Mycophenolic Acid Synergizing with Lipopolysaccharide to Induce Interleukin-1β Release via Activation of Caspase-1

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Abstract

Background: The previous study showed that mycophenolic acid (MPA) synergizing with lipopolysaccharide (LPS) promoted interleukin (IL)-1β release, but the mechanism is unclear. This study aimed to investigate the mechanism of MPA synergizing with LPS to induce IL-1β release.

Methods: Undiluted human blood cells, THP-1 human myeloid leukemia mononuclear cells (THP-1) cells, or monocytes were stimulated with LPS and treated with or without MPA, and the supernatant IL-1β was detected by enzyme-linked immunosorbent assay. The mRNA levels of IL-1β were detected by real-time quantitative polymerase chain reaction. The intracellular protein levels of nuclear factor kappa B (NF-κB) phospho-p65 (p-p65), precursor interleukin-1β (pro-IL-1β), NOD-like receptor pyrin domain containing-3 (NLRP3), and cysteine aspartic acid-specific protease-1 (caspase-1) p20 in THP-1 cell were measured by Western blot.

Results: The MPA alone failed to induce IL-1β, whereas MPA synergized with LPS to increase IL-1β in a dose-dependent manner (685.00 ± 20.00 pg/ml in LPS + 5 μmol/L MPA group, P = 0.035; 742.00 ± 31.58 pg/ml in LPS + 25 μmol/L MPA group, P = 0.017; 1000.00 ± 65.59 pg/ml in LPS + 75 μmol/L MPA group, P = 0.024; versus 408.00 ± 35.50 pg/ml in LPS group). MPA alone has no effect on the IL-1β mRNA expression, LPS induced the expression of IL-1β mRNA 2761 fold, and LPS + MPA increased the IL-1β expression 3018 fold, which had the same effect with LPS group (P = 0.834). MPA did not affect the intracellular NF-κB p-p65 and pro-IL-1β protein levels but activated NLRP3 inflammasome. Ac-YVAD-cmk blocked the activation of caspase-1 and subsequently attenuated IL-1β secretion (181.00 ± 45.24 pg/ml in LPS + MPA + YVAD group vs. 588.00 ± 41.99 pg/ml in LPS + MPA group, P = 0.014).

Conclusions: Taken together, MPA synergized with LPS to induce IL-1β release via the activation of caspase-1, rather than the enhanced production of pro-IL-1β. These findings suggested that patients immunosuppressed with mycophenolate mofetil may have overly activated caspase-1 during infection, which might contribute to a more sensitive host defense response to invading germs.

Key words: Autoimmune Diseases; Caspase-1 Host Defense; Interleukin-1β; Lipopolysaccharide Mycophenolic Acid

Introduction

Mycophenolic acid (MPA) is the active metabolite of mycophenolate mofetil (MMF) that inhibits type II inosine monophosphate dehydrogenase, a rate-limiting enzyme during the *de novo* biosynthesis of guanosine nucleotide.[1] The inhibition in nucleotide synthesis eventually leads to the inhibition of T and B lymphocyte proliferation. MMF is an immunosuppressive agent that is widely used in the treatment of allogeneic transplantations and autoimmune diseases, especially in systemic lupus erythematosus (SLE) and lupus nephritis.[2-4]

Intravenous cyclophosphamide (CYC) plus steroid has been recognized as the standard protocol for severe lupus nephritis.[5] Unfortunately, this strategy has been usually

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correlated with severe opportunistic infections.[6,7] Recent studies suggested that patients treated with MMF plus glucocorticoid exhibited better clinical outcomes as well as lower infection incidences than patients with glucocorticoid in combination with CYC or azathioprine (AZA).[6-8]

Previous results demonstrated that cytokine levels could be used to evaluate the immune status in patients with chronic heart failure or those exposed to immunosuppressants.[9-11] The previous study showed that dexamethasone (DEX) reduced the release of multiple cytokines, including interleukin (IL)-1β, IL-2, IL-5, IL-6, IL-8, IL-17, IL-10, IL-13 and interferon-γ, granulocyte-colony stimulating factor. Surprisingly, MPA in association with phytohemagglutinin (PHA) increased the secretion of IL-1β even though under the condition with DEX,[9] which contradicted the traditional conception that immunosuppressants inhibit the secretion of inflammatory factors.

IL-1β is the "master" cytokine mainly produced by monocytes and macrophages in response to inflammatory stimuli such as lipopolysaccharide (LPS) and plays an essential role in innate and adaptive immune responses.[12,13] As IL-1β is required for the efficient clearance of infected bacteria, we believe that increased IL-1β may contribute to less infection in MMF-treated patients. However, it is unknown how MPA leads to the increased IL-1β release.

Different from most canonically released cytokines such as tumor necrosis factor (TNF)-α, IL-2, and IL-12 that are dependent on the endoplasmic reticulum and Golgi apparatus, IL-1β is more slowly released through a nonclassical secretory pathway.[13,14] However, the production of IL-1β is tightly controlled by at least three distinct steps. The initial events involve the production of the NOD-like receptor pyrin domain containing-3 (NLRP3) and precursor interleukin-1β (pro-IL-1β) through the activation of Toll-like receptor 4. Second, the activation of inflammasome mediates the autoactivation of pro-caspase-1 (p45) to active caspase-1 (p20 and p10). Third, the cleavage of the 31,000 pro-IL-1β by caspase-1 (p20 or p10) results in the production and secretion of active form of 17,000 IL-1β.[15] In addition to IL-1β, IL-18 and IL-33 were also identified as the caspase-1 substrates.[15,16] NLRP3 inflammasome is the most widely studied intracellular multiprotein complex that contains an NLRP3 sensor, an apoptosis-associated speck-like protein containing a caspase recruitment domain adaptor and a pro-caspase-1. The complex serves as a molecular platform for pro-caspase-1 autoactivation and regulates caspase-1 (p20)-mediated cleavage of pro-IL-1β. It has been known that NLRP3 inflammasome plays a crucial role in both immunity and inflammation.[17,18]

In this study, we first determined the effects of MPA in association with LPS on IL-1β production. Then, we explored whether the synergized effect on IL-1β production was mediated through the nuclear factor kappa B (NF-κB) pathway to produce more pro-IL-1β or through the triggering of NLRP3 inflammasome to enhance activation of caspase-1 that resulted in degradation of pro-IL-1β into mature IL-1β.

Methods

Ethical approval
This study was approved by the Ethics Committee of the Third Affiliated Hospital of Southern Medical University (No. 201501001).

Materials
MPA, DEX, 6-mercaptopurine (6-MP, the active metabolite of AZA), CYC, LPS, adenosine triphosphate (ATP), and the specific inhibitor of caspase-1 ac-YVAD-cmk were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-human pro-IL-1β, phospho-NF-κB p65 (p-p65), total NF-κB p65, and β-actin antibodies were purchased from cell signaling technology (Danvers, USA). The antibodies against human caspase-1 and NLRP3 were purchased from Abcam (Cambridge, UK). The horseradish peroxidase (HRP)-conjugated secondary antibodies against mouse and rabbit were purchased from Cell Signaling Technology (Danvers, USA). Enzyme-linked immunosorbent assay (ELISA) kits for human cytokine detection were purchased from eBioscience (San Diego, USA). All cell culture reagents were from Gibco (Paisley, UK).

Isolation of human peripheral blood monocytes
Peripheral blood was obtained from healthy donors with informed consents. Peripheral blood mononuclear cells were isolated using Ficoll density gradient centrifugation and CD14+ monocytes separated with anti-CD14-conjugated microbeads (Miltenyi Biotec, Germany).

Cell culture
Monocytes and THP-1 cells (the human monocyte-like cells; 1×10^6 cells/ml) were cultured in Roswell Park Memorial Institute-1640 media containing 10% fetal bovine serum and 100 U/ml penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2.

Whole blood treatment
Heparinized undiluted whole blood (200 μl) was cocultured with or without DEX (0.25, 2.5, 25 μmol/L), MPA (5, 25, 75 μmol/L), 6-MP (0.8, 8, 80 μmol/L), CYC (0.4, 4, 40 μmol/L), and stimulated with LPS (1 μg/ml) for 12 h at 37°C in a humidified atmosphere containing 5% CO_2. Then, the blood was centrifuged, and the supernatants collected and stored at −80°C until analysis.

Cell treatment
Cells were treated with LPS (1 μg/ml) and/or MPA (5, 25, 75 μmol/L), ATP (5 mmol/L), and ac-YVAD-cmk (100 μmol/L). After 12 h, the supernatants were harvested and stored at −80°C for ELISA. One hour before LPS and MPA or ATP stimulation, ac-YVAD-cmk was added.

For Western blot analyses, the THP-1 cells were cultured in 6-well plates (4×10^6 cells/ml) in the absence or presence

of LPS (1 µg/ml), MPA (75 µmol/L), ATP (5 mmol/L), and ac-YVAD-cmk (100 µmol/L) as indicated.

Cytotoxicity assay

The cytotoxicity detection kit (Roche Diagnostics GmbH, Mannheim, Germany) was used for measuring the lactate dehydrogenase (LDH) activity. THP-1 cells were cultured for 12 h in the presence or absence of MPA (5, 25, and 75 µmol/L) and LPS (1 µg/ml). The assays were performed according to the manufacturer's instructions.

Cytokine measurements

Culture supernatants were collected after 3 and 12 h, and IL-1β was determined by ELISA according to the manufacturer's instructions.

Real-time quantitative polymerase chain reaction

The expression levels of IL-1β to β-actin RNA were measured with the LightCycler® system (Roche, Mannheim, Germany). The total RNA was isolated from THP-1 cells with trizol extraction. The first strand cDNA synthesis kit for real-time polymerase chain reaction (RT-PCR) (TOYOBO, Japan) was used to prepare the first-strand cDNA according to the manufacturer's instructions. The IL-1β fragment was amplified using primers: forward, 5'-AAACCCTCTGTCATTCGCTCCC-3'; reverse, 5'-ACACTGCTACTTCTTGCCCCCT-3'. The β-actin fragment was amplified using the following primer: forward, 5'-TGTTCCCCTTGGTATTTG-3'; reverse, 5'-CAAGACAAAACAACTGGT-3'. Data were analyzed using LightCycler Software, version 3.5 (Roche) and the program LinRegPCR, version 7.5 for analysis of RT-PCR data. To adjust for variations in the amount of input RNA, the IL-1β levels were normalized against the mRNA levels of the β-actin using the calculation $2^{-\Delta Ct}$.

Western Blot analysis

Proteins were extracted from THP-1 cell lysates in radioimmunoprecipitation buffer (Sigma Aldrich) containing phosphatase and proteinase inhibitors for 30 min before centrifugation (13,200×*g*, 4°C, 10 min). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were then blocked with 5% nonfat milk, washed with TBS plus Tween briefly, and incubated with primary antibodies (anti-pro-IL-1β, anti-p-p65, anti-p65, anti-caspase-1, and anti-NLRP3) at 4°C overnight. The anti-β-actin monoclonal antibody served as a loading control. Afterward, the membranes were incubated with secondary HRP-conjugated anti-rabbit/mouse IgG antibodies for 1 h at room temperature. Finally, an enhanced chemiluminescence plus chemiluminescent kit was used for the detection of the signal (Advansta, USA).

Statistical analysis

Data are presented as the mean ± standard error (SE). Significant differences between the mean values were evaluated using one-way analysis of variance followed by Student-Newman-Keuls multiple test or Dunnett's test for multigroups. All data were analyzed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered as statistically significant.

RESULTS

Immunosuppressants distinctively influenced interleukin-1β secretion in undiluted whole blood, while only mycophenolic acid enhanced lipopolysaccharide-induced interleukin-1β release

The previous studies showed that MPA increased the secretion of IL-1β, while decreased the secretion of IL-2 and IL-13, in whole blood stimulated with PHA.[9] Therefore, we determined whether MPA, DEX, CYC, and AZA, the immunosuppressants that are commonly used in the clinical treatment of SLE, have different effects on IL-1β secretion. We analyzed IL-1β secretion in human whole blood treated with LPS or the immunosuppressants or the combination of different doses of immunosuppressants with LPS for 12 h. The results showed that the level of IL-1β in control group was 10.78 ± 0.78 pg/ml, and incubation with immunosuppressants (DEX, MPA, 6MP, and CYC) alone did not induce detectable levels of IL-1β release (11.56 ± 1.61 pg/ml in DEX group, $P = 1.000$; 11.91 ± 0.95 pg/ml in MPA group, $P = 0.980$; 11.56 ± 2.79 pg/ml in 6MP group, $P = 1.000$; 11.56 ± 1.61 pg/ml in CYC group, $P = 1.000$). Compared to LPS stimulation alone (499.00 ± 67.11 pg/ml), DEX dose dependently inhibited the secretion of LPS-induced IL-1β (249.00 ± 28.91 pg/ml in LPS + 0.25 µmol/L DEX group, $P = 0.024$; 117.00 ± 10.35 pg/ml in LPS + 2.5 µmol/L DEX group, $P = 0.010$; 72.00 ± 3.61 pg/ml in LPS + 25.0 µmol/L DEX group, $P = 0.010$; Figure 1a). In contrast to DEX, MPA significantly and dose dependently increased LPS-induced IL-1β release (686.00 ± 55.46 pg/ml in LPS + 5 µmol/L MPA group, $P = 0.023$; 1356.00 ± 70.65 pg/ml in LPS + 25 µmol/L MPA group, $P < 0.001$; 1938.00 ± 1.46 pg/ml in LPS + 75 µmol/L MPA group, $P < 0.001$; Figure 1b), compared to LPS stimulation alone. However, 6-MP or CYC did not have any effects on the LPS-induced release of IL-1β (all $P > 0.05$; Figure 1c and 1d). These data demonstrated that different immunosuppressants had distinctive effects on IL-1β secretion, while only MPA enhanced LPS-induced IL-1β release.

Mycophenolic acid enhanced lipopolysaccharide-induced interleukin-1β release in human monocytes and THP-1 cells

To further verify this phenomenon, monocytes and THP-1 cells were tested. Freshly isolated human monocytes and THP-1 cells were cultured for 12 h in the presence or absence of MPA or LPS and then the concentration of IL-1β in the supernatant detected by ELISA. Indeed, MPA synergistically enhanced LPS-stimulated IL-1β release in monocytes (669.00 ± 30.30 pg/ml in LPS + MPA group versus 284.00 ± 7.09 pg/ml in LPS
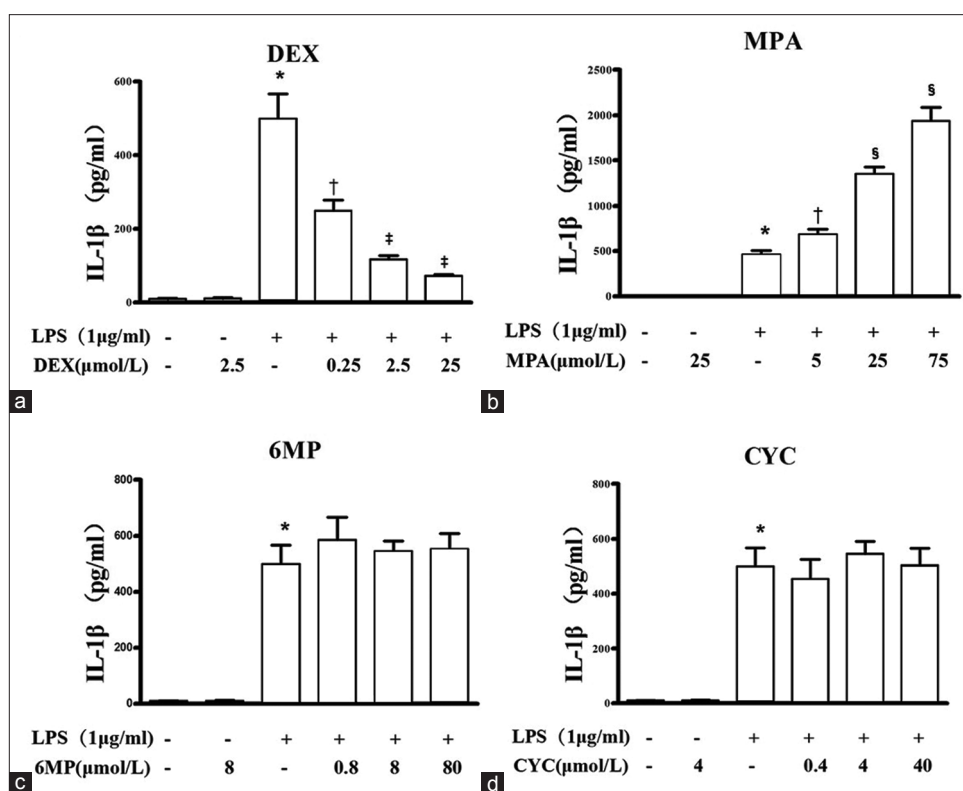
Figure 1: DEX (a), MPA (b), 6MP (c), CYC (d) distinctively influenced IL-1βsecretion in undiluted whole blood. Immunosuppressants distinctively influenced IL-1β secretion in undiluted whole blood. *$P < 0.010$, compared with control; while †$P < 0.050$, ‡$P < 0.010$, and §$P < 0.001$ versus LPS alone group. LPS: Lipopolysaccharide; MPA: Mycophenolic acid; DEX: Dexamethasone; 6-MP: 6-mercaptopurine; AZA: Azathioprine; CYC: Cyclophosphamide; IL: Interleukin.

group, $F = 391.455$, $P = 0.014$; Figure 2a). Again, compared with LPS group (408.00 ± 35.50 pg/ml), the effects displayed an obviously dose-dependent pattern in THP-1 cells (685.00 ± 20.00 pg/ml in LPS + 5 μmol/L MPA group, $P = 0.035$; 742.00 ± 31.58 pg/ml in LPS + 25 μmol/L MPA group, $P = 0.017$; 1000.00 ± 65.59 pg/ml in LPS + 75 μmol/L MPA group, $P = 0.024$; Figure 2b). MPA alone has no effect on the IL-1β mRNA expression (1.28 ± 0.20 fold, $P > 0.05$); LPS induced the expression of IL-1β mRNA 2761 fold after 3 h, compared with the control cells ($F = 3.975$, $P = 0.048$). However, the combination of LPS and MPA increased the IL-1β expression 3018 fold, which had the same effect with LPS group ($F = 3.975$, $P = 0.834$; Figure 2c). In addition, the enhanced IL-1β release in the presence of MPA plus LPS was not due to passive "leakage" of IL-1β because there were no significant changes in LDH release ($P > 0.05$; Figure 2d), indicating that the cell membrane was not impaired.

Mycophenolic acid did not affect the intracellular nuclear factor kappa B phospho-p65 and precursor interleukin-1β protein levels

To investigate the mechanisms by which MPA enhanced LPS-mediated IL-1β release, we next determined whether a combined treatment of MPA with LPS also increased the production of pro-IL-1β. The amount of phospho-p65 (p-p65) is commonly used as an indicator of NF-κB activation that is crucial for the production of pro-IL-1β.[19] Thus,

we determined whether the treatment of MPA with LPS influenced the activation of p-p65. THP-1 cells were cultured as before in the presence or absence of LPS with or without MPA for 3 h. The cells were harvested, cell extracts prepared as previously described. The results showed that although LPS alone dramatically increased p-p65 and pro-IL-1β, MPA alone did not affect the expression of both molecules [Figure 3a and 3b]. Nevertheless, MPA further dramatically increased the secretion of IL-1β as compared to LPS-treated cells (677.00 ± 13.98 pg/ml in LPS + MPA group vs. 378.00 ± 13.43 pg/ml in LPS group, $F = 110.6$, $P = 0.001$; Figure 3c). These findings indicated that synergy of MPA with LPS to induce IL-1β release did not result from the enhancement of pro-IL-1β synthesis.

Mycophenolic acid augmented lipopolysaccharide -induced interleukin-1β secretion via activation of caspase-1

The experiments above prompted us to hypothesize that MPA induced IL-1β release through the activation of NLRP3 inflammasome. Hence, we investigated the effect of MPA on the NLRP3 inflammasome in THP-1 cells. Cells were cultured as before in the presence or absence of LPS with or without MPA for 3 h. LPS in association with ATP served as a positive control.[20-22] The results showed that both MPA and LPS increased the levels of NLRP3. Although LPS alone induced moderate amount expression of caspase-1 (p20), MPA dramatically increased its expression.
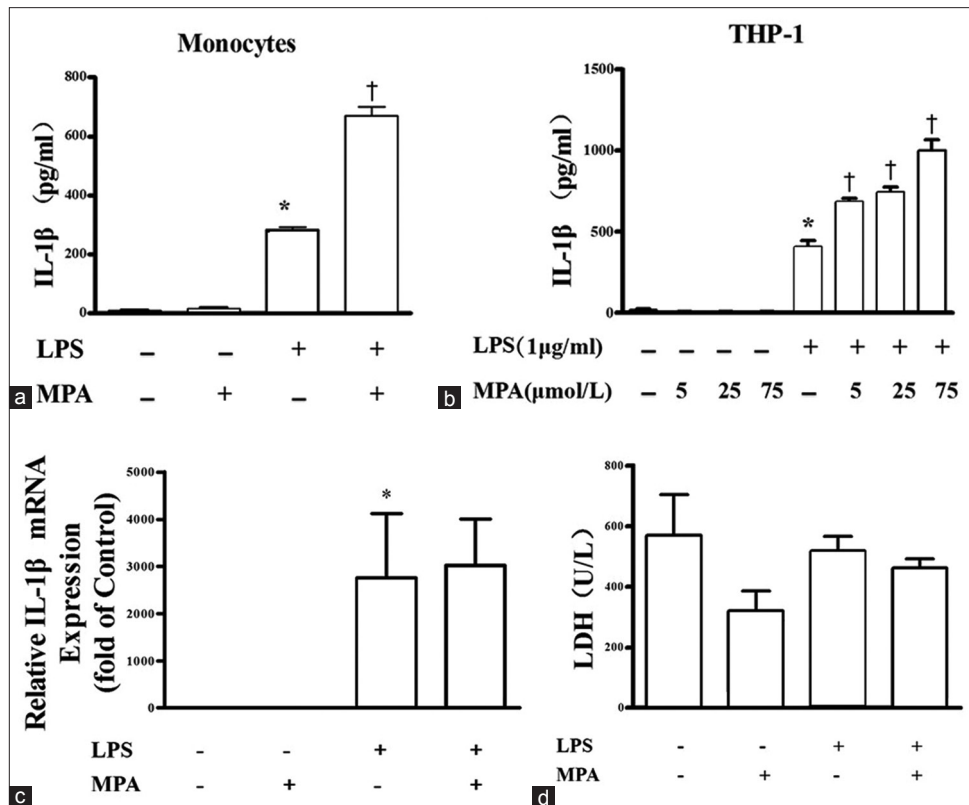
Figure 2: MPA enhanced LPS-induced IL-1β release in human monocytes (a) and THP-1 cells (b). (c) Expression of mRNA for IL-1β. (d)Cell viability. *$P < 0.010$ compared with control; while †$P < 0.050$ versus LPS alone group. LPS: Lipopolysaccharide; MPA: Mycophenolic acid; IL: Interleukin.
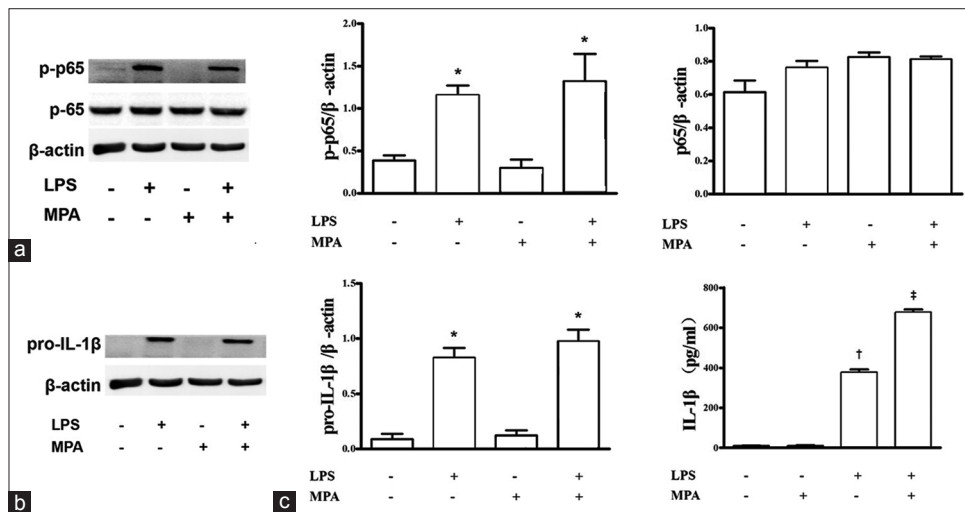

Figure 3: MPA did not affect the protein levels of intracellular p-p65 (a) and pro-IL-1β (b). (c) THP-1 cells were cultured with LPS and/or MPA; the supernatant IL-1β was determined 3 h later. *$P < 0.05$, †$P < 0.01$ versus control, ‡$P < 0.001$ versus LPS alone group. LPS: Lipopolysaccharide; MPA: Mycophenolic acid; IL: Interleukin; pro-IL-1β: Precursor interleukin-1β.

Therefore, MPA in association with LPS constituted the strongest stimulation for caspase-1 (p20) [Figure 4a]. Accordingly, LPS alone induced a moderate release of IL-1β (329.00 ± 28.28 pg/ml), whereas coincubation of LPS with MPA constituted a very strong stimuli for IL-1β production (588.00 ± 41.99 pg/ml, $F = 91.639$, $P = 0.035$; Figure 4b). Similar synergistic effects were also observed in the group treated with LPS and ATP (692.00 ± 3.91 pg/ml,

$F = 91.639$, $P = 0.024$; Figure 4b). Since caspase-1 (p20) has also been known to degrade pro-IL-18 into mature IL-18, we also tested IL-18 and found that MPA synergized with LPS to increase IL-18 release (1051.00 ± 44.79 pg/ml in LPS + MPA vs. 421.00 ± 28.74 pg/ml in LPS group, $F = 263.46$, $P = 0.007$; Figure 4c). To further investigate whether MPA-activated caspase-1 was related to IL-1β and IL-18 secretion, THP-1 cells were preincubated
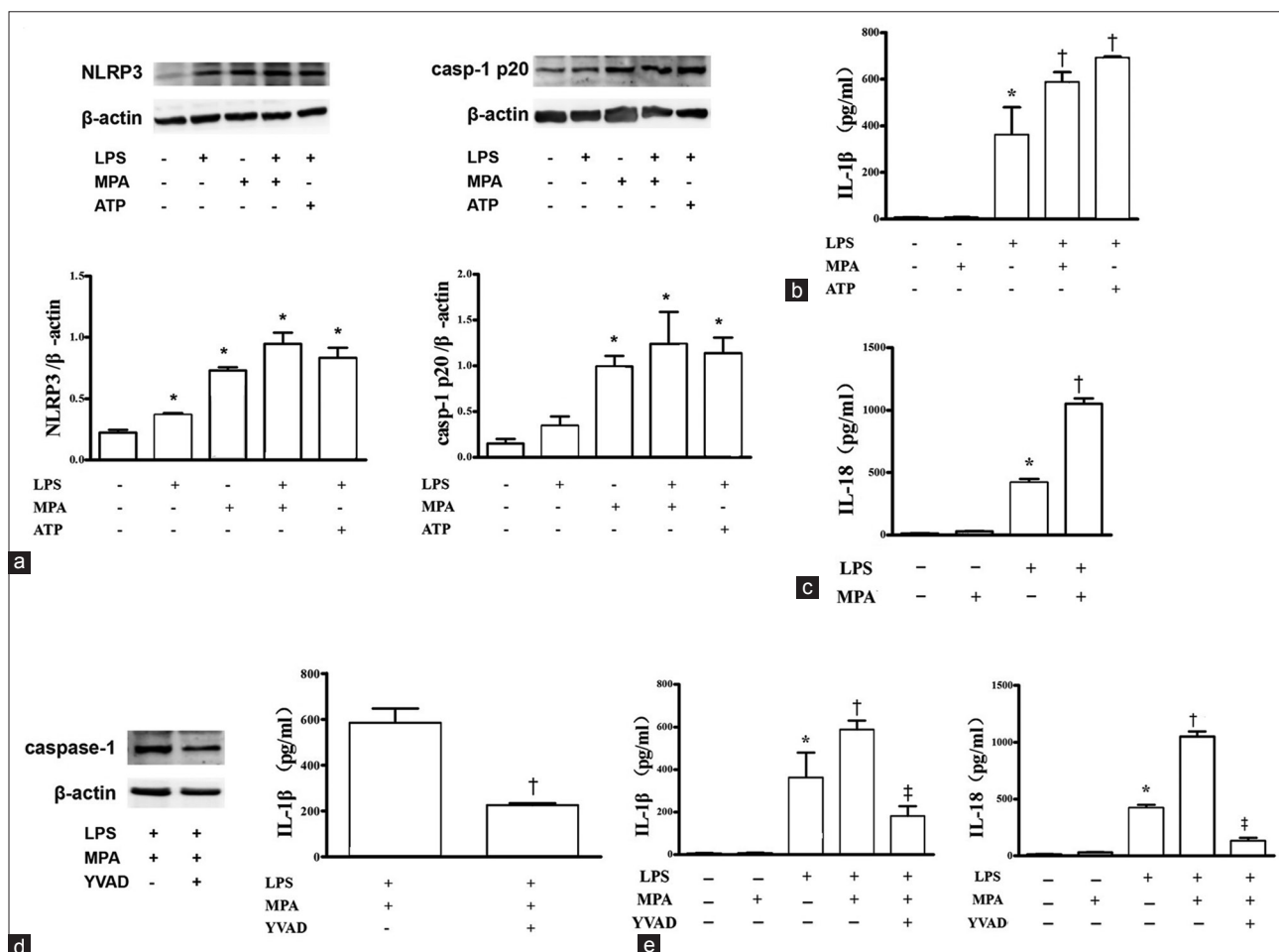
Figure 4: MPA augmented LPS-induced IL-1β release by activation of NLRP3 inflammasome (a). IL-1β (b) and IL-18 (c) were determined. Ac-YVAD-cmk was added (d). Casp-1 (p20), (e) IL-1β and IL-18 were detected. *$P < 0.05$ versus control, †$P < 0.05$ versus LPS group, ‡$P < 0.05$ versus LPS + MPA group. LPS: Lipopolysaccharide; MPA: Mycophenolic acid; IL: Interleukin; NLRP3: NOD-like receptor pyrin domain containing-3; IL-1β: Interleukin-1β.

with a specific caspase-1 inhibitor, ac-YVAD-cmk, for 1 h before stimulation with MPA and LPS. The results showed that ac-YVAD-cmk inhibited the activation of caspase-1 (p20) [Figure 4d] and significantly abrogated IL-1β ($181.00 ± 45.24$ pg/ml, $F = 91.639$, $P = 0.014$) and IL-18 ($130.00 ± 28.58$ pg/ml, $F = 263.46$, $P = 0.002$) release induced by LPS in combination with MPA [Figure 4e]. Therefore, caspase-1 was the key mediator in the synergy of MPA and LPS to stimulate IL-1β and IL-18 secretion.

DISCUSSION

In the last few decades, extensive researches have demonstrated that immunosuppressants such as CYC, AZA, glucocorticoids, MMF, and cyclosporin A are capable of reducing lymphocyte proliferation as well as altering cytokine secretion such as IL-2, IL-6, IL-17, IL-5, and IL-13.[23-27] However, immune suppression is commonly associated with opportunistic infections in clinical practice. Intravenous CYC plus steroid has been recognized as the standard protocol for severe lupus nephritis.[5] Unfortunately, this strategy has been usually correlated with severe opportunistic infections.[6,7] Several studies have reported that in lupus nephritis induction therapy,

MMF has been shown to be equivalent in effectiveness to intravenous CYC and superior to AZA with a more safety profile,[6-8] but the mechanism is still unclear. Previous results demonstrated that cytokine levels could be used to evaluate the immune status in patients with chronic heart failure or those exposed to immunosuppressants.[9-11] MPA is the active metabolite of MMF, which affects the *de novo* purine synthesis and consequently decreases lymphocyte proliferation. Surprisingly, although MPA decreased the secretion of IL-2 and IL-13 in PHA-treated whole blood, it dramatically promoted the secretion of IL-1β,[9] which contradicted the traditional conception that immunosuppressants inhibited the secretion of inflammatory factors. This study showed that whole blood cells, monocytes, and THP-1 cells treated with MPA in combination with LPS obviously augmented IL-1β [Figures 1b, 2a, 2b, 3c and 4b] and IL-18 release [Figure 4c].

Therefore, we determined to investigate the mechanisms of MPA-mediated IL-1β release. We found that MPA had no effect on the expression of NF-κB p-p65 and intracellular pro-IL-1β protein [Figure 3]. MPA treatment induced an increase in intracellular NLRP3 and activation of
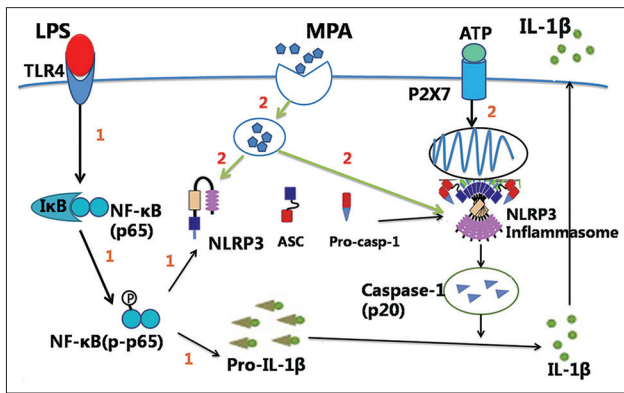
Figure 5: The "two-signal" model of MPA synergizing with LPS induces IL-1β release. (1) Priming. LPS initiates the synthesis of NLRP3 and pro-IL-1β. (2) Activation. MPA activates the NLRP3 inflammasome which contains active caspase-1 and degrades pro-IL-1β into IL-1β. LPS: Lipopolysaccharide; MPA: Mycophenolic acid; IL: Interleukin; NLRP3: NOD-like receptor pyrin domain containing-3; ATP: Adenosine triphosphate; pro-IL-1β: Precursor interleukin-1β; NF-κB: Nuclear factor kappa B; TLR4: Toll-like receptor 4; ASC: Apoptosis-associated speck-like protein containing a caspase recruitment domain.

caspase-1 [Figure 4a–4c]. As expected, the MPA-mediated IL-1β and IL-18 production could be blocked by the caspase-1 inhibitor ac-YVAD-cmk [Figure 4d and 4e]. All these data indicated that the enhanced secretion of IL-1β by MPA in association with LPS was due to increased caspase-1 activity to accelerate the degradation of pro-IL-1β, rather than result from the increased generation of pro-IL-1β. Taken together, the data substantiated a "two-signal" model where LPS stimulation acted as the first signal for efficient production of intracellular pro-IL-1β and NLRP3 proteins. In addition, MPA or ATP activated the NLRP3 inflammasome as the second signal to induce proteolytic activity of caspase-1 that subsequently degraded the pro-IL-1β protein into mature IL-1β [Figure 5]. This model could also account for increased IL-18 release after MPA treatment.

IL-1β could induce the synthesis of chemokines that can modulate macrophage, neutrophil, and T cell activity.[13,28] IL-18 also potentially primed neutrophil and promotes early innate immune responses.[29] IL-1β and IL-18 indirectly participated in antiviral responses.[30] The survival of mice pretreated recombinant IL-1β was higher than control after LPS challenged.[31] The mice deficient in IL-18 exhibited a higher mortality than wild-type mice while challenged with infection.[32] IL-1 receptor-deficient mice (IL-1R–/–) showed fatal outcome during *Staphylococcus aureus* sepsis, and the number of *Staphylococci* was increased as compared with IL-1R+/+ mice.[33] Mice that were treated with IL-1R antagonist anakinra significantly increased the mortality induced by *S. aureus*.[34] All of these findings indicated that IL-1β as well as IL-18 played important roles in host defense. Therefore, we believed that the synergy of MPA with LPS to increase IL-1β and IL-18 release might contribute to a better infection defense in patients treated with MMF than those treated with CYC.

It was reported that prolonged production of IL-1β could lead to severe tissue and organ damage.[35] In this study,

the finding that MPA induced IL-1β production was not contradictory to its immunosuppressive effect since the induction of IL-1β by MPA only happened in the presence of LPS, while MPA alone failed to do so [Figures 1 and 2]. In other words, patients with autoimmune diseases treated with MMF could not produce IL-1β unless exposed to pathogens, for example, bacteria, indicating that the immune system of those patients receiving MMF might be properly suppressed while maintaining alert to bacterial infection.

In conclusion, this study has clarified that MPA synergized with LPS to enhance IL-1β release via the activation of caspase-1. These findings suggested that patients with autoimmune diseases who are treated with MMF might have highly activated caspase-1, which causes macrophages or monocytes to more readily release IL-1β in response to bacterial components, such as LPS. The data of this study provided experimental evidence as well as theoretical rationales for MMF to be included in immunosuppressive protocols that are not only more effective for transplant recipients or patients with autoimmune disorders but also enable the patients to be less liable to some bacterial infections.

Conflicts of interest

There are no conflicts of interest.

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霉酚酸协同脂多糖通过活化caspase-1促进白介素-1β分泌

摘要

背景： 我们的既往研究表明MMF的活性代谢产物霉酚酸（MPA）协同LPS可促进白介素-1β分泌，但其机制不明确。本研究拟探讨MPA协同LPS促进白介素-1β的机制。

方法： 未稀释的人外周血用地塞米松、霉酚酸（MPA，MMF的活性代谢产物）、硫唑嘌呤或CYC单独使用或联合脂多糖（LPS）培养12小时后，用ELISA方法检测培养上清的白细胞介素（IL）-1β水平。THP-1细胞或单核细胞，MPA单独使用或与LPS联用处理细胞后，用ELISA方法检测培养上清的IL-1β水平。实时荧光定量PCR的方法检测THP-1细胞的IL-1β mRNA水平。Western blot法检测THP-1细胞中NF-κB p-p65，pro-IL-1β，NLRP3和caspase-1（p20）的胞内蛋白水平。

结果： MPA单独使用不能诱导IL-1β产生，而MPA与LPS协同可增加IL-1β的分泌，并呈剂量依赖性 (685.00±20.00 pg/ml in LPS+5 μmol/L MPA group, *P*=0.035; 742.00±31.58 pg/ml in LPS+25 μmol/L MPA group, *P*=0.017; 1000.00±65.59 pg/ml in LPS+75 μmol/L MPA group, *P*=0.024; vs. 408.00±35.50 pg/ml in LPS group)。 MPA对细胞内IL-1β的mRNA转录水平无影响，LPS可使IL-1β的mRNA转录水平增加2761倍，而LPS+MPA组IL-1β的mRNA转录水平与对照组比较升高3018倍，与LPS组比较无统计学差异 (*P*=0.834)。MPA对细胞内NF-κB p-p65和pro-IL-1β蛋白水平无影响，但可以激活NLRP3炎性体。Caspase-1的特异性抑制剂Ac-YVAD-cmk可阻断caspase-1的活化并减少IL-1β的分泌，LPS+MPA组IL-1β浓度为588.00±41.99 pg/ml，而LPS+MPA+ YVAD组为181±45.24 pg/ml (*P*= 0.014)。

结论： MPA协同LPS促进IL-1β产生是通过激活caspase-1使pro-IL-1β转化为IL-1β增多所致，而不是通过增加pro-IL-1β的产生。这些发现表明，用MMF免疫抑制的患者可能在感染期间过度激活caspase-1，可能有助于宿主对入侵的细菌产生更敏感的防御反应。