Involvement of CD14 in the inhibitory effects of dimethyl-α-cyclodextrin on lipopolysaccharide signaling in macrophages

Keiichi Motoyama a, Hidetoshi Arima a, Yoji Nishimoto a, Kensuke Miyake b, Fumitoshi Hirayama a, Kaneto Uekama a,∗

a Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862-0973, Japan
b Division of Infectious Genetics, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Tokyo 108-8639, Japan

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Abstract The potential use of α-cyclodextrin and its hydrophobic α-cyclodextrin derivatives (α-CyDs) as antagonists against lipopolysaccharide (LPS), which stimulates the nitric oxide (NO) and tumor necrosis factor-α (TNF-α) production as well as nuclear factor-kB (NF-kB) activation in macrophages was examined. Of three α-CyDs used in the present study, 2,6-di-O-methyl-α-CyD (DM-α-CyD) had greater inhibitory activity than did the other CyDs against NO and TNF-α production through an impairment of gene expression in macrophage cell lines and primary macrophages stimulated with LPS and lipid A in a concentration-dependent manner. Concomitantly, DM-α-CyD inhibited NF-κB translocation into nucleus. These inhibitory effects of DM-α-CyD could be attributed to the release of CD14 from lipid rafts caused by an efflux of phospholipids, but not cholesterol. These results suggest that DM-α-CyD may have promise as a potent and unique antagonist for excess activation of macrophages stimulated with LPS.

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1. Introduction

Lipopolysaccharide (LPS) is an integral component of the outer membrane of gram-negative bacterial, consisting of a hydrophilic polysaccharide and a highly conserved hydrophobic lipid A portion [1]. LPS is conserved microbial molecules (pathogen-associated molecular patterns), which can stimulate both innate and adaptive immune responses, particularly on macrophages and dendritic cells. Actually, serum LPS is carried in the body by a specific carrier protein, LPS-binding protein (LBP), and exposes cells of the innate immune system through a distinct receptor, CD14 [2]. CD14 is a 55-kDa glycosphosphatidylinositol (GPI)-anchored protein expressed on monocytes, macrophages and neutrophils [3,4]. The LPS-CD14 complex then engages a specific receptor, the Toll-like receptor 4 (TLR4) and MD-2 complex (TLR4/MD-2 complex), to afford a variety of biological responses, i.e., macrophages can be elicited to produce proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1, interleukin-6, and nitric oxide (NO), as well as to express cell surface antigens such as MHC class II, CD80 and CD86 ([5,6]). These functions are important for establishing innate immunity against the microorganism. However, excess amounts of LPS engender a septic shock and life-threatening organ dysfunction as described above [7].

Cyclodextrins (CyDs) are host molecules, which form inclusion complexes with lipophilic drugs as guests and thus have been utilized for improving their water solubility and dissolution rates [8,9]. CyDs have also been reported to interact with membrane constituents such as cholesterol, phospholipids and phosphatidylinositol, depending on their cavity sizes, resulting in not only the induction of hemolysis of erythrocytes [10,11] but also the disruption of the structures of lipid rafts [12–14], lipid microdomains formed by lateral assemblies of cholesterol and sphingolipids in the cell membrane [15]. Recently, Cuscheri et al. reported that cholesterol depletion with methyl-β-CyD (M-β-CyD) in THP-1 cells is associated with a significant attenuation of LPS-mediated mitogen-activated protein kinases activation [16]. We recently reported that 2,6-di-O-methyl-α-CyD (DM-α-CyD) inhibited NO production in RAW264.7 cells, a mouse macrophage cell line, stimulated with LPS from Escherichia coli (serotype O111:B4) [17]. In the following study, we examined whether DM-α-CyD inhibits NO and TNF-α production as well as NF-κB activation in various mouse macrophage cell lines and primary peritoneal exudate cells (PECs) stimulated with LPS from E. coli (serotype O111:B4 and O55:B5) and lipid A. In addition, the involvement of CD14 and TLR4/MD-2 complex in the inhibitory mechanism of DM-α-CyD was investigated.

2. Materials and methods

2.1. Materials

CyDs used in this study are depicted in Table 1. α-CyD, 2-hydroxypropyl-α-CyD (HP-α-CyD) and DM-α-CyD were donated from Nihon Shokuhin Kako (Tokyo, Japan). LPS from E. coli (serotype O111:B4...
and O55:B5), fluorescein isothiocyanate (FITC)-conjugated LPS (O111:B4), lipid A and M-β-CyD were purchased from Sigma (St. Louis, MO). RPMI-1640 culture medium and fetal calf serum (FCS) were obtained from Nissui Pharmaceutical (Tokyo, Japan) and JRH Biosciences (Renexa, KS), respectively. CpG phosphorothioate oligonucleotide (CpG-ODN, 5′-TCCATGACGTTCCTGACGTT-3′) was obtained from Hokkaido System Science (Sapporo, Japan). Deoxyribonuclease I and ribonuclease inhibitor were purchased from Nippon Gene (Toyama, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. Reverse transcriptase (SuperScript II) and Taq polymerase (Gene) and Nippon Gene (Toyama, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. Reverse transcriptase (SuperScript II) and Taq polymerase (Applied Biosystems, Tokyo, Japan), respectively. Thioglycollate was obtained from Difco Laboratories (Detroit, MI). All other chemicals and solvents were of analytical reagent grade.

2.2. Cell culture

RAW264.7, J774.1 and P5U-18 cells (Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan), murine macrophage-like cell lines and PEC isolated from C3H/HeN mice (4–5 weeks old, Nihon SLC, Shizuoka, Japan) stimulated with 3% thioglycollate were used for this assay. In addition, low-density lipid raft-enriched and HRP-goat anti-rat IgG Ab (Amersham Bioscience, Tokyo, Japan) and HRP-goat-anti-rat IgG Ab (Amersham Bioscience, Tokyo, Japan) were used for this assay. In addition, low-density lipid raft-enriched domains were isolated by a carbonate-based fractionation method as described previously [21].

2.3. Cell viability and nitrite determination

Cell viability and nitrite production were assayed using a Cell Counting Kit (WST-1 method) [18,19] from Wako Pure Chemical Industries (Osaka, Japan) and using Griess reagent [20], respectively, as reported previously [17].

2.4. Western blot and enzyme-linked immunosorbent assay (ELISA)

Inducible nitric oxide (iNOS) in the RAW264.7 cells and TNF-α in cell-free supernatant were detected by Western blot and ELISA, respectively. Briefly, RAW264.7 cells (3 × 10⁶ cells/dish) were incubated with 10 mM LPS for 4 h with or without α-CyDs, then washed with culture medium and incubated for 4 h in culture medium. Then, the cells were scraped and lysed. After determining protein concentrations, samples (20 μg as protein) were separated with 7.5% SDS–PAGE and transferred onto Immobilon P membranes (Nihon Millipore, Tokyo, Japan). The membranes were blocked and incubated with rabbit anti-mouse iNOS antibody (Affinity Bioreagent, Neshanic Station, NJ) or rabbit anti-goat actin antibody (Santa Cruz Biotechnology, CA) for 2 h. After washing, the membranes were incubated with peroxidase-conjugated goat anti-rabbit IgG antibody (Cappel, Durham, NC) or peroxidase-conjugated anti-rabbit IgG antibody (Amersham Biosciences, Tokyo, Japan). Specific bands were detected using an ECL Western blot analysis kit (Amersham Biosience, Tokyo, Japan). The level of TNF-α in cell-free supernatants was routinely assayed by a sandwich ELISA using pairs of purified capture and detection monoclonal antibodies recognizing murine TNF-α (Pharmingen, San Diego, CA), according to the manufacturer’s protocols.

2.5. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

The levels of iNOS mRNA and TNF-α mRNA in cells were assayed by semiquantitative RT-PCR method as reported previously [17]. Total RNA was isolated from RAW264.7 cells (3 × 10⁶ cells/dish) following the manufacturer’s instructions. cDNA was synthesized using a reverse primer and SuperScript II. The primer sequences were as follows: mouse β-actin: forward, 5′-TTGGGATAGGTTCCTTTACCGGA-3′; reverse, 5′-CACCCAACCCCTCCTACAGG-3′; mouse TNF-α forward, 5′CTCTGGTATGAGATGACAGAAACGCA-3′; and reverse, 5′-CAAGGGAATGAGAATCCCTAAA-3′; iNOS forward, 5′-ACA-GGGAAGCTTGAACACTAG-3′; reverse, 5′-CATGCAAGGAA-GGGAACTCCTC-3′.

2.6. NF-κB activation

RAW264.7 cells (2 × 10⁶ cells/dish) were stimulated with LPS (1000 ng/ml) with or without α-CyDs for 15 min. After LPS treatment, the cells were fixed in methanol, incubated with mouse anti-NF-κB p65 Ab for 1 h. Primary antibody was detected with FITC-conjugated goat anti-mouse IgG2a. Samples were viewed and photographed by using a confocal fluorescence microscopic system (Olympus FV300-BX, Tokyo) with an argon ion laser (excitation wavelength, 488 nm).

2.7. Flow cytometry

RAW264.7 cells (2 × 10⁶ cells/dish) were incubated with or without 10 mM α-CyDs at 4°C for 30 min. The cells were washed with HBBS and incubated with FITC-conjugated anti-CD14 or anti-TLR4/MD-2 complex antibody (MTS510) at 4°C for 30 min. After washing, the cells were incubated with PBS twice and incubated with FITC-conjugate anti-IgG2a Ab (Pharmingen, San Diego, CA) and HRP-goat anti-mouse IgG Ab (Amersham Bioscience, Tokyo, Japan) were used for this assay. In addition, low-density lipid raft-enriched domains were isolated by a carbonate-based fractionation method as described previously [21].

2.8. Release of CD14 from lipid rafts

RAW264.7 cells (3 × 10⁶ cells/dish) were incubated with α-CyDs at the designated concentration at 37°C for 1 h. The CD14 released from the cells into the cell supernatant was concentrated with 10% trichloroacetic acid and was then assayed by Western blot as described above. Rat anti-mouse CD14 monoclonal Ab (Pharmingen, San Diego, CA) and HRP-goat-anti-rat IgG Ab (Amersham Bioscience, Tokyo, Japan) were used for this assay. In addition, low-density lipid raft-enriched domains were isolated by a carbonate-based fractionation method as described previously [21].

2.9. Released cholesterol and phospholipids determination

RAW264.7 cells (2 × 10⁶ cells/dish) were incubated for 10 h in growth medium supplemented with 10% FCS containing [3H]cholesterol (5 μCi/ml of serum). Prior to experiments, the cells were incubated in HBBS containing various concentrations of α-CyDs and M-β-CyD. The total amount of [3H]cholesterol in the cells was detected by the same method after lysed in 1 N NaOH. The concentrations of [3H]cholesterol in HBBS were determined with an Aloka LSC-3500 liquid scintillation counter. The concentrations of phospholipids released from the cells in the buffer were determined using a Phospholipids Kit Wako® (Wako Pure Chemical Industries, Osaka, Japan).

2.10. Statistical analysis

Data are given as means ± S.E.M. Statistical significance of means for the studies was determined by analysis of variance followed by Scheffe’s test. P-values for significance were set at 0.05.
3. Results

3.1. Cytotoxicity of CyDs

We examined the effects of α-CyDs on the viability of macrophages using the WST-1 method. In the range of low concentrations, none of the α-CyDs showed cytotoxicity for RAW264.7 cells (Fig. 1A), but in the range of high concentrations the viability was decreased by adding α-CyDs and LD_{50} values lowered in the order of HP-α-CyD > α-CyD > DM-α-CyD (Fig. 1B). In addition, similar cytotoxic effects of α-CyDs were observed on other mouse macrophages such as PU5-18, J774.1 and PEC (data not shown). These results indicate that of α-CyDs used here DM-α-CyD interacts to the greatest extent with these macrophages.

3.2. DM-α-CyD suppressed NO and TNF-α production in macrophages stimulated with LPS

To reveal whether DM-α-CyD inhibits NO and TNF-α production in various mouse macrophages stimulated with LPS, we assayed nitrite and TNF-α levels in the cell supernatant 24 h after stimulation with LPS by the Griess method [20] and ELISA, respectively. DM-α-CyD had a superior

![Fig. 1. Cytotoxicity of α-CyDs in RAW264.7 cells. RAW264.7 cells (1 × 10^5) were incubated for 1 h with 150 μl of RPMI-1640 culture medium supplemented with 10% FCS containing CyDs at the designated concentrations. The cell viability was assayed using a Cell Counting Kit. The concentrations of α-CyDs were up to 10 mM (A) and 100 mM (B), respectively. Each point represents means ± S.E.M. of three experiments.

![Fig. 2. DM-α-CyD inhibits NO production in RAW264.7 cells and PEC stimulated with LPS (O111:B4), LPS (O55:B5) or lipid A, but not with CpG-ODN. Macrophages (1 × 10^5) were incubated for 1 h with 150 μl of RPMI-1640 culture medium supplemented with 10% FCS containing LPS, lipid A or CpG-ODN with or without α-CyDs. After washes with PBS to remove their ligands and CyDs, the cells were incubated for 24 h. The nitrite level in the culture supernatant was assayed by the Griess method. (A, B) The concentration of LPS and lipid A was 1000 ng/ml, and that of α-CyDs was 10 mM, respectively. (C) The concentrations of LPS were 0 ng/ml (closed circle), 1 ng/ml (open circle), 10 ng/ml (closed triangle), 100 ng/ml (open triangle) and 1000 ng/ml (closed square), respectively. Each value represents means ± S.E.M. of five experiments. *P < 0.05, compared to LPS alone. (D) The concentrations of CpG-ODN were 5 and 10 μg/ml and that of α-CyDs was 10 mM, respectively. Each value represents means ± S.E.M. of five experiments.]}
inhibitory effect on NO production in RAW264.7 cells as well as PEC stimulated with LPS (serotype O111:B4), LPS (serotype O55:B5) or lipid A, to α-CyD and HP-α-CyD (Fig. 2A and B). Almost the same inhibitory effect of DM-α-CyD was elicited in PU5-18 and J74.1 cells (data not shown). In addition, the inhibitory effect of DM-α-CyD was concentration-dependent when stimulated with LPS (serotype O111:B4) at the various concentrations in RAW264.7 cells (Fig. 2C). These results suggest that DM-α-CyD provides an inhibitory effect on NO and TNF-α production not only in mouse macrophage-like cell lines but also in primary macrophages with stimulated with two types of LPSs and lipid A. Furthermore, α-CyDs did not allow to change NO production in RAW264.7 cells stimulated with CpG-ODN, a TLR9 ligand (Fig. 2 D). It is possible that the inhibitory effects of α-CyDs are due to the interaction with the lipid A moiety of LPS and/or cell surface, because α-CyDs fail to enter cells.

Next, we examined whether DM-α-CyD suppresses iNOS and TNF-α expression. In subsequent experiments, we used LPS (serotype O111:B4) as a stimulant. As shown in Fig. 3, an iNOS band at 130 kDa was observed in extracts of cells stimulated with LPS, whereas the treatment with 5 mM DM-α-CyD attenuated iNOS expression. However, in the case of other α-CyDs treatments, the band densities were almost the same as that of the control (Fig. 3A). In addition, the three α-CyDs used here did not alter the control actin band densities (Fig. 3A). Moreover, the inhibitory effects of α-CyDs on iNOS expression, but not actin expression, were concentration-dependent (Fig. 3B). Fig. 3C shows the iNOS mRNA levels determined by RT-PCR of total RNA in RAW264.7 cells. DM-α-CyD caused a substantial reduction in the density of the 1033 bp band derived from iNOS mRNA but not in that of the 630-bp band of control β-actin mRNA (Fig. 3C). Likewise, the band densities after treatment with DM-α-CyD lowered in a concentration-dependent manner (Fig. 3D). Therefore, these results suggest that DM-α-CyD suppresses iNOS gene expression in RAW264.7 cells stimulated with LPS. Similarly, DM-α-CyD inhibited TNF-α production in RAW264.7 cells, but other α-CyDs did not (Fig. 4A). In addition, the TNF-α mRNA levels were lowered by the addition of DM-α-CyD (Fig. 4B), whereas the suppressive effect was concentration-dependent (Fig. 4C). Thus, DM-α-CyD is likely to attenuate iNOS and TNF-α expression at a pre-transcriptional level.

3.3. DM-α-CyD blocked NF-κB translocation into nucleus

LPS signaling mediated by myeloid differentiation factor 88 requires NF-κB activation and involves the production of inflammatory cytokines such as TNF-α, interleukin-1, interleukin-6 and NO. Then, we investigated whether DM-α-CyD prevents NF-κB activation when stimulated with LPS. In the absence of LPS, NF-κB was observed in cytoplasm (Fig. 5A). Upon LPS stimulation, NF-κB obviously translocated to nucleus (Fig. 5B). The addition of DM-α-CyD significantly inhibited NF-κB translocation (Fig. 5C). Therefore, the inhibitory effects of DM-α-CyD on NO and TNF-α production may result from the inhibitory effects on NF-κB activation.

3.4. DM-α-CyD released CD14 from lipid rafts

To gain insight into the inhibitory mechanism of DM-α-CyD for the LPS signaling, we examined the CD14 and TLR4/MD-2 complex expressions on the surface of
RAW264.7 cells by a flow cytometry (Fig. 6). The treatment with DM-α-CyD shifted the curve corresponding to CD14 to the left-hand side, while treatment with the other α-CyDs did not (Fig. 6A). Additionally, the shifts in the DM-α-CyD system were dose-dependent (Fig. 6B). While none of the α-CyDs shifted the curve corresponding to TLR4/MD-2 complex on the RAW264.7 cells (Fig. 6C). Thus, the suppressive effects of DM-α-CyD on LPS signaling may result from impairment of CD14 expression on the plasma membranes.

It was recently reported that CD14 and TLR4/MD-2 complex reside in lipid rafts [22–24]. We reported that DM-β-CyD released P-glycoprotein and MRP2 from caveolae, flask-shaped lipid microdomains, of vinblastine-resistant Caco-2 cells [25]. Therefore, we examined whether α-CyDs release CD14 and TLR4/MD-2 complex from the lipid rafts.

Consistent with the finding reported by Pfeiffer et al. [23], CD14 resided in lipid raft fractions (Fig. 7A) irrespective of LPS, whereas the treatment of RAW264.7 cells with DM-α-CyD markedly lowered the CD14 band density (Fig. 7A). To determine the effect of DM-α-CyD on CD14 expression in and outside of lipid raft fractions, samples were loaded in each lane after adjusting the protein content. Determining the band density using the NIH image, the treatment with DM-α-CyD decreased CD14 levels in rafts to 27.2% relative to the control (Fig. 7B). These results suggest that DM-α-CyD attenuates the CD14 level in lipid rafts. Indeed, the band corresponding to CD14 was clearly observed in culture medium after treatment with DM-α-CyD compared with that with α-CyD and α-CyDs.
HP-α-CyD (Fig. 7C). In addition, the effect of DM-α-CyD to release CD14 in the medium was in a concentration-dependent manner (Fig. 7D). However, DM-α-CyD neither released TLR4/MD-2 complex from the cells nor translocated outside of lipid rafts (data not shown). Taken together, it is evident that DM-α-CyD uniquely extracts CD14, but not TLR4/MD-2 complex, from lipid rafts into culture medium.

3.5. DM-α-CyD released phospholipids rather than cholesterol from cells

CyDs are known to extract membrane lipids such as cholesterol and phospholipids from liposomes and cellular membranes [26]. To address the question of how DM-α-CyD releases CD14 from lipid rafts of RAW264.7 cells, we investigated the effects of α-CyDs and M-β-CyD, a novel cholesterol depletion agent, on the release of membrane components. As expected, M-β-CyD released cholesterol, but other α-CyDs did not (Fig. 8A). To the contrary, DM-α-CyD markedly released phospholipids from the cells, compared with other α-CyDs (Fig. 8B). Taken together, these results suggest that membrane perturbation elicited by DM-α-CyD through extraction of phospholipids leads to CD14 release from lipid rafts.

4. Discussion

This is the first report showing that of three α-CyDs, DM-α-CyD inhibits LPS signaling in murine macrophage cell lines and primary PEC stimulated with two types of LPS and lipid A, likely due to the release of CD14 from the lipid rafts of these cells to supernatants.

HP-α-CyD and DM-α-CyD have higher solubility in water than α-CyD, and they are surface active [27]. Besides, DM-α-CyD has hemolytic activity higher than α-CyD and HP-α-CyD, suggesting the superior interaction with erythrocytes to HP-α-CyD and α-CyD. Thus, we hypothesized that of these α-CyDs, DM-α-CyD markedly suppresses LPS signaling through the interaction with LPS and/or cell membranes. As predicted, DM-α-CyD had the most potent inhibitory effects on the LPS signaling in macrophages without cytotoxicity, compared with other α-CyDs used here (Figs. 1–5).

The ability of DM-α-CyD to release CD14 from lipid rafts may result in LPS signaling in macrophages. In our previous study, DM-α-CyD indeed ameliorated cellular binding of LPS to RAW264.7 cell [16], and CyDs are generally known to be impermeable through lipid bilayers owing to their hydrophilic properties with high molecular weight [27]. Additionally, CD14 was extracted from cell membranes to a supernatant in response to DM-α-CyD (Figs. 6 and 7). Besides, CD14 was found to localize in lipid rafts as mentioned by Miyake [28] and was released from the microdomains to supernatant by treatment with DM-α-CyD in a concentration-dependent manner (Fig. 7). Taken together, it is likely that CD14 released by DM-α-CyD from lipid rafts is involved in the impairment of LPS signaling.

DM-α-CyD is likely to release CD14 through indirect interaction with proteins because CyDs are known to interact with proteins to a lesser extent and prefers the inclusion complexation with phospholipids to cholesterol [27]. Our results also showed that DM-α-CyD released CD14 from lipid rafts (Fig. 7), irrespective of the very slight interaction with cholesterol.

An addition of free phosphatidylcholine, not cholesterol, to DM-α-CyDs produced buffer completely inhibited the release of CD14 into the culture medium (data not shown). Interestingly, M-β-CyD neither released CD14 nor blocked NF-κB activation under the present experimental conditions (data not shown). These results indicate that the effects of DM-α-CyD on LPS signaling in macrophages are totally different from that of M-β-CyD. Furthermore, CD14 release induced by DM-α-CyD seems to be exerted by the secondary effect of lipid extraction from the membranes.

However, DM-α-CyD and other α-CyDs failed to release TLR4/MD-2 complex from the cell membranes (Fig. 6), partially consistent with the results reported by Cuschi et al. [16,29]. This fact might be related to the difference in membrane topology between CD14 and TLR4/MD-2 because CD14 and TLR4/MD-2 complex are a GPI-anchored protein.
and transmembrane protein, respectively. Studies are currently underway to investigate whether DM-α-CyD specifically releases GPI-anchored proteins from lipid rafts.

Recently, there have been some interesting reports that (1) LPS is predominantly taken up via CD14-mediated pathways, not TLR4 pathway [30], (2) LPS is rapidly delivered from the plasma membrane to an intracellular site [31], (3) LPS is internalized and then rapidly encounters intracellular TLR4, and downstream signaling is triggered [32]. These lines of evidence as well as our present results make it tempting to speculate that DM-α-CyD first extracts membrane lipids, and then releases CD14 from lipid rafts, and then LPS endocytosis and/or LPS transfer to TLR4 disappears, resulting in blocking TLR4 signaling pathway towards NF-κB activation. Another possibility may be proposed that DM-α-CyD may disturb a cluster of receptors comprising TLR4, CD11b/CD18, CD55, CD81, hsp70, hsp90, GDF5 and CXCR4 which are crucial for TLR4 signal following LPS stimulation [33]. Thus, elaborate studies are further required to decipher the detailed mechanism by which DM-α-CyD affects LPS signaling from lipid rafts.

DM-α-CyD is likely to have different properties from various LPS antagonists, e.g., polymyxin B, Eritoran (E5564), HA-1A, E5 and surfactant protein A [34–37], since the inhibitory effect of DM-α-CyD is likely to be associated with CD14. Our preliminary spectrophotometric study demonstrates the direct interaction of LPS with DM-α-CyD was observed only very slightly (data not shown). Thus, it is possible that DM-α-CyD is a highly potent and unique endotoxin antagonist.

CD14 is well known to be associated with various diseases, e.g., asthma bronchial, systemic lupus erythematosus, atopic dermatitis, chronic hepatitis B and C infection, alcoholic liver cirrhosis and rheumatoid arthritis [38]. Recently, Fassebender et al. have suggested that CD14 links innate immunity with Alzheimer’s disease (AD) [39]; CD14 interacts with amyloid peptide, and then promotes neuroinflammation in AD. Therefore, our results lead us to expect the possibility that DM-α-CyD may be useful for an inhibitor of CD14 to reduce the central and peripheral inflammation.

In conclusion, the present study suggests that DM-α-CyD impaired LPS signaling in murine macrophages stimulated with LPS and lipid A. The inhibitory effect of DM-α-CyD could be attributed to the release of CD14 from lipid rafts caused by efflux of phospholipids, but not by cholesterol. These results suggest that DM-α-CyD may be useful as a
potent antagonist for excess activation of macrophages stimulated with LPS.

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