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Induction of THP-1 Cells into M1 Macrophages Under Certain Conditions

Bo Zhang[1], Huaixin Pan[1], Jinling Li[1], Ruiyi Jia[1], Linying Yang[1], Guifen Pang[1], and Jiming Bai[2]

[1] Department of Respiratory and Critical Care Medicine, Affiliated Hospital of Chengde Medical University, Chengde, Hebei, China
[2] Department of Thyroid, Breast, and Rectal Surgery, Chengde Central Hospital, Chengde, Hebei, China

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ABSTRACT

Macrophages are essential components of the immune system but, unlike many other immune cells, are nonregenerative. The THP-1 cell line is therefore widely used as a surrogate for primary macrophages because THP-1 monocytes can be induced to differentiate into M1 macrophages under specific conditions. In this study, we induced M1 macrophages from THP-1 monocytes and assessed polarization by measuring CD80 (B7-1) expression with flow cytometry and interleukin-6 (IL-6) secretion in the culture supernatant with enzyme-linked immunosorbent assay (ELISA).

THP-1 monocytes were treated with phorbol 12-myristate 13-acetate (PMA; 150 ng/mL) for 24 hours to generate M0 macrophages and then stimulated with lipopolysaccharide (LPS; 100 ng/mL) and interferon-γ (IFN-γ; 20 ng/mL) for a further 24 hours to induce M1 polarization. CD80 expression was detected by flow cytometry. Culture supernatants were assayed by ELISA according to the manufacturer's instructions to determine IL-6 concentration.

Approximately 37.7% of induced M1 macrophages expressed the costimulatory marker CD80. IL-6 levels in the culture supernatant increased significantly after induction. Nearly all THP-1 cells became spherical, adherent M0 macrophages after 24 hours of PMA treatment, whereas only a subset of M0 cells developed the characteristic dendritic, antenna-like morphology after a further 24 hours of LPS and IFN-γ treatment.

This indicated that partial THP1 cells can be polarized into M1 macrophages under this experimental condition. Nevertheless, the induced M1 cell population retained the capacity to release the inflammatory cytokine IL-6.

Keywords: B7-1 antigen; Inflammation; Interleukin-6; Macrophages; Monocytic leukemia cell line THP-1; Phorbol 12-myristate 13-acetate

Corresponding Authors: Guifen Pang, MMed;
Department of Respiratory and Critical Care Medicine, Affiliated Hospital of Chengde Medical University, Chengde, Hebei, China.
Tel: (+86 156) 3314 2975, Email: Pang-gf@sohu.com

Jiming Bai, MMed;
Department of Thyroid, Breast, and Rectal Surgery, Chengde Central Hospital, Chengde, Hebei, China. Tel: (+86 152) 3341 8518, Email: 505638942@qq.com

The first and second authors contributed equally to this study

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INTRODUCTION

Macrophages are essential components of the immune system and participate in numerous physiological and pathological processes,[1] but they are nonregenerative cells. The THP-1 cell line is therefore commonly used as a surrogate for primary macrophages. THP-1 cells, which were derived from a patient with acute monocytic leukemia,[2] proliferate faster than primary cells, are easy and safe to obtain, and have a homogeneous genetic background that minimizes variability in macrophage phenotype.[3] These properties make THP-1 cells a widely used model for studying macrophage-related mechanisms. THP-1 cells grow as spherical cells in suspension, are continuously renewable, and can be induced to differentiate into M1 or M2 macrophages under different stimulation conditions.[4]

In this study, THP-1 monocytes were first treated with phorbol 12-myristate 13-acetate (PMA) for 24 hours to generate M0 macrophages, after which lipopolysaccharide (LPS) and interferon-γ (IFN-γ) were added to induce M1 polarization. M1 macrophages promote inflammation and pathogen clearance. To confirm the polarization of THP-1-derived M1 macrophages under these induction conditions, we compared expression of the costimulatory factor CD80 by flow cytometry and interleukin-6 (IL-6) secretion in the culture supernatant by enzyme-linked immunosorbent assay (ELISA) between M1 macrophages and THP-1 monocytes. These analyses provided experimental evidence for M1 polarization from THP-1 cells. The novelty of this study lies in providing an accurate conversion rate of THP-1 cells to M1 macrophages and a defined IL-6 concentration under these specific conditions.

MATERIALS AND METHODS

Cell Culture and Stimulation

THP-1 cells were purchased from Punosai Life Science and Technology Co, Ltd (Wuhan, China) and cultured in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS; Viva Cell, China) and 1% penicillin-streptomycin (Solarbio, China) at 37 °C in 5% CO_2. Because THP-1 cells proliferate faster in an acidic environment,[5] they were passaged whenever the culture medium turned yellowish. Cells were seeded in 6-well plates at a density of 2×10^5 cells/mL. According to a previous study, THP-1 cells stimulated with 80 ng/mL or 160 ng/mL PMA showed CD14-positive rates of 54.7±1.1% and 42.0±3.0%, respectively, 24 hours after induction. We therefore selected a PMA concentration of 150 ng/mL, intermediate between these 2 values, and incubated cells with PMA for 24 hours at 37°C to generate M0 macrophages. LPS is a potent inflammatory stimulus that is widely used to induce inflammation in vitro and closely simulate inflammatory conditions.[6,7] We subsequently incubated M0 macrophages with LPS (100 ng/mL; Sigma, USA) and IFN-γ (20 ng/mL; Thermo Fisher Scientific, USA) for 24 hours to obtain the M1 phenotype, after which the treated cells were harvested.

This study used exclusively the widely accepted commercial THP-1 cell line obtained from Punosai Life Science and Technology Co, Ltd. No human participants, fresh human tissue, or experimental animals were involved in any aspect of the experimental design or procedures. On this basis, the study was exempt from institutional ethical review and ethics committee approval. All cell culture procedures and related experimental operations were performed in strict accordance with laboratory biosafety regulations and standard practice requirements.

Flow Cytometry

Previous studies have shown that expression of the costimulatory factor CD80 (B7-1; clone 2D10.4, Thermo Fisher Scientific) is significantly upregulated in M1 macrophages and can serve as a M1 marker.[8] In this study, CD80 antibody and isotype control (0.5 mg/mL) were incubated at 4°C in the dark for 30 minutes, and CD80 expression was then detected by flow cytometry using Cytomics FC 500 flow cytometer (Beckman Coulter). Detection was performed with the fluorescein isothiocyanate (FITC) fluorescence signal following excitation with a 488-nm laser. At least 10 000 valid cellular events were collected per tube. The live-cell population was gated using forward scatter (FSC) and side scatter (SSC) to exclude debris and dead cells. The negative gate (mean fluorescence intensity [MFI] $<10^2$) was set using the isotype control, and the percentage of CD80-positive cells among all live cells in the experimental group was used as the polarization efficiency index for M1 macrophages.

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ELISA

M1 macrophages participate in numerous physiological and pathological processes and play an important role in maintaining tissue homeostasis.[9] They secrete pro-inflammatory cytokines and contribute to anti-infective defense by upregulating reactive oxygen species (ROS) and SOD2,[10] generating reactive nitrogen species, and increasing antimicrobial peptide production.[11] In vitro, M1 macrophages exert pro-inflammatory effects in part by releasing IL-6 and other inflammatory mediators into the culture supernatant.[12] In this study, culture supernatants were collected and stored at −80 °C until cytokine assays were performed, and IL-6 concentrations were determined by ELISA according to the manufacturer's instructions. Briefly, culture supernatants from each group were centrifuged at 4000 rpm for 5 minutes to remove cell debris; the clarified supernatants were either assayed immediately or stored at −80 °C. The lyophilized standard was reconstituted in standard diluent and serially diluted (2000, 1000, 500, 250, 125, 62.5, and 31.25 pg/mL) according to the kit instructions to generate a standard curve. Fifty microliters of standard, sample, or negative control was added to antibody-coated wells of the ELISA plate, followed by 50 µL of biotin-labeled detection antibody, the plate was sealed and incubated at 37 °C for 60 minutes. Wells were then washed 5 times with 350 µL of wash buffer per well (30 seconds per wash) and blotted dry on absorbent paper. One hundred microliters of horseradish peroxidase (HRP)-labeled streptavidin was added to each well, incubated at 37 °C in the dark for 30 minutes, and washed and dried as before. One hundred microliters of tetramethylbenzidine (TMB) substrate was then added to each well and incubated at 37 °C in the dark for 15 minutes. Once a clear blue color gradient developed in the standard wells, 50 µL of stop solution was added to each well, turning the solution from blue to yellow. Absorbance, reported as optical density (OD), was measured immediately at 450 nm using a microplate reader, and a standard curve was generated by plotting standard concentration against OD value. Cytokine concentrations in the samples were calculated from the resulting curve equation.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics. Cytokine concentrations were calculated from the ELISA standard curve equation. All experiments were performed independently in triplicate, and results are expressed as mean±standard deviation. Data normality were assessed before statistical testing. An independent-samples *t* test was used to compare the 2 groups. $p<0.05$ was considered statistically significant.

RESULTS

CD80 Expression

THP-1-derived macrophages treated with PMA (150 ng/mL) for 24 hours and then with LPS (100 ng/mL) and IFN-γ (20 ng/mL) for a further 24 hours showed approximately 37.7±3.6% CD80-positive cells (Figure 1).

IL-6 Expression in the Culture Supernatant

Across 3 independent measurements, the mean IL-6 concentration in the supernatant of induced M1 cells was 460.72±73.80 pg/mL by ELISA, compared with 214.42±56.65 pg/mL in THP-1 monocytes ($p=0.007$). This significant increase in IL-6 following induction was consistent with the expected pro-inflammatory phenotype of M1 macrophages.

Cell Morphology

Nearly all THP-1 cells became spherical, adherent M0 cells after 24 hours of PMA treatment, whereas only a subset of M0 cells developed the characteristic antenna-like morphology after a further 24 hours of LPS and IFN-γ treatment (Figure 2). Because clear morphological differences were evident among THP-1 cells, M0 macrophages, and M1 macrophages on visual inspection, quantitative morphological analysis was not performed.

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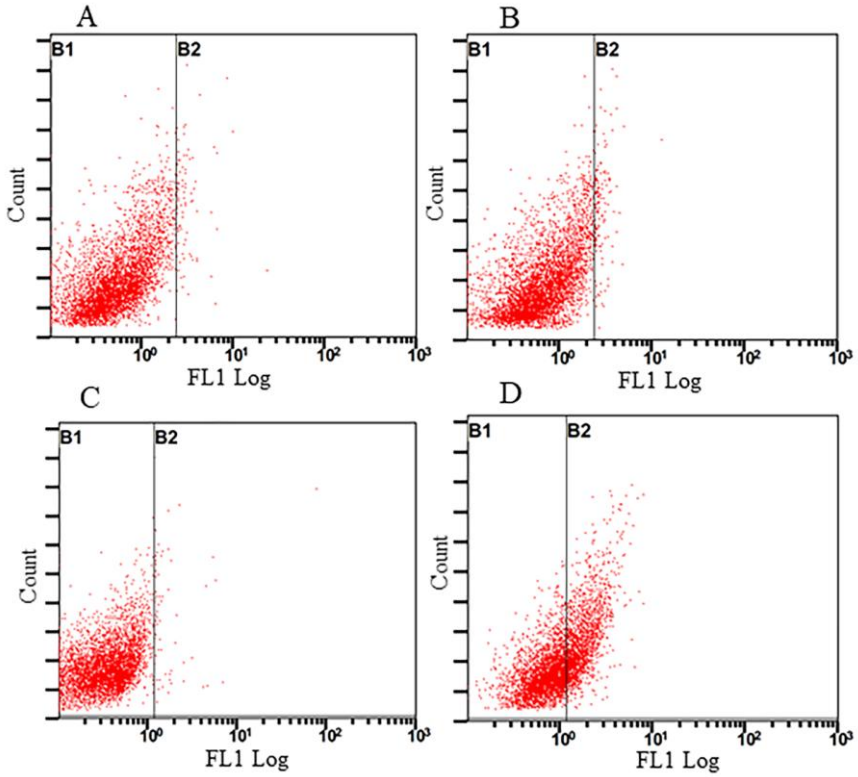

Figure 1. CD80 Expression in THP-1 and M1 Cells (B2 denotes the CD80-positive population). A. Isotype control staining showed a mean CD80 positivity rate of 1.39% in THP-1 cells. B. CD80 antibody staining showed a mean positivity rate of 2.81% in THP-1 cells. C. Isotype control staining showed a mean CD80 positivity rate of 1.06% in M1 cells. D. CD80 antibody staining showed a mean positivity rate of 37.41% in M1 cells.

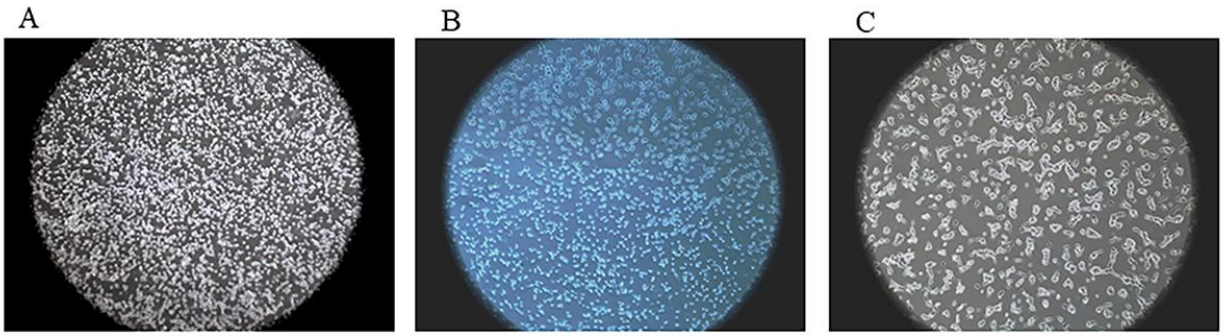

Figure 2. Morphology of THP-1, M0, and M1 Cells. A. Characteristic morphology of THP-1 cells growing in suspension (total magnification, 100×). B. Characteristic morphology of M0 cells, which were THP-1 macrophages induced with PMA for 24 hours and shown as adherent cells (total magnification, 200×). C. Characteristic morphology of M1 cells, which were M0 cells induced with LPS (100 ng/mL) and IFN-γ (20 ng/mL) for 24 hours and shown as adherent cells with slender pseudopods (total magnification, 200×).

DISCUSSION

Macrophage polarization is currently a major focus of research. THP-1 monocytes can be differentiated into 3 macrophage subtypes: M0, M1, and M2.[13] In our study of M0/M1 polarization, THP-1 cells were treated with PMA (150 ng/mL) for 24 hours and then with LPS (100 ng/mL) and IFN-γ (20 ng/mL) for a further 24 hours to regulate macrophage polarization and function. We found that only a subset of M0 cells acquired the antenna-like morphology after this treatment, possibly reflecting the induction time and drug concentration.

M1 macrophages can be identified by a range of markers, including the surface costimulatory molecules CD80 and CD86, inducible nitric oxide synthase (iNOS), and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 in the culture supernatant. In this study, we did not assess all of these markers but focused on CD80 expression and IL-6 secretion. Approximately 37.7% of cells expressed CD80 under the present induction protocol, indicating that only a subset of M0 macrophages was converted to M1 macrophages. These cells are characterized by secretion of pro-inflammatory cytokines and increased CD80 expression, which promote antigen-driven T-cell responses and support anti-infective immunity.[14]

Approximately 37.7% of cells expressed the costimulatory marker CD80 in our study, whereas other reports have described CD80 positivity rates exceeding 60% following induction. This discrepancy may reflect differences in PMA, LPS, and IFN-γ concentrations, cell density, incubation times, THP-1 heterogeneity, and methodological variations. Likewise, the 37.7% polarization rate we observed was lower than the approximately 62.5%±2.0% reported in some other studies. We attribute this difference in part to variation in experimental methods: the referenced study used a THP-1 cell density of 5×10^5 cells/mL and a PMA concentration of 100 ng/mL, compared with 2×10^5 cells/mL and 150 ng/mL in our experiments.[15] These differences suggest that both cell density and PMA concentration may influence the M1 transformation rate. Future experiments will examine the effects of different stimulation durations (24, 48, and 72 hours), cell density, and methodological variations.

We also examined IL-6 concentration in the culture supernatant and found that IL-6 levels were significantly higher than those in untreated THP-1 cells, further supporting successful induction of a subset of M1 macrophages. These findings may be useful for researchers using THP-1-derived macrophage models.

This study has several limitations, including its relatively simple design, reliance on a single cell line, limited panel of biomarkers, and absence of functional assays. In future work, we plan to further investigate the specific molecular mechanisms underlying macrophage polarization and metabolism-related immunomodulatory function.

STATEMENT OF ETHICS

Because this study was an in vitro experiment using a commercial cell line (THP-1) and did not involve human or animal participants, ethical approval was not required.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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All authors have read and agreed to the published version of the manuscript. The content of Figure 1D comes from Huaixin Pan's degree research work.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

There were no artificial intelligence (AI) tools used in preparing this manuscript.

REFERENCES

1. Liu L, Stokes JV, Tan W, Pruett SB. An optimized flow cytometry panel for classifying macrophage polarization. J

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Immunol Methods. 2022;511:113378.
2. Zhou X, Lie L, Liang Y, Xu H, Zhu B, Huang Y, et al, GSK-3α/β activity negatively regulates MMP-1/9 expression to suppress Mycobacterium tuberculosis infection. Front Immunol. 2022;12:752466.
3. Miljkovic M, Lozano S, Castellote I, de Cózar C, Villegas-Moreno AI, Gamallo P, et al, Novel inhibitors that target bacterial virulence identified via HTS against intra-macrophage survival of Shigella flexneri. mSphere. 2023; 8(5):e0015423.
4. Yunna C, Mengru H, Lei W, Weidong C. Macrophage M1/M2 polarization. Eur J Pharmacol. 2020;877:173090.
5. Wang T, Lu H. Ganoderic acid A inhibits ox-LDL-induced THP-1-derived macrophage inflammation and lipid deposition via Notch1/PPARγ/CD36 signaling. Adv Clin Exp Med. 2021;30(10):1031-41.
6. Izvolskaia M, Sharova V, Zakharova L. Prenatal programming of neuroendocrine system development by lipopolysaccharide: long-term effects. Int J Mol Sci. 2018;19(11):3695.
7. Haileselassie B, Joshi AU, Minhas PS, Mukherjee R, Andreasson KI, Mochly-Rosen D. Mitochondrial dysfunction mediated through dynamin-related protein 1 (Drp1) propagates impairment in blood brain barrier in septic encephalopathy. J Neuroinflammation, 2020;17(1):36.
8. Yamaguchi M, Nakao S, Arima M, Little K, Singh A, Wada I, et al, Heterotypic macrophages/microglia differentially contribute to retinal ischaemia and neovascularization. Diabetologia. 2024;67(10):2329-2345.
9. Razi S, Yaghmoorian Khojini J, Kargarijam F, Panahi S, Tahershamsi Z, Tajbakhsh A, et al, Macrophage efferocytosis in health and disease. Cell Biochem Funct. 2023;41(2):152-65.
10. Fu W, Fang Y, Wang T, Lu Q, Wu J, Yang Q: Low-protein diet inhibits the synovial tissue macrophage pro-inflammatory polarization via NRF2/SIRT3/SOD2/ROS pathway in K/BxN rheumatoid arthritis mice. Inflammation. 2025;48(4):1689-703.
11. Singh PK, Shiha MJ, Kumar A. Antibacterial responses of retinal Müller glia: production of antimicrobial peptides, oxidative burst and phagocytosis. J Neuroinflammation.2014;11:33.
12. Zhang B, Yang Y, Yi J, Zhao Z, Ye R: Hyperglycemia modulates M1/M2 macrophage polarization via reactive oxygen species overproduction in ligature-induced periodontitis. J Periodontal Res. 2021;56(5):991-1005.
13. Yang L, Han P, Cui T, Miao Y, Zhao T, Cui Z, et al, M2 macrophage inhibits the antitumor effects of lenvatinib on intrahepatic cholangiocarcinoma. Front Immunol. 2023;14:1251648.
14. Gao H, Zhang W, Li Z, Liu W, Liu M, Zhuo Q, et al, Distinctive grade based on Ki67 index and immune microenvironment of metastatic pancreatic neuroendocrine tumors responding to capecitabine plus temozolomide. BMC Cancer. 2024;24(1):1362.
15. Li M, Xiong YF, Huang XJ, Chen TA, Li JD. CCAAT/enhancer binding protein δ inhibits invasion and metastasis of liver cancer by regulating M1 type macrophages polarization. Zhonghua Gan Zang Bing Za Zhi 2021;29(8):794-8.

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