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Oxysterols induce transition of monocytic cells to phenotypically mature dendritic cell-like cells

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ABSTRACT

Dendritic cells (DCs) activate adaptive immune responses in atherosclerotic plaques; however, the origin of DCs is in question. We attempted to determine whether cholesterol or its oxide forms, which are detected in abundance in atheromatous lesions, could induce differentiation or transition of monocytic cells to DCs. Treatment of THP-1 cells with 27-hydroxycholesterol (27OH-Chol) and 7α-hydroxycholesterol (7αOH-Chol) resulted in an increase in the numbers of adherent cells, and, in contrast to PMA, decreased uptake of FITC-conjugated dextran. In addition, treatment with 27OH-Chol and 7αOH-Chol induced expression of mDC-specific molecules, including CD40, CD80, CD83, and CD88. Of the two oxysterols, 27OH-Chol enhanced expression of MHC class I and II molecules as well as CCR7. However, treatment with an identical concentration of cholesterol and 7-ketocholesterol did not influence adherence, uptake of FITC-conjugated dextran, and expression of the aforementioned molecules. This is the first study to report on change of monocytic cells by oxysterols to phenotypically atypical cells with some characteristics of mDCs detected in atherosclerotic lesions. We propose that a certain type of oxysterol would contribute to immune responses in atherosclerotic lesions by enhancing expression of multiple CD molecules as well as MHC molecules by monocytic cells.

1. Introduction

Activation of innate and adaptive immune responses that follow inflammation in the artery, which is mediated by functional changes in endothelial cells, T lymphocytes, monocytes/macrophages, and smooth muscle cells (SMC), predisposes blood vessels to complication of atherosclerosis [1,2]. Among different types of immune cells, dendritic cells (DCs) play potent roles in directing adaptive immune responses against antigens and altered self antigens [3]. In general, $CD34^+$ DC progenitor cells circulating the bloodstream give rise to immature DCs when taking residence in target tissue at sites of potential antigen entry. Immature DCs efficiently take up antigens and undergo transition into mature DCs (mDCs) with down-regulation of endocytic activity and up-regulation of molecules such as CCR7 (CD197), CD83, and CD86 [4]. In comparison with normal arteries, increased numbers of DCs are detected from advanced atherosclerotic plaques, as identified by markers that were expressed [5,6]. Most DCs are mature and are found in clusters with T lymphocytes [7,8], and some DCs are immunoreactive for CD14 [6,9]. CD14 is a marker of the monocyte lineage; therefore, co-localization of DC markers with CD14 suggests the possibility of transition of monocytes to DCs. However, it is not known whether differentiation of monocytic lineage cells into DC-like cells occurs under a cholesterol-rich milieu.

Atherosclerosis is characterized by accumulation of lipids, including free cholesterol and oxidized low density lipoproteins (oxLDL) [10,11]. Cholesterol oxides, oxysterols, derived non-enzymatically from *in vivo* oxidation or formed enzymatically during cholesterol catabolism, are also present in abundance in atherosclerotic lesions. The most abundant oxysterol is 27-hydroxycholesterol (27OH-Chol), followed by 7-ketocholesterol (7K), 7β-hydroxycholesterol (7βOH-Chol), and 7α-hydroxycholesterol (7αOH-Chol) [12,13]. Oxysterols are active constituents of oxLDL; many of the atherogenic characteristics of oxLDL are attributed to oxysterol content of the lipoprotein [14]. OxLDL induces differentiation of monocytes into macrophages and enhances mDC transition of monocytes differentiating in the presence of granulocyte macrophage colony-stimulating factor and interleukin-4 *in vitro* [15,16]. 7K, one of the cholesterol oxides in oxLDL, is able to induce differentiation of monocytes into macrophages [17]. These results indicate that oxysterols participate in differentiation of monocytic cells. However, roles of the aforementioned oxysterols in differentiation to DCs have not been reported.

The numbers of DCs showed a marked increase in atherosclerotic plaques [4,7]. Circulating DCs may migrate from the

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bloodstream in response to chemoattractants secreted from cells in atherosclerotic lesions and/or monocytic cells may undergo differentiation into DCs after stimulation by local factors [6]. We investigated the question of whether lipid components present in atherosclerotic lesions affected differentiation of monocytic cells into DC-like cells. In the current study, we demonstrated for the first time that treatment of THP-1 monocytic cells with particular types of oxysterols yielded a phenotype similar to that of mDCs detected in atherosclerotic lesions.

2. Materials and methods

2.1. Cell culture and reagents

THP-1 cells purchased from American Type Culture Collection (ATCC, Manassas, VA 20108) were maintained with RPMI 1640 containing 10% fetal bovine serum (FBS) in the presence of penicillin and streptomycin. Cholesterol, 7K, 7αOH-Chol, 27OH-Chol, and oxLDL were purchased from Research Plus, Inc. (Bayonne, NJ, USA). Primary antibodies used in the current study were purchased from Santa-Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell counting

THP-1 cells (2×10^4 cells) seeded into each well of a 96-well plate were serum-starved for 24 h in the presence of RPMI1640 containing 0.1% bovine serum albumin (BSA). After serum-starvation, cells were treated with cholesterol, 7K, 7αOH-Chol, 27OH-Chol, and phorbol myristate acetate (PMA) in the presence of 10% FBS in RPMI 1640 for 48 h. Cells in suspension were removed from the wells, and the number of adherent cells was calculated using a Cell Counting Kit-8 following the manufacturer's instructions (DOJINDO Molecular Technologies, Inc., Japan).

2.3. Dextran–FITC uptake assay

After incubation with cholesterol, oxysterols, and PMA, THP-1 cells were resuspended in culture medium containing fluorescein isothiocyanate (FITC)-conjugated dextran (40 kDa) and incubated for 30 min at 37 or 4 °C (for background control). Cells were washed with cold phosphate buffered saline (PBS) containing 1% FBS, and flow cytometry was performed for analysis of uptake of FITC–dextran.

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

RT-PCR was performed as previously described [18]. In brief, total RNAs were reverse-transcribed for 1 h at 42 °C with Moloney Murine leukemia virus reverse transcriptase, followed by PCR analysis. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as a control with primers of 5′-gagtcaacggatttggtcgt-3′ (forward) and 5′-tgtggtcatgagtccttcca-3′ (reverse). Primer pairs of CD molecules examined in the current study were designed using free online primer design tool primer3 (Table 1). PCR products were visualized using ethidium bromide after electrophoresis on agarose gels.

2.5. Flow cytometric analysis

After incubation with cholesterol and oxysterols as described above, THP-1 cells were harvested and incubated for 1 h at 4 °C with antibodies against CD40, CD80, CD83, CD88, CCR7, and major histocompatibility complex (MHC) class I and II, followed by washing and incubation with fluorescent dye-conjugated secondary antibodies. Cells were washed and resuspended in 1% paraformaldehyde in PBS. Flow cytometry was performed for analysis of fluorescence.

Table 1
Sequence of primers.

	Sequence (5′ to 3′′)	Gene accession number (NCBI)
CD1c	F: tctccaggctgtggaagttt R: gatgatgtcctggcctccta	NM 001765
CD11a	F: ggggaggacaagaagtgtga R: gatggggatgatggtagtgg	NM 001114380
CD11b	F: ggatgacctcagcatcacct R: ggttctgggcatgttgttct	NM 000632
CD11c	F: gagcttcacctggtctggag R: gaacagcatcacaccaccac	NM 000887
CD14	F: aggcctcaaggtactgagca R: ctgttgcagctgagatcgag	NM 000591
CD39	F: gcatcctggagctcttcaac R: aggtggagtgggagagaggt	NM 001098175
CD40	F: cagaagggcacctcagaaac R: tcgggaaaattgatctcctg	NM 001250
CD49a	F: gaaaatgggcctgttcttga R: ttcaggaggttcttcgctgt	NM 181501
CD72	F: ggagaccttgcaaagtgagg R: cagtgtccaccatgaccaag	NM 001782
CD80	F: cacgtgaccaaggaagtgaa R: cacgtgaccaaggaagtgaa	NM 005191
CD83	F: cggtctcctgggtcaagtta R: gagaaaagctcgttccatgc	NM 004233
CD86	F: acaaaaagcccacaggaatg R: atccaaggaatgtggtctgg	NM 006889
CD88	F: gccttggtcatctttgcagt R: caggaaggagggtatggtca	NM 001736
CD105	F: agaggtgcttctggtcctca R: agttccaccttcaccgtcac	NM 000118
CD112	F: cagaggaggacgaagacctg R: ctttgccctggtaggaatca	NM 001042724
CD123	F: aggatgaaagcaaaggctca R: actttgagaaccgctggaga	NM 002183
CCR7	F: tgttttcaggaccaggaagg R: tttctgccccgtttaacaac	NM 001561
CD163	F: aggaaggacccacttcctgt R: acgaaaatggccaacagaac	NM 004244
CD166	F: tacaagtgctgccaccaaaa R: cgcagacatagtttccagca	NM 001627
CD180	F: catccaccacatttcagtgc R: gtaagcgggtaaatgccaaa	NM 005582
CD197	F: ggctggtcgtgttgacctat R: taaaggcctccacatgctct	NM 001838
CD204	F: tttgatgctcgctcaatgac R: ccctggactgaggaaaacaa	NM 002445
CD281	F: tagtgtgctgccaattgctc R: tccagctgaccctgtagctt	NM 003263
CD304	F: atagacctgggggaggagaa R: cacctgtgagctggaagtca	NM 001024628
S-100	F: ggagaccctcatcaacgtgt R: cagccacaagcaccacatac	NM 006271
HLA-A	F: attacatcgccctgaacgag R: ctccagtgatcacagctcca	NM 002116
HLA-B	F: gacacccagttcgtgaggtt R: gtgggtcacgtgtgtctttg	NM 005514
HLA-DQα1	F: ccctgtggaggtgaagacat R: catcagcagaagggaggaag	NM 002122
HLA-DQβ1	F: cgggtagcaactgtcaccttt R: aaccaccggactttgatctg	NM 002123

F: forward; R: reverse.

2.6. Immunofluorescence staining

Frozen sections of aortic roots were prepared from wild type and *apoE*$^{-/-}$ mice fed a high cholesterol diet for 12 weeks and fixed for 20 min with 4% paraformaldehyde. After washing with wash buffer (0.1% Tween 20 in PBS), sections were incubated for 1 h with blocking solution (20% horse serum in washing buffer), followed by incubation at 4 °C with antibodies against CD40, CD80, CD83, and

CD88 diluted in blocking solution (1:50) overnight. After three washes, sections were incubated for 40 min with Alexa Fluor 488-conjugated secondary antibody diluted in blocking solution (1:100) at room temperature in darkness. After three washes, sections were mounted and visualized by confocal microscopy.

2.7. Statistical analysis

Statistical analyses were performed using one-way ANOVA, followed by Tukey's multiple comparison tests, using GraphPad PRISM (version 5.0).

3. Results

3.1. 27OH-Chol and 7αOH-Chol induced morphological changes in THP-1 cells

We investigated the effects of oxysterols on adherence of monocytic cells. THP-1 cells were treated with cholesterol, 7K, 7αOH-Chol, and 27OH-Chol in parallel with PMA prior to visualization by microscopy. Treatment with 27OH-Chol and 7αOH-Chol, as well as PMA, resulted in cell attachment (Fig. 1A, B). The numbers of attached cells showed a significant increase in the order of PMA, 27OH-Chol, and 7αOH-Chol, compared with control. However, treatment with cholesterol and 7K did not result in an increase in cell attachment. We also investigated morphology of THP-1 cells. Cells attached to the bottom after treatment with PMA showed morphological characteristics of macrophages. The morphology of oxysterol-treated cells appeared to differ from that of PMA-treated cells. Treatment with oxysterols, primarily 27OH-Chol, resulted in change of THP-1 cells into dendritic shape and formation of large piles of unattached cells by self-aggregation (Fig. 1A, 100× of lower panel). These results indicated that treatment with oxysterols resulted in increased adherence and morphological changes in monoctytic cells.

3.2. 27OH-Chol and 7αOH-Chol changed function of THP-1 cells opposite to that by PMA

Immune cells, including monocytes, macrophages, and immature DCs are able to take up antigens via endocytosis [3]. We investigated the effects of cholesterol and oxysterols on endocytosis. THP-1 cells were incubated with FITC-conjugated dextran after treatment with cholesterol, oxysterols, and PMA, and uptake of FITC–dextran was determined after measurement of fluorescence by flow cytometry (Fig. 2A, B). Treatment with PMA resulted in significant enhancement of endocytosis. The percentage of THP-1 cells exhibiting endocytic activity was 11.7% and the percentage increased to 63.7% in the presence of PMA. In contrast, treatment with 7αOH-Chol and 27OH-Chol resulted in reduction of uptake. The percentage of THP-1 cells exhibiting endocytic activity decreased to 2.4% and 3.8% in the presence of 7αOH-Chol and

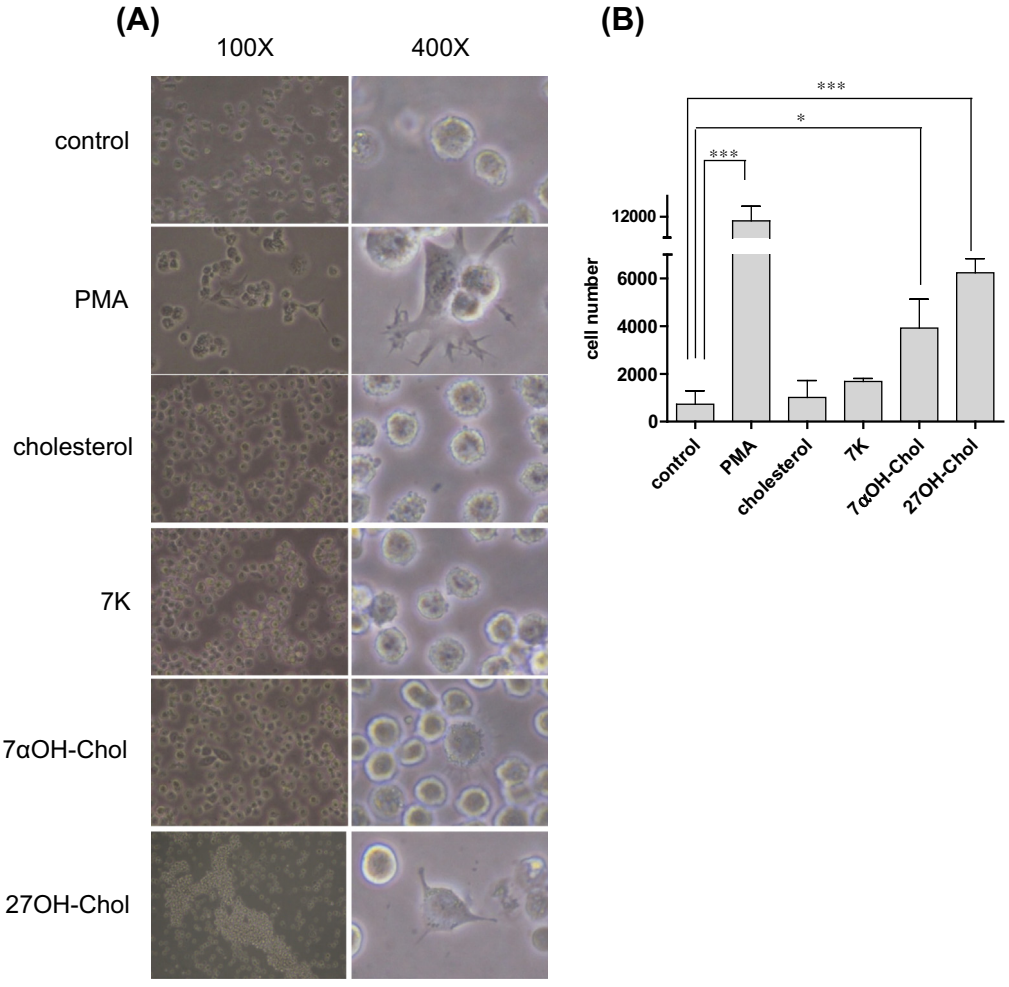

Fig. 1. Morphology of THP-1 cells treated with oxysterols and PMA. THP-1 cells (1×10^6 cells/60 mm culture dish) were cultured for 48 h in the presence of PMA (200 nM), cholesterol, 7K, 7αOH-Chol (5 μg/ml each), and 27OH-Chol (2.5 μg/ml). (A) Cells were visualized through a microscope and photographed. (B) After removal of cells in suspension, numbers of adherent cells were determined. Data are expressed as mean ± SD ($n = 3$ replicates/group). $^{*}p < 0.05$, $^{***}p < 0.001$.

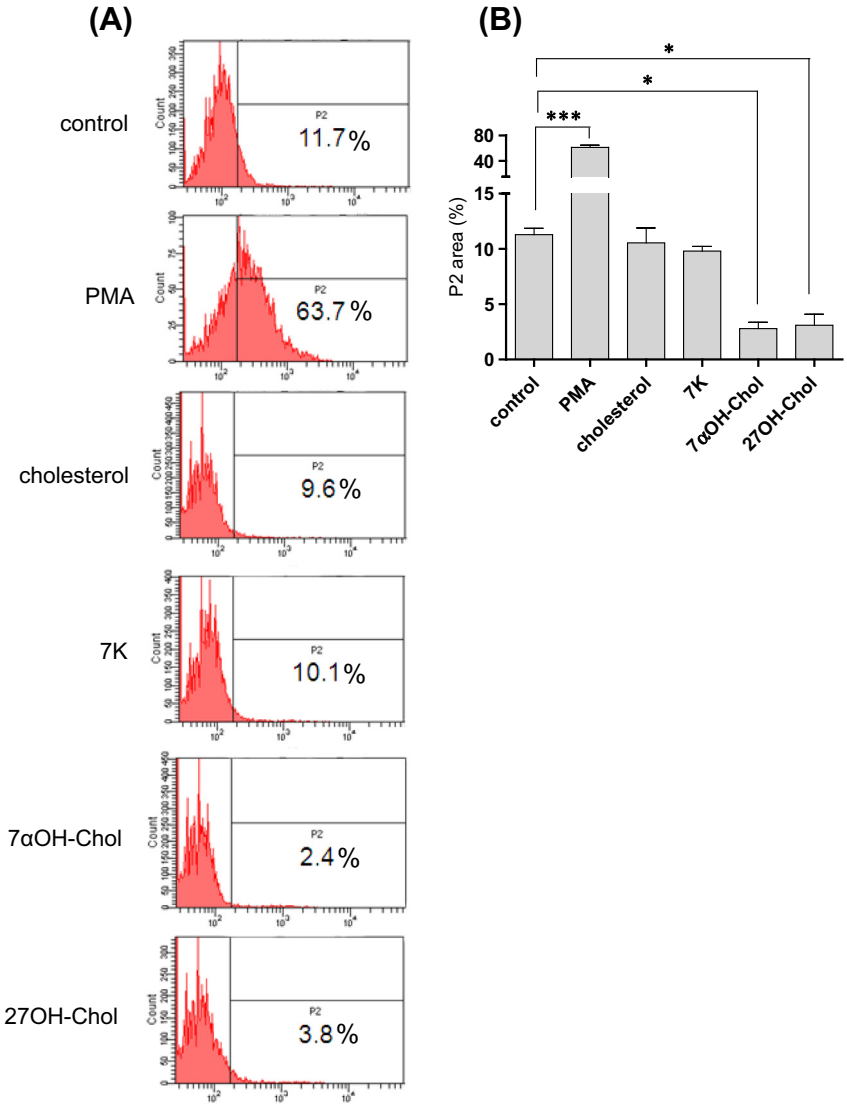

Fig. 2. Endocytosis by THP-1 cells treated with oxysterols and PMA. After incubation for 48 h with PMA (200 nM), cholesterol, 7K, 7αOH-Chol (5 μg/ml each), and 27OH-Chol (2.5 μg/ml), THP-1 cells were exposed to 1 mg/ml of FITC-conjugated dextran for 1 h. (A) Percentage of cells that showed high fluorescence intensity was determined by flow cytometry and (B) the percentage was described using a bar graph for comparison. Data are expressed as mean ± SD ($n = 3$ replicates/group). Results are representative of three independent experiments. $^{*}p < 0.05$, $^{***}p < 0.001$.

27OH-Chol, respectively. However, cholesterol and 7K did not influence uptake. These results indicated that treatment of THP-1 cells with 27OH-Chol and 7αOH-Chol induced a functional change that was distinct from that induced by PMA.

3.3. Oxysterol enhanced expression of DC markers by THP-1 cells

The above results suggest that 27OH-Chol and 7αOH-Chol induced transition of monocytic cells into unidentified type of cells. In an attempt to characterize the cell type to which THP-1 cells were driven, expression profiles of CD molecules were determined by RT-PCR after treatment with cholesterol and oxysterols. We examined expression of 139 genes and found 20 genes whose transcripts were induced or enhanced in the presence of 27OH-Chol or 7αOH-Chol, compared with control cells treated with medium alone (Supplementary data Fig. 1). In pairwise comparisons with control, markers of mDCs, such as CD40, CD80, CD83, CD86, and CD88, as well as a pro-atherogenic molecule CD137 were among the genes that showed differential expression at twofold or higher. Expression of six gene transcripts was slightly decreased, whereas, expression of 113 gene transcripts was not changed by treatment with oxysterols (see Supplementary data for the list of genes).

Expression of some DCs markers was analyzed at the protein level. In agreement with results of RT-PCR, flow cytometric analyses revealed an increase of CD molecules in the presence of 27OH-Chol and 7αOH-Chol (Fig. 3). The percentages of control THP-1 cells that expressed a high level of CD40, CD80, CD83 and CD88 were 3.1%, 3.3%, 0.2% and 0.1%, respectively. The percentage showed a significant increase to 11.8%, 21.6%, 50.1% and 44.7%, respectively, in the presence of 27OH-Chol, and to 9.3%, 13.0%, 35% and 38.2%, respectively, in the presence of 7αOH-Chol. However, cholesterol and 7K did not affect expression of CD molecules. The question of whether oxLDL influenced expression of the CD molecules in parallel with oxysterols was also examined. We observed that oxLDL had little influence on expression of CD molecules (data not shown).

We investigated the question of whether oxysterols affected expression of molecules involved in antigen presentation and lymph node-homing. Expression of MHC class I, MHC class II, and CCR7 was determined by flow cytometry after treatment with cholesterol and oxysterols (Fig. 4). In comparison with control, treatment with 27OH-Chol resulted in enhanced expression of

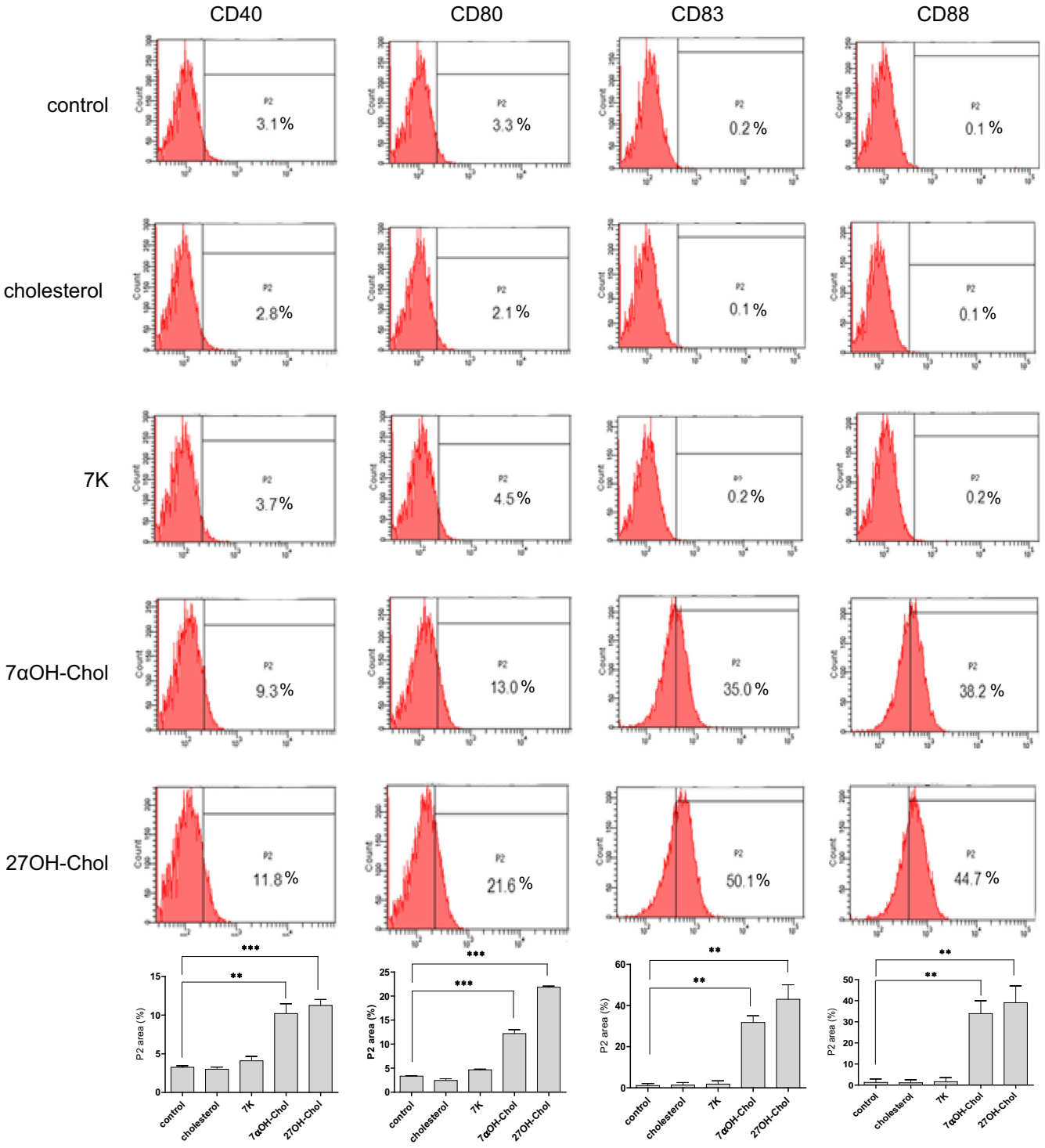

Fig. 3. Effects of oxysterols on expression of mDC markers. After incubation for 48 h in the presence of cholesterol and oxysterols, THP-1 cells (1×10^6 cells/60 mm culture dish) were immunostained for CD molecules indicated. Fluorescence was analyzed by flow cytometry, and the percentage of cells that showed enhanced fluorescence was determined and expressed using a bar graph for comparison. Data are expressed as mean ± SD ($n = 3$ replicates/group). Results are representative of three independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$.

MHC I, MHC II, and CCR7. The control cells showing positivity for MHC I, MHC II, and CCR7 were 2.5%, 2.6%, and 2.0%, respectively, which were increased to 10.7%, 13.3%, and 6.2% in the presence of 27OH-Chol. In the meantime, treatment with 7αOH-Chol, cholesterol, and 7K did not affect expression of the molecules. These results indicated that treatment with 27OH-Chol and 7αOH-Chol resulted in enhanced expression of mDC markers, even if both oxysterols had differential effects on proteins involved in antigen presentation and homing.

4. Discussion

Monocytes function as precursor cells with capacity to differentiate into DCs subset in response to appropriate stimulants. Human

blood CD14$^+$ monoctyes differentiate into DCs when cultured in combination with granulocyte–macrophage colony-stimulating factor (GMC-SF) and interleukin-4 (IL-4), and addition of tumor necrosis factor-α (TNF-α) enhances maturation with expression of CD83 [19,20]. Bacterial lipopolysaccharide (LPS) or calcium ionophore induces differentiation of blood CD14$^+$ monocytes [21,22]. Human monocytic leukemia cell line THP-1 also differentiates into DCs. Treatment of THP-1 cells with the cytokines and inonomycin results in a complete differentiation of the cells into mDCs displaying morphologic, phenotypic, molecular and

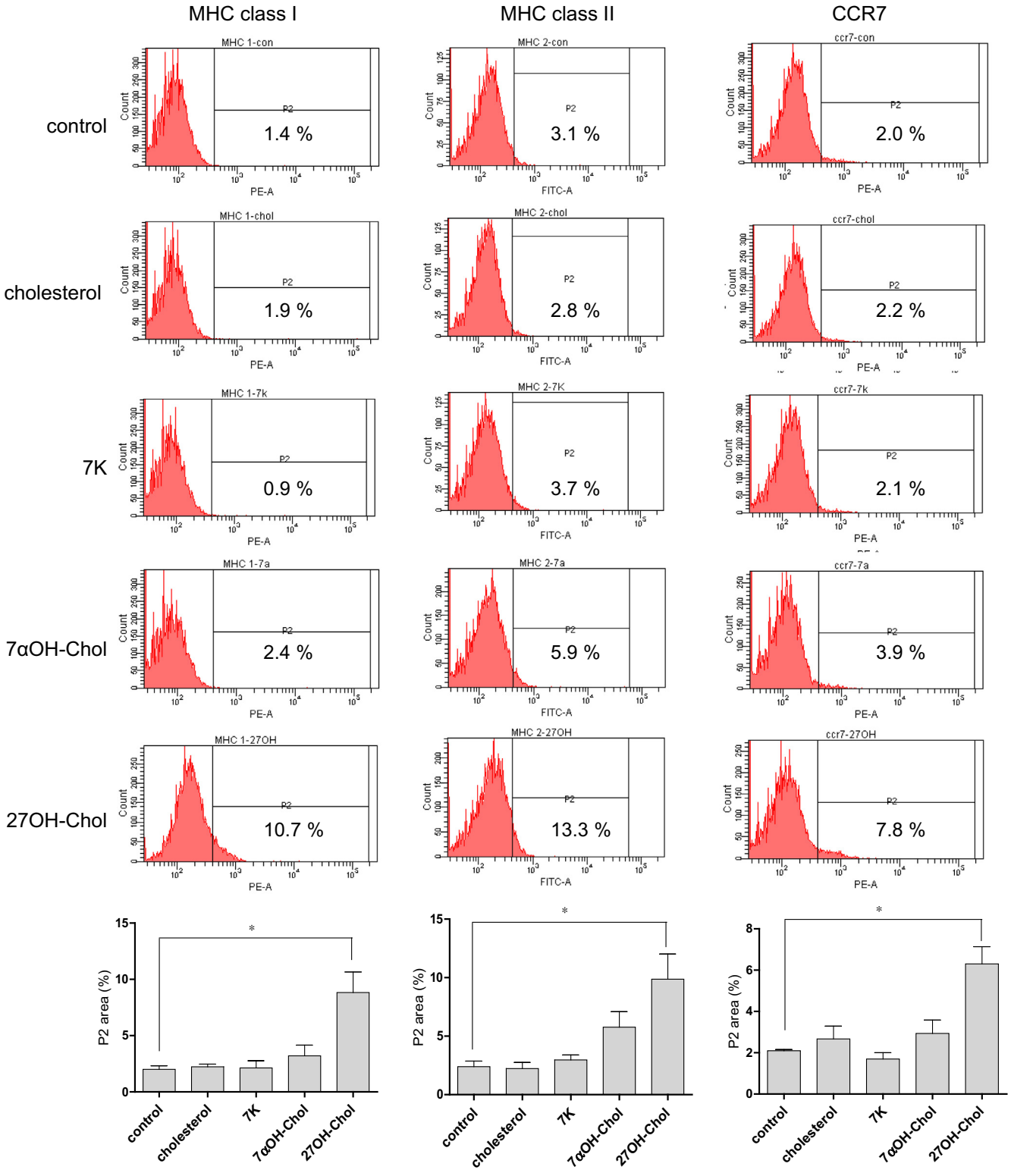

Fig. 4. Effects of oxysterols on expression of MHC molecules and CCR7. THP-1 cells were immunostained for MHC molecules and CCR7 after incubation for 48 h in the presence of cholesterol and oxysterols. The percentage of cells with enhanced fluorescence was determined by flow cytometry, which was expressed using a bar graph for comparison. Data are expressed as mean ± SD (n = 3 replicates/group). Results are representative of three independent experiments. $^*p < 0.05$.

functional properties of the DCs generated from human donor-derived $CD14^+$ monocytes or $CD34^+$ hematopoietic cells derived cells (HPCs) [23].

Because THP-1 has been proposed as a highly reproducible model for investigation of DC differentiation [23], we exposed THP-1 cells to oxysterols and PMA and compared morphological changes in order to investigate the question of whether oxysterols are proatherogenic molecules favoring DC differentiation of monocytic cells to the phenotype with functional DCs. Treatment of THP-1 cells with PMA has been reported to increase adherence and cause morphological changes to macrophages [24]. In agreement a previous study, PMA induced an increase in the numbers of adherent cells and induced differentiation into macrophages. 27OH-Chol and 7αOH-Chol also increased the numbers of adherent cells. Of the two oxysterols, 27OH-Chol was more efficient in increasing adherence. However, the morphology yielded after treatment with 27OH-Chol was distinct from that observed after treatment with PMA as 27OH-Chol-treated cells exhibited dendritic morphology. We also investigated the question of whether oxysterols affected endocytic function of monocytic cells. Uptake of FITC–dextran by THP-1 cells was significantly enhanced by treatment with PMA, which is consistent with a previous study reporting increased uptake of FITC–dextran after treatment with PMA [25]. In contrast, 27OH-Chol and 7αOH-Chol induced a significant decrease in uptake of FITC–dextran. The decrease was not due to cytotoxicity of the oxysterols because 27OH-Chol and 7αOH-Chol did not change the viability of THP-1 cells, as determined by trypan blue exclusion (data not shown). We observed that 7K did not affect adherence of THP-1 cells. The observation might be confusing because Hayden et al. reported that treatment with 7K promoted adherence of THP-1 cells and induced morphologic features of differentiated macrophages [17]. We think that the differences resulted from concentration of 7K used and duration of treatment with 7K as effects of 7K on adherence and differentiation were time and dose dependent [17]. Taken together, results for 27OH-Chol and 7αOH-Chol indicate that oxysterols induced differentiation of monocytic cells into a cell type that is distinct from that induced by PMA .

As DCs are mature, uptake of antigens via endocytosis is significantly reduced while the antigen presentation and homing processes to secondary lymph nodes are upregulated [3,4]. Since 27OH-Chol increased the numbers of adherent cells with dendritic appearance as well as 27OH-Chol and 7αOH-Chol reduced endocytosis, we investigated the question of whether monocytic cells were in transition to DCs by examining expression of characteristic markers of DCs. Treatment with 27OH-Chol and 7αOH-Chol resulted in increased expression of CD40, CD80, CD83, and CD88. Of the molecules, expression of mDC markers such as CD83 and CD88 was more effectively induced, compared with the other two molecules. In addition, treatment with 27OH-Chol resulted in increased expression of another mDC-specific marker, CCR7, a key homing molecule for DCs to lymph node, indicating that oxysterols are able to induce expression of multiple mDC markers by monocytic cells. These results suggest that monocytes are likely to change to phenotypically mDC-like cells in the presence of oxysterols.

We determined whether expression of CD molecules was different between adherent cells (27OH-Chol-ad.) and cells in suspension (27OH-Chol-sus.) after treatment with 27OH-Chol (Supplementary data Fig. 2). In comparison with cells in suspension, adherent cells showed stronger expression of markers of DCs, such as CD1c, CD80, CD86, and CD88. In addition, expression of CD197 (CCR7) was detected mainly from adherent cells. These results indicate that adherent cells expressed high levels of DC-markers in comparison with cells in suspension. We found that treatment with 27OH-Chol also resulted in increased expression of CD14, which was reported to be highly expressed by some DCs in the atherosclerotic plaques [6,9]. The enhanced expression of the DC markers as well as CD14 may explain detection of DCs expressing CD14 in atherosclerotic plaques .

Expression of CD40, CD80, CD83, and CD88 was investigated using atherosclerotic arteries obtained from $apoE^{-/-}$ mice (Supplementary data Fig. 3). Because infection could affect expression of co-stimulatory molecules by peripheral DCs [26,27], mice were housed under specific pathogen free conditions throughout the experiment in order to avoid accidental exposure to infectious agents. The immunoreactivity of each molecule was increased in atherosclerotic plaques from $apoE^{-/-}$ mice in comparison with normal artery obtained from wild type mice, which was consistent with a previous study reported by Liu et al., who reported overexpression of CD40 in atherosclerotic plaques of ApoE-deficient mice [28]. These results suggest that expression of CD80, CD83, and CD88 is affected under an *in vivo* cholesterol-rich milieu as well.

A number of studies have demonstrated differentiation of monocytic cells into imDCs or mDCs.

Treatment with 27OH-Chol resulted in increased expression of MHC class I and II molecules, as well as co-stimulatory molecules CD80 and CD86. MHC class I molecules present endogenous antigens and interact with $CD8^+$ T cells, and MHC class II molecules present exogenous antigens and interact with $CD4^+$ T cells [29,30]. Co-stimulatory molecules are essential for T cell activation and increase antigen presentation efficiency of antigen-presenting molecules such as MHC I and II molecules [31]. Elevated expression of MHC class molecules and co-stimulatory molecules may contribute to activation of T cells in atherosclerotic lesions.

We report that 27OH-Chol and 7αOH-Chol induce differentiation of THP-1 cells into the subtype of mDCs expressing CD molecules, including CD40, CD80, CD83, and CD86, with reduced endocytic ability, which are similar to that obtained after treatments with cytokines, LPS, or inonomycin, despite some variation in the level of the CD molecules expressed [19,21–23]. In comparison with cytokines which takes several days to drive differentiation, oxysterols trigger differentiation in two days. We think that oxidation of cholesterol to a certain type of oxysterols would be a critical event leading to DC differentiation from monocytes in the atherosclerotic lesions.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.07.046.

References

[1] G. Millonig, G.T. Malcom, G. Wick, Early inflammatory-immunological lesions in juvenile atherosclerosis from the pathobiological determinants of atherosclerosis in youth (PDAY)-study, Atherosclerosis 160 (2002) 441–448.
[2] J. Ozmen, Y.V. Bobryshev, R.S. Lord, K.W. Ashwell, Identification of dendritic cells in aortic atherosclerotic lesions in rats with diet-induced hypercholesterolaemia, Histol. Histopathol. 17 (2002) 223–237.
[3] J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.J. Liu, B. Pulendran, K. Palucka, Immunobiology of dendritic cells, Annu. Rev. Immunol. 18 (2000) 767–811.
[4] Y.V. Bobryshev, Dendritic cells and their role in atherogenesis, Lab. Invest. 90 (2010) 970–984.
[5] C. Erbel, K. Sato, F.B. Meyer, S.L. Kopecky, R.L. Frye, J.J. Goronzy, C.M. Weyand, Functional profile of activated dendritic cells in unstable atherosclerotic plaque, Basic Res. Cardiol. 102 (2007) 123–132.

[6] E.A. Van Vre, J.M. Bosmans, I. Van Brussel, M. Maris, G.R. De Meyer, P.E. Van Schil, C.J. Vrints, H. Bult, Immunohistochemical characterisation of dendritic cells in human atherosclerotic lesions: possible pitfalls, Pathology 43 (2011) 239–247.
[7] A. Yilmaz, M. Lochno, F. Traeg, I. Cicha, C. Reiss, C. Stumpf, D. Raaz, T. Anger, K. Amann, T. Probst, J. Ludwig, W.G. Daniel, C.D. Garlichs, Emergence of dendritic cells in rupture-prone regions of vulnerable carotid plaques, Atherosclerosis 176 (2004) 101–110.
[8] A. Link, M. Bohm, Potential role of dendritic cells in atherogenesis, Cardiovasc. Res. 55 (2002) 708–709.
[9] E.J. Soilleux, L.S. Morris, J. Trowsdale, N. Coleman, J.J. Boyle, Human atherosclerotic plaques express DC-SIGN, a novel protein found on dendritic cells and macrophages, J. Pathol. 198 (2002) 511–516.
[10] J.A. Berliner, J.W. Heinecke, The role of oxidized lipoproteins in atherogenesis, Free Radic. Biol. Med. 20 (1996) 707–727.
[11] L.J. Jara, G. Medina, O. Vera-Lastra, M.C. Amigo, Accelerated atherosclerosis, immune response and autoimmune rheumatic diseases, Autoimmun. Rev. 5 (2006) 195–201.
[12] A.J. Brown, W. Jessup, Oxysterols and atherosclerosis, Atherosclerosis 142 (1999) 1–28.
[13] K.L. Carpenter, S.E. Taylor, C. van der Veen, B.K. Williamson, J.A. Ballantine, M.J. Mitchinson, Lipids and oxidised lipids in human atherosclerotic lesions at different stages of development, Biochim. Biophys. Acta 1256 (1995) 141–150.
[14] G. Leonarduzzi, B. Sottero, G. Poli, Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects, J. Nutr. Biochem. 13 (2002) 700–710.
[15] B. Fuhrman, A. Partoush, N. Volkova, M. Aviram, Ox-LDL induces monocyte-to-macrophage differentiation in vivo: possible role for the macrophage colony stimulating factor receptor (M-CSF-R), Atherosclerosis 196 (2008) 598–607.
[16] L. Perrin-Cocon, F. Coutant, S. Agaugue, S. Deforges, P. Andre, V. Lotteau, Oxidized low-density lipoprotein promotes mature dendritic cell transition from differentiating monocyte, J. Immunol. 167 (2001) 3785–3791.
[17] J.M. Hayden, L. Brachova, K. Higgins, L. Obermiller, A. Sevanian, S. Khandrika, P.D. Reaven, Induction of monocyte differentiation and foam cell formation in vitro by 7-ketocholesterol, J. Lipid Res. 43 (2002) 26–35.
[18] S.M. Kim, H. Jang, Y. Son, S.A. Lee, S.S. Bae, Y.C. Park, S.K. Eo, K. Kim, 27-Hydroxycholesterol induces production of tumor necrosis factor-alpha from macrophages, Biochem. Biophys. Res. Commun. 430 (2013) 454–459.
[19] F. Sallusto, A. Lanzavecchia, Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha, J. Exp. Med. 179 (1994) 1109–1118.
[20] L.J. Zhou, T.F. Tedder, $CD14^+$ blood monocytes can differentiate into functionally mature $CD83^+$ dendritic cells, Proc. Natl. Acad. Sci. USA 93 (1996) 2588–2592.
[21] G.K. Koski, L.A. Lyakh, N.R. Rice, Rapid lipopolysaccharide-induced differentiation of CD14(+) monocytes into CD83(+) dendritic cells is modulated under serum-free conditions by exogenously added IFN-gamma and endogenously produced IL-10, Eur. J. Immunol. 31 (2001) 3773–3781.
[22] L.A. Lyakh, G.K. Koski, W. Telford, R.E. Gress, P.A. Cohen, N.R. Rice, Bacterial lipopolysaccharide, TNF-alpha, and calcium ionophore under serum-free conditions promote rapid dendritic cell-like differentiation in $CD14^+$ monocytes through distinct pathways that activate NK-kappa B, J. Immunol. 165 (2000) 3647–3655.
[23] C. Berges, C. Naujokat, S. Tinapp, H. Wieczorek, A. Hoh, M. Sadeghi, G. Opelz, V. Daniel, A cell line model for the differentiation of human dendritic cells, Biochem. Biophys. Res. Commun. 333 (2005) 896–907.
[24] S. Tsuchiya, Y. Kobayashi, Y. Goto, H. Okumura, S. Nakae, T. Konno, K. Tada, Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester, Cancer Res. 42 (1982) 1530–1536.
[25] E. Ibeas, L. Fuentes, R. Martin, M. Hernandez, M.L. Nieto, Secreted phospholipase A2 type IIA as a mediator connecting innate and adaptive immunity: new role in atherosclerosis, Cardiovasc. Res. 81 (2009) 54–63.
[26] K. Hoebe, E.M. Janssen, S.O. Kim, L. Alexopoulou, R.A. Flavell, J. Han, B. Beutler, Upregulation of costimulatory molecules induced by lipopolysaccharide and double-stranded RNA occurs by trif-dependent and trif-independent pathways, Nat. Immunol. 4 (2003) 1223–1229.
[27] V. Verhasselt, C. Buelens, F. Willems, D. De Groote, N. Haeffner-Cavaillon, M. Goldman, Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells: evidence for a soluble CD14-dependent pathway, J. Immunol. 158 (1997) 2919–2925.
[28] G. Liu, B. Wang, J. Zhang, H. Jiang, F. Liu, Total panax notoginsenosides prevent atherosclerosis in apolipoprotein E-knockout mice: role of downregulation of CD40 and MMP-9 expression, J. Ethnopharmacol. 126 (2009) 350–354.
[29] E.W. Hewitt, The MHC class I antigen presentation pathway: strategies for viral immune evasion, Immunology 110 (2003) 163–169.
[30] T.M. Holling, E. Schooten, P.J. van Den Elsen, Function and regulation of MHC class II molecules in T-lymphocytes: of mice and men, Hum. Immunol. 65 (2004) 282–290.
[31] D.J. Lenschow, T.L. Walunas, J.A. Bluestone, CD28/B7 system of T cell costimulation, Annu. Rev. Immunol. 14 (1996) 233–258.