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Suppressive Effect on MDC and IP-10 Expression in Monocytes by Endocrine Disruptor Chemicals

Ching-Hui Yeh,[1,2] Hsaing-Chi Wu,[1] Thai-Hung Kuo,[1] Chang-Hung Kuo,[3] San-Nan Yang,[3,4,5] Wei-Li Wang,[3] Huan-Nan Chen,[6] Wan-Ju Wei,[6] and Chih-Hsing Hung[3,4,5,6,7]

Abstract—The expression of chemokines is critical in leukocyte recruitment and inflammation, but the regulatory mechanisms involved remain incompletely defined. While endocrine disrupter chemicals (EDCs) are known to be ubiquitous in the environment and often associated with altered inflammatory response, their potential impact on chemokine expression in monocytes is at present unknown. To this end, the effects of EDCs on the expression of Th1- and Th2-related chemokines in a human monocytic cell line, THP-1, were investigated. THP-1 cells were pre-treated with varying concentrations of EDCs (nonylphenol and 4-octylphenol) with or without the addition of an estrogen receptor (ER) antagonist, ICI 182,780 and then stimulated by lipopolysaccharide (LPS). The levels of chemokines, CXCL10/IFN-α-inducible protein 10 (IP-10, a Th1 chemokine) and monocyte-derived chemokine (MDC)/CCL22, a Th2 chemokine) were measured by ELISA. EDC-mediated signaling events and histone modifications were examined by the use of Western blotting and chromatin immunoprecipitation (ChIP) assay. Nonylphenol and 4-octylphenol were able to suppress LPS-induced MDC and IP-10 expression. This suppressive effect was not reversed by the addition of ICI 182,780. Nonylphenol and 4-octylphenol reduced LPS-induced activation of MAPK signaling pathway, MKK1/2 and ERK, concomitant with decreased levels of LPS-induced acetylated histone 4 (H4) at the IP-10 and MDC gene loci. Nonylphenol and 4-octylphenol suppressed LPS-induced MDC expression in monocytes via, at least in part, the MKK1/2-ERK MAPK pathway and histone H4 acetylation, but not the estrogen receptor.

KEY WORDS: endocrine disruptor chemical; MDC; IP-10; monocyte; chemokine.

INTRODUCTION

Polarization with cytokines influences every aspect of the immune response from the innate to the adaptive. For monocytes, cytokines influence the production of chemokines, the expression of costimulatory molecules, and the execution of effector programs. T helper (Th)1- and Th2-polarization with IFN-r and IL-4 are two well-studied systems in both the mouse and the human [1, 2]. IL-4 polarization, referred to as either alternative or M2a

[1] Department of Family Medicine, Zuoying Armed Force General Hospital, Kaohsiung, Taiwan, Republic of China
[2] Department of Business Management, National Sun Yat-Sen University, Kaohsiung, Taiwan, Republic of China
[3] Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, Republic of China
[4] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China
[5] Department of Pediatrics, Faculty of Pediatrics, College of Medicine, Kaohsiung, Taiwan, Republic of China
[6] Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China
[7] To whom correspondence should be addressed at Department of Pediatrics, Kaohsiung Medical University Hospital, #100, Tz-You 1st Road, Kaohsiung 807, Kaohsiung, Taiwan, Republic of China. E-mail: pedhung@hotmail.com

ABBREVIATIONS: EDCs, endocrine disrupting chemicals; NP, nonylphenol; 4-OP, 4-octylphenol; ChIP, chromatin immunoprecipitation assay; MDC, monocyte-derived chemokine; IP-10, IFN-γ-inducible protein 10; LPS, lipopolysaccharide.

activation, promotes a response characteristic of wound healing and parasite immunity, whereas IFN-r polarization, known as classical or M1 activation, programs monocytes for intracellular killing, tumor resistance, and IL-12 production [3]. Chemokines are a family of small (8- to 10-kDa), secreted, usually inducible proteins which are important for leukocyte recruitment and critical for leukocyte homeostasis and for mediation of immune and inflammatory responses [4]. Chemokines also play critical roles during inflammatory responses and pathogenesis [4]. Many viral infections induce expression of chemokines, which are most likely involved in orchestrating recruitment of effector leukocytes to the sites of infection [5, 6]. The CXCL10/IFN-r-inducible protein 10 (IP-10) can compete with dengue virus for binding to heparan sulfate on the cell surface and block viral uptake and replication [7]. Macrophage-derived chemokine (MDC)/CCL22 is a Th2-related chemokine involved in recruitment of chemokine CC chemokine receptor (CCR) 8- and CCR4-bearing cells. MDC play an important role in innate immunity during sepsis and may aid in an adjunct therapy in sepsis [8]. MDC could also enhance the phagocytic and killing activities of peritoneal macrophages to *Escherichia coli* and induced both a respiratory burst and the release of lysozomal enzyme from macrophages [8].

Endocrine-disrupting chemicals (EDCs) are ubiquitous in environment and may have undesirable effects on human health via estrogen receptor (ER). Since the endocrine and immune systems share portions of some intracellular signaling pathways, EDCs are considered as potential agents for influencing inflammatory responses. Some EDCs, such p-n-nonylphenol (NP) and p-n-octylphenol (OP), suppressed LPS-induced nitric oxide production in mouse macrophage cell line. Thus, EDCs may interfere with host defense system against foreign pathogens [9]. It is at present unknown, however, as to whether NP and 4-OP, two EDCs, have any effect on chemokines' expression in monocytes. We investigated whether NP and 4-OP could suppress the LPS-induced MDC and IP-10 expression of monocytes and intracellular mechanisms, including epigenetic regulation, of EDC-regulated chemokines expression in monocytes were also explored.

MATERIALS AND METHODS

Cell Preparation

The human monocytic cell line THP-1 (American Type Culture Collection, Rockville, MD) was cultured in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37°C and 5% CO_2 in a humidified incubator. Cells were centrifuged and resuspended in fresh media in 24-well plates at a concentration of 10^6/mL for 24 h before experimental use. For human primary monocyte study, peripheral blood samples were collected from healthy individuals after obtaining informed consent, who had no personal or family history of allergy (n=3). The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital. Blood samples were diluted with an equal volume of phosphate-buffered saline. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation (Lymphoprep, Oslo, Norway). Human primary blood monocytes were isolated from PBMCs by magnetic bead sorting with anti-CD14 monoclonal antibody (mAb) (MACS, Milteny Biotec, Germany). The cells were pre-treated with NP and 4-OP 2 h before LPS (0.2 μg/ml) (*E. coli*; Sigma Chemical Co., St. Louis, MO) stimulation. Cell supernatant was collected at 24 h after LPS stimulation. To examine the involvement of the estrogen receptor axis, THP-1 cells were pre-treated with an estrogen receptor antagonist, ICI 182,780, 1 h before the treatment of the cells with NP and 4-OP.

ELISA Assay

The MDC, IP-10 and TNF-α concentrations of cell supernatants were determined using commercially available ELISA-based assay systems (R&D System, Minneapolis, MN). Assays were performed using the protocols recommended by the manufacturer.

Western Blotting

After treatment for 2 h with or without NP and 4-OP (10^{-8}–10^{-6} M), the cells were stimulated with LPS (0.2 μg/ml) and lysed with equal volumes of ice-cold 150-μl lysis buffer 1 h later. After centrifugation at 13,000×g for 15 min, equal amounts of cell lysates were analyzed by Western blot with anti-MAPK (p38, ERK and JNK) and anti-phospho-MAPK (pp38, p-ERK and p-JNK) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibody and the enhanced chemiluminescence (ECL) system (Amersham Pharmacia Biotech).

Chromatin Immunoprecipitation Assay

Chromatin Immunoprecipitation Assay (ChIPs) were performed as described previously [10] with minor modifications. 5×10^5 cells were treated with 1% formaldehyde for 10 min at RT, followed by sonication of DNAs and immunoprecipitation of chromatins overnight with Abs for H4 (Upstate Biotechnology, Waltham, MA). Immune complexes were collected using a protein A slurry (Invitrogen, Carlsbad, CA), and the DNA was reverse cross-linked, extracted, and quantified on a Taqman SDS 7900HT. For PCR amplification of ChIP products, primers and probes were designed to analyze the proximal promoter regions of the IP-10 gene as previously described [11], encompassing the following subregions relative to the transcription start sites (+9/−172): sense, 5′-GAGGGAAATTCC GTAACTTGG-3′; anti-sense, 5′-TCAGAAAACG TGGGGCTAGT-3′ with two NFκB binding site [11]. The sequence of PCR primer for MDC used in this study was as follows: sense, 5′-GACATTAAAGGC CAGGGACA-3′; anti-sense, 5′-CCATTCACCTAAA GGCAGGT-3′, encompassing the following subregions (−841/−999). PCRs were run on the ABI 7700 Taqman thermocycler. All Taqman reagents were purchased from Applied Biosystems. The relative intensities of the amplified products were normalized to the input DNAs.

Statistic Analysis

All data are presented as mean ± SD. Change in chemokines at different doses of NP, and 4-OP were analyzed by using Student's *t*-test for each pairwise comparison and one-way ANOVA was used for multiple group comparisons. A P value <0.05 was considered indicative of significant between-group differences.

RESULTS

NP and 4-OP Inhibited LPS-mediated Expression of MDC and IP-10 in THP-1 Cells and Human Primary Monocytes

To examine the potential effect of NP and 4-OP on the expression of Th1- and Th2-related chemokines in monocytes, THP-1 cells were treated with varying doses of NP and 4-OP (10^{-8}–10^{-6} M) either alone or in combination with varying concentrations of LPS. The results showed first that NP and 4-OP were capable of suppressing the LPS-induced MDC production in THP-1 cells (Fig. 1a, b). Pretreatment with NP could also suppress LPS-induced MDC production in human primary monocytes (Fig. 1c). Th1 immunity is important for viral or intracellular infection. We next evaluated whether NP and 4-OP could influence LPS-induced IP-10 expression in

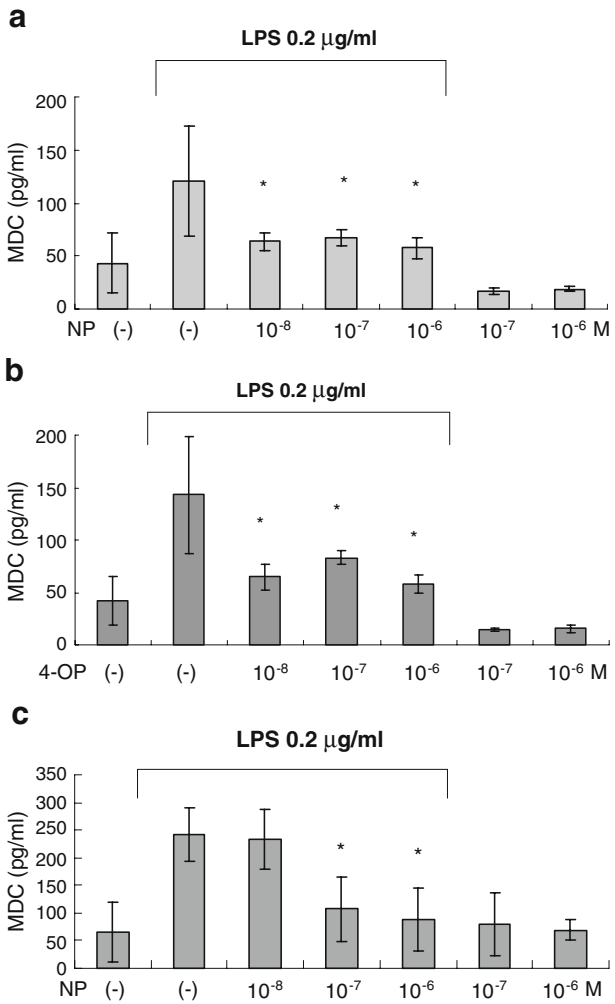


Fig. 1. Suppressive effect of NP and 4-OP on LPS-induced MDC production in THP-1 cells and human primary monocytes. NP (**A**) and 4-OP (**B**) were capable of suppressing, the LPS-induced MDC production in THP-1 cells. Similarly, NP also suppressed the LPS-induced MDC production in human primary monocytes (**C**) (*p<0.05).

THP-1 cells. There was only higher dose (10^{-6} M) of NP and 4-OP could significantly suppress LPS-induced IP-10 in THP-1 cells (Fig. 2a, b). NP could also suppress the LPS-induced IP-10 production in human primary monocytes (Fig. 2c). However, NP had no effect on the LPS-induced TNFα expression in THP-1 cells (Fig. 2d).

NP and 4-OP Suppressed MDC Production in Monocytes Not Via Estrogen Receptor

EDCs are thought as inflammatory inducer via partly via estrogen receptor [12]. Therefore, we next examined whether EDCs could decrease MDC expression in monocytes was through estrogen receptor. An estrogen receptor antagonist, ICI 182,780 could not reverse NP and 4-OP-suppressed MDC expression in monocytes (Fig. 3a, b), but further decrease MDC concentrations. We checked the viability of ICI 182,780-treated THP-1 cells to exclude the possibility of decreased viability of THP-1 cells by ICI 182,780. The viability of THP-1 cells after ICI 118,551 treatment was more than 90% (data not showed). These data suggested these NP and 4-OP suppressed LPS-induced MDC expression in monocytes was not through estrogen receptor.

NP and 4-OP Suppressed MDC Production in Monocytes via ERK MAPK Pathway

MAPK pathways are important for LPS-induced MDC expression in monocytes [13]. Next, we explored whether NP and 4-OP suppressed LPS-induced MDC production in monocytes via MAPKs pathway. Figure 4a showed that NP suppressed LPS-induced ERK, JNK and p38 expression, suggesting that ERK-, JNK- and p38-MAPK signaling is an important factor of NP suppressing LPS-induced MDC expression in monocytes. 4-OP could decrease LPS-induced ERK and JNK, but not p38 MAPK expression in monocytes (Fig. 4b). Therefore, suppression of ERK MAPK expression is important for the reduction of LPS-induced MDC expression in THP-1 cells. MKK1/2 is the upstream signaling of ERK [14]. Therefore, we next evaluated the upstream pathway of NP. The results showed NP could also decrease p-MKK1/2 expression, suggesting NP suppressed MDC and IP-10 expression, at least, in part through MKK 1/2-ERK pathway (Fig. 4c).

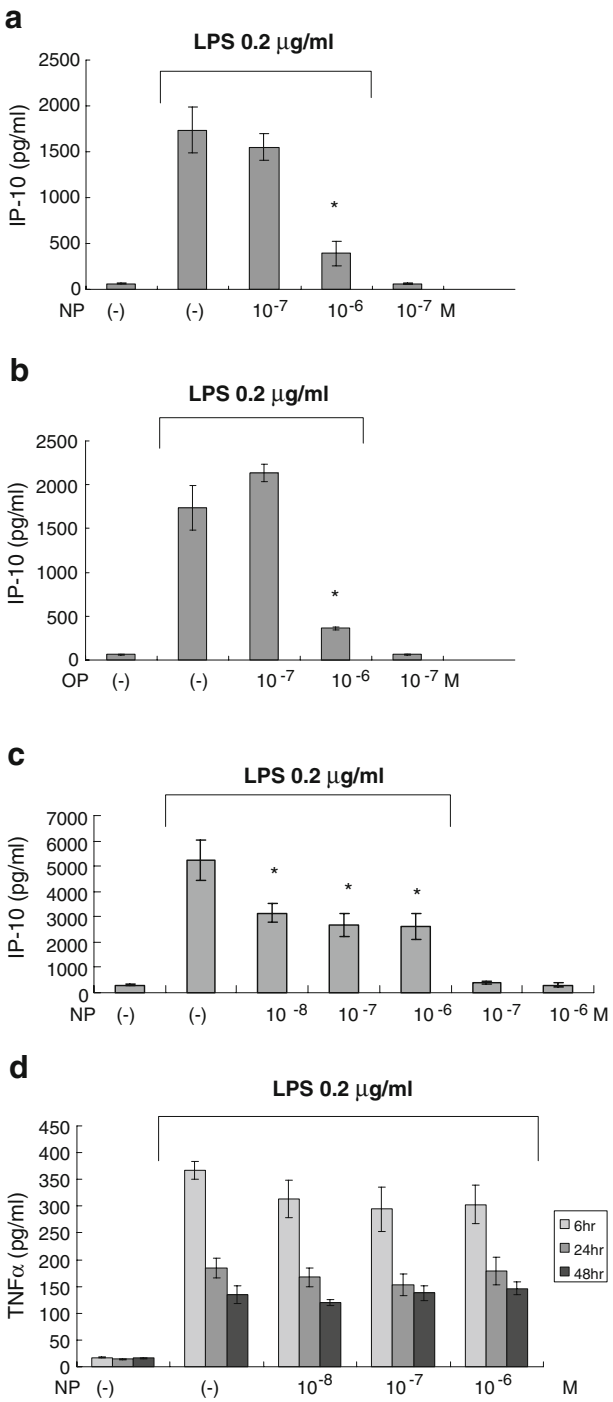


Fig. 2. Suppressive effect of NP and 4-OP on LPS-induced IP-10 production in THP-1 cells and human primary monocytes. NP (**A**) and 4-OP (**B**) could significantly reduce LPS-induced IP-10 in THP-1 cells. NP also suppressed LPS-induced IP-10 in human primary monocytes (**C**). But NP showed no effect on LPS-induced TNFα in THP-1 cells (**D**) (*$p<0.05$).

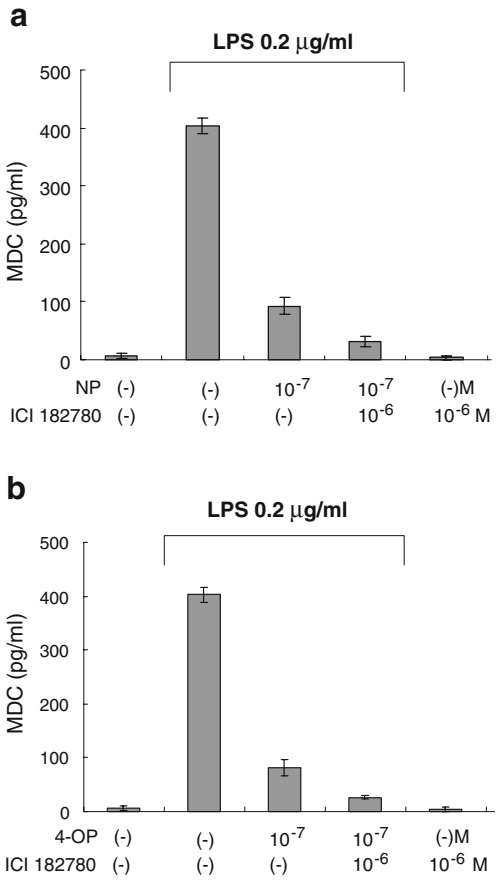


Fig. 3. Effect of NP and 4-OP on LPS-induced MDC and IP-10 expression in monocytes could not be reversed by ER receptor antagonist. THP-1 cells were pre-treated with an ER receptor antagonist, ICI 182780, 1 h before the treatment of the cells with EDCs. ICI 182780 could not reverse NP (**A**) and 4-OP (**B**)-suppressed MDC expression in THP-1 cells (*$p<0.05$).

Effect of NP on H4 Acetylation at the IP-10 and MDC Promoter in THP-1 Cells

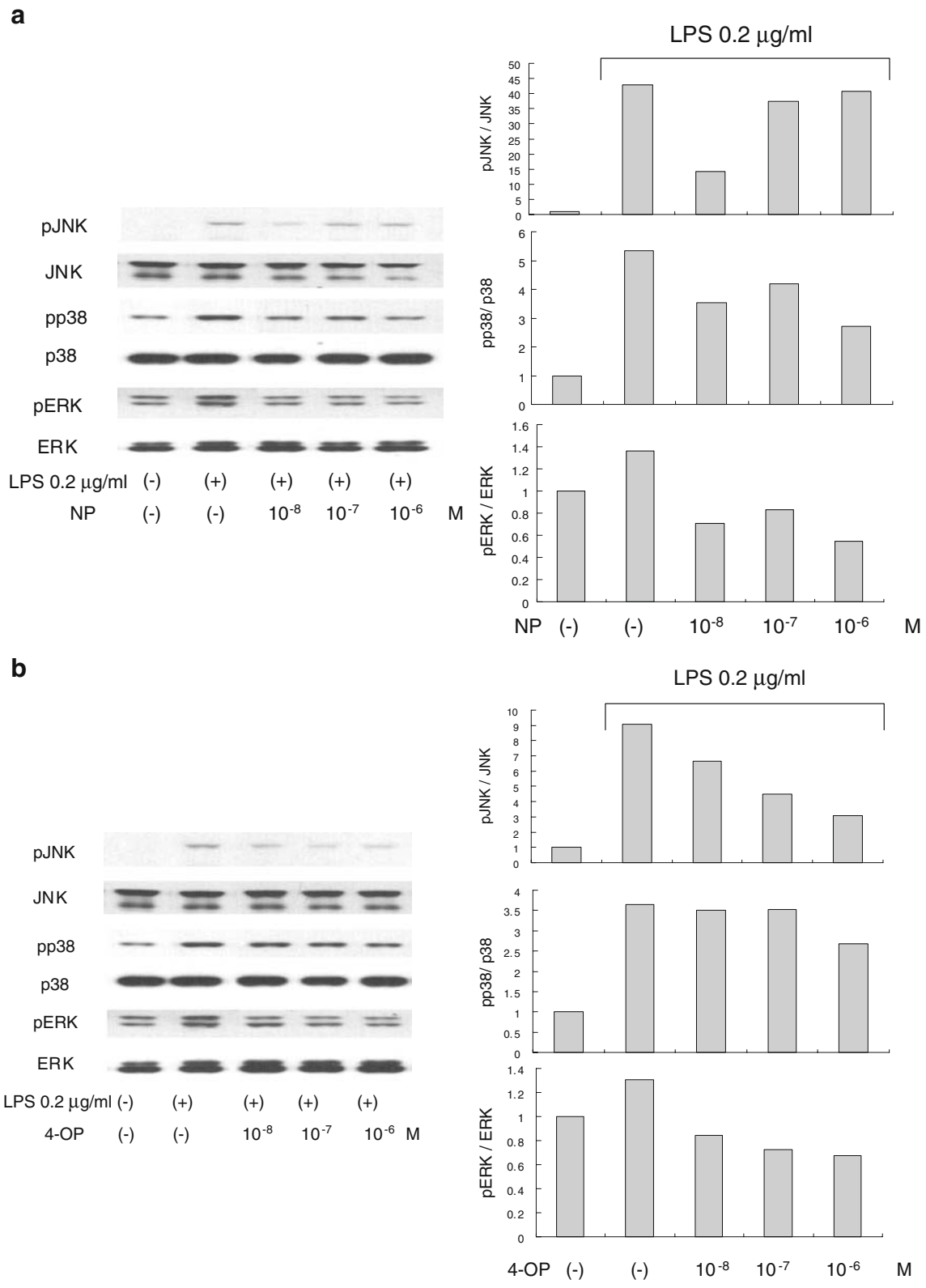
Chromatin carries several histone and DNA modifications that are associated with gene transcription. Histone acetylation is correlated with either positive transcriptional state. While the detailed mechanisms and the extent to which the modifications occur upon exposure to EDCs remain unclear, a role of EDCs in the regulation of monocytes function through epigenetic modulation on the expression of cytokines should be addressed. Therefore, THP-1 cells were treated with NP for 1 h. The H4 acetylation at the IP-10 and MDC promoter were analyzed by ChIP assay with specific total H4 acetylation antibodies. Interestingly, NP suppressed LPS-induced histone H4 acetylation at promoter of the IP-10 and MDC gene (Fig. 5a and b). Therefore, these data suggested NP-suppressed IP-10 and MDC production at least, in part, via suppressing LPS-induced histone H4 acetylation at IP-10 and MDC promoters.

DISCUSSION

The impact of EDCs exposure on human health is increasingly focused. EDCs can disturb development of the endocrine system and of the organs responding to endocrine signals in organisms indirectly exposed during prenatal and early postnatal life. Effects of exposure during development are permanent and irreversible [15]. EDCs exposure has been associated with several reproductive disorders as well as cancerogenesis both in animals and humans. EDCs are also reported to interfere with synthesis of cytokines, immunoglobulins, and cell mediators as well as immune cell activation and survival. Modulation by EDCs of interleukin-4 production, Th1/Th2 balance and IgE production suggest their potential effect on allergic immune responses [16]. Some EDCs (benzophenone, p-octylphenol, and tributyltin chloride) modulate cytokine production of antigen presenting cells through reduction of intracellular glutathione levels [17].

Th1 and Th2 immunity play important roles for intracellular infection and the defense of parasite infection, respectively [18]. LPS is ubiquitously present in the environment and induces Th1- and Th2-related chemokines expression. IP-10 is a chemokine that attracts Th1 lymphocytes through its receptor CXCR3 [19]. IP-10 is induced in a variety of cells in response to the Th1 cytokine IFN-r and important for protective immunity to viral infections such as dengue viral infection [7, 20]. MDC are Th2 chemokines involved in the recruitment of CC chemokine receptor (CCR) 4-bearing Th2 cells [21]. MDC is important in innate immunity during sepsis and enhancing the phagocytic and killing activities of peritoneal macro-

Fig. 4. NP and 4-OP suppressed MAPKs and MKK1/2 expression in ▶ monocytes. NP suppressed LPS-induced ERK, JNK and p38 expression in monocytes (**A**). 4-OP could also decrease LPS-induced ERK and JNK, but not p38 MAPK expression in monocytes (**B**) (*$p<0.05$). MKK1/2 are the upstream signaling of ERK. Therefore, we next evaluated whether NP could also suppress the expression of MKK 1/2 in THP-1 cells (**C**).


a
LPS 0.2 μg/ml
pJNK
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LPS 0.2 μg/ml (-) (+) (+) (+) (+)
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NP (-) (-) 10-8 10-7 10-6 M
b
LPS 0.2 μg/ml
pJNK
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p38
pERK
ERK
LPS 0.2 μg/ml (-) (+) (+) (+) (+)
4-OP (-) (-) 10-8 10-7 10-6 M
pJNK / JNK
pp38/ p38
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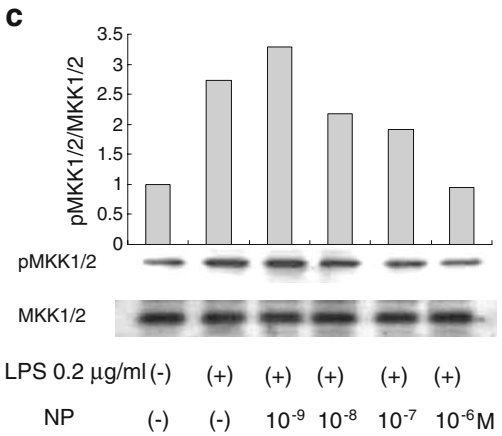
4-OP (-) (-) 10-8 10-7 10-6 M

phages to *E. coli* [8] and also plays roles in parasitic infection [22]. In the present study, two EDCs could suppress LPS-induced Th1- and Th2-related chemokines expression in THP-1 cells. These data suggested EDCs may interfere with Th1- and Th2-related immunity.

EDCs are ubiquitous in environment and may have some undesirable effects on human health. EDCs have been shown to undergo significant bioaccumulation [23], and tissue concentrations of NP have been measured in the 1–20 μM range in aquatic organisms [24, 25]. In the present study, the effective concentration of NP and 4-OP on TNF-α expression was less than 10^{-6} M. NP has been demonstrated to be able to stimulate pregnane X receptor (PXR; a member of the nuclear receptor superfamily) [26]-mediated gene transcription, through its interaction with PXR, whereby facilitating the receptor-coactivator interaction. It is tempting to speculate that NP (or 4-OP)-mediated TNF-α expression in THP-1 cells may involve ER-independent pathway. In addition, there is still a possibility that similar to other EDCs, NP or 4-OP may regulate hormonal responses by directly modulating cell-signaling pathways rather than interacting with hormone receptors. It is particularly relevant from the findings that the suppressive effect of NP and 4-OP on the expressions of MDC and IP-10 appears to be independent of estrogen receptor, suggesting the existence of alternative pathways. The evidence provided herein supports a role of EDCs in the regulation of monocytes' function through epigenetic modulation on the expression of cytokines, adding a new dimension to the existing regulatory network in monocytes. It has been demonstrated that epigenetic modification at the inflammatory gene locus results from a concerted and complex network of regulation involving histone modification and chromatin remodeling. It is known that acetylation of core histones allows the transformation of chromatin structure from a resting, closed conformation to an activated open form and subsequent initiation of gene transcription [27]. Further detailed investigation with emphasis on defining the epigenetic mechanisms associated with EDC's function would be important in defining the molecular basis of EDC's involvement in regulating immune response.



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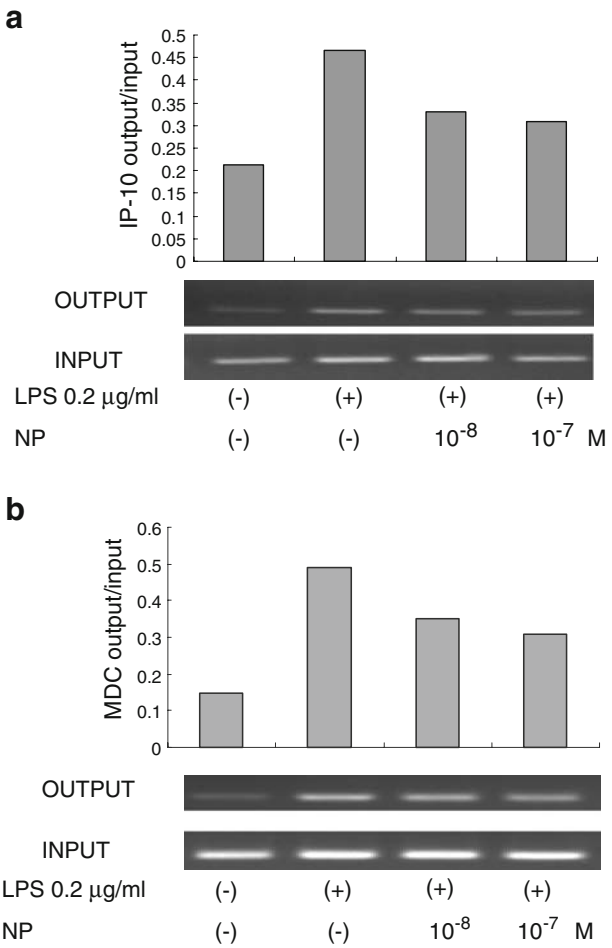

Fig. 5. Effect of NP on H4 acetylation at the IP-10 and MDC promoter in THP-1 cells. NP suppressed LPS-induced histone H4 acetylation at IP-10 promoter (**A**). NP also reduced LPS-induced H4 acetylation at the proximal promoter region of the MDC gene (**B**).

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Conflict of Interest Statement. There is no potential conflict of interest in this manuscript.

REFERENCES

1. Raes, G., R. Van den Bergh, D. P. Baetselier, G. H. Ghassabeh, S. Scotton, M. Locati, A. Mantovani, and S. Sozzani. 2005. Arginase-1 and Ym1 are markers for murine, but not human, alternatively activated myeloid cells. *J. Immunol.* **174**:6561–6562.
2. Martinez, F. O., S. Gordon, M. Locati, and A. Mantovani. 2006. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J. Immunol.* **177**:7303–7311.
3. Mantovani, A., A. Sica, S. Sozzani, P. Allavena, A. Vecchi, and M. Locati. 2004. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* **25**:677–686.
4. Luster, A. D. 1998. Chemokines: chemotactic cytokines that mediate inflammation. *N. Engl. J. Med.* **338**:436–445.
5. Melchjorsen, J., L. N. Sorensen, and S. R. Paludan. 2003. Expression and function of chemokines during viral infections: from molecular mechanisms to *in vivo* function. *J. Leukoc. Biol.* **74**:331–333.
6. Chensue, S. W. 2001. Molecular machinations: chemokine signals in host-pathogen interactions. *Clin. Microbiol. Rev.* **14**:821–835.
7. Chen, J. P., H. L. Lu, S. L. Lai, G. S. Campanella, J. M. Sung, M. Y. Lu, B. A. Wu-Hsieh, Y. L. Lin, T. E. Lane, A. D. Luster, and F. Liao. 2006. Dengue virus induces expression of CXC chemokine ligand 10/IFN-gamma-inducible protein 10, which competitively inhibits viral binding to cell surface heparan sulfate. *J. Immunol.* **177**:3185–3192.
8. Matsukawa, A., C. M. Hogaboam, N. W. Lukacs, P. M. Lincoln, H. L. Evanoff, and S. L. Kunkel. 2000. Pivotal role of the CC chemokine, macrophage-derived chemokine, in the innate immune response. *J. Immunol.* **164**:5362–5368.
9. Yoshitake, J., K. Kato, D. Yoshioka, Y. Sueishi, T. Sawa, T. Akaike, and T. Yoshimura. 2008. Suppression of NO production and 8-nitroguanosine formation by phenol-containing endocrine-disrupting chemicals in LPS-stimulated macrophages: involvement of estrogen receptor-dependent or -independent pathways. *Nitric Oxide*. **18**:223–228.
10. Lee, J. Y., N. A. Kim, A. Sanford, and K. E. Sullivan. 2003. Histone acetylation and chromatin conformation are regulated separately at the TNF alpha promoter in monocytes and macrophages. *J. Leukoc. Biol.* **73**:862–871.
11. Majumder, S., L. Z. Zhou, P. Chaturvedi, G. Babcock, S. Aras, and R. M. Ransohoff. 1998. Regulation of human IP-10 gene expression in astrocytoma cells by inflammatory cytokines. *J. Neurosci. Res.* **54**:169–180.
12. Chalubinski, M., and M. L. Kowalski. 2006. Endocrine disrupters–potential modulators of the immune system and allergic response. *Allergy.* **61**:1326–1335.
13. Hung, C. H., J. L. Suen, H. C. Chang, Y. M. Hua, and Y. J. Jong. 2007. Suppressive effects of ketotifen on Th1- and Th2- related chemokines of monocyte. *Pediatr. Allergy Immunol.* **18**:378–384.
14. Jones, N. C., Y. V. Fedorov, R. S. Rosenthal, and B. B. Olwin. 2001. ERK1/2 is required for myoblast proliferation but is dispensable for muscle gene expression and cell fusion. *J. Cell. Physiol.* **186**:104–115.
15. Colborn, T., F. S. vom Saal, and A. M. Soto. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Perspect.* **101**:378–384.
16. Chalubinski, M., and M. L. Kowalski. 2006. Endocrine disrupters–potential modulators of the immune system and allergic response. *Allergy.* **61**:1326–1335.
17. Kato, T., S. Tada-Oikawa, K. Takahashi, K. Saito, L. Wang, A. Nishio, R. Hakamada-Taguchi, S. Kawanishi, and K. Kuribayashi. 2006. Endocrine disruptors that deplete glutathione levels in APC promote Th2 polarization in mice leading to the exacerbation of airway inflammation. *Eur. J. Immunol.* **36**:1199–1209.
18. Akubzick, C., H. Wen, A. Matsukawa, M. Keller, S. L. Kunkel, and C. M. Hogaboam. 2004. Role of CCR4 Ligands, CCL17 and CCL22, during Schistosoma mansoni egg-induced pulmonary granuloma formation in mice. *Am. J. Pathol.* **165**:1211–1221.
19. Siveke, J. T., and A. Hamann. 1998. T helper 1 and T helper 2 cells respond differentially to chemokines. *J. Immunol.* **160**:550–554.
20. Hsieh, M. F., S. L. Lai, J. P. Chen, J. M. Sung, Y. L. Lin, B. A. Wu-Hsieh, C. Gerard, A. Luster, and F. Liao. 2006. Both CXCR3 and CXCL10/IFN-inducible protein 10 are required for resistance to primary infection by dengue virus. *J. Immunol.* **177**:1855–1863.
21. Imai, T., M. Nagira, S. Takagi, M. Kakizaki, M. Nishimura, J. Wang, P. W. Gray, K. Matsushima, and O. Yoshie. 1999. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int. Immunol.* **11**:81–88.
22. Hübner, M. P., B. J. Manfras, M. C. Margos, D. Eiffler, W. H. Hoffmann, H. Schulz-Key, P. Kern, and P. T. Soboslay. 2006. Echinococcus multilocularis metacestodes modulate cellular cytokine and chemokine release by peripheral blood mononuclear cells in alveolar echinococcosis patients. *Clin. Exp. Immunol.* **145**:243–251.
23. Lalah, J. O., A. Behechti, G. F. Severin, D. Lenoir, K. Gunther, A. Kettrup, and K. W. Schramm. 2003. The bioaccumulation and fate of a branched ^{14}C-p-nonylphenol isomer in *Lymnaea stagnalis* L. *Environ. Toxicol. Chem.* **22**:1428–1436.
24. Wenzel, A., W. Bohmer, J. Muller, H. Rudel, and C. Schroter-Kermani. 2004. Retrospective monitoring of alkylphenols and alkylphenol monoethoxylates in aquatic biota from 1985 to 2001: results from the German Environmental Specimen Bank. *Environ. Sci. Technol.* **38**:1654–1661.
25. Lalah, J. O., K. W. Schramm, G. F. Severin, D. Lenoir, B. Henkelmann, A. Behechti, K. Guenther, and A. Kettrup. 2003. *In vivo* metabolism and organ distribution of a branched ^{14}C-nonylphenol isomer in pond snails, *Lymnaea stagnalis* L. *Aquat. Toxicol.* **62**:305–319.
26. Masuyama, H., Y. Hiramatsu, M. Kunitomi, T. Kudo, and P. N. MacDonald. 2000. Endocrine disrupting chemicals, phthalic acid and nonylphenol, activate Pregnane X receptor-mediated transcription. *Mol. Endocrinol.* **14**:421–428.
27. Atanaskova, N., V. G. Keshamouni, J. S. Krueger, J. A. Schwartz, F. Miller, and K. B. Reddy. 2002. MAP kinase/estrogen receptor cross-talk enhances estrogen-mediated signaling and tumor growth but does not confer tamoxifen resistance. *Oncogene.* **21**:4000–4008.