Mycobacterium tuberculosis MPT64 stimulates the activation of murine macrophage modulated by IFN-γ

Q. FAN, M. LU, Z.-Y. XIA, L. BAO

Laboratory of Infection and Immunity, School of Basic Medical Sciences, West China Center of Medical Science, Sichuan University, No. 17, 3rd Section, Ren Min Nan, Chengdu, Sichuan, China

Abstract. – BACKGROUND AND AIMS: MPT64, a major target of cell-mediated immunity in tuberculosis, is proposed to be a promising candidate for novel vaccines and diagnostic tests; it also involves in virulence mechanism of *Mycobacterium tuberculosis*. In this study, we investigated the effect of MPT64 on activation of RAW264.7 macrophage, and explored the possible mechanism.

MATERIALS AND METHODS: MPT64 protein was prepared by cloning, expression and purification from *Escherichia (E.) coli*. Cytokines (IL-1β, IL-6, IL-10 and TNF-α) expression in RAW264.7 macrophage induced by MPT64 was evaluated by xMAP technology. Cell counting kit (CCK8) assay was employed to detect the viability of MPT64-treated macrophage; moreover, flow cytometric analysis and Western Blot analysis were used to measure the effect of MPT64 on apoptosis in macrophage and cleaved caspase-3 expression respectively.

RESULTS: MPT64 significantly promoted the levels of IL-1β, IL-6, IL-10 and TNF-α; pretreatment of MPT64 followed by IFN-γ stimulation further up-regulated IL-6 production ($p < 0.01$, compared with MPT64-treated group). In CCK8 assay, significantly increased absorbance of MPT64-treated macrophage suggested that cell viability might be enhanced. Additionally, MPT64 modulated the apoptosis of RAW264.7 macrophage partly through caspase-3 pathway.

CONCLUSIONS: MPT64 activated RAW264.7 macrophage to secrete IL-1β, IL-6, IL-10 and TNF-α; it might down-regulate apoptosis of macrophage partially via caspase-3 pathway. Furthermore, the effect of MPT64 on RAW264.7 macrophage could be modulated by IFN-γ, and co-stimulation of MPT64 with IFN-γ was conducive to the host immune, which might be applied for vaccine design and provide a reference for the treatment of tuberculosis.

Key Words:
   Mycobacterium tuberculosis, MPT64, Macrophage, IFN-γ, Apoptosis

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb), remains one of the most widespread and deadly diseases, accounting for nearly two million deaths annually[1]. It is estimated that one third of the population worldwide is latently infected with Mtb, and the explosion of the HIV epidemic greatly increases the rate of active, infectious TB progression from latent TB[2]. For nearly a century, *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) has been used as the only live attenuated vaccine to prevent TB, but the efficacy is suboptimal, especially for adults. Genome comparison have identified that 16 regions, designated as Regions of Difference (RD1-16), are absent from BCG substrains[3,4]. Among these deletions, RD1 and RD2 appear to be of special importance. Loss of RD1 during the original propagation of BCG is associated with the attenuation of vaccine; however, reintroduction of RD1 did not fully restore the pathogenicity[5,6]. Therefore, it was hypothesized that other deleted genes might also be responsible for the attenuation. Further investigation explored that the deletion of RD2 during 1927 to 1931 coincided with further attenuation of BCG vaccine[7].

MPT64 (also termed as Rv1980c), a well characterized antigen located in RD2, is deleted from nearly all BCG strains and expressed only when *Mycobacteria* cells are actively divided[8]. Owing to its strong immunogenicity and relatively restricted distribution, this protein has been proposed a promising candidate antigen[9]. In addition, MPT64 elicits strong delayed-type hypersensitivity (DTH) reactions in animal models of TB, and can be recognized by Th1 cells from BCG-vaccinated and Mtb-infected healthy subjects; thus, it is considered as a predominant diagnostic reagent for TB[10]. Recently, a series of

Corresponding Author: Lao Bang, MD; e-mail: baolang@scu.edu.cn

rapid diagnostic assays based on MPT64 have been developed. Excellent sensitivity, specificity and low cost of MPT64 antigen detection make it a rapid, reliable and economical method in clinical laboratories[11-13].

As a dominant target for cell-mediated immunity in early infection of TB, MPT64 induces specific T cell immune response; however, like some early secretory antigens, it also involves in the virulence mechanism of Mtb. Recent reports revealed the correlation between MPT64 expression and apoptosis inhibition in multinucleated giant cells[14,15]. Although it is reported that MPT64 can induce high level of IFN-γ, less is known about the relationship between MPT64 and IFN-γ in macrophage. Our previous study showed that in the presence of IFN-γ, CFP-10/ESAT-6, secretory antigens of RD1 from Mtb, represented a new immunogenicity and protection[16]. To determine whether IFN-γ would affect the function of MPT64 in macrophage, we designed experiment of MPT64 on RAW264.7 cells. The investigation showed that MPT64 itself activated macrophage to secrete considerable cytokines. In addition to promote the expression of IL-6 significantly, co-stimulation of IFN-γ with MPT64 also strengthened the expression of cleaved caspase-3, which is served as the executor at the most distal stage of apoptosis pathway. These results implied that the function of MPT64 in RAW264.7 macrophage could be modulated by IFN-γ.

Materials and Methods

Cloning, Expression and Purification of MPT64 Protein

The gene encoding MPT64 was amplified using Mtb H37Rv genomic DNA as a template along with sense (5'-AT CATATG GCC CCG AAG ACC TAC TGC GAG-3') and antisense (5'-GT GGATCC TTA GGC CAG CAT CGA GTC GAT-3') primers respectively. After purification, the PCR product was digested with Ned I and BamH II, subcloned into corresponding sites of PET-19b (+) vector, and sequenced in its entirety. *Escherichia (E.) coli* BL21 (DE3) PlysS was chosen for T7-polymerase based over expression of MPT64. After DNA sequencing, the transformant was cultured in LB (lysogeny broth) medium containing ampicillin sodium (50 μg/ml) at 37°C. This culture was grown with shaking (200 rpm/min) until the OD600nm reached approximately 0.6, target protein was induced with 0.5 mM isopropyl-1-thio-β-galactopyranoside (IPTG) and grown for another 6 h. Cells were harvested by centrifugation at 4°C and lysed by sonication, soluble MPT64 protein was purified by Ni Sepharose High Performance purification columns according to the manufacturer's instructions. The molecular weight and purity of this protein was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Purified protein was concentrated by ultrafiltration through Amicon Centrifugal Filter Devices and stored at –80°C. The protein concentration was determined by BCA reagent (Thermo Scientific, Waltham, MA, USA) with bovine serum albumin (BSA) as the standard.

RAW264.7 Cell Culture

RAW264.7 cells were maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal calf serum, sodium bicarbonate, 0.03% L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C with 5% CO_2. RAW264.7 cells were incubated with different concentration of MPT64 or medium (control group) for 24, 30, 36 and 42h. Alternatively, cells were incubated with or without MPT64 (25 μg/ml) for 6, 12, 18 and 24 h respectively, then stimulated with IFN-γ (30 ng/ml) till 24 to 42h. Cell culture supernatants were collected for IL-1β, IL-6, IL-10 and TNF-α measurement. As for flow cytometry and Western Blot analysis, RAW264.7 cells were incubated with different inducers designed, then harvested and treated for corresponding detection.

IL-1β, IL-6, IL-10, and TNF-α Measurement by Multiplex Technology

Four cytokines of cell culture supernatants were measured by xMAP (also known as Suspension Array, SA) technology developed by Luminex Corporation. Mouse Fluorokine MAP Multiplex Kit and corresponding beads set (antibody-coated microparticles and biotinylated detection antibody) were used according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA). Cytokine standard cocktail was dissolved and diluted in Calibrator Diluent RD5K, which was also used as assay blank control. All washes were performed in 96 well filter-bottom microplate and removed by vacuum filtration to avoid any loss of microparticles. Incubations were performed at room temperature on a horizontal orbital shaker set at 500 ± 50 rpm, and the plate should be covered by foil plate sealer during incu-

bations. Microparticles, biotin antibody and strep-tavidin-PE concentrate were diluted with corresponding diluents included in base kit. Briefly, pre-wet the plate with 100 μl Wash Buffer and removed the liquid. Resuspended diluted microparticle mixture of cytokines mentioned above, added 50 μl of the mixture to each well of the plate. After addition of 50 μl standard or sample within 20 min, the plate was incubated at room temperature for 3 h. Removed the liquid and washed the plate with 100 μl Wash Buffer thrice, added 50 μl diluted Biotin antibody cocktail to each well and incubated the plate for 1 h. After the subsequent washes, 50 μl diluted Streptavidin-PE were added to each well and incubated for another 30 min. Repeated washing step trice, resuspended the microparticles by adding 100 μl of Wash Buffer, incubated the plate for 2 min and then read with Luminex 100 analyzer.

Cell Counting Kit 8 (CCK8) Assay

Cell viability was measured by Cell counting kit 8 (CCK8, Boster, Wuhan, China) based on tetrazolium salt WST-8. Mitochondrial dehydrogenases of living cells can induce the conversion of WST-8 (water-soluble tetrazolium salt-8) into a yellow water-soluble formazan, and the amount of formazan dye is proportional to the number of viable cells. Due to the high water-solubility and sensitivity of WST-8, CCK8 is much more sensitive and reliable than other tetrazolium salts like MTT, XTT or MTS. RAW264.7 cells were seeded at 5×10^3 per well into 96 well microplate and incubated for 24h peripheral wells of microplate were filled with sterile phosphate buffered saline (PBS). After addition of different concentrations of MPT64 respectively, cells were incubated for another 48 h until testing. 10 μl of the CCK8 (Boster) solution was added to each well and the plate was incubated for an additional 1 h. Finally, the absorbance of each well was measured by multiskan spectrum microplate reader (Thermo, Walthan, MA, USA) at 490 nm wavelength.

Flow Cytometry Analysis of Apoptosis

To investigate the cytotoxic effect of MPT64 on RAW264.7 macrophage, cell-death pattern of Raw264.7 macrophage was analyzed after being treated by different inducers designed. Cells grown in 6-well plates (2×10^5 cells per well) were incubated with or without inducers, then harvested by trypsinization and rinsed with PBS twice. After centrifugation at 4°C, 1000 g for 5 min, cells were suspended by 100 μl binding buffer, and then stained using fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kit I (BD Pharmingen, San Jose, CA, USA) at room temperature for 15 min. All procedures should be carried on softly. Thereafter, flow cytometric analysis of cells was performed with FACSAsia flow cytometer (BD, San Jose, CA, USA) within 1 h. Cytometric analysis was repeated three times.

Western Blot Analysis

RAW264.7 cells cultured in different conditions were lysed by RIPA buffer (150 mM NaCl, 50 mM Tri-HCl, pH 7.4, 0.5% sodium deoxycholate, 1% TritonX-100, 1 mM EDTA) with protease and phosphatase inhibitors. After centrifugation at 4°C, 12,000 g for 15 min, cell supernatants was recovered and the protein content was determined with DC protein assay (Bio-Rad, Carlsbad, CA, USA). Fifty micrograms of total proteins were separated by 12% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membrane (PALL Life Science, Washington, NY, USA). After blocking with 5% non-fat dry milk in Tris-buffered saline (TBS) with 0.05% Tween20 (TBST) at room temperature for 1 h, membrane was washed with TBST solution for 10 min trice, and then probed with antibodies of anti-cleaved caspase-3 or anti-β-actin (Cell Signaling Technology, Boston, MA, USA) at a dilution of 1:1000 at 4°C overnight. PVDF membrane was washed with TBST for another three times and incubated with horseradish peroxidase conjugated secondary anti-rabbit (Boster) at a dilution of 1:1000 at room temperature for 1 h. Finally, blots detection was performed using ECL chemiluminescence (Beyotime, Shanghai, China) and Multilmage System (Alpha Innotech, San Leandro, CA, USA).

Statistical Analysis

Data were evaluated using the SPSS 16.0 statistical software package (SPSS Inc., Chicago, IL, USA). Data were analyzed using one-way ANOVA. Results were expressed as the mean ± standard deviation, and $p < 0.05$ was considered statistically significant.

Results

Purification of MPT64 Expressed in Escherichia coli

Induced *E. coli* expressing MPT64 was sonicated and centrifugated to generate a soluble pro-

tein lysate, which contained almost all MPT64 protein. The soluble protein was purified as described in Materials and Methods, and the purity of this protein was detected by SDS-PAGE and confirmed by Western Blot assay. Purified MPT64 protein has a molecular mass of 28 kDa, which is equivalent to predicted molecular weight (Figure 1A, B). The endotoxin content of purified protein did not exceed 0.4 ng/mg as determined by E-Toxate kit (Sigma Aldrich, Saint Louis, MO, USA.

MPT64 Induced Cytokines Production by RAW24.7 Cells

To determine whether MPT64 could activate RAW264.7 cells to produce inflammatory cytokines, RAW264.7 cells were cultured with MPT64 for detection. 25 μg/ml of MPT64 significantly enhanced the production of IL-1β, IL-6, IL-10 and TNF-α (Figure 2A-D) when compared with control group. The expression of IL-1β was in a dose and time-dependent manner. 25 μg/ml of MPT64 significantly triggered TNF-α and IL-6 secretion, higher concentration of MPT64 had similar effect on their production. IL-6 expression increased in a time-dependent way; the highest secretion of TNF-α in response to MPT64 happened at 24 h, and then it declined softly. Dose-dependent IL-10 generation induced by MPT64 was also detected, with a distinct peak

occurring at 24 h. In contrast to consistent increasing of IL-1β, IL-10 production decreased gradually after that peak (Figure 2D).

30 ng/ml of IFN-γ induced considerable IL-6 at 6h and 12h time point ($p < 0.05$ and $p < 0.01$ compared to control group); pretreatment of MPT64 followed by IFN-γ induction dramatically enhanced IL-6 production, the difference between co-stimulation group and MPT64-treated group was significant ($p < 0.01$, Figure 2E). These results suggested that MPT64 potentiated RAW264.7 cells to generate several cytokines; co-stimulation of MPT64 with IFN-γ had synergistic effect on IL-6 secretion, especially at 6 h, 12 h time point.

Vitality of RAW264.7 Cells Might be Enhanced by MPT64

The effect of MPT64 on RAW264.7 cell viability was assessed by CCK8 assay. Absorbance in macrophage was enhanced by all concentrations of MPT64 (25, 50, 75, 100, 125 and 150 μg/ml) designed (Figure 3). 25 μg/ml of MPT64 significantly increased the absorbance of RAW264.7 cells ($p < 0.05$), and strengthened difference between MPT64 treated and control group was induced by 50 μg/ml or higher concentration of MPT64 ($p < 0.01$). These results demonstrated that the viability of RAW264.7 macrophage might be effectively promoted by MPT64.
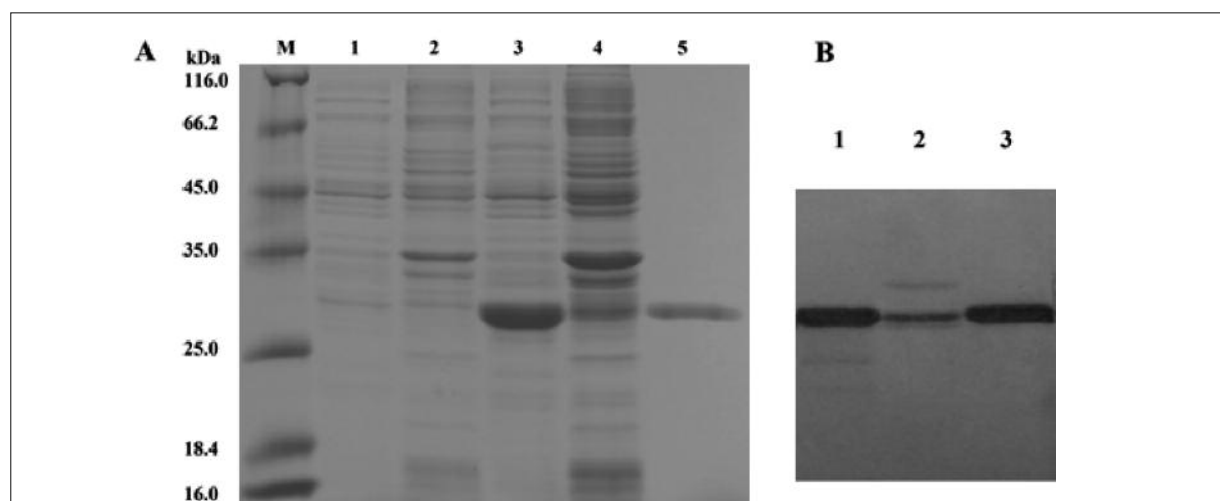

Figure 1. Characterization of purified MPT64 by SDS-PAGE and Western Blot. *A,* Purified MPT64 was analyzed by Coomassie Blue stained 12% SDS-PAGE. Lanes: M, molecular weight marker; 1, supernatant of *E. coli* lysate harboring pET19b(+) induced by IPTG; 2, pellet of *E. coli* lysate harboring pET19b(+) induced by IPTG; 3, supernatant of *E. coli* lysate harboring pET-MPT64 induced by IPTG; 4, pellet of *E. coli* lysate harboring pET-MPT64 induced by IPTG; 5, purified 28 kDa MPT64 protein. *B,* Western Blot analysis of MPT64 performed with mouse anti-his tag monoclonal antibody. Lanes: 1, supernatant of *E. coli* lysate harboring pET-MPT64 induced by IPTG; 2, pellet of *E. coli* lysate harboring pET-MPT64 induced by IPTG; 3, purified MPT64 protein.
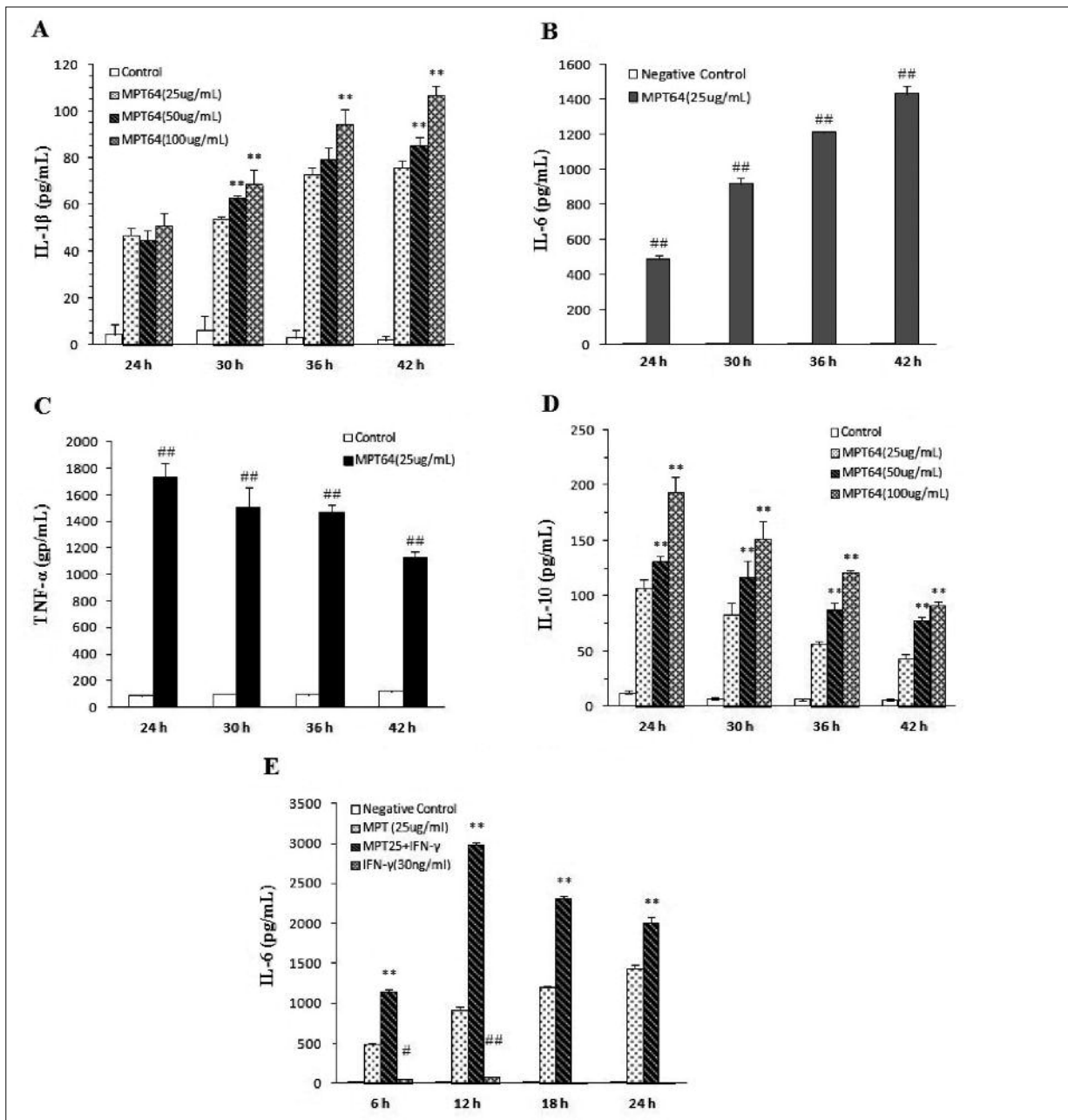
Figure 2. MPT64 potentiated cytokines secretion in RAW264.7 macrophage. Cells were incubated with different concentrations of MPT64 (25, 50, 100 μg/ml) for 24, 30, 36 and 42 h respectively, the level of IL-1β *(A)*, IL-6 *(B)*, TNF-α *(C)* and IL-10 *(D)* in cell culture supernatant were detected by xMAP technology. *E,* The complex treatment significantly enhanced IL-6 release. RAW264.7 cells were pretreated with MPT64 (25 μg/ml) for 6, 12, 18 and 24 h respectively, then incubated with or without IFN-γ (30 ng/ml) till 24 to 42 h, cell culture supernatant was collected for IL-6 detection. Control and IFN-γ (30 ng/ml) induced groups were also tested. Values are means ± SD from 3 independent experiments. *$p < 0.05$, **$p < 0.01$ compared to group stimulated by MPT64 (25 μg/ml); #$p < 0.05$, ##$p < 0.01$ compared to control group.

MPT64 Inhibited Apoptosis in Both Normal and INF-γ Induced RAW264.7 Cells

Apoptosis of RAW274.7 cells in different experimental conditions was assayed by flow cytometry analysis. Apoptosis in control group increased gradually during the period of study (Figure 4A-C). Treatment of MPT64 decreased both early and late apoptosis of RAW264.7 macrophage when compared with control group, but the difference was significant only at 24 h
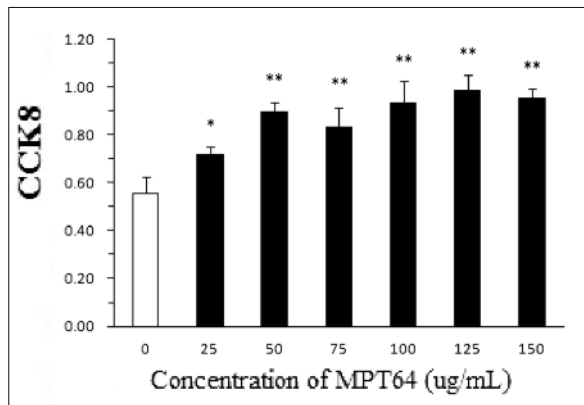
Figure 3. Vitality of RAW264.7 cells might be enhanced by MPT64. RAW264.7 macrophage were seeded into 96 well flat-bottomed microplate and incubated with various concentrations of MPT64 (25, 50, 75, 100, 125 and 150 μg/ml) for 48 h. Absorbance of each well was measured at 490 nm by microplate reader. Data are expressed as means ± SD. n = 6 wells for all CCK-8 assay. *$p < 0.05$, **$p < 0.01$, compared with control group.

time point ($p < 0.05$). On the contrary, apoptosis in IFN-γ stimulated group was higher than other groups, and at 18 and 24 h time point, almost all early apoptosis turned into late apoptosis. Pretreatment of MPT64 down-regulated the apoptosis induced by IFN-γ, and the difference between co-stimulated group and IFN-γ induced group was significant at 18 and 24 h time point ($p < 0.05$). These results indicated that adequate stimulation of MPT64 could inhibit apoptosis of RAW264.7 macrophage, neither the macrophages was stimulated by IFN-γ or not.

MPT64 Pretreatment Affected the Expression of Cleaved Caspase-3 of RAW264.7 Cells in Both Control and IFN-γ Treated Groups

Western Blot analysis showed that, apparent promotion of cleaved caspase-3 in most groups was observed at 18 h time point. MPT64 down-regulated the expression of cleaved caspase-3 at 12, 18 and 24 h time point when compared with control group, but the difference was not significant. This result was consistent with apoptosis tendency measured by flow cytometry analysis. However, the level of cleaved caspase-3 in IFN-γ induced macrophage kept on a low level. Activated caspase-3 in IFN-γ induced group was much lower than that of co-stimulated group at all time point ($p < 0.05$), and even lower than control group ($p < 0.05$ at 12 and 18 h time point). Since

highest apoptosis of RAW264.7 cells induced by IFN-γ was confirmed by flow cytometry assay, we supposed that cleaved caspase-3 might not play a major role in apoptosis induced by IFN-γ. Cleaved caspase-3 expression in MPT64 and IFN-γ co-stimulated group, however, was higher than that of both control and MPT64 induced group.

Discussion

In this study, we found that MPT64 induced RAW264.7 macrophage to secrete high levels of proinflammatory cytokines IL-1β, IL-6 and TNF-α. Efficient induction of classic cytokines is decisive for the defense against Mtb infection, immune mediators like IL-1β, IL-6 and TNF-α can activate macrophage to induce early bacterial killing[17,18]. In mouse model, IL-1β from infected macrophage involved in the granuloma formation, deficient of this cytokine enhanced mycobacterial growth at infection site[19]. Absence of IL-6 promoted delayed IFN-γ response in the lung and increased Mtb burden[20,21]. As the regulator of inflammatory response, TNF-α mediates early inflammatory responses against Mtb, it also stimulates the secretion of IL-1 and IL-6[22]. High level of TNF-α contributed to the control of Mtb growth and granuloma formation, on the contrary, TNF-α deficient mouse produced decreased levels of cytokines, resulting in defective granuloma formation[23]. Moreover, increased susceptibility to BCG infection caused by TNF-α blockers was also demonstrated[24,25]. Therefore, relatively high expression of TNF-α induced by MPT64 was, at least partly, responsible for the persistently increasing of IL-1β and IL-6. And these cytokines induced by MPT64 might contribute to the early immunity against Mtb infection.

MPT64 alone also induced considerable IL-10, which is considered to be associated with apoptosis inhibition of cells infected with virulent mycobacteria strains[26-28]; while TNF-α has been reported to play opposite roles in different experimental systems[29-31]. Mustafa et al[14,15] revealed a positive correlation of IL-10 and TNF-α, and suggested the cooperation of these two cytokines might be responsible for apoptosis inhibition[14,15]. In this study, enhanced viability and decreased apoptosis in RAW264.7 cells stimulated by MPT64 were observed (Figures 3 and 4), similar expression tendency of IL-10 and TNF-α
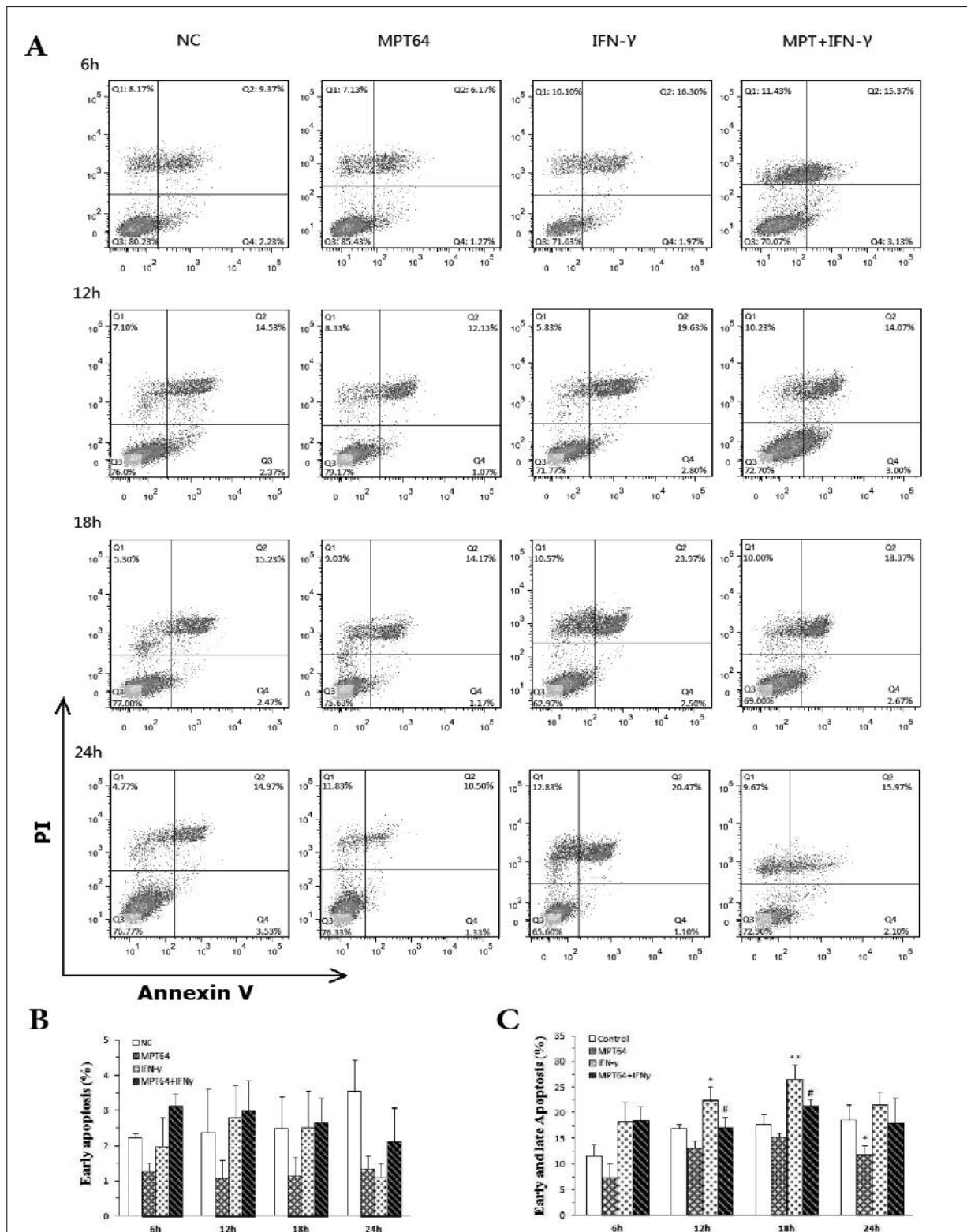
Figure 4. Apoptosis Inhibition mediated by MPT64 in RAW264.7 cells. **A,** Representative AnnexinV/PI flow cytometry analysis under different experimental conditions at 6, 12, 18 and 24 h time points. Cells in the early stage of apoptosis are FITC AnnexinV+ PI−; and cells in late apoptosis or already dead are FITC AnnexinV+ PI+. **B, C,** Quantitative analysis of Annexin V+ PI+ (Q2) and AnnexinV+ PI− (Q4) RAW264.7 macrophage by flow cytometry. Values are means ± SD from 3 independent experiments. $*p < 0.05$, $**p < 0.01$ compared to control group, $\#p < 0.05$ compared to groups stimulated by IFN-γ (30 ng/ml).

was also detected. These results indicated that IL-10 induced by MPT64 might contribute to apoptosis inhibition in this model. Therefore, we concluded that MPT64 induced RAW264.7 cells to secrete classic immune mediators IL-1β, IL-6 and TNF-α; these cytokines in turn activated macrophage. On the other hand, IL-10 induced by MPT64 might involve in virulent mechanism of apoptosis inhibition[14].

Absorbance induced by MPT64 in CCK8 assay increased significantly (Figure 3), the result was in agreement with the promotion of IL-10 induced by MPT64[27,32]; flow cytometry analysis further revealed the effect of MPT64 on apoptosis inhibition. Moreover, it seemed that adequate pretreatment of MPT64 might also down-regulated IFN-γ induced apoptosis, since apoptosis ratio induced by co-stimulation of IFN-γ with MPT64 was lower than that by IFN-γ alone (Figure 4).

Cleaved caspase-3, the activated form of lethal protease, is served as an executor at the most distal stage of apoptosis pathway[33-35]. In some extent, it is a reliable indicator of apoptosis. Expression of cleaved caspase-3 in both normal and MPT64-treated RAW264.7 cells suggested that these immune cells were eliminated by apoptosis to prevent their destructive potential (Figure 5). Interestingly, although the most serious apoptosis induced by IFN-γ was confirmed by flow cytometry assay, the expression of cleaved caspase-3 in this group was much lower than that of other groups. Consistent low level of cleaved caspase-3 in this condition demonstrated that IFN-γ induced apoptosis mainly through a caspase-3 independent pathway. Furthermore, pretreatment of MPT64 slightly decreased apoptosis induced by IFN-γ, but co-stimulation of MPT64 with IFN-γ strengthened cleaved caspase-3 expression. Based on these findings, we supposed that MPT64 exerted its effect on apoptosis regulation partly through caspase-3 pathway.

Conclusions

This study showed that MPT64 could activate RAW264.7 macrophage to induce considerable cytokines; but it also contributed to the virulence
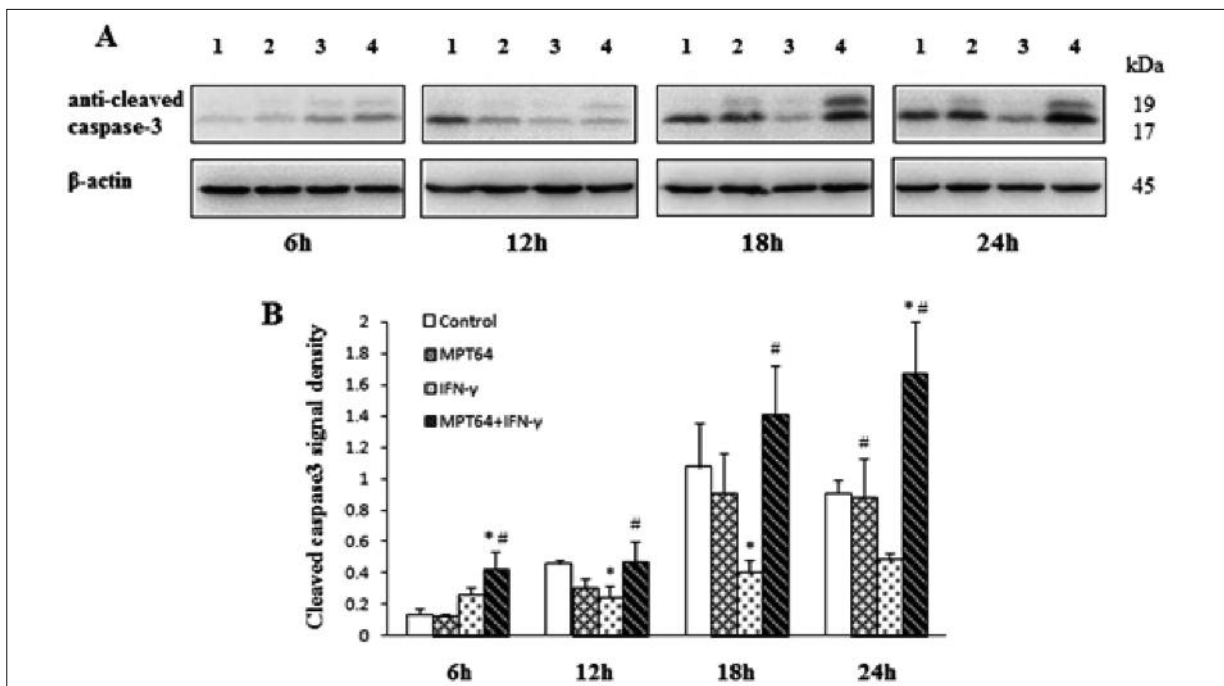

Figure 5. Expression of cleaved caspase-3 with different stimulations in RAW264.7 macrophage. **A,** Western Blot analysis of cleaved caspase-3 in RAW264.7 cells. Western Blot revealed that INF-γ enhanced the apoptosis in macrophage pre-incubated with MPT64 (25 µg/ml) for different time. Lanes: 1, untreated control; 2, MPT64 (25 µg/ml) treated groups; 3, IFN-γ (30 ng/ml) treated groups; 4, RAW264.7 cells were pretreated with MPT64 (25 µg/ml) for 6, 12, 18 and 24 h, and then stimulated with IFN-γ (30 ng/ml). **B,** Densitometric analysis of cleaved caspase-3 in Figure 5A. Data are the mean ± SD of three independent experiments with similar results. *$p < 0.05$ compared to control group; #$p < 0.05$ compared to compared to groups stimulated by IFN-γ (30 ng/ml).

mechanism of apoptosis inhibition. Co-stimulation of MPT64 and IFN-γ, however, not only promoted IL-6 production significantly but also modulated apoptosis of RAW264.7 cells, which might be beneficial for host immune. These findings implied that the effect of MPT64 on macrophage could be optimized by IFN-γ. And we supposed our investigation might provide a reference for vaccine design and treatment of TB.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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