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Cyclosporin A inhibits differentiation and activation of monocytic cells induced by 27-hydroxycholesterol

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ABSTRACT

27-Hydroxycholesterol (27OHChol) is a bioactive molecule that induces monocytic cell activation and differentiation and thereby participates in immune responses under hypercholesterolemic condition. However, it is unknown whether cyclosporin A (CsA), an immunosuppressant, affects biological effects of 27OHChol. In this study, we investigated whether CsA alters 27OHChol-induced cellular and molecular responses using the human monocyte/macrophage THP-1 cells. Treatment of the cells with CsA resulted in decreased expression of the mDC-specific markers (CD80, CD83 and CD88) induced by 27OHChol. Reduced endocytic activity recovered in the presence of CsA. The drug also inhibited the expressions of MHC class I and II molecules and CD197, a homing molecule of mDCs. We further investigated the outcomes of CsA treatment on the expression of M1 polarization markers and CD14, a component of the innate immune system. The drug decreased transcript levels of genes associated with the M1 polarization of monocytic cells, including CCL2, as well as expression of CD14 and MMP-9 which is involved in soluble CD14 shedding. Taken together, these results indicate that CsA inhibits the 27OHChol-induced differentiation and activation of monocytic cells into a mature dendritic cell (mDC) type and an immuno-stimulatory M1 subset, respectively, thereby modifying immune responses in a milieu rich in cholesterol and oxidized cholesterol molecules.

1. Introduction

Cyclosporin A (CsA), a lipophilic drug, is widely used as an immunosuppressant reagent in tissue transplantation [1,2]. CsA acts as an inhibitor of calcineurin, a calcium/calmodulin-dependent serine/threonine protein phosphatase. Calcineurin dephosphorylates the regulatory sites on several transcription factors, most notable being the nuclear factor of activated T-lymphocytes (NFATs). The inhibition of calcineurin by CsA prevents activation of promoters of T-cell activation, and consequently the overall immune response [3]. CsA is also involved in cell differentiation, having varied roles depending on the cell types involved. Cardiac differentiation is reported to be induced by CsA, whereas the drug inhibits the hemato-endothelial differentiation of P19 cells [4]. These reports suggest that, in addition to suppressing immune responses, CsA also affects cell differentiation.

Oxysterols, including 27-hydroxycholesterol (27OHChol), are oxidative derivatives of cholesterol involved in inflammatory responses and immune modulations [5,6]. Monocytic cells stimulated by 27OHChol get activated and produce inflammatory chemokines, which in turn induce the recruitment of immune cells, thereby amplifying inflammation [7–9]. Monocytic cells are further induced by 27OHChol to differentiate into a mature dendritic cell (mDC) type [10]. Dendritic cells (DCs) are involved in the initiation of cellular immune responses and autoimmune responses [11,12]. Activated DCs cause inflammatory immune responses, including the recruitment of T lymphocytes and the infiltration of inflammatory cells by secretion of various cytokines/chemokines [13,14]. Taken together, these findings suggest that 27OHChol alters the inflammatory and immune responses by activating and differentiating monocytic cells.

The involvement of oxysterols in immune cell function is well-documented. However, little is known regarding the drugs that regulate biological activity of oxysterols. This study was undertaken to see

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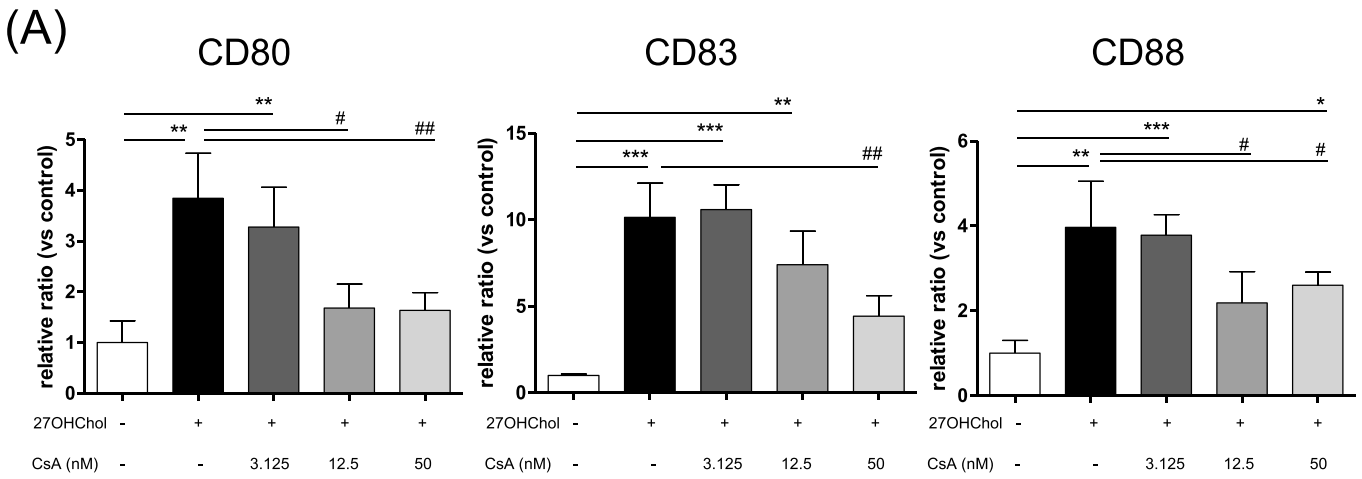

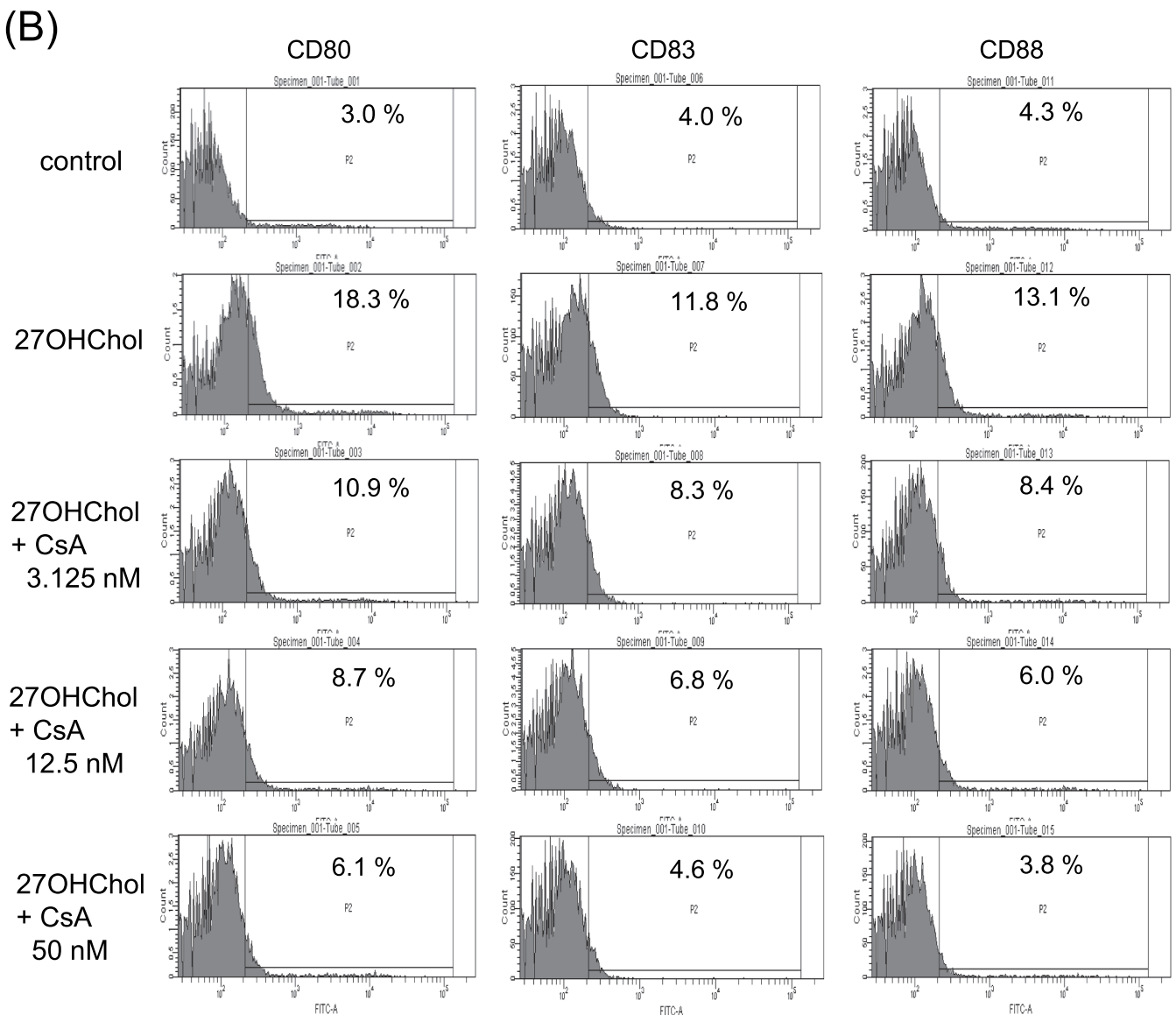

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Fig. 1. Effects of CsA on the transcription and surface expression of mDC-markers induced by 27OHChol. THP-1 cells (1×10^6 cells/60 mm culture dish) were cultured for 48 h with 2.5 μg/ml 27OHChol in the presence or absence of 3.125, 12.5 and 50 nM of CsA.
(A) The transcript levels of CD80, CD83, and CD88 genes were assessed by real-time PCR. Data are expressed as mean ± SD ($n = 3$ replicates/group). $^*P < 0.05$ vs. control; $^{**}P < 0.01$ vs. control; $^{***}P < 0.001$ vs. control; $\#P < 0.05$ vs. 27OHChol; $\#\#P < 0.01$ vs. 27OHChol.
(B) CD80, CD83, and CD88 on cell surface were immunostained by using antibodies conjugated with a fluorescent dye, and the fluorescence was analyzed by flow cytometry. Results are the representative of three independent experiments.

whether CsA can exert its effects in an environment rich in oxidized cholesterol. We report new biological activities of CsA that contribute to pharmacological action of the drug after investigation of whether CsA influences the differentiation of monocytic cells into mDCs, the polarization to immuno-stimulatory subtype, and the innate immune response that are enhanced by 27OHChol, the most abundant oxysterol in plasma and tissues.

2. Materials and methods

2.1. Cell culture and reagents

THP-1 cells purchased from the American Type Culture Collection (ATCC, Manassas, VA 20108, USA) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin. 27OHChol was purchased from Research Plus, Inc. (Bayonne, NJ, USA). CsA was purchased from ENZO Life Science, Inc. (Farmingdale, NY). Fluorescein isothiocyanate (FITC)-conjugated dextran (40 kDa) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies for CD14, CD80, CD83, CD88, and CD197 were purchased from Santa-Cruz Biotechnology (Santa Cruz, CA, USA). Alexa Fluor 488-conjugated secondary antibodies for FACS analysis were purchased from Invitrogen (Eugene, Oregon, USA).

2.2. Dextran-FITC uptake assay

The assay was performed as previously described [15]. Briefly, after incubation with indicated concentration of CsA and 27OHChol, the

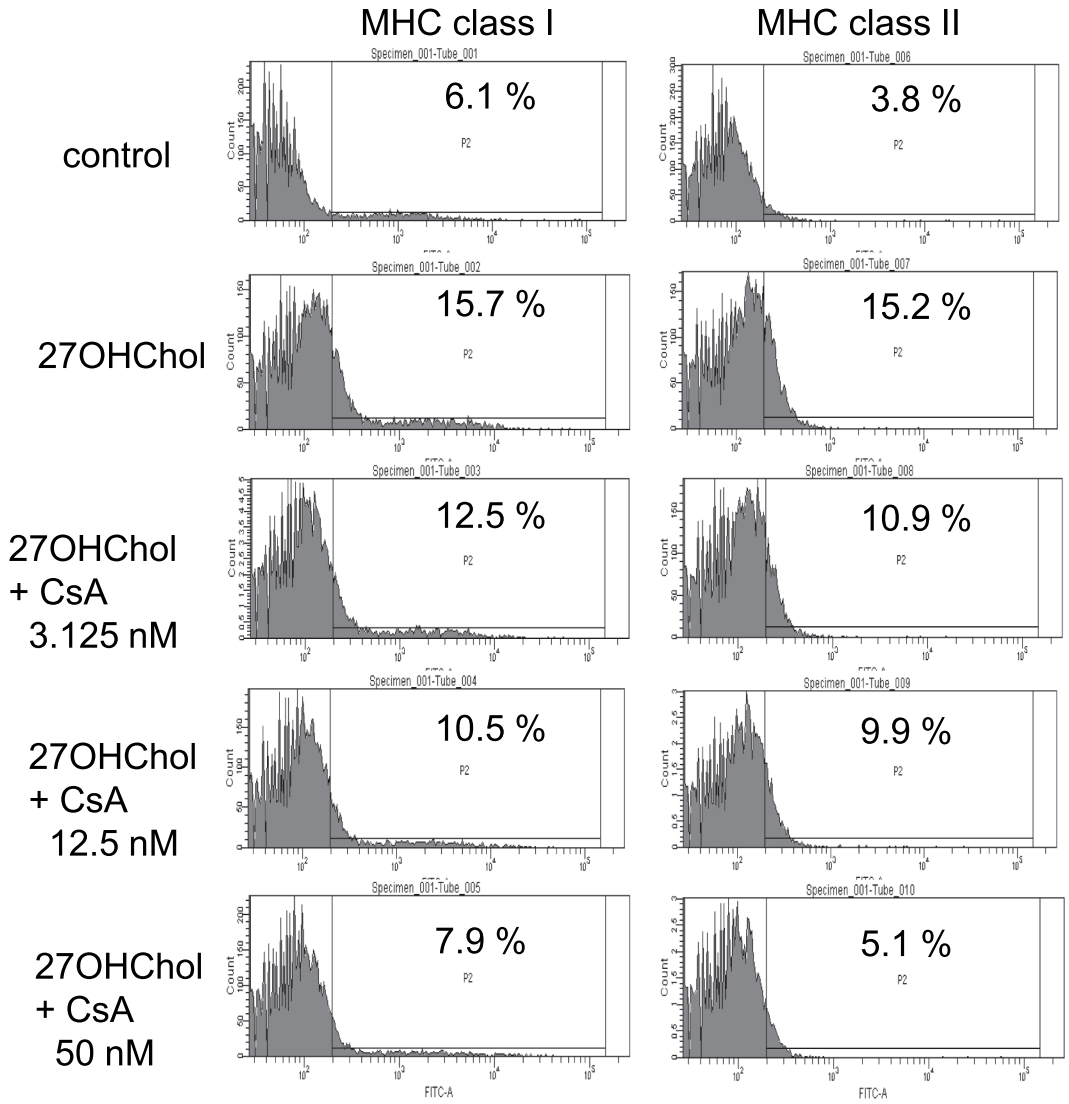

Fig. 2. Effects of CsA on expression of MHC class molecules induced by 27OHChol. MHC class I and II markers on cell surface were immunostained using antibodies conjugated with a fluorescent dye following incubation of THP-1 cells (1×10^6 cells/60 mm culture dish) for 48 h with 27OHChol (2.5 μg/ml) in the presence or absence of 3.125, 12.5 and 50 nM of CsA. Fluorescence was analyzed by flow cytometry. Results are the representative of three independent experiments.

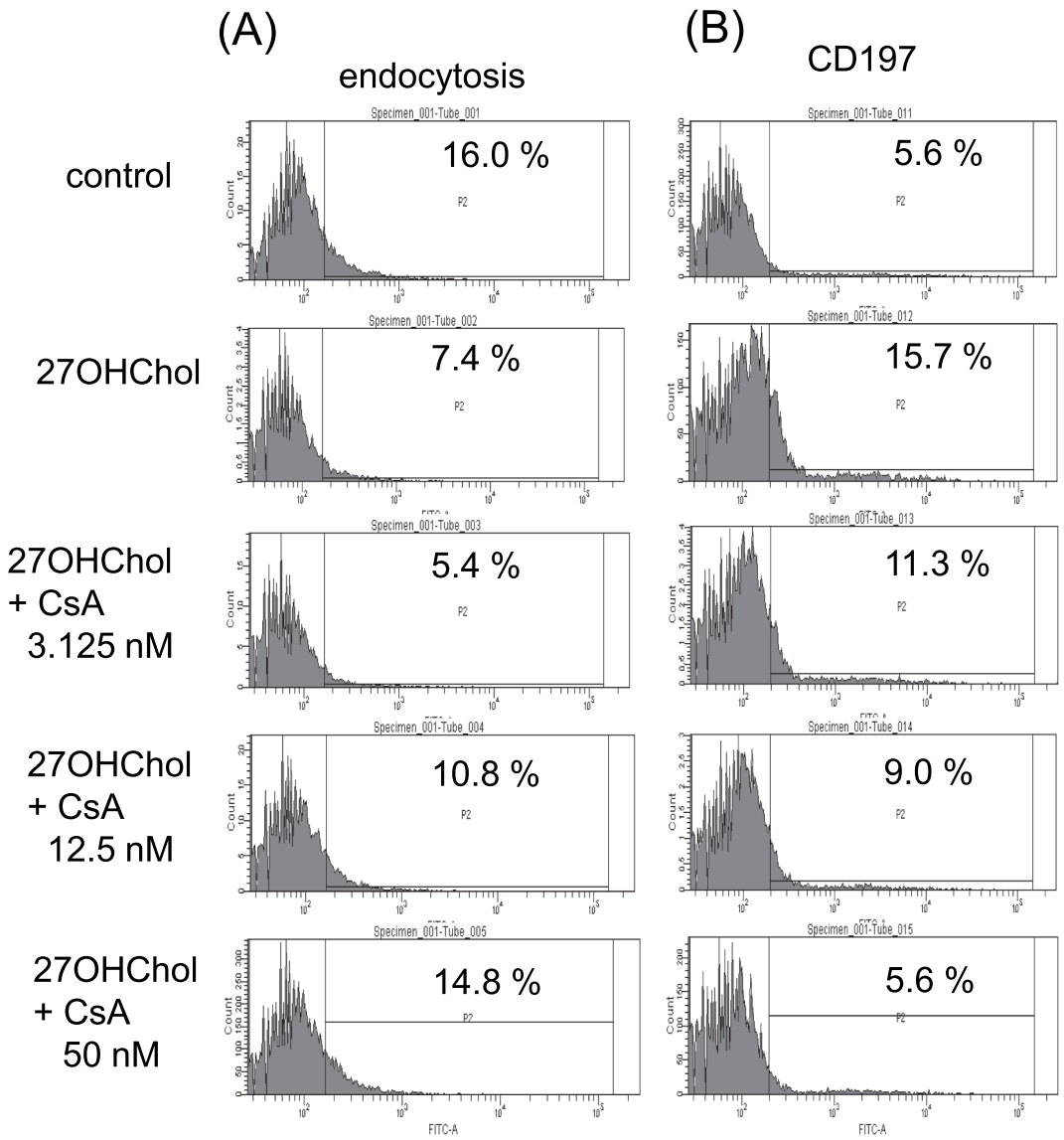

Fig. 3. Effects of CsA on the expression of CD197 and functional changes induced by 27OHChol. THP-1 cells (1×10^6 cells/60 mm culture dish) were cultured for 48 h with 2.5 μg/ml 27OHChol in the presence or absence of 3.125, 12.5 and 50 nM of CsA.
(A) After further incubation for 1 h with 1 mg/ml of FITC-conjugated dextran, cells were analyzed by flow cytometry. Results are the representative of three independent experiments. (B) CD197 on cell surface was immunostained with fluorescent dye-conjugated antibody, after which fluorescence was analyzed by flow cytometry. Results are the representative of three independent experiments.

monocytic cells were incubated in culture medium containing 1 mg/ml of FITC-conjugated dextran for 30 min at 37 °C or at 4 °C (for background control). The cells were washed with cold PBS containing 1% FBS, and fluorescence due to dextran up-take was analyzed by flow cytometry.

2.3. Quantitative real-time polymerase chain reaction

Quantitative real-time PCR was performed in triplicate, as previously described [16]. Briefly, the assay was performed in 96-well plates containing SYBR Green PCR Master Mix, and 10 pM forward primer and reverse primer for CD molecules and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The sequences of CD molecule primers were forward 5′-TGGTGCTGGCTGGTCTTTC and reverse 5′-CTGTGCCACTTCTTTCACTTCC (CD80); forward 5′-TCCTGAGCTGC GCCTACAG and reverse 5′-GCAGGGCAAGTCCACATCTT (CD83); forward 5′-GTGGTCCGGGAGGAGTACTTT and reverse 5′-GCCGTTTGTCG TGGCTGTA (CD88). Primers for GAPDH were forward 5′-ATGGGGAA GGTGAAGGTCG and reverse 5′-GGGGTCAT TGATGGCAACAATA.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The levels of chemokine CCL2, sCD14, and MMP-9 secreted from monocytic cells were determined using ELISA kits (BD Biosciences, San Diego, CA, USA) as per the manufacturer's instructions.

2.5. Flow cytometric analysis

The analysis was performed as previously described [15]. Briefly, the monocytic cells were harvested and incubated for 2 h at 4 °C with antibodies against indicated antigens, followed by washing with PBS and incubation with fluorescent dye-conjugated secondary antibodies. The cells were washed with PBS and resuspended in 1% paraformaldehyde in PBS. Flow cytometry was performed to analyze the fluorescence.

2.6. Chemotaxis assay

Migration assay was performed using the Transwell Permeable

Supports (Costar, Cambridge, MA) as per the protocol suggested in the user's manual, as described in the previous study [17].

2.7. Secreted MMP-9 gelatinolytic activity

MMP-9 activity in the culture media was measured by zymography using 8% polyacrylamide-SDS gels containing 0.2% type A gelatin, as described previously [8].

2.8. Statistical analysis

Statistical analyses were performed using one-way ANOVA, followed by Tukey's multiple comparison tests, using GraphPad PRISM (version 5.0). A P-value < 0.05 is considered to be significant.

3. Results

3.1. CsA down-regulates expression of mDC markers

To evaluate whether CsA affected 27OHChol-induced differentiation of monocytic cells, we analyzed expression levels of mDC-specific markers. Data of real-time PCR revealed that stimulation with 27OHChol caused significant increases in the transcription of mDC-specific markers (CD80, CD83, and CD88). Compared with the unstimulated control, the levels of CD80, CD83, and CD88 transcripts increased by 3.7-, 10.1-, and 3.9-folds, following stimulation with 27OHChol, respectively. But, the increases were significantly suppressed by treatment with CsA as treatment with this drug resulted in dose-dependent decreases in the transcripts levels of these markers (Fig. 1A). We further investigated the effects of CsA on the surface expression of mDC markers (Fig. 1B). In agreement with the results of real time-PCR, flow cytometric data showed increased expression of the markers following stimulation with 27OHChol. The percentages of control cells positive for CD80, CD83, and CD88 were 3.0%, 4.0%, and 4.3%, and they increased to 18.3%, 11.8%, and 13.1%, respectively, after stimulation with 27OHChol. The increases, however, were suppressed by treatment with CsA in a dose-dependent manner. These date indicated that CsA inhibits the expressions of mDC markers induced by 27OHChol.

3.2. CsA down-regulates MHC molecules

It was determined whether CsA influences expression of MHC class I/II molecules induced by 27OHChol by flow cytometric assay (Fig. 2). The percentage of control cells positive for MHC class I and class II molecules were 6.1% and 3.8%, and they significantly increased to 15.7% and 15.2%, respectively, after stimulation with 27OHChol. The increases in levels of both MHC class molecules were suppressed by treatment with CsA, which suggested that CsA down-regulates the surface levels of MHC class I and II molecules.

3.3. CsA suppresses 27OHChol-induced alteration of endocytic function and expression of homing factor in monocytic cells

To determine whether CsA affects the functional changes of monocytic cells, we analyzed the endocytic activity (Fig. 3A). The percentage of cells exhibiting endocytic activity, which was 16.0% without any treatment, was reduced to 7.4% following stimulation with 27OHChol. However, treatment with CsA resulted in recovery of endocytic activity. In addition, we examined whether CsA influences expression of CD197, which is involved in migration of cells to the secondary lymph nodes. The surface expression of CD197 increased from 5.6% to 15.7% by stimulation with 27OHChol, but the increase was dose-dependently suppressed by treatment with CsA (Fig. 3B). These results suggested that CsA recovers endocytic function and is likely to suppress homing activity of 27OHChol-stimulated cells.

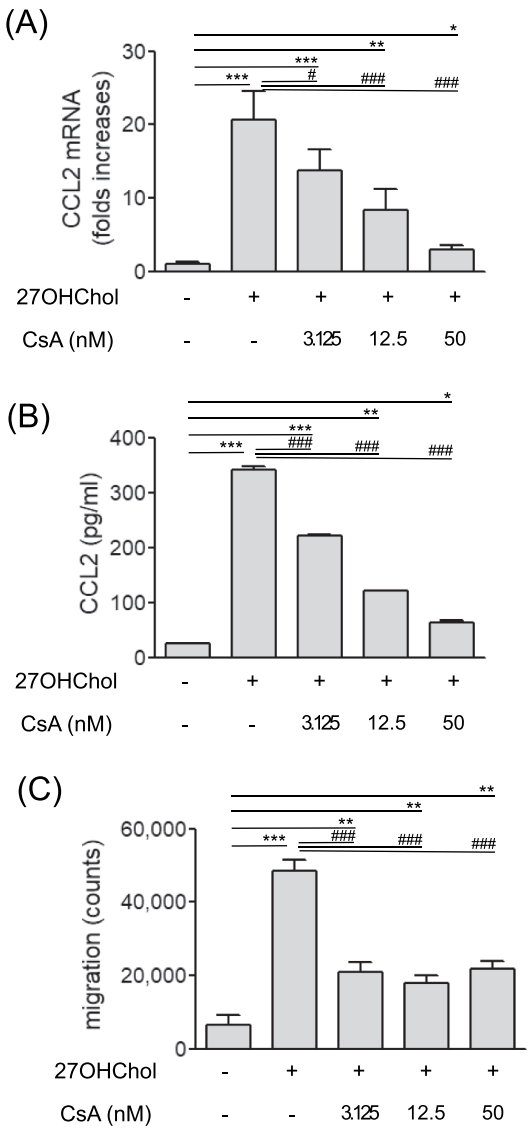

Fig. 4. Effects of CsA on the expression of CCL2 and monocytic cell migration induced by 27OHChol. THP-1 cells (1×10^6 cells/60 mm culture dish) were cultured for 48 h with 2.5 μg/ml 27OHChol in the presence or absence of 3.125, 12.5 and 50 nM of CsA.

(A) The levels of CCL2 transcripts were assessed by real-time PCR. Data are expressed as mean ± SD ($n = 3$ replicates/group). $^*P < 0.05$ vs. control; $^{**}P < 0.01$ vs. control; $^{***}P < 0.001$ vs. control; $\#P < 0.05$ vs. 27OHChol; $\#\#\#P < 0.001$ vs. 27OHChol.

(B) The amount of CCL2 protein secreted into culture media was analyzed by ELISA. Data are expressed as mean ± SD ($n = 3$ replicates/group). $^*P < 0.05$ vs. control; $^{**}P < 0.01$ vs. control; $^{***}P < 0.001$ vs. control; $\#\#\#P < 0.001$ vs. 27OHChol.

(C) Monocytic cells were exposed to conditioned media isolated from THP-1 cells treated with 2.5 μg/ml 27OHChol with or without indicated concentration of CsA. Migration of monocytic cells was determined by chemotaxis assays. Data are expressed as mean ± SD (n = 3 replicates for each group). $^{**}P < 0.01$ vs. control; $^{***}P < 0.001$ vs. control; $\#\#\#P < 0.001$ vs. 27OHChol.

3.4. CsA inhibits the production of biologically active CCL2

The inflammatory chemokine CCL2 (also known as monocyte chemoattractant protein-1; MCP-1) is a well-known product of the innate immune response [18]. To determine the effects of CsA on the oxysterol-induced inflammatory response, we analyzed expression of CCL2. Transcript levels of CCL2 gene were elevated to 20-fold after

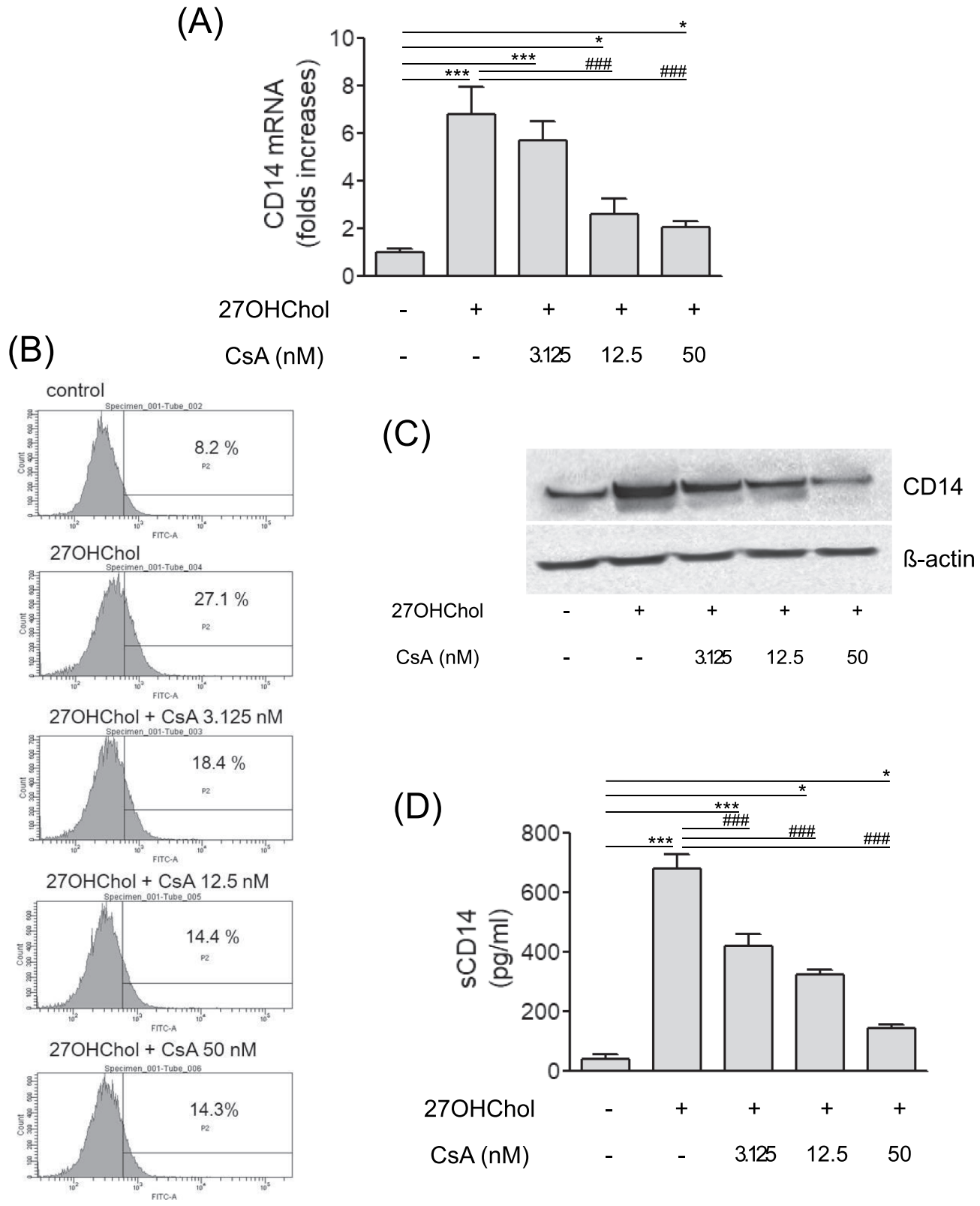

Fig. 5. Effects of CsA on the expression of CD14 induced by 27OHChol.
THP-1 cells (1×10^6 cells/60 mm culture dish) were cultured for 48 h with 2.5 μg/ml 27OHChol in the presence or absence of 3.125, 12.5 and 50 nM of CsA.
(A) The levels of CD14 transcripts were assessed by real-time PCR. Data are expressed as mean ± SD ($n = 3$ replicates/group). *$P < 0.05$ vs. control; ***$P < 0.001$ vs. control; ###$P < 0.001$ vs. 27OHChol.
(B) CD14 on cell surface was immunostained with fluorescent dye-conjugated antibody, and fluorescence was analyzed by flow cytometry. Results are the representative of three independent experiments.
(C) Whole cell extracts were isolated from the cells and subjected to immunoblotting for CD14 and β-actin.
(D) The amount of sCD14 released from the cells to culture media was measured by ELISA. Data are expressed as mean ± SD ($n = 3$ replicates/group). *$P < 0.05$ vs. control; ***$P < 0.001$ vs. control; ###$P < 0.001$ vs. 27OHChol.

stimulation with 27OHChol as compared to unstimulated cells, but the levels were dose-dependently reduced by treatment with CsA (Fig. 4A). We also observed that secretion of CCL2 protein enhanced following stimulation with 27OHChol was inhibited in a dose-dependent fashion by treatment with CsA (Fig. 4B). Furthermore, 27OHChol enhanced chemotactic cell migration, which was impaired by CsA (Fig. 4C). These results indicated that CsA effectively inhibits the expression of functional CCL2.

3.5. CsA down-regulates CD14 expression

The CD14 receptor of lipopolysaccharide (LPS) is involved in the innate immune response [19]. It was investigated whether CsA affected the expression of CD14. The transcript levels of CD14 were enhanced following stimulation with 27OHChol, but the levels were dose-dependently reduced by treatment with CsA (Fig. 5A). The CD14 protein levels on cell surface, which were elevated following stimulation with 27OHChol, were down-regulated by treatment with CsA (Fig. 5B and C). Secretion of soluble CD14 (sCD14) was inhibited in a similar fashion as that observed in membrane-bound CD14 (Fig. 5D). These results indicated that CsA down-regulates CD14.

3.6. CsA inhibits expression of MMP-9

MMP-9 plays roles in both innate and adaptive immune responses [20–22]. Therefore, we investigated the effects of CsA on 27OHChol-induced expression of MMP-9. As indicated by quantitative real-time PCR, transcription of MMP-9 increased by stimulation with 27OHChol, but the increase was dose-dependently suppressed by treatment with CsA (Fig. 6A). Secretion of MMP-9 protein was also inhibited in a similar pattern as that observed with its transcripts (Fig. 6B). The activity of MMP-9 was further analyzed by zymographic analysis. The elevated gelatin-lysis activity of the secreted MMP-9 was observed following 27OHChol stimulation, but the activity was dose-dependently reduced by treatment with CsA (Fig. 6C). These results indicated that CsA inhibits expression of MMP-9 induced by 27OHChol.

3.7. CsA affects expression of M1/M2 markers of monocytic cells

To see whether CsA affects M1/M2 polarization of monocytic cells, we investigated effects of CsA on expression of M1/M2 markers regulated by 27OHChol. Markers of M1/M2 polarization were analyzed by real-time PCR (Fig. 7). Transcripts levels of M1 markers (CCL2, CXCL10, CXCL11, IL-1β, TNF-α, CD80 and CD86) were enhanced following stimulation with 27OHChol, but the expression was inhibited by treatment with CsA, except IL-1β (Fig. 7A). Transcripts levels of M2 markers of LXRα, CD163 and CD206 were increased after stimulation with 27OHChol. Whereas CD206 expression was suppressed by CsA, expression of LXRα and CD163 was not changed (Fig. 7B). These results suggested that CsA preferentially affects expression of M1 markers.

3.8. CsA does not affect viability and basal expression of genes

It was investigated whether CsA and 27OHChol changed viability of monocytic cells and whether CsA influenced the basal expression of CCL2, CD14, and MMP-9. Compared with cells treated with 27OHChol alone, viability of cells was not decreased by additions of CsA (Fig. 8A). Moreover, CsA alone did not elevate or decrease the basal transcript levels of the genes aforementioned (Fig. 8B).

3.9. CsA inhibits LPS responses further enhanced in the presence of 27OHChol

Because CsA down-regulated CD14, we investigated the effects of the drug on LPS response by measuring CCL2 expression. Levels of the CCL2 transcripts increased following stimulation with 27OHChol or

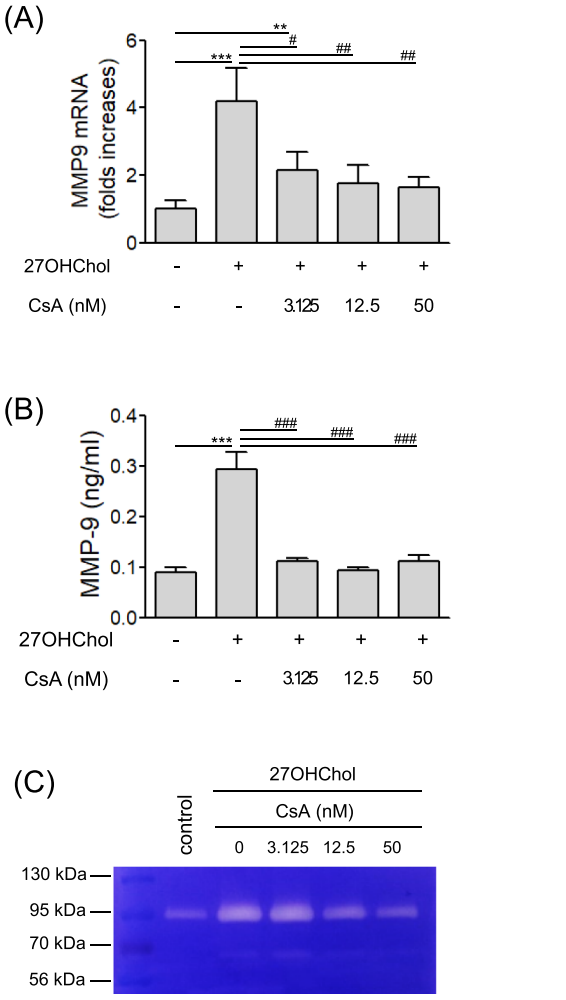

Fig. 6. Effects of CsA on the expression of MMP-9 induced by 27OHChol.
THP-1 cells (1×10^6 cells/60 mm culture dish) were cultured for 48 h with 2.5 μg/ml 27OHChol in the presence or absence of 3.125, 12.5 and 50 nM of CsA.
(A) The levels of MMP-9 transcripts were assessed by real-time PCR. Data are expressed as mean ± SD (n = 3 replicates/group). $^{**}P < 0.01$ vs. control; $^{***}P < 0.001$ vs. control; $\#P < 0.05$ vs. 27OHChol; $\#\#P < 0.01$ vs. 27OHChol.
(B) The amount of MMP-9 protein in culture media was measured by ELISA. Data are expressed as mean ± SD (n = 3 replicates/group). $^{***}P < 0.001$ vs. control; $\#\#\#P < 0.001$ vs. 27OHChol.
(C) Gelatinolytic activity of culture media was determined via zymography. Results are the representative of three independent experiments.

LPS, alone and additions LPS to 27OHChol-stimulated cells resulted in further elevation of CCL2 transcripts, but the elevation was suppressed in the presence of CsA (Fig. 9A). CsA also affected expression of CCL2 protein in a similar fashion as that observed with transcripts of the gene, as determined by ELISA (Fig. 9B).

4. Discussion

It is well-known that oxysterols are bioactive molecules that alter cell differentiation, gene expression and cell functions. However, drugs that affect the physiological/immunological effects of oxysterols are poorly known. Previously, we reported that monocytic cells differentiate in response to oxysterols, including 27OHChol [10] and that dexamethasone inhibits the 27OHChol-induced differentiation [15]. In the current study, we investigated the overall effects of CsA on cellular and molecular changes in monocytic cells induced by 27OHChol. We

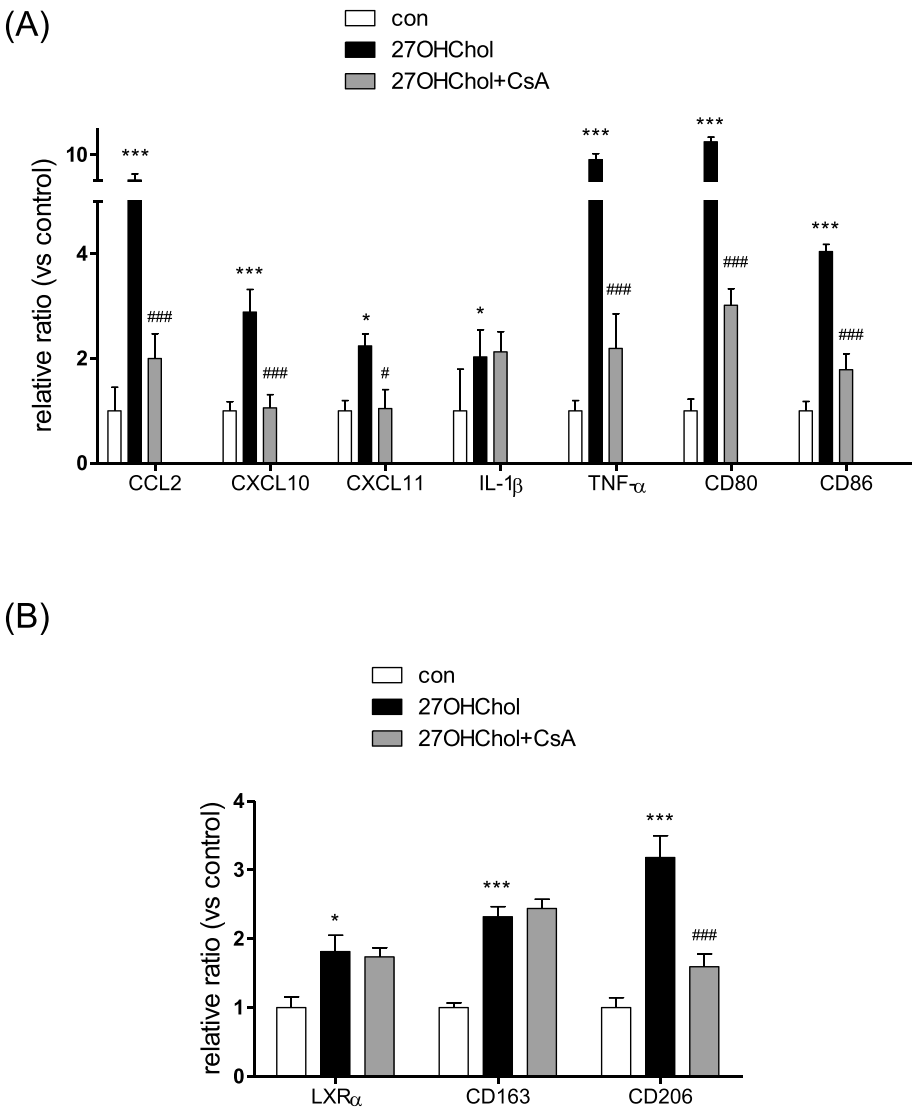

Fig. 7. Effects of CsA on M1/M2 polarization of the 27OHChol-stimulated monocytic cells.
THP-1 cells (1×10^6 cells/60 mm culture dish) were cultured for 48 h with 2.5 μg/ml 27OHChol in the presence or absence of 50 nM of CsA. The transcript levels of the markers for M1 polarization (A) and M2 polarization (B) were assessed by real-time PCR. Data are expressed as mean ± SD (n = 3 replicates/group). $^*P < 0.05$ vs. control: $^{***}P < 0.001$ vs. control; $\#P < 0.05$ vs. 27OHChol; $\#\#\#P < 0.001$ vs. 27OHChol.

are positive that the effects of CsA are specific and not due to cytotoxicity because 27OHChol and CsA caused no toxicity at the concentrations and duration of treatment used in this study and because CsA did not influence the basal gene expression of CCL2, CD14, and MMP-9.

Results of this study suggest that CsA effectively affects cell differentiation induced by 27OHChol. CsA down-regulates mDC-specific markers such as CD80, CD83, CD88, MHC class I/II molecules and endocytosis activity. In addition, the levels of CD197 (CCR7; a homing molecule of mDCs) on cell surface of 27OHChol-stimulated monocytic cells was reduced by treatment with CsA. CD197 is involved in the migration of mDCs to the secondary lymph nodes, such as spleen and interacts with CCL19 and CCL21 that are expressed in endothelial cells and in the T cell zone of lymphoid organs [23–25]. The inhibition of CD197 by CsA may result in the down-regulation of cell migration. Hence, these results suggest that CsA can attenuate the T cell activation via down-regulation of the MHC class molecules and subsequent impairment in the migration of mDCs.

Oxysterols induce inflammatory cytokines and chemokines such as CCL2, CCL3, TNF-α, CXCL8 and IL-23 through various pathways [7–9,26,27]. Therefore, we investigated the effects on molecules that can stimulate inflammation and immunity. MMP-9 is involved in inflammatory responses and innate immunity [20,22], and MMP-9 protein release is increased in mDCs than in immature DCs [28]. These reports are in line with our finding of increased MMP-9 release following oxysterol-induced differentiation into mDCs. CD14 is a co-activator of toll-like receptor-4 (TLR-4; a receptor of LPS), which is expressed on macrophages, neutrophils and dendritic cells [29]. Previous reports indicate the involvement of CD14 in innate immunity and in diseases including Alzheimer's disease, allergic responses, and atherosclerosis [19,30,31]. We demonstrated that MMP-9 and CD14 are increased under inflammatory conditions caused by oxysterols, and these increases were inhibited by CsA. In addition, super-induction of CCL2 observed in the presence of LPS plus 27OHChol through the increased levels of CD14 was significantly and dose-dependently reduced by CsA. The concurrent inhibition of MMP-9 and CD14 and CCL2 expression suggests that CsA simultaneously regulates multiple molecules involved in inflammation and innate immunity in a milieu rich in 27OHChol and that CsA impairs the 27OHChol-induced responses.

The M1 polarization of monocytic cells, which is known as the classical activation, leads to a Th1-type immune response like inflammation [32]; M2 polarization, also called the alternative activation,

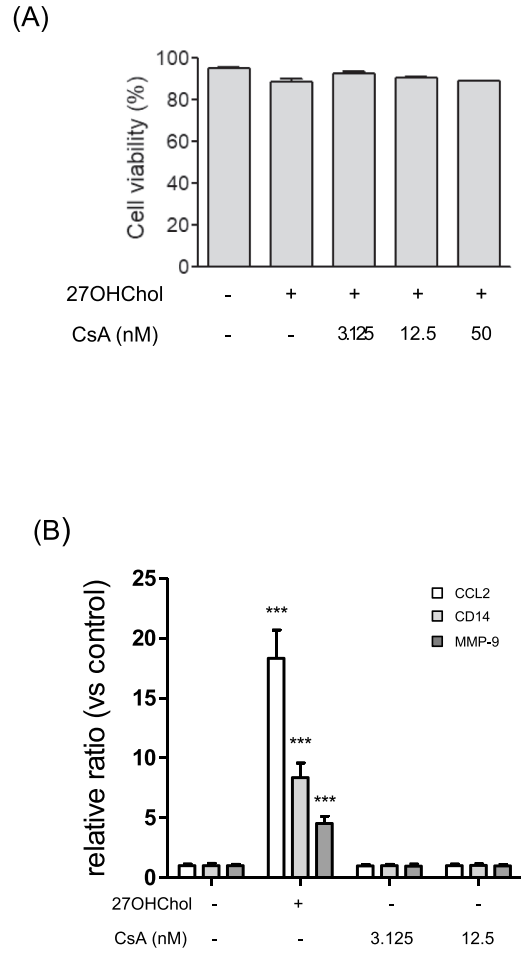

Fig. 8. Effects of 27OHChol and/or CsA on cell viability and the basal gene expression.
THP-1 cells were incubated with or without 2.5 μg/ml of 27OHChol in the presence of the indicated concentration of CsA for 48 h.
(A) Viability of the cells was measured by using cell counting kit-8. Results are the representative of three independent experiments.
(B) The transcript levels of CCL2, CD14 and MMP-9 genes were assessed by real-time PCR. Data are expressed as mean ± SD (n = 3 replicates/group). Results are the representative of three independent experiments. ***$P < 0.001$ vs. control (cultured in media alone).

induces a Th2-type immune response [33]. We demonstrated that, in addition to inhibition of CCL2, CsA inhibits expression of primarily M1 markers. These results suggest that CsA acts as a component of the anti-Th1 immune response, which, we think, agrees with the previous study that reported the aforementioned mechanisms and anti-inflammatory effects of CsA [34]. We consider that the preferential inhibition of M1 markers by CsA will cause imbalance of M1/M2 polarization in 27OHChol-rich tissues.

We tried to understand the molecular mechanisms underlying the inhibitory effects of CsA. The PI3 kinase/Akt pathway has been reported to be involved in the 27OHChol-induced differentiation and inflammatory responses [35], and the NF-κB play a role in the 27OHChol-induced chemokine production [9]. Therefore, we investigated whether CsA affected Akt and NF-κB by examining phosphorylation of the protein. We, however, were unable to obtain conclusive data demonstrating involvement of the molecules in CsA action because the drug did not affect the phosphorylation of Akt or p65 (a subunit of NF-κB) (Fig. 10). The signaling molecules that are responsible for inhibitory effects of CsA should be elucidated through further studies to understand mechanisms of action of the drug.

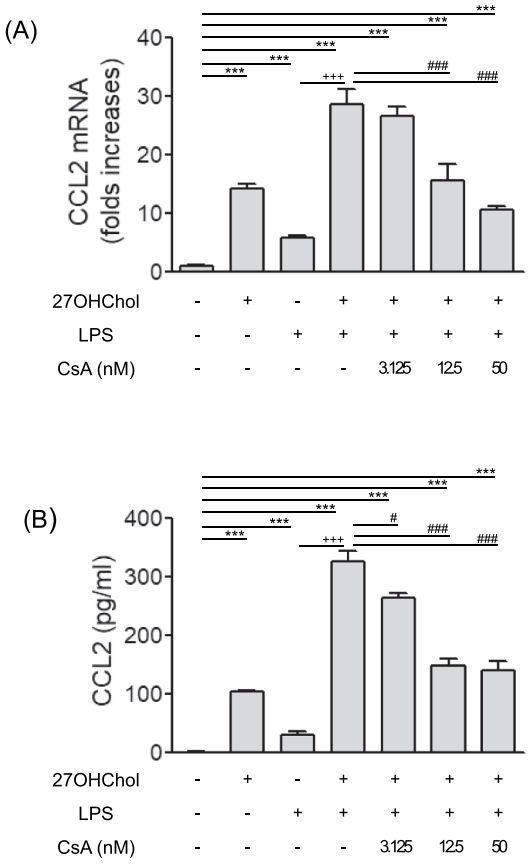

Fig. 9. Effects of CsA on enhanced-induction of CCL2 caused by 27OHChol and LPS.
THP-1 cells (1×10^6 cells/60 mm culture dish) were cultured for 24 h with 27OHChol (2.5 μg/ml) in the presence of indicated concentrations of CsA (3.125, 12.5 and 50 nM), followed by stimulation for 9 h with or without 100 ng/ml of LPS.
(A) The levels of CCL2 transcripts were assessed by real-time PCR. Data are expressed as mean ± SD (n = 3 replicates/group). ***$P < 0.001$ vs. control; +++$P < 0.001$ vs. LPS; ###$P < 0.001$ vs. 27OHChol plus LPS.
(B) The amount of CCL2 protein secreted into the media was measured by ELISA. Data are expressed as mean ± SD (n = 3 replicates/group). ***$P < 0.001$ vs. control; +++$P < 0.001$ vs. LPS; #$P < 0.05$ vs. 27OHChol plus LPS; ###$P < 0.001$ vs. 27OHChol plus LPS.

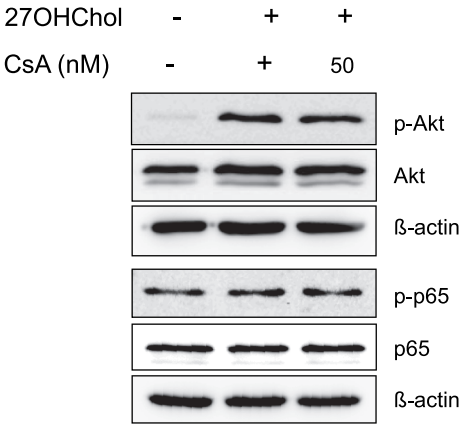

Fig. 10. Effects of CsA on phosphorylation of Akt and NF-κB p65 subunit.
THP-1 cells were incubated for 9 h with 27OHChol (2.5 μg/ml) in the presence or absence of 50 nM of CsA. Whole cell extracts were isolated from the cells and subjected to immunoblotting for Akt, phosphorylated Akt, p65, phosphorylated p65, and β-actin. Results are the representative of three independent experiments.

We have demonstrated inhibitory effects of CsA on the 27OHChol-induced cell responses. The drug inhibits differentiation into mDCs, polarization to the immuno-stimulatory M1 subset, LPS response, and expression of molecules involved in inflammation. Because 27OHChol plays important roles in onset and progression of chronic diseases, results of the current study suggest that CsA can modify pathophysiology of the diseases whose pathogenesis is associated with the oxidized cholesterol.

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Conflict of interest

The authors declare that they have no conflict of interests.

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