Differential susceptibility to lipopolysaccharide affects the activation of toll-like-receptor 4 signaling in THP-1 cells and PMA-differentiated THP-1 cells

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Abstract
Monocytes and macrophages that originate from common myeloid progenitors perform various crucial roles in the innate immune system. Stimulation with LPS combined with TLR4 drives the production of pro-inflammatory cytokines through MAPKs and NF-κB pathway in different cells. However, the difference in LPS susceptibility between monocytes and macrophages is poorly understood. In this study, we found that pro-inflammatory cytokines—IL-1β, IL-6 and TNFα showed greater induction in phorbol-12-myristate-13-acetate (PMA)-differentiated THP-1 cells than in THP-1 cells. To determine the difference in cytokine expression, the surface proteins such as TLR4-related proteins and intracellular adaptor proteins were more preserved in PMA-differentiated THP-1 cells than in THP-1 cells. MyD88 is a key molecule responsible for the difference in LPS susceptibility. Moreover, MAPKs and NF-κB pathway-related molecules showed higher levels of phosphorylation in PMA-differentiated THP-1 cells than in THP-1 cells. Upon MyD88 depletion, there was no difference in the phosphorylation of MAPK pathway-related molecules. Therefore, these results demonstrate that the difference in LPS susceptibility between THP-1 cells and PMA-differentiated THP-1 cells occur as a result of gap between the activated MAPKs and NF-κB pathways via changes in the expression of LPS-related receptors and MyD88.

Keywords
monocyte, macrophage, toll-like-receptor 4, MyD88, MAPK

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Introduction
Mononuclear phagocytes, monocytes, and macrophages have crucial and diverse roles in the regulation of the innate immune system and act as an antigen presenting cells in adaptive immunity.¹ They are also responsible for the initiation and resolution of inflammation against invading antigens and antigen presentation.²,³ Monocytes and macrophages originate from common myeloid progenitor cells but their specialized roles differ because monocytes differentiate into tissue specific macrophages.⁴ However, The diversity in terms of the sensitivity of antigens (e.g. bacterial lipopolysaccharide (LPS) and endotoxins observed in immune cells) has not been explored well. LPS, a pathogen-associated molecular patterns, induces immune responses by binding to the CD14/TLR4/MD2 receptor complex in various cell types.⁵ It stimulates monocytes and macrophages, preserves the LPS receptor, and induces pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-8, inducible nitric oxide synthase, and tumor necrosis factor α (TNFα).⁶ TLR4 signaling is separated into myeloid differentiation primary response 88 (MyD88)-dependent and TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent pathways. MyD88 induces phosphorylation of interferon regulatory factor 5, mitogen-activated protein kinase (MAPK), and nuclear factor (NF)-κB pathways through IRAKs/TRAF6; TRIF activates IRF3, in the late-phase of the MAPK and NF-κB pathways by recruiting RIP1 and TRAF3.⁷ These pathways play a critical role in

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activating MAPKs and NF-κB pathways induce pro-inflammatory cytokines. In addition, the secreted IL-1β, IL-6, and TNFα are abolished in MyD88−/− macrophages when the MAPK and NF-κB pathways were inactivated.

In a previous report, LPS sensitivity was found to differ between monocytes and macrophages. The TLR4 expression were greater in M-CSF induced monocyte-derived macrophages compared to monocytes. However, it is still not clear of how TLR4 signal transduction is related to the induction of pro-inflammatory cytokines in monocytes and macrophages. In this study, we investigated the differences in susceptibility to the effects of LPS between THP-1 cells, human monocytic cell line and phorbol-12-myristate-13-acetate (PMA)-differentiated THP-1 cells. We also examined the changes in activation of MAPKs and NF-κB pathway according to LPS sensitivity in these cells.

### Materials and methods

#### Cell culture

The human monocytic leukemia cell line THP-1 (American Type Culture Collection, Manassas, VA, USA) was cultured in RPMI 1640 (SH30027.01, Hyclone, Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (SH30919.03, Hyclone), 1% MEM non-essential amino acid solution (11140, Gibco, Grand Island, NY, USA), and 1% penicillin/streptomycin (P/S, 15140, Gibco) at 37°C in a 5% CO2 incubator. THP-1 cells were differentiated by incubating the cells with 10 ng/mL phorbol-12-myristate-13-acetate (PMA) for 24 h, with the media changed on the next day followed by further incubation for 24 h. To stimulate the cells, the cells were seeded into 6-well culture plates (140675, Thermo Fisher Scientific, Waltham, MA, USA) at 1 × 10⁶ cells/well and treated with 1, 10, 100 and 1000 ng/mL LPS (L2637, Sigma, St. Louis, MO, USA). THP-1 cells depleted of MyD88 were purchased from InvivoGen (thpd-komyd, Carlsbad, CA, USA).

#### Isolation of mRNA and Rt-PCR

Total RNA was isolated using Trizol (15596018, Life Technologies, Carlsbad, CA, USA). Total cellular RNA was used to synthesize cDNA using a Quantitect Reverse Transcription Kit (205313, Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

#### Real-time quantitative PCR

Quantitative polymerase chain reaction (qPCR, Power SYBR™ Green PCR Master Mix, 4368702, Applied Biosystems, Foster City, CA, USA) was conducted using human primers for GAPDH, IL-1β, TNFα, IL-6, CD14, TLR4, MD-2, LBP, MyD88, TIRAP, TRAM, and TRIF. All the qPCR primers were designed by Primer 3V0.4.0 (Table 1). qPCR was as performed as follows: 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min on a PCR machine (A28134, Applied Biosystems). mRNA levels were calculated using GAPDH ($\triangle\triangle C_t$ = $C_t$ gene of interest – $C_t$ GAPDH) and reported as relative mRNA expression ($\triangle\triangle C_t$ = $2^{\triangle C_t}$ sample–$\triangle C_t$ control) or the fold-change.

#### Flow cytometry

The cells in each group were collected into FACS tube (352063, BD) and washed in ice-cold phosphate-buffered saline (PBS) twice. THP-1 and PMA-differentiated THP-1 cells were stained with fluorescein isothiocyanate (FITC)-conjugated CD14 (555397, BD Biosciences, Franklin Lakes, NJ, USA) and phycoerythrin (PE)-conjugated TLR4 (564215, BD Biosciences) for 1 h at room temperature. The stained cells were analyzed by flow cytometry (cytoFLEX, A00-1-1102, Beckman Coulter, Brea, CA, USA) and software (CytExpert, Beckman Coulter). For each sample, the count in 10,000 cells was measured. The region of each sample was selected in the forward scatter and side scatter, and then a histogram was used to measure the mean fluorescence intensity of the FITC or PE, which represented the CD14 or TLR4, respectively. FITC-rat IgG2a (BD, 555843) and PE-mouse IgG1 (BD, 559320) were used as isotype controls.

#### ELISA

Cells were seeded at 1 × 10⁶ cells/well in 6-well culture plates and stimulated with 10 ng/mL LPS in serum-deprived culture media for 24 h. The cell culture supernatants were collected by centrifugation at 2000 rpm for

### Table 1. The primers for real-time PCR.

| Genes     | Sequence (5’ to 3’)   |
|-----------|-----------------------|
| GAPDH     | Forward: CCACCTCTCCACCTTTGAC Revers: ACCCTGTTGCTTGAGCCA |
| IL-1β     | Forward: AGCCTGATGGCCCTAAACAGA Revers: TCGGAGATTTGAGTGAGAT |
| TNFα      | Forward: AACCTCCCTCTGACATCAAA Revers: GGAAGACCCCTCCAGATAG |
| IL-6      | Forward: AAAAGAGGCACTGGCAAGAAA Revers: TTCTCCAGGCAAAGTCTCCT |
| CD14      | Forward: TTAGCTTGCAACCTTTCCAGAG Revers: CTTTTAGGCCACCTTCCAGAG |
| TLR4      | Forward: ATGTCCTGCAACCTCTTCGTTG Revers: GGATGACAAACTCCAAGCAA |
| MD-2      | Forward: TATTGTCGGAGATGATGAGT Revers: GGATGACAAACTCCAAGCAG |
| LBP       | Forward: ATTCCTCCGAACTTTCCAGAG Revers: GGAATGTGACTTCCAGACCA |
| MyD88     | Forward: GGAATGGTGACTTCCAGACCA Revers: GATGCTGCCAGAACTTTCC |
| TIRAP     | Forward: ATGTCCTCAGCCTACCTCTCA Revers: TCTTTGCAGTCAGGACTACT |
| TRAM      | Forward: AAGGCTCTCAGGCTACCTCAAA Revers: GTCATGCAAGACCACTTAC |
| TRIF      | Forward: CGCCACTCCACCTTTCTGTA Revers: TCTGTTCGGATGATGATC |

- **GAPDH Forward:** CCACCTCTCCACCTTTGAC
  **GAPDH Reverse:** ACCCTGTTGCTTGAGCCA
- **IL-1β Forward:** AGCCTGATGGCCCTAAACAGA
  **IL-1β Reverse:** TCGGAGATTTGAGTGAGAT
- **TNFα Forward:** AACCTCCCTCTGACATCAAA
  **TNFα Reverse:** GGAAGACCCCTCCAGATAG
- **IL-6 Forward:** AAAAGAGGCACTGGCAAGAAA
  **IL-6 Reverse:** TTCTCCAGGCAAAGTCTCCT
- **CD14 Forward:** TTAGCTTGCAACCTTTCCAGAG
  **CD14 Reverse:** CTTTTAGGCCACCTTCCAGAG
- **TLR4 Forward:** ATGTCCTGCAACCTCTTCGTTG
  **TLR4 Reverse:** GGATGACAAACTCCAAGCAA
- **MD-2 Forward:** TATTGTCGGAGATGATGAGT
  **MD-2 Reverse:** GGATGACAAACTCCAAGCAG
- **LBP Forward:** ATTCCTCCGAACTTTCCAGAG
  **LBP Reverse:** GGAATGTGACTTCCAGACCA
- **MyD88 Forward:** GGAATGGTGACTTCCAGACCA
  **MyD88 Reverse:** GATGCTGCCAGAACTTTCC
- **TIRAP Forward:** ATGTCCTCAGCCTACCTCAAA
  **TIRAP Reverse:** TCTTTGCAGTCAGGACTACT
- **TRAM Forward:** AAGGCTCTCAGGCTACCTCAAA
  **TRAM Reverse:** GTCATGCAAGACCACTTAC
- **TRIF Forward:** CGCCACTCCACCTTTCTGTA
  **TRIF Reverse:** TCTGTTCGGATGATGATC
10 min and cytokine levels were detected using specific ELISA kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer’s instructions.

**Western blotting**

Protein concentration was determined by the BCA assay (23225, Thermo Fisher Scientific, Waltham, MA, USA). Proteins (1 μg/well) were added to 2 × Laemmlı sample buffer (1610737, Bio-Rad, Hercules, CA, USA). Samples were separated by electrophoresis (041BR89916, Bio-Rad) on 4–20% Bis-Tris polyacrylamide gels (456-1093, Bio-Rad), and the proteins were transferred to a polyvinylidene fluoride membrane (1620177, Bio-Rad). The membranes were blocked in PBST/5% skim milk (232100, BD Biosciences) for 1 h at room temperature. Primary and secondary antibodies (Abs) were diluted at appropriate concentrations for blotting. Quantitation and imaging of the western blots were performed using a Bio-Rad imaging system (ChemiDoc MP) following the manufacturer’s instructions.

**Antibody and reagents**

For ELISA, flow cytometry, and western blotting, the following materials were used: IL-1β (DLB50, R&D Systems), IL-6 (D6050, R&D Systems), TNFα (DTA00D, R&D Systems), Anti-Rabbit IgG – HRP (1:10000, PI-2000, Vector), FITC-rat IgG2a (555843, BD Biosciences), PE-mouse IgG1 (559320, BD Biosciences), MyD88 (1:5000, ab133739, Abcam, Cambridge, UK), TRIF (1:1000, ab13810, Abcam), p-p38 (1:1000, ab4822, Abcam), p-p38 (1:1000, ab124956, Abcam), p-ERK1/2 (1:5000, ab201015, Abcam), p-NF-κB (1:1000, 3033, Cell Signaling Technology, Danvers, MA, USA), and β-actin (1:5000, A1978, Sigma).

**Statistical analysis**

All results are presented as the mean ± standard deviation (SD). All experiments were performed three or more times. Statistical significance was determined by Student’s t-test (two-tailed) or analysis of variance using GraphPad Prism 8 software (GraphPad, Inc., La Jolla, CA, USA). Values of *p < .05, **p < .005, ***p < .001, and ****p < .0001 were considered to indicate significant results.

**Result**

**Differences in the induction of pro-inflammatory cytokines between THP-1 cells and PMA-differentiated THP-1 cells**

A previous study showed that the expression of pro-inflammatory cytokines differs between THP-1 cells and PMA-differentiated THP-1 cells. To further investigate whether these differences depend on the LPS concentration in THP-1 cells and PMA-differentiated THP-1 cells, the expression levels of mRNA and protein were analyzed following treatment with 0, 1, 10, 100, and 1000 ng/mL LPS for 24 h. The mRNA expression levels of IL-1β, IL-6, and TNFα showed greater upregulation in PMA-differentiated THP-1 cells than in THP-1 cells after LPS treatment (Figure 1(a)–(c)). In addition, the protein expression levels of these cytokines were significantly higher in PMA-differentiated THP-1 cells than in THP-1 cells at all doses (Figure 1(d)–(f)). Cytokines released by THP-1 cells and PMA-differentiated THP-1 cells increased in a dose-dependent manner at each time point. Except for IL-6, IL-1β and TNFα were basally released from PMA-differentiated THP-1 cells but not from THP-1 cells. These data demonstrate that PMA-differentiated THP-1 cells are more susceptible than THP-1 cells to the effects of LPS.

**The mRNA expression of LPS-related-signaling molecules in THP-1 cells and PMA-differentiated THP-1 cells**

To investigate whether the differential expression of LPS-related-receptors causes a difference in LPS susceptibility between THP-1 cells and PMA-differentiated THP-1 cells, these cells were treated with 10 ng/mL LPS for 24 h. Compared with those of THP-1 cells, the mRNA levels of LPS-related-receptors, CD14, TLR4, and MD-2, were upregulated in PMA-differentiated THP-1 cells in the presence and absence of LPS, whereas those of LBP did not significantly differ (Figure 2(a)–(d)). We also found that the mRNA levels of TLR4 signal adapter proteins, MyD88, TIRAP, TRAM, and TRIF, were higher in PMA-differentiated THP-1 cells than in THP-1 cells (Figure 2(e)–(h)). This finding suggests that PMA-differentiated THP-1 cells have a greater capacity for LPS signaling than THP-1 cells.

**Higher capacity for LPS-affinity-effect in PMA-differentiated THP-1 cells than in THP-1 cells**

To determine whether THP-1 macrophages have larger amounts of LPS-related-receptors and intracellular proteins, CD14 and TLR4 were characterized by flow cytometry in THP-1 cells and PMA-differentiated THP-1 cells with or without LPS. Cell surface expression of CD14 and TLR4 was higher in PMA-differentiated THP-1 cells than in THP-1 cells. Additionally, CD14 expression was lower in macrophages treated with LPS than in macrophages not treated with LPS (Figure 3(a)). Western blot analysis showed that compared with that in THP- cells, MyD88 expression was remarkably upregulated in THP-1 macrophages; TRIF expression showed no dynamic change
Taken together, these data demonstrate that the difference in LPS sensitivity between THP-1 cells and PMA-differentiated THP-1 cells occurs because of a mount of the expression level of CD14, TLR4 and MyD88. MAPK and NF-κB pathway-related molecules were more activated by MyD88 axis in PMA-differentiated THP-1 cells than in THP-1 cell.

LPS signaling leads to the activation of MAPK and NF-κB pathways in monocytes and macrophages. To investigate whether the phosphorylation of the MAPK and NF-κB pathway-related molecules is more active in THP-1 cells or PMA-differentiated THP-1 cells, the cells were treated with 10 ng/mL LPS for different times, and the phosphorylation of p38 (p-p38), JNK (p-JNK), and ERK (p-ERK) was analyzed by immunoblotting. The MAPK pathway was activated starting at 15 min, and pathway-related molecules were more phosphorylated in PMA-differentiated THP-1 cells than in THP-1 cells (Figure 4(a)). NF-κB was phosphorylated by LPS stimulation in THP-1 cells and PMA-differentiated THP-1 cells, and this increase was initiated at 15 min in both cells (Figure 4(b)). To investigate whether MyD88 induces differential susceptibility to LPS between THP-1 cells and PMA-differentiated THP-1 cells, MyD88−/− THP-1 cells were employed. MyD88 deletion was detected in MyD88−/− THP-1 cells and PMA-differentiated THP-1 cells and the LPS receptors CD14 and TLR4

Figure 1. LPS-induced mRNA and protein expression levels in THP-1 cells and PMA-differentiated THP-1 cells. The cells were treated with LPS 0, 1, 10, 100, and 1000 ng/mL for 24 h. mRNA expression levels of (a) IL-1β, (b) TNFα, and (c) IL-6, and protein expression levels of (d) IL-1β, (e) IL-6, and (f) TNFα were analyzed by qPCR and ELISA, respectively. Mean values represent the mean ± SD of three independent experiments. * p ≤ .05, ** p ≤ .005, *** p ≤ .001, and **** p ≤ .0001, nd: Not detected.

Figure 2. Increased mRNA expression of LPS-signaling-related molecules in PMA-differentiated THP-1 cells versus THP-1 cells. The cells were treated with LPS 10 ng/mL for 24 h or not treated. mRNA expression levels of (a) CD14, (b) LBP, (c) MD-2, (d) TLR4, (e) MyD88, (f) TIRAP, (g) TRAM, and (h) TRIF were analyzed by qPCR. Mean values represent the mean ± SD of three independent experiments. * p ≤ .05, ** p ≤ .005, *** p ≤ .001, and **** p ≤ .0001, ns: Not significant.
Figure 3. Up-regulated protein expression of LPS-signaling-related molecules in PMA-differentiated THP-1 cells compared with that in THP-1 cells. (a) Expression levels of CD14 and TLR4 were analyzed by flow cytometry. Black: Isotype control, red: CD14, blue: TLR4. (b) MyD88 and TRIF in THP-1 cells and PMA-differentiated THP-1 cells were detected by western blotting. Mean values represent the mean ± SD of three independent experiments. * p ≤ .05, ** p ≤ .005, *** p ≤ .001, and **** p ≤ .0001, ns: Not significant, #compared to the untreated group.

Figure 4. Comparison of LPS-induced MAPK and NF-κB pathway-related molecule phosphorylation in THP-1 cells and PMA-differentiated THP-1 cells. Representative immunoblots and quantification of (a) phosphorylation of p38, JNK and ERK, (b) NF-κB, and (c) p38, JNK, and ERK and (d) NF-κB in MyD88−/− cells. Mean values represent the mean ± SD of three independent experiments. * p ≤ .05, ** p ≤ .005, *** p ≤ .001, and **** p ≤ .0001, #compared to the non-treated group. T: THP-1, P: PMA-THP-1.
were more preserved in MyD88−/− PMA-differentiated THP-1 cells than in MyD88−/− THP-1 cells (Figure S1). This suggests that MyD88−/− THP-1 cells have similar levels of surface molecules, CD14 and TLR4, as WT THP-1 cells. In contrast to WT THP-1 cells and PMA-differentiated THP-1 cells, the MAPK and NF-κB pathways were abolished in MyD88−/− THP-1 cells and PMA-differentiated THP-1 cells (Figure 4(c) and (d)). These results suggest that MyD88 induces differences in LPS sensitivity between THP-1 cells and PMA-differentiated THP-1 cells through sequential signaling, MAPKs and NF-κB pathway.

Discussion

In order to study how LPS susceptibility differs between monocytes and macrophages, we investigated THP-1 cells, human leukemia monocytic cell line. It is frequently used in different studies.14 Human primary monocytes secreted higher amount of IL-1β, TNFα, and IL-6 than THP-1 cells because of the upregulation of CD14 in monocytes.15,16 However, the tendency of signal transduction of MAPK and NF-κB pathway between monocytes and THP-1 cells is comparable despite the difference in the amount of pro-inflammatory cytokine secretion by LPS.17 In the case of primary macrophage and PMA-differentiated THP-1 cells, it was reported that cell viability and the level of cytokine expression were comparable when TLR4 signal induction was performed with *Mycobacterium tuberculosis*.18 Macrophages and PMA-differentiated THP-1 cells showed a close relation as a result of gene expression profile and CD surface marker expression analysis.19

PMA is one of the molecules used to differentiate THP-1 cells into macrophages as a model of human macrophages. PMA has been used in studies at various concentrations. This study was conducted at 10 ng/ml of PMA, and the concentration was set based on other studies that CD14 expression was sufficiently induced, and that more than 90% of cells were attached and spread.20,21 When LPS was treated at different concentration, it was found that inflammation-related molecules increased in a LPS dose-dependent manner.20 In addition, when macrophage polarization was induced after obtaining PMA-differentiated THP-1 cells with 100 ng/ml of PMA, M2 macrophage polarization was not sufficiently induced.22 However, 10 ng/ml of PMA induced M2 macrophage polarization.23 Based on these studies, we used 10 ng/ml of PMA to implement the state of M0 macrophage in PMA-differentiated THP-1 cells. PMA has the effect of inducing cytokine release in THP-1 cells on their own. CD14 and TNFα increase in a time-dependent manner when THP-1 cells are continuously treated with PMA23,24 through protein kinase C and NF-κB signaling activated by PMA.25 In order to block constant PMA stimulation, the differentiation media of THP-1 cells was replaced with normal media for a day. This study was carried out by selecting the treatment of PMA 24 hours with a high cell collection rate to accurately compare LPS sensitivity between THP-1 cells and PMA-differentiated THP-1 cells.21

In this study, levels of pro-inflammatory cytokines IL-1β, TNFα, and IL-6 increased in a dose-dependent manner in THP-1 cells and PMA-differentiated THP-1 cells (Figure 1). However, LPS had no dose-dependent effect on the mRNA expression levels of TNFα (Figure 1(b)). Fundamental biological differences can occur between transcription and translation processes.26 Cytokine expression at the mRNA and protein levels was consistent with previous results.10,12 Accordingly, PMA-differentiated THP-1 cells are more sensitive to LPS compared to THP-1 cell, resulting in higher release of cytokines for LPS stimulation.

In previous studies, reactions to inflammation, injury, and repair and populations of TLRs were found to differ between monocytes and macrophages.27,28 As shown in Figures 2 and 3, the levels of LPS-related-receptors present on the cell surface and intracellular adaptor proteins, MyD88 and TRIF, were higher on PMA-differentiated THP-1 cells than on THP-1 cells, except for LBP. It has...
been predicted that LPS transfer does not differ between THP-1 cells and PMA-differentiated THP-1 cells; however, the quantity of receptors and intracellular proteins, particularly MyD88, are more conserved in macrophages. As a result of confirming TLR4 expression patterns of tissue-resident macrophages and monocytes, monocytes expressed more TLR4 in the intestine and lung, but monocyte-derived macrophages expressed more TLR4.\textsuperscript{11,29,30} These results are thought to be potential differences due to the involvement of other transcription factors responsible for immunity and tissue supporting activities of tissue-resident macrophages in terms of differentiation.\textsuperscript{31} As shown in Figure 3(a), the surface protein CD14 was downregulated in LPS-treated-PMA-differentiated THP-1 cells, as reported in a previous study in which the expression of CD14 was decreased in macrophages exposed to LPS in vivo and in vitro.\textsuperscript{32} It could be caused that the ERK pathway controls the expression of membrane CD14 on macrophages.\textsuperscript{33} Therefore, our data suggest that the difference in LPS susceptibility between THP-1 cells and PMA-differentiated THP-1 cells occurs because of altered expression of LPS-related receptors and MyD88.

The MyD88-dependent pathway is more essential than the MyD88-independent pathway for LPS-induced cytokine production in immune cells.\textsuperscript{34} In addition, pro-inflammatory cytokines generated by LPS-TLR4 signaling are regulated by the MAPK and NF-κB pathways.\textsuperscript{35,36} To confirm that LPS susceptibility was dependent on MyD88 expression, the MAPK and NF-κB pathways involved in the transcription of pro-inflammatory cytokines induced by TLR4 signal were evaluated (Figure 4). Although phosphorylation of NF-κB occurs comparably in both cells, the initiation of NF-κB activation is more active in PMA-differentiated THP-1 cells than THP-1 cells. Additionally, MAPK pathway-related molecules were more highly phosphorylated in PMA-differentiated THP-1 cells than in THP-1 cells. We used MyD88\textsuperscript{-/-} THP-1 cells to confirm that MyD88 is a key molecule inducing differential susceptibility to LPS in TLR4 signaling. In a previous study, activation of the MAPK and NF-κB pathways was abrogated in MyD88\textsuperscript{-/-} mice.\textsuperscript{37} In our study, the MAPK and NF-κB pathways were inactivated in MyD88\textsuperscript{-/-} THP-1 cells and PMA-differentiated THP-1 cells. Therefore, the MAPK and NF-κB pathways show greater activation in PMA-differentiated THP-1 cells than in THP-1 cells in a MyD88-dependent manner.

This study focus on the expression of TLR4 between THP-1 cells and PMA-differentiated THP-1 cells. THP-1 cells have limitations in fully implementing monocyte functions or responses in vivo. Also, PMA-differentiated THP-1 cells did not correctly represent primary macrophages. To overcome short lifespan and individual variation that provoke limited studies, THP-1 cells frequently were employed in research.\textsuperscript{14} THP-1 cells were considered as a tool of human macrophages.\textsuperscript{38} In addition to differences in LPS sensitivity, the expression patterns of pattern recognition receptors (PRRs), including other TLRs, differ from cell to cell.\textsuperscript{30,39,40} Identification of PRRs expression that differ from cell to cell is worth studying to precisely target and develop the therapy for sepsis or other diseases that arise from innate immunity.

Conclusion
We observed differentiation in sensitivity to LPS between THP-1 cells and PMA-differentiated THP-1 cells. PMA-differentiated THP-1 cells showed higher levels of LPS-related surface receptors and MyD88. In addition, activation of MAPKs and NF-κB pathways caused by differences in MyD88 expression induced differential expression of pro-inflammatory cytokines (Figure 5). Therefore, LPS susceptibility between THP-1 cells and PMA-differentiated THP-1 cells differs, because of differences in the expression levels of LPS-related receptors and activation of MyD88-dependent pathways.

Declaration of conflicting interests
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Supplemental material
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