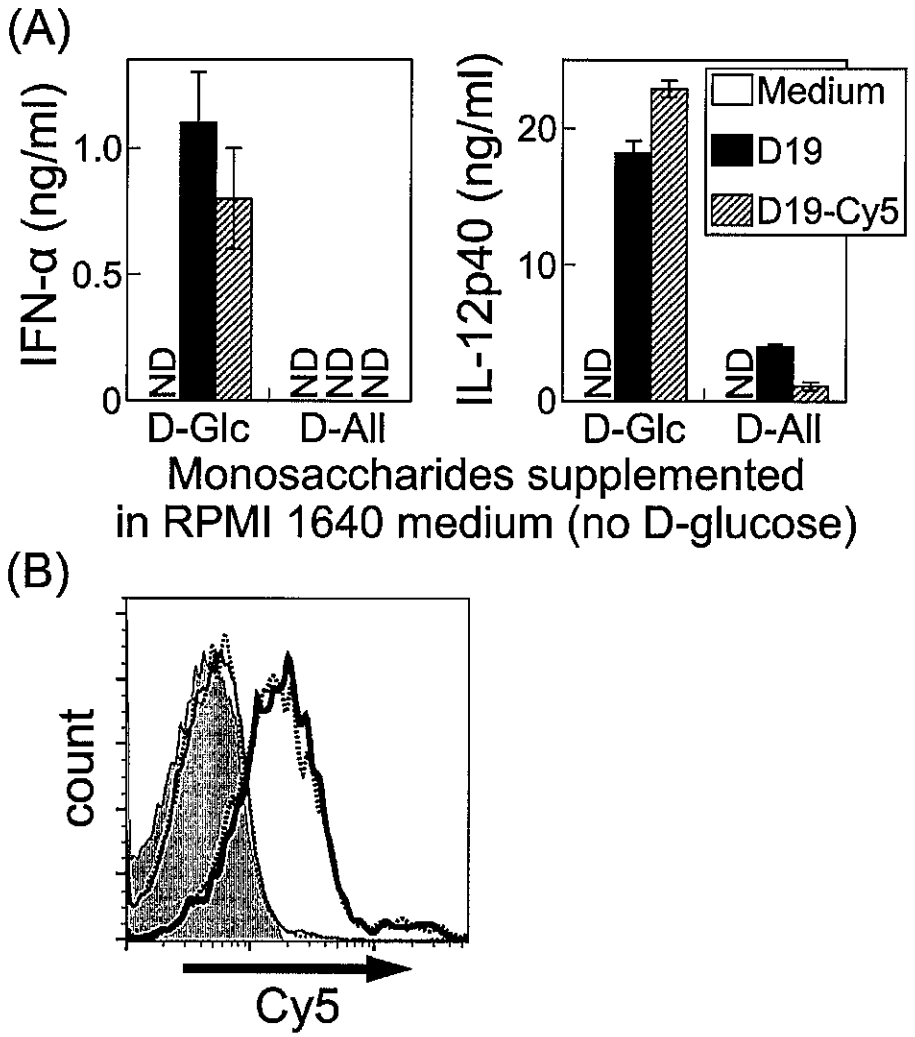

Fig. 3. Incorporation of Cy5-labeld CpG DNA into Flt3L-pDCs. (A) Flt3L-pDCs were stimulated with 3.0 μM Cy5-labeled D19 or control D19 for 24 h. The concentrations of IFN-α and IL-12p40 were measured by ELISA. Data are representative of three independent experiments (mean ± SD). ND: below the detectable limit. **(B)** Flt3L-pDCs were incubated with 1.0 μM Cy5-D19 for 30 min at 37 °C or on ice, followed by flow cytometric analyses. *Filled histograms:* Negative control cells without incubation with Cy5-D19. *Open histograms with thick lines:* The cells incubated for 30 min at 37 °C in medium containing D-allose (*solid lines*) or D-glucose (*dotted lines*). *Open histograms with thin lines:* The cells incubated for 30 min on ice in medium containing D-allose (*solid lines*) or D-glucose (*dotted lines*). Representative profiles of three independent experiments are shown.

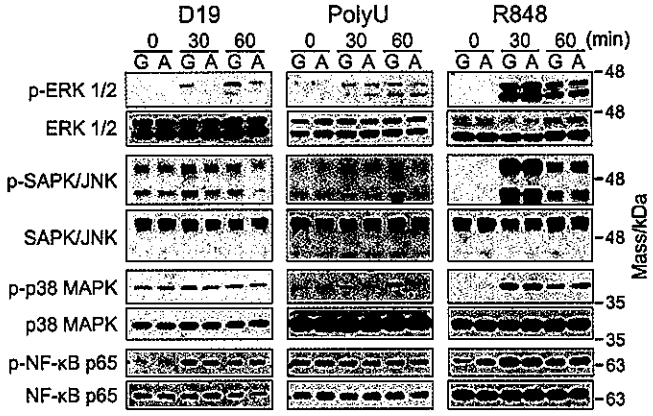

Fig. 4. Western blot analysis of the signaling molecules in Flt3L-pDCs stimulated with TLR ligands. Flt3L-pDCs were incubated in medium containing D-glucose (G) or D-allose (A). Subsequently, Flt3L-pDCs were stimulated with 3.0 μM D19, 2.5 μg/ml polyU, or 100 nM R848 for the indicated times and analyzed for the phosphorylation levels of Erk1/2, JNK/SAPK, p38 and NF-kB p65. Data are representative of at least three independent experiments.

attenuated in the presence of D-allose. When polyU was used to stimulate pDCs, the results were similar to those observed when D19 was used for stimulation. In contrast, when pDCs were stimulated with R848—which induced the phosphorylation of these signaling molecules most strongly among the TLR7/9 ligands we used (Suppl. Fig. S5)—there were no differences in the phosphorylation status of Erk1/2, p38 MAPK, SAPK/JNK, or NF-κB p65 between the media containing D-allose and D-glucose (Fig. 4).

4. Discussion

We investigated the effects of D-allose on cytokine production by DCs, and our results demonstrated that D-allose had a selective inhibitory effect on cytokine production by pDCs. This inhibitory effect was observed only when pDCs were stimulated with polyU or CpG DNA. In contrast, the stimulation of pDCs with synthetic TLR7 ligands such as imidazoquinolines and a guanosine analog did not result in decreased cytokine production. We thus speculate that these inhibitory effects of D-allose to pDCs are not due to a suppression of cellular glucose metabolism, nor due to induction of cell death by D-allose.

Because the endocytic uptake of CpG DNA into Flt3L-pDCs was observed in the presence of D-allose, the inhibitory effect of D-allose was not attributed to an inhibition of the endocytic activity of DCs. We suspect that the internalization of polyU into pDCs is not decreased by D-allose, either. In contrast, the internalization of BCG-*anti*-BCG IgG immune complexes into cDCs has been reported to be inhibited in the presence of D-allose [12]. Our present findings showed that there was a difference in reactivity to D-allose between the Fc receptor-mediated phagocytosis of antigens and the endocytosis of CpG DNA in cDCs. The mechanisms underlying this difference is unknown and beyond the scope of this study.

In order to analyze the molecular mechanism of the inhibitory effect of D-allose on cytokine production by pDCs, we evaluated the phosphorylation status of intracellular signaling molecules in pDCs. The results demonstrated that when pDCs were stimulated with polyU or CpG DNA, the phosphorylation status of members of the MAPK family was moderately attenuated in the presence of D-allose compared to the D-glucose controls. This moderate inhibition of MAPK signaling was linked to a marked decrease in cytokine production by pDCs. In contrast, the R848-induced activation of MAPK signaling was not inhibited in the presence of D-allose. These results indicate that the activation of MAPK signaling is important for polyU or CpG DNA-induced cytokine production by pDCs. The R848-signaling most strongly induced phosphorylation of the signaling molecules as compared to polyU or CpG DNA-signaling. Further studies are necessary to clarify why R848-signaling strongly induces phosphorylation of the signaling molecules, and how cytokine production by R848-stimulated pDCs is not inhibited in the presence of D-allose.

It has been reported that the CpG DNA-induced production of IFN-α and TNF-α by human pDCs is suppressed by p38 MAPK inhibitors [15,16]. Our present study also demonstrated the importance of MAPK signaling in cytokine production by pDCs. In contrast to MAPK activation, we observed that the polyU or CpG DNA-induced phosphorylation of NF-κB p65 was not inhibited by the presence of D-allose. The transcription factor NF-κB is required for the induction of the proinflammatory cytokines including IL-12 in pDCs [17,18]. Our analyses revealed that in the presence of D-allose, IL-12 production was substantially decreased, even though the phosphorylation of NF-κB p65 was induced by stimulation with polyU or CpG DNA. This outcome suggested that the phosphorylation of NF-κB p65 was not sufficient for IL-12 production by pDCs stimulated with polyU or CpG DNA. The transcription factor IRF-7 plays an essential role in IFN-α production by pDCs [17]. We are unable to present data regarding the phosphorylation status of IRF-7 because there were no antibodies recognizing phosphorylated IRF-7.

The stimulation of pDCs with CpG DNA or R848 is reported to induce not only cytokine production but also ROS production. The TLR7/9 ligand-induced productions of both IFN-α and ROS are inhibited by a pretreatment of pDCs with N-acetylcysteine, which is an ROS scavenger [19]. It has been reported that D-allose inhibits the production of ROS by rat neutrophils and mouse neuroblastoma Neuro2A cells [20,21]. We suspect that the ROS-scavenging activity of D-allose cannot explain the reason why D-allose inhibits cytokine production by pDCs but not by cDCs, or why D-allose exerts two contradictory effects on cytokine production by pDCs when stimulated with two different TLR7 ligands, R848 or polyU.

It has also been reported that supplementation of a high-salt diet with D-allose reduces the phosphorylation levels of Erk1/2 in the renal cortex of Dahl salt-sensitive hypertensive rats [5]. Although the detailed molecular mechanism remains unknown, the decrease in the Erk1/2 phosphorylation level in the renal cortex is considered to be due to the antioxidant activity of D-allose [5]. Further studies are necessary to clarify the molecular mechanisms by which D-allose exerts its inhibitory effect on MAPK phosphorylation in pDCs.

Our present findings demonstrate that D-allose has a unique immunomodulatory property, i.e., the inhibition of cytokine production by pDCs stimulated with ssRNA or CpG DNA. Type I IFN production by pDCs is involved not only in antiviral immunity but also in the pathogenesis of autoimmune diseases. Our findings contribute to a further understanding of the biological activity of D-allose and may lead to the development of new approaches to the treatment of autoimmune disorders or pathological conditions in which cytokine production by pDCs are critically involved.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2022.08.037.

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