Indonesian Journal of Medical Laboratory Science and Technology
Open Access
p-ISSN 2684-6748
e-ISSN 2656-9825

RESEARCH ARTICLE

# The baseline levels of receptors and cytokines in phorbol 12-myristate 13-acetate-induced THP-1 monocyte to macrophage cells

Sri Wulandari [1], Tri Agusti Sholikah [2], Sinu Andhi Jusup [1]

[1]Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
[2]Department of Histology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

*Correspondence:*
Sri Wulandari
Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret,
Ir. Sutami Street 36 Kentingan, Jebres,
Surakarta, Central Java – 57126, Indonesia

Email: rajsriwulandari@staff.uns.ac.id

*Article history:*
Received: 2025-08-15
Revised: 2026-01-13
Accepted: 2026-01-20
Available online: 2026-0428

*Keywords:*
Advanced glycation end-product
Macrophages
Phorbol 12-myristate 13-acetate
THP-1
Toll-like receptors

https://doi.org/10.33086/ijmlst.v8i1.7917

## Abstract

Monocyte-derived macrophages regulate various aspects of inflammation. Phorbol 12-myristate 13-acetate (PMA)-induced THP-1 monocytes are widely used as monocyte-derived macrophage models suitable for studying the pathophysiology of inflammation and developing therapies. However, there is insufficient data concerning the baseline expression of pro-inflammatory receptors and the associated cytokine levels in THP-1-derived macrophage-like cells compared with their monocyte precursors. In this study, PMA-treated THP-1 cells were employed as models for monocyte-derived macrophage-like cells (M0). The mRNA levels of Toll-like receptors (TLR)4, receptor for advanced glycation end-product (RAGE), and TLR7 in THP-1 cells, both with and without PMA treatment, were evaluated using quantitative real-time polymerase chain reaction (RT-qPCR). ELISA was performed to quantify IL-6, TNF-α, and IL-18 levels in the THP-1 culture supernatant. The mRNA expression of TLR4, RAGE, and TLR7 was increased twofold in PMA-induced THP-1-derived macrophage-like cells compared to monocytes. IL-6 ($p = 0.0277$) and TNF-α ($p = 0.0105$) levels were significantly elevated in the culture supernatants of THP-1-derived macrophages. However, IL-18 levels did not show a significant increase. The upregulation of TLR4, RAGE, and TLR7 transcription was accompanied by elevated secretion of IL-6 and TNF-α during PMA-induced differentiation of THP-1-derived macrophage-like cells (M0), highlighting the polarization state and the specific role of macrophages in producing pro-inflammatory cytokines. This study provides baseline data on pro-inflammatory receptor expression and cytokine production in the THP-1-derived macrophage-like cells model for inflammatory research.

## 1. INTRODUCTION

Monocytes and macrophages are fundamental in regulating all phases of inflammation. Under inflammatory conditions, circulating monocytes in the bloodstream are recruited to the tissue by cytokines and chemokines released by damaged and endothelial cells, where they further differentiate into monocyte-derived macrophages. The balance between tissue-resident and monocyte-derived macrophages is consistently maintained to ensure tissue homeostasis (1). Disrupting this balance may adversely affect disease progression. Monocyte-derived macrophages, characterized by their highly inflammatory features, dominate the cell population in the bronchoalveolar lavage fluid of patients with severe COVID-19 (2). They also promote low-grade inflammation in diabetes mellitus, and macrophage-induced inflammation is a significant factor in the progression of both insulin resistance and hyperinsulinemia (3).

The activation of monocytes and macrophages is initiated when pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) interact with their specific pattern recognition receptors (PRRs) on the cell surface. PAMPs are exogenous molecules, such as nucleic acids, lipopolysaccharides, flagellin, and peptidoglycan, produced by pathogens as part of their life cycle. In contrast, DAMPs are self-molecules of the host, including heat shock protein, high mobility group box 1, and ATP, which are liberated into the extracellular space during various forms of cell

**Citation:** Wulandari S, Sholikah TA, Jusup SA. The baseline levels of receptors and cytokines in phorbol 12-myristate 13-acetate-induced THP-1 monocyte to macrophage cells. Indones J Med Lab Sci Technol. 2026;8(1):24–32. https://doi.org/10.33086/ijmlst.v8i1.7917

death (4). Change in the metabolic homeostasis of lipids, amino acids, glucose, and nucleic acids can also lead to the formation of DAMPs, which in turn trigger specific PRRs and provoke an inflammatory response (5).

Toll-like receptors (TLRs) are a family of PRRs that recognize DAMPs and PAMPs, triggering the secretion of interferon, chemokines, cytokines, and antimicrobials as part of the host's first line of defense. Ten human TLRs have been identified and categorized by their function and localization into cell surface and intracellular TLRs (6). Cell surface TLR4 is a crucial receptor, capable of inducing both myeloid differentiation primary response gene 88 (MyD88)-dependent and independent signaling pathways, underlining its relevance in both infectious and non-infectious inflammation (7). TLR7 is an endosomal receptor that functions as part of the nucleic acid sensor system, recognizing fragments of single-stranded RNA (ssRNA) from viruses, bacteria, fungi, and protozoa (8). It can also detect self-derived ssRNA, playing a fundamental part in the pathophysiology of systemic lupus erythematosus (SLE) (9) and Parkinson's disease (10). TLR7 activation prompts the release of inflammatory cytokines regulated by NF-κB and interferons, further activating various cells to sustain inflammation (11).

The receptor for advanced glycation end-product (RAGE) is a member of the immunoglobulin superfamily that functions as a PRR. It serves as a ligand for AGEs, harmful molecules formed when reducing sugars reacts with proteins, lipids, or DNA. RAGE activation leads to heightened inflammation and accelerates the progression of vascular complications in neurological disease, diabetes, cardiovascular disease, and cancer (12,13). The lungs exhibit the highest expression of RAGE (14), underscoring its impact on the pathophysiology of COVID-19 (15-17).

There are considerable discrepancies regarding TLR expression in human primary monocytes and macrophages. TLR4 expression has been reported to be significantly higher in monocyte-derived macrophages compared to their precursors; however, other studies have found no such differences. Similar inconsistencies have been observed for TLR2 and TLR9. These variations are likely due to differences in the conditions used to induce monocytes to differentiate into macrophages (18,19). RAGE is upregulated in macrophages; however, differences in expression levels between monocytes and macrophages remain undefined (20).

Primary monocyte cells isolated from human blood serve as an ideal model for studying inflammation. However, they present several challenges: each experiment requires fresh specimens; they cannot be stored long-term; and their genetic diversity is wide, as they originate from multiple donors. As an alternative, the immortalized THP-1 cell line, originally derived from a patient with acute monocytic leukemia, is commonly used for investigating pathways and functions associated with human monocytes and macrophages. These cells can be cultured in vitro, conveniently preserved in liquid nitrogen, and exhibit a genetically consistent background. The genetic stability of THP-1 cells offers the advantage of minimizing individual variability in various experiments (21).

Exposure to phorbol 12-myristate 13-acetate (PMA) induces the conversion of THP-1 monocyte cells into macrophage-like cells (M0), which can subsequently be polarized into inflammatory macrophages (M1) or anti-inflammatory macrophages (M2) models (22,23). Exposure to PMA, followed by a PMA-free rest period, induces THP-1-derived macrophage-like cells that exhibit morphology, organelles, surface markers, phagocytic capacity, and cytokine patterns resembling those of human monocyte-derived macrophages (24). PMA concentrations ranging from 5 to 100 ng/ml have been shown to induce differentiation with minimal toxicity (25). Previous studies have characterized the expression of specific markers in PMA-differentiated THP-1 cells, including surface markers cluster of differentiation (CD)14, CD35, CD36, CD55, CD59, CD163, and CD204 (24,26,27).

However, data regarding the expression of TLRs and RAGE in PMA-induced THP-1 macrophage-like cells (M0) compared to undifferentiated THP-1 monocytes remains limited. Studies have reported receptor and cytokine expression in PMA-induced THP-1 cells under stimulated conditions, typically using LPS (27,28). Establishing receptor expression under unstimulated conditions is not only essential for understanding the differentiation of monocytes into macrophages (M0) but also serves as a baseline reference for detecting receptor and cytokine upregulation or downregulation in response to various stimuli. This study followed a previously validated protocol (27), which utilized a short period of PMA exposure followed by a rest period to induce THP-1 differentiation effectively. This protocol has been shown to produce THP-1-derived macrophage-like cells with optimal innate immune and inflammatory responses. Using this approach, we evaluated the baseline mRNA expression of key receptors involved in inflammatory signaling in both monocytes and macrophages, with TLR4 and RAGE representing surface receptors and TLR7 representing intracellular (endosomal) receptors. The production of cytokines interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-18 as downstream effectors of receptor activation was also assessed. IL-6, TNF-α, and IL-18 are key pro-inflammatory cytokines produced by monocytes and macrophages whose elevated levels reflect increased inflammatory activity. IL-18 is produced as an inactive precursor (pro-IL-18) and requires inflammasome activation for maturation and secretion (4). This study aimed to provide valuable data for the development of monocyte and macrophage-like cells (M0) models using THP-1 cells. Specifically, TLR4, TLR7, and RAGE mRNA expression, along with IL-6, TNF-α, and IL-18 levels, were compared between undifferentiated THP-1 monocytes and PMA-induced THP-1 macrophage-like cells (M0) without further stimulation. Without baseline data on receptor mRNA expression and cytokine levels, it would be difficult to distinguish whether observed effects are attributable to external stimuli or merely reflect changes induced by monocyte differentiation into macrophages.

# 2. MATERIALS AND METHODS

## 2.1. THP-1 Cell Culture and Differentiation

Human monocytic THP-1 cells, obtained from the European Collection of Authenticated Cell Cultures (ECACC), were cultured in T25 flasks with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 µg/mL Primocin® (Invivogen, San Diego, CA) at 37 °C in a 5% $CO_2$ atmosphere. Cell density was maintained between 3 and 8 × $10^5$ cells/mL to ensure cells were in the exponential growth phase at the time of harvest. THP-1 monocytes and THP-1-derived macrophage-like cells (5 × $10^5$ cells/well) were seeded into 24-well plates in 2 mL of complete medium. Following seeding, the medium was refreshed and cells were incubated for 24 hours prior to treatment. PMA-induced differentiation was performed as previously described (27) with minor modifications: differentiation into THP-1-derived macrophage-like cells (M0) (2.5 × $10^5$ cells/mL) was induced using 62.5 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich, St. Louis, MO) for 16 hours, followed by a 48-hour resting phase in PMA-free medium. As the THP-1 macrophage-like cells would serve as a negative control for subsequent treatments, cells were maintained in fresh medium for an additional 24 hours before harvesting to allow adequate equilibration. Before adding fresh medium, cells were washed with PBS to remove residual PMA and cell debris. Differentiation of monocytes into macrophage-like cells (M0) was confirmed by visual inspection using an inverted microscope, as evidenced by the transition from a round, suspension morphology to an adherent, spread morphology characteristic of macrophage-like cells. The experiment was performed in three independent biological replicates, each conducted in duplicate.

## 2.2. Real-time PCR

Total RNA from THP-1 monocytes and THP-1-derived macrophage-like cells was extracted using the rSYNC™ RNA Isolation Kit (Geneaid, Shijr, New Taipei City, Taiwan) following the manufacturer's protocol. RNA concentration and purity were determined using a NanoDrop spectrophotometer, and samples with a 260/280 absorbance ratio between 1.8 and 2.1 were considered acceptable for downstream analysis. First-strand cDNA was synthesized from 500 ng of total RNA using the Excel™ Reverse Transcription Kit II (Smobio, Hsinchu City, Taiwan). A 20 µLreaction mix was prepared using the SensiFAST™ SYBR® Lo-ROX Kit (#BIO-94005, Bioline, London, UK) with the following components: cDNA template (4.4 µL), 2× SensiFAST™ SYBR® Lo-ROX mix (10 µL), forward primer (0.8 µL/10 µM), reverse primer (0.8 µL/10 µM), and $H_2O$ (4 µL). Reactions were performed using an Agilent Technologies Stratagene Mx3005P real-time PCR system. Real-time quantitative polymerase chain reaction (real-time qPCR) was performed using the following thermal cycling programme: initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of 95 °C for 5 seconds, 60 °C for 11 seconds (for GAPDH and TLR4) or 62 °C for 11 seconds (for RAGE and TLR7), and 72 °C for 19 seconds. A no-template control (NTC) was included in every experiment to detect potential contamination. The cycle threshold (Ct) values were analysed using the 2^−ΔΔCt method (29). Primer amplification efficiencies ranged from 90% to 110% for all target and housekeeping gene (GAPDH). The primers used in this study, based on previously published sequences (26-29), are presented in Table 1.

## 2.3. ELISA

Cell supernatants were collected by centrifugation, re-centrifuged at 5000 rpm for 2 minutes, and stored at –80 °C until analysis. Cell supernatants were diluted with complete medium before measuring IL-6 (1:100), TNF-α (1:100), and IL-18 (1:4). The levels of proinflammatory cytokines IL-6, IL-18, and TNF-α in THP-1 monocyte and THP-1-derived macrophage-like cells supernatants were measured using ELISA, following the manufacturer's protocol (FineTest, Wuhan, China). The detection range for the human IL-6 kit was 4.688–300 pg/mL, whereas for the human IL-18 and human TNF-α kits, it was 15.625–1000 pg/mL. All kits demonstrated intra-assay precision with CV < 8% and inter-assay precision with CV < 10%. Cytokine concentrations were determined from ELISA absorbance readings using MyAssay Analysis Software (http://www.myassays.com) with a four-parameter logistic (4PL) curve fit.

## 2.4. Statistical Analysis

All data were analyzed using Stata 17.0. TLR4, RAGE, and TLR7 mRNA levels were normalized to GAPDH as an endogenous control. For statistical analyses, n refers to three independent biological replicates, each conducted in duplicate. A normality test performed on the qPCR data (ΔΔCt values) yielded $p > 0.05$, indicating that the data were normally distributed. A paired t-test was conducted to compare mRNA expression in PMA-induced THP-1-derived macrophage-like cells relative to their monocyte precursors, using the ΔΔCt method. The relative expression levels (2^−ΔΔCt) are presented graphically, with monocyte expression levels set to 1. IL-6, TNF-α, and IL-18 concentrations were tested for normality using the Shapiro–Wilk test. A paired t-test was used for normally distributed data, and the Wilcoxon signed-rank test was applied to non-normally distributed data. All graphs were generated using GraphPad Prism 8.0.2 and display data as mean ± standard error of the mean (SEM). A p-value $< 0.05$ was considered statistically significant.

**Table 1.** The primers used for real-time PCR

| Gene | Forward | Reverse | References |
|---|---|---|---|
| GAPDH | GAAGGTGAAGGTCGGAGTC | GAAGATGGTGATGGGATTTC | (26) |
| TLR4 | AAGCCGAAAGGTGATTGTTG | CTGAGCAGGGTCTTCTCCAC | (27) |
| RAGE | CCCTGCTCATTGGGGTCATC | GTACTACTCTCGCCTGCCTC | (28) |
| TLR7 | GCTCTGTGGGAGTTCTGTCC | ACCGTTTCCTTGAACACCTG | (29) |

## 3. RESULTS AND DISCUSSION

*Ex vivo*, fully functional monocyte-derived macrophages can be obtained during the differentiation phase, when monocytes transform into macrophages (M0), and the activation phase, when monocyte-derived macrophages undergo polarization into pro-inflammatory M1 and anti-inflammatory M2 macrophages (1). In this study, we aimed to establish baseline data for receptors TLR4, TLR7, and RAGE, as well as the downstream cytokines IL-6, IL-18, and TNF-α, during the differentiation phase of PMA-induced THP-1 cells into macrophage-like cells (M0). Baseline data provide a reference point for accurately measuring changes in receptor expression and related cytokines upon stimulation, thereby avoiding misinterpretation with changes that occur during monocyte-to-macrophage differentiation.

### 3.1. PMA-Induced THP-1 Cells Exhibited Macrophage-Like Characteristics

To facilitate the differentiation of THP-1 cells into macrophage-like cells, the cells were treated with 62.5 ng/mL PMA for 16 hours, followed by a 48-hour resting period in PMA-free medium and an additional 24 hours in fresh medium. Differentiation into THP-1-derived macrophage-like cells was characterized by cell adherence to the bottom of the well, an elongated morphology, and increased cytoplasmic volume. These features are consistent with those previously reported (27) (Figure 1B).

### 3.2. mRNA Expression Levels of TLR4, RAGE, and TLR7 in THP-1-Derived Macrophages Compared with Monocytes

To gain further insight into the expression of specific receptors in THP-1-derived macrophage-like cells, the mRNA levels of TLR4, RAGE, and TLR7 in PMA-induced THP-1 macrophage-like cells (M0) were compared to those of their monocytic precursors. The mRNA expression of the three receptors observed in THP-1-derived macrophage-like cells showed a trend toward increased expression relative to their monocyte precursors, though this increase did not reach statistical significance. Following PMA stimulation, THP-1-derived macrophage-like cells exhibited a 2.1-fold increase in TLR4 mRNA levels ($p = 0.1495$), a 2.3-fold increase in RAGE mRNA levels ($p = 0.1525$), and a 2.4-fold increase in TLR7 mRNA levels ($p = 0.1616$). Overall, TLR4, RAGE, and TLR7 transcription was elevated during the differentiation of PMA-induced THP-1 monocytes into macrophage-like cells (M0) (Figure 2). The average Ct values of genes observed in this study are shown in Table 2.

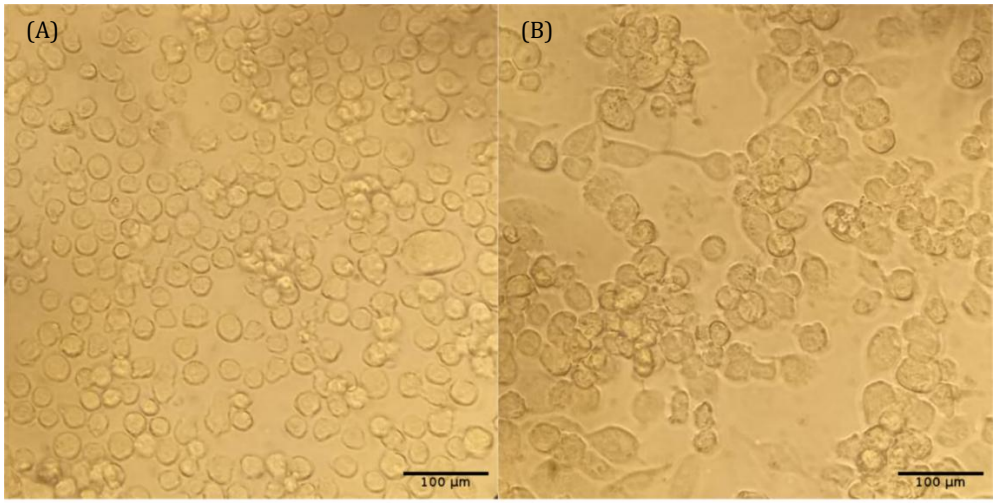


**Figure 1.** Differentiation of THP-1 cells into macrophage-like cells. (A) THP-1 cells. (B) PMA-induced THP-1-derived macrophage-like cells. The THP-1-derived macrophage-like cells demonstrated enhanced attachment to the bottom of the well and an increase in cytoplasmic volume

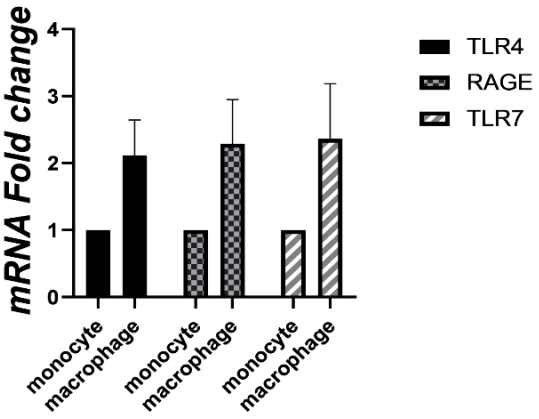


**Figure 2.** Level of mRNA expression of TLR4, RAGE, and TLR7 in THP-1-derived macrophage-like cells and THP-1 monocytes. Statistical analysis was performed using a paired t-test, comparing receptor mRNA expression in THP-1-derived macrophage-like cells relative to monocytes (ΔΔCt). Data are presented as the relative expression ratio (2^−ΔΔCt), with monocyte expression set to 1. Results are shown as mean ± SEM.

**Table 2**. Average Ct value of GAPDH, TLR4, RAGE, and TLR7 genes

| Gene | Ct value Mean ± SEM | |
|---|---|---|
| | THP-1 cells | THP-1-derived macrophage cells |
| GAPDH | 22.78 ± 0.60 | 23.92 ± 0.58 |
| TLR4 | 23.54 ± 0.07 | 23.89 ± 0.15 |
| RAGE | 22.77 ± 0.14 | 23.08 ± 0.22 |
| TLR7 | 24.45 ± 0.10 | 24.84 ± 0.19 |

Previous research showed that 16 hours of PMA treatment, followed by 48 hours in PMA-free medium, induced a twofold increase in TLR2 expression in THP-1-derived macrophage-like cells (M0). Upon activation by TLR agonists, a greater release of pro-inflammatory cytokines IL-6, TNF-α, and IL-8 was observed in THP-1-derived macrophage-like cells. This referenced study used 0.2 × $10^6$ cells/mL with 50 ng/mL PMA (27). This study used a modified protocol and adjusted PMA concentration of 62.5 ng/mL PMA to account for a cell density of 2.5 × $10^5$ cells/mL. An additional 24 hours of incubation in PMA-free medium before harvesting were also added. Another study reported increased TLR4 mRNA expression in PMA-differentiated THP-1 cells compared with THP-1 cells in the presence or absence of LPS (28). This previous study used a lower concentration of PMA (10 ng/mL), a longer exposure time (24 hours), and shorter resting periods compared to our study. Differences in PMA concentrations and resting periods might influence receptor expression and signaling pathways (27). We also investigated RAGE and TLR7 as additional proinflammatory receptors, and IL-18 as a pro-inflammatory cytokine of the IL-1 family. In agreement with previous studies findings, our investigation revealed a trend of elevated transcription for TLR4, TLR7, and RAGE in THP-1-derived macrophage-like cells compared with their monocyte counterparts. Our results indicate that the increase in receptor transcription occurs during the differentiation phase, not solely during the activation phase, as previously reported. Following stimulation by DAMPs or PAMPs, activated TLR4 and TLR7 utilize the adaptor molecule myeloid differentiation primary response gene 88 (MyD88) at the membrane surface to trigger the transcription factor nuclear factor κ-light-chain enhancers of activated B cells (NF-κB), thereby promoting proinflammatory cytokine production (6).

RAGE stimulation by its ligand induces sustained activation of NF-κB. Beyond AGEs, RAGE ligands are diverse and include high mobility group box 1, S100 proteins, phosphatidylserine, and complement protein C1q (12). Activation of RAGE by its specific ligand results in ligand internalization through endocytosis, which is essential in the clearance of harmful substances in both professional and non-professional phagocytes, and also promotes angiogenesis (30–32).

## 3.3. The Levels of Pro-Inflammatory Cytokines IL-6, TNF-α, and IL-18 in THP-1-Derived Macrophages Compared with Monocytes

Building on the observed trend of increased receptor mRNA expression in PMA-induced THP-1 macrophage-like cells (M0), we next investigated the secretion of proinflammatory cytokines as the final downstream products of TLR4, RAGE, and TLR7 activation. We observed a significant increase in IL-6 levels from 1,305.57 pg/mL to 3,162.67 pg/mL ($p = 0.0277$) and in TNF-α levels from 2,428.12 pg/mL to 44,878.45 pg/mL ($p = 0.0105$) in the supernatant of THP-1-derived macrophage-like cells compared with monocytes. In contrast, although an upward trend was noted, the levels of IL-18, a proinflammatory cytokine belonging to the IL-1 family, did not differ significantly between macrophages and their

monocyte precursors (112.13 pg/mL vs. 163.98 pg/mL; p = 0.3454). These findings suggest that PMA-induced differentiation of THP-1 into macrophage-like cells promotes increased secretion of IL-6 and TNF-α, while minimally affecting IL-18 levels (Figure 3A–B).

As expected, our observation indicated that the upregulated TLR4, RAGE, and TLR7 transcription in THP-1-derived macrophage-like cells corresponded with increased release of the pro-inflammatory cytokines IL-6 and TNF-α. Both TLR4 and TLR7 activate MyD88-dependent pathways. TLRs engage the adaptor protein MyD88 at the plasma membrane. MyD88, along with interleukin-1 receptor-associated kinase (IRAK) 1, 2, and 4, assembles the Myddosome to recruit tumor necrosis factor receptor-associated factor 6 (TRAF6). This signaling cascade triggers transforming growth factor beta-activated kinase 1 (TAK1) activation, followed by the induction of transcription factor NF-κB, and mitogen-activated protein kinase (MAPK). Ultimately, pro-inflammatory cytokines, including the IL-1 family, TNF-α, and IL-6, are produced and released (6).

RAGE is associated with multiple cellular signaling pathways that activate transcription factors, such as NF-κB, early growth response-1 (Egr-1), activator protein-1 (AP-1), and signal transducer and activator of transcription 3 (STAT3). These pathways are also responsible for the enhanced production and secretion of inflammatory cytokines (12).

Considering that all measurements of receptors and cytokines were taken from unstimulated or resting PMA-induced THP-1 macrophage-like cells (M0), these data may represent baseline levels relevant to the differentiation process into M0, rather than being influenced by additional stimulation (e.g., LPS). A modest twofold increase in receptor mRNA expression was observed in this study. Previous research showed that a slight increase in receptor could be sufficient to induce a biological effect. A 2.2-fold increase in TGFB2 ligand mRNA has been observed in low-grade gliomas (LGG) compared to healthy brain tissue (33). Angiotensin II type 1 receptor (AT1R) mRNA expression in stress-stimulated human coronary VSMCs increased twofold compared to unstimulated cells, followed by a corresponding increase in protein expression (34). mRNA levels do not always correlate with protein levels, which more directly reflect biological effects. Several factors influence this correlation, including translation efficiency, protein degradation, and feedback regulation of mRNA levels by protein abundance (35). The lack of correlation between mRNA and protein levels does not mean that changes in transcript levels are biologically irrelevant. For example, mRNA levels of 17 mediators involved in wound healing, measured under the same conditions, were associated with their protein levels; however, such a correlation was not seen when each gene and its corresponding protein were analyzed individually (36). The twofold increase in receptor mRNA expression observed in our study indicates an enhanced ability of macrophages to engage and respond to DAMPs and PAMPs relative to monocytes. This is supported by increased levels of IL-6 and TNF-α reflecting amplified downstream signaling leading to proinflammatory cytokine production and secretion.

In contrast, we observed no significant increase in IL-18 levels in the culture supernatant of THP-1-derived macrophage-like cells. Following TLR activation and signaling, IL-18 is produced as an inactive precursor, pro-IL-18. The activated protease caspase-1 cleaves pro-IL-18 (as well as pro-IL-1β) into its mature active form. Caspase-1 activation induces pore formation in the plasma membrane, leading to pyroptotic cell death and the subsequent release of IL-18 into the extracellular space (37). Therefore, IL-18 secretion relies on inflammasome activation. The inflammasome is a multiprotein complex comprising PRRs such as NOD-like receptor-pyrin-containing proteins (NLRPs) or absent in melanoma 2 (AIM2), the protease caspase-1, and the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC). PAMPs and DAMPs are the primary triggers of inflammasome assembly and activation (38). In the absence of PAMPs and/or DAMPs in this study, it is likely that most IL-18 remained in its immature precursor form and was not released into the culture supernatant of THP-1-derived macrophages.

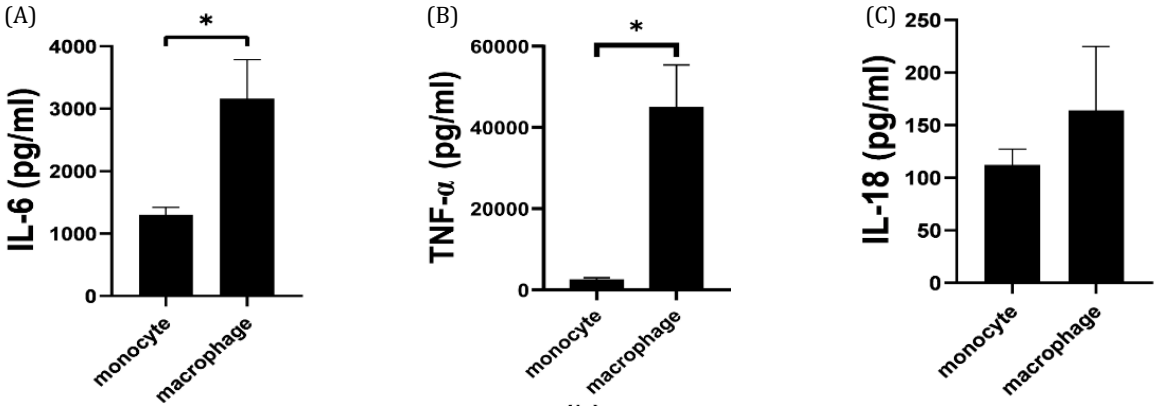


**Figure 3.** Levels of IL-6 (A), TNF-α (B), and IL-18 (C) in the supernatants of THP-1-derived macrophage-like cells and monocyte cultures. The levels of pro-inflammatory cytokine were analyzed using a paired t-test or Wilcoxon signed-rank test, as appropriate. Data are presented as mean ± SEM.

This study has limitations. The PMA-induced differentiation of THP-1 into macrophage-like cells was defined based on changes in morphology and increased adhesion observed under light microscopy. For a more comprehensive and objective assessment, expression of the cell surface marker cluster of differentiation 14 (CD14) is often used to evaluate THP-1-derived macrophage differentiation. CD14 is dispensable for LPS-stimulated TLR4 internalization and activation (39). However, its expression may vary depending on THP-1 seeding density, PMA concentration, and duration of exposure (40). To date, there is no standardized method for PMA-induced differentiation of THP-1-derived macrophage-like cells. This study followed a detailed protocol validated in a previous study that compared three methods varying in concentration, duration of exposure, and resting periods of PMA-stimulated THP-1-derived macrophage-like cells (27). The data obtained from this study provide valuable insight into the characteristics of monocytes and macrophages, particularly with respect to receptor expression and cytokine production.

## 4. CONCLUSIONS

This study provides baseline data on pro-inflammatory receptor expression and cytokine production using a specific PMA-induced THP-1-derived macrophage-like cells differentiation protocol. The upregulation of TLR4, RAGE, and TLR7 transcription, along with increased levels of IL-6 and TNF-α during the differentiation of THP-1-derived macrophage-like cells (M0), supports the role of macrophages as pro-inflammatory cytokine-secreting cells primed to recognize and respond to DAMPs and/or PAMPs. This approach enhances understanding of THP-1-derived macrophage-like cell polarization and lays the foundation for developing a macrophage model for inflammatory studies. The baseline mRNA expression data for receptors and cytokines obtained using this protocol will serve as a reference to distinguish changes caused by external stimuli from those induced by monocyte-to-macrophage differentiation.

**Author contributions:** SW: Conceptualization, Formal Analysis, Writing—Original Draft Preparation. TAS, SAJ: Data Curation, Writing—Review and Editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Universitas Sebelas Maret Strengthening Research Group Capacity (PKGR-UNS A) Grant number 371/UN27.22/PT.01.03/2025.

**Acknowledgements**: None.

**Ethics statement:** None.

**Conflict of interest:** The authors have no conflicts of interest.

## REFERENCES

1. Chaintreuil P, Kerreneur E, Bourgoin M, Savy C, Favreau C, Robert G, Jacquel A, Auberger P. The generation, activation, and polarization of monocyte-derived macrophages in human malignancies. Front Immunol. 2023;14:1178337. https://doi.org/10.3389/fimmu.2023.1178337
2. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, Cheng L, Li J, Wang X, Wang F, Liu L, Amit I, Zhang S, Zhang Z. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med. 2020;26(6):842–4. https://doi.org/10.1038/s41591-020-0901-9
3. Püschel GP, Klauder J, Henkel J. Macrophages, low-grade inflammation, insulin resistance and hyperinsulinemia: A mutual ambiguous relationship in the development of metabolic diseases. J Clin Med. 2022;11(15):4358. https://doi.org/10.3390/jcm11154358
4. Cicchinelli S, Pignataro G, Gemma S, Piccioni A, Picozzi D, Ojetti V, Franceschi F, Candelli M. PAMPs and DAMPs in Sepsis: A review of their molecular features and potential clinical implications. Int J Mol Sci. 2024;25(2):962. https://doi.org/10.3390/ijms25020962
5. Kang N, Ji Z, Li Y, Gao J, Wu X, Zhang X, Duan Q, Zhu C, Xu Y, Wen L, Shi X, Liu W. Metabolite-derived damage-associated molecular patterns in immunological diseases. FEBS J. 2024;291(10):2051–67. https://doi.org/10.1111/febs.16902
6. Aluri J, Cooper MA, Schuettpelz LG. Toll-like receptor signaling in the establishment and function of the immune system. Cells. 2021(6);10:1374. https://doi.org/10.3390/cells10061374
7. Kim HJ, Kim H, Lee JH, Hwangbo C. Toll-like receptor 4 (TLR4): New insight immune and aging. Immun Ageing. 2023;20(1):67. https://doi.org/10.1186/s12979-023-00383-3
8. Lind NA, Rael VE, Pestal K, Liu B, Barton GM. Regulation of the nucleic acid-sensing Toll-like receptors. Nat Rev Immunol. 2022;22(4):224–35. https://doi.org/10.1038/s41577-021-00577-0
9. David C, Badonyi M, Kechiche R, Insalaco A, Zecca M, De Benedetti F, Orcesi S, Chiapparini L, Comoli P, Federici S, Gattorno M, Ginevrino M, Giorgio E, Matteo V, Moran-Alvarez P, Politano D, Prencipe G, Sirchia F, Volpi S, Masson C, Rice GI, Frémond ML, Lepelley A, Marsh JA, Crow YJ. Interface gain-of-function mutations in TLR7 cause systemic and neuro-inflammatory disease. J Clin Immunol. 2024;44(2):60. https://doi.org/10.1007/s10875-024-01660-6
10. Campolo M, Filippone A, Biondo C, Mancuso G, Casili G, Lanza M, Cuzzocrea S, Esposito E, Paterniti I. TLR7 / 8 in the pathogenesis of parkinson's disease †. Int J Mol Sci. 2020;21(24):9384. https://doi.org/10.3390/ijms21249384

11. Bender AT, Tzvetkov E, Pereira A, Wu Y, Kasar S, Przetak MM, Vlach J, Niewold TB, Jensen MA, Okitsu SL. TLR7 and TLR8 differentially activate the IRF and NF-κB pathways in specific cell types to promote inflammation. Immunohorizons. 2020;4(2):93–107. https://doi.org/10.4049/immunohorizons.2000002

12. Dong H, Zhang Y, Huang Y, Deng H. Pathophysiology of RAGE in inflammatory diseases. Front Immunol. 2022;13:931473. https://doi.org/10.3389/fimmu.2022.931473

13. Egaña-Gorroño L, López-Díez R, Yepuri G, Ramirez LS, Reverdatto S, Gugger PF, Shekhtman A, Ramasamy R, Schmidt AM. Receptor for advanced glycation end products (Rage) and mechanisms and therapeutic opportunities in diabetes and cardiovascular disease: Insights from human subjects and animal models. Front Cardiovasc Med. 2020;7:37. https://doi.org/10.3389/fcvm.2020.00037

14. Pang S, Jia T, Yang Z. Unraveling the RAGE axis in pulmonary disorders: Mechanisms and therapeutical potential. Biocell. 2024;48(12):1721–34. https://doi.org/10.32604/biocell.2024.055753

15. Chen R, Huang Y, Quan J, Liu J, Wang H, Billiar TR, Lotze MT, Zeh HJ, Kang R, Tang D. HMGB1 as a potential biomarker and therapeutic target for severe COVID-19. Heliyon. 2020;6(12):e05672. https://doi.org/10.1016/j.heliyon.2020.e05672

16. Butcher L, Zaldua JC, Carnicero JA, Hawkins K, Whitley J, Mothukuri R, Evans PA, Morris K, Pillai S, Erusalimsky JD. High levels of soluble RAGE are associated with a greater risk of mortality in COVID-19 patients treated with dexamethasone. Respir Res. 2022;23(1):303. https://doi.org/10.1186/s12931-022-02220-5

17. Angioni R, Bonfanti M, Caporale N, Sánchez-Rodríguez R, Munari F, Savino A, Pasqualato S, Buratto D, Pagani I, Bertoldi N, Zanon C, Ferrari P, Ricciardelli E, Putaggio C, Ghezzi S, Elli F, Rotta L, Scardua A, Weber J, Cecatiello V, Iorio F, Zonta F, Cattelan AM, Vicenzi E, Vannini A, Molon B, Villa CE, Viola A, Testa G. RAGE engagement by SARS-CoV-2 enables monocyte infection and underlies COVID-19 severity. Cell Reports Med. 2023;4(11):101266. https://doi.org/10.1016/j.xcrm.2023.101266

18. O'Mahony DS, Pham U, Iyer R, Hawn TR, Liles WC. Differential constitutive and cytokine-modulated expression of human Toll-like receptors in primary neutrophils, monocytes, and macrophages. Int J Med Sci. 2008;5(1):1–8. http://doi.org/10.7150/ijms.5.1

19. Juarez E, Nuñez C, Sada E, Ellner JJ, Schwander SK, Torres M. Differential expression of Toll-like receptors on human alveolar macrophages and autologous peripheral monocytes. Respir Res. 2010;11(1):2. http://doi.org/10.1186/1465-9921-11-2

20. Schmidt AM. 2016 ATVB plenary lecture receptor for advanced glycation endproducts and implications for the pathogenesis an treatment of cardiometabolic disorders: Spotlight on the macrophage. Arterioscler Thromb Vasc Biol. 2017;37(4):613–21. https://doi.org/10.1161/ATVBAHA.117.307263

21. Tedesco S, De Majo F, Kim J, Trenti A, Trevisi L, Fadini GP, Bolego C, Zandstra PW, Cignarella A, Vitiello L. Convenience versus biological significance: Are PMA-differentiated THP-1 cells a reliable substitute for blood-derived macrophages when studying in vitro polarization? Front Pharmacol. 2018;9:71. http://doi.org/10.3389/fphar.2018.00071

22. Hoppenbrouwers T, Bastiaan-Net S, Garssen J, Pellegrini N, Willemsen LEM, Wichers HJ. Functional differences between primary monocyte-derived and THP-1 macrophages and their response to LCPUFAs. PharmaNutrition. 2022;22:100322. https://doi.org/10.1016/j.phanu.2022.100322

23. Mohd Yasin ZN, Mohd Idrus FN, Hoe CH, Yvonne-Tee GB. Macrophage polarization in THP-1 cell line and primary monocytes: A systematic review. Differentiation. 2022;128:67–82. https://doi.org/10.1016/j.diff.2022.10.001

24. Daigneault M, Preston JA, Marriott HM, Whyte MKB, Dockrell DH. The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. PLoS One. 2010;5(1):e8668. https://doi.org/10.1371/journal.pone.0008668

25. Browne E, Kavanagh S, Devery S. The cytotoxicity of phorbol 12- myristate 13-acetate and lipopolysaccharide on THP-1 cells and an optimized differentiation protocol. Med Sci Forum. 2022;11:5. https://doi.org/10.3390/BiTaP-12785

26. Forrester MA, Wassall HJ, Hall LS, Cao H, Wilson HM, Barker RN, Vickers MA. Similarities and differences in surface receptor expression by THP-1 monocytes and differentiated macrophages polarized using seven different conditioning regimens. Cell Immunol. 2018;332:58–76. http://doi.org/10.1016/j.cellimm.2018.07.008

27. Pinto SM, Kim H, Subbannayya Y, Giambelluca MS, Bösl K, Ryan L, Sharma A, Kandasamy RK. Comparative proteomic analysis reveals varying impact on immune responses in phorbol 12-myristate-13-acetate-mediated THP-1 monocyte-to-macrophage differentiation. Front Immunol. 2021;12:679458. http://doi.org/10.3389/fimmu.2021.679458

28. Kim YK, Hwang JH, Lee HT. Differential susceptibility to lipopolysaccharide affects the activation of toll-like-receptor 4 signaling in THP-1 cells and PMA-differentiated THP-1 cells. Innate Immun. 2022;28(3–4):122–9. https://doi.org/10.1177/17534259221100170

29. Mahboudi H, Heidari NM, Rashidabadi ZI, Anbarestani AH, Karimi S, Darestani KD. Prospect and competence of quantitative methods via real-time PCR in a comparative manner: An experimental review of current methods. Open Bioinforma J. 2018;11:1–11. https://doi.org/10.2174/1875036201811010001

30. Lan J, Luo H, Wu R, Wang J, Zhou B, Zhang Y, Jiang Y, Xu J. Internalization of HMGB1 (high mobility group box 1) promotes angiogenesis in endothelial cells. Arterioscler Thromb Vasc Biol. 2020;40(12):2922–40. https://doi.org/10.1161/ATVBAHA.120.315151

31. Lan J, Luo H, Wu R, Wang J, Zhou B, Zhang Y, Jiang Y, Xu J. Receptor for advanced glycation end-products (RAGE) mediates phagocytosis in nonprofessional phagocytes. Commun Biol. 2022;5(1):824. https://doi.org/10.1038/s42003-022-03791-1

32. Chang YF, Huang YP, Chou CH, Ho MW, Lin HJ, Chen CY, Wu HY, Lai YR, Lee YH, Chiu CH, Lai CH. RAGE participates in the intracellular transport of *Campylobacter jejuni* cytolethal distending toxin. J Microbiol Immunol Infect. 2024;57(5):709–19. https://doi.org/10.1016/j.jmii.2024.07.007

33. Trieu V, Maida AE, Qazi S. Transforming Growth Factor Beta 2 (TGFB2) mRNA levels, in Conjunction with interferon-gamma receptor activation prognostic markers in low-grade gliomas. Cancers (Basel). 2024;16(16):1202. https://doi.org/10.3390/cancers16061202

34. Backlund M, Paukku K, Kontula KK, Lehtonen JYA. Endoplasmic reticulum stress increases

AT1R mRNA expression via TIA-1-dependent mechanism. Nucleic Acids Res. 2015;44(7):3095–104. https://doi.org/10.1093/nar/gkv1368

35. Munro V, Kelly V, Messner CB, Kustatscher G. Cellular control of protein levels: A systems biology perspective. Proteomics. 2024;24(12-13):2200220. https://doi.org/10.1002/pmic.202200220

36. Prabahar A, Zamora R, Barclay D, Yin J, Ramamoorthy M, Bagheri A, Johnson SA, Badylak S, Vodovotz Y, Jiang P. Unraveling the complex relationship between mRNA and protein abundances: A machine learning-based approach for imputing protein levels from RNA-seq data. NAR Genomics Bioinforma. 2024;6(1):lqae019. https://doi.org/10.1093/nargab/lqae019

37. Prabahar A, Zamora R, Barclay D, Yin J, Ramamoorthy M, Bagheri A, Johnson SA, Badylak S, Vodovotz Y, Jiang P. Interleukin-18 cytokine in immunity, inflammation, and autoimmunity: Biological role in induction, regulation, and treatment. Front Immunol. 2022;13:919973. http://doi.org/10.3389/fimmu.2022.919973

38. Zheng D, Liwinski T, Elinav E. Inflammasome activation and regulation: Toward a better understanding of complex mechanisms. Cell Discov. 2020;6:36. https://doi.org/10.1038/s41421-020-0167-x

39. Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci. 2021;78(4):1233–61. https://doi.org/10.1007/s00018-020-03656-y

40. Liu T, Huang T, Li J, Li A, Li C, Huang X, Li D, Wang S, Liang M. Optimization of differentiation and transcriptomic profile of THP-1 cells into macrophage by PMA. PLoS One. 2023;18(7):e0286056. http://dx.doi.org/10.1371/journal.pone.0286056