Inflamm. Res. (2014) 63:91–98
DOI 10.1007/s00011-013-0676-2

Inflammation Research

ORIGINAL RESEARCH PAPER

Microgravity inhibition of lipopolysaccharide-induced tumor necrosis factor-α expression in macrophage cells

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Received: 28 July 2013 / Revised: 3 October 2013 / Accepted: 8 October 2013 / Published online: 6 November 2013

Abstract
Objective and design Microgravity environments in space can cause major abnormalities in human physiology, including decreased immunity. The underlying mechanisms of microgravity-induced inflammatory defects in macrophages are unclear.
Material or subjects RAW264.7 cells and primary mouse macrophages were used in the present study. Lipopolysaccharide (LPS)-induced cytokine expression in mouse macrophages was detected under either simulated microgravity or 1*g* control.
Methods Freshly isolated primary mouse macrophages and RAW264.7 cells were cultured in a standard simulated microgravity situation using a rotary cell culture system (RCCS-1) and 1*g* control conditions. The cytokine expression was determined by real-time PCR and ELISA assays. Western blots were used to investigate the related intracellular signals.
Results LPS-induced tumor necrosis factor-α (TNF-α) expression, but not interleukin-1β expression, in mouse macrophages was significantly suppressed under simulated microgravity. The molecular mechanism studies showed that LPS-induced intracellular signal transduction including phosphorylation of IKK and JNK and nuclear translocation of NF-κB in macrophages was identical under normal gravity and simulated microgravity. Furthermore, TNF-α mRNA stability did not decrease under simulated microgravity. Finally, we found that heat shock factor-1 (HSF1), a known repressor of TNF-α promoter, was markedly activated under simulated microgravity.
Conclusions Short-term treatment with microgravity caused significantly decreased TNF-α production. Microgravity-activated HSF1 may contribute to the decreased TNF-α expression in macrophages directly caused by microgravity, while the LPS-induced NF-κB pathway is resistant to microgravity.

Keywords Microgravity · Macrophages · Toll-like receptors · TNF-α · Heat shock factor-1

Introduction

Exposure to the microgravity environment can cause severe abnormalities in human physiology, including fluid shift, anemia, osteoporosis, immunosuppression, etc. [1, 2]. Dysfunction of immune cells may underlie the immunosuppression under microgravity. Decreased response of T lymphocytes to potent activators and reduced cytotoxic activity of natural killer cells have been observed under microgravity [2]. Monocytes and macrophages are important components of the immune system. Impaired cytokine secretion by activated monocyte/macrophages has been reported under microgravity. For example, less interleukin-1 (IL-1) was produced when the human monocytic cell lines, THP1 and U937, were stimulated with phorbol esters

Responsible Editor: Michael J. Parnham.

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in space [3, 4], and less tumor necrosis factor-α (TNF-α), IL-6 and IL-12 were secreted when the murine macrophage cell line, RAW264.7, was treated with lipopolysaccharide (LPS; constituents of the outer membrane of Gram-negative bacteria) plus interferon-γ (IFN-γ) under simulated microgravity [5]. While the inhibition of protein kinase C (PKC) translocation on phorbol ester stimulation under microgravity may explain the impaired IL-1 production by the phorbol ester-stimulated U937 cells in this environment [6, 7], how microgravity directly affects the macrophage activation pathway is unclear to date.

Here we studied the effect of simulated microgravity on LPS-induced proinflammatory cytokine expression in primary mouse peritoneal macrophages and mouse macrophage cell line RAW264.7 cells and explored the relevant molecular mechanisms. LPS-induced TNF-α expression, but not IL-1β expression, in mouse macrophages was found to be suppressed under simulated microgravity. Molecular mechanism studies showed that neither signal transduction downstream of Toll-like receptor 4 (TLR4, the receptor for LPS) that activates the transcription of cytokine genes nor TNF-α mRNA stability was affected by the simulated microgravity environments. Instead, the master transcription factor of the heat-shock response, heat shock factor-1 (HSF1), was found to be activated under simulated microgravity, which may in turn suppress LPS-induced TNF-α expression. Thus, microgravity-reduced TNF-α production by macrophages may be mediated by the induction of HSF1 activity.

Materials and methods

Cell culture and LPS treatment

Murine RAW264.7 cells were generally cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37 °C under a 5 % CO_2 humidified atmosphere. For the simulated microgravity condition, RAW264.7 cells were cultured in a rotary cell culture system (RCCS-1) manufactured by Synthecon, Inc. Disposable vessels (10 ml) were used in the RCCS. Cytodex 3 microcarriers (GE Healthcare) were supplemented in the rotary cell culture for RAW264.7 cells to adhere to. For the normal gravity control condition, RAW264.7 cells were cultured in plastic plates coated with gelatin/Attachment Factor (Life Technologies). After 24 h culture under normal gravity and simulated microgravity, RAW264.7 cells were stimulated with 100 ng/ml LPS (Sigma) for subsequent assays.

Thioglycolate-elicited mouse peritoneal macrophages were prepared as previously reported with slight modifications [8]. In brief, 10–12-week-old male C57BL/6 mice, purchased from Beijing University Experimental Animal Center (Beijing, China), were each injected intraperitoneally with 1 ml of 3 % Brewer thioglycollate medium. Five days later, the total peritoneal cells were harvested, plated on plastic plates, and cultured at 37 °C for 30 min. Non-adherent cells were washed away with warm phosphate-buffered saline (PBS). The adherent cells (mostly macrophages) were detached from the plastic plates with an ice-cold solution of 1 mM EDTA in PBS. The collected macrophages were cultured under normal gravity and simulated microgravity and then stimulated with LPS, as described above. Animal protocols were approved by the Animal Ethics Committee of the Institute of Zoology, Beijing, China.

RNA extraction, reverse transcription and real-time PCR

Total RNA was extracted from cell samples by using E.Z.N.A.® MicroElute Total RNA Kit (Omega Bio-Tek). One microgram of total RNA was reverse-transcribed into cDNA by using oligo (dT) primers and AMV Reverse Transcriptase XL (Takara). Real-time PCR was performed with a CFX96 real-time PCR detection system (Bio-Rad). The PCR mixture was at a volume of 20 μl containing 10 μl SYBR Premix Ex Taq (Takara), 0.5 μM of each of the primers, and 1 μl cDNA prepared as described above. PCRs were cycled 40 times after initial denaturation (95 °C, 2 min) with the following parameters: denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s, and extension at 72 °C for 15 s. The $2^{-\Delta\Delta C_T}$ method was used to compare gene expression in different cell samples, with HPRT (hypoxanthine phosphoribosyltransferase) expression as the internal control [9]. The following primers were used: HPRT, sense 5′-AGTACAGCCCCAAAATGGTTAAG-3′ and antisense 5′-CTTAGGCTTTGTATTTGGCTTTTC-3′; TNF-α, sense 5′-CTGAACTTCGGGGTGATCGG-3′ and antisense 5′-GGCTTGTCACTCGAATTTTGAGA-3′; IL-1β, sense 5′-TGTGTAATGAAAGACGGCACA-3′ and antisense 5′-CTGCTTGTGAGGTGCTGATG-3′; and IFN-β, sense 5′-CAGCTCCAAGAAAGGACGAA-3′ and antisense 5′-CATCTCTTGGATGGCAAAGG-3′.

Flow cytometry

For analysis of TLR4 expression, cells were stained with PE-anti-mTLR4/MD-2 (clone MTS510, eBioscience) in PBS containing 0.1 % (wt/vol) bovine serum albumin (BSA) and 0.1 % NaN_3. Flow cytometry data were acquired on a Beckman Coulter Epics XL benchtop flow cytometer. The data were analyzed with FlowJo software (Tree Star, San Carlos, CA, USA).

For analysis of TNF-α synthesis after LPS treatment, RAW264.7 cells were stimulated with LPS for 4 h in the presence of BD GolgiPlug (BD Biosciences). The cells were fixed and permeabilized with BD Cytofix/Cytoperm solution (BD Biosciences), and stained intracellularly with PE-anti-mTNF (clone MP6-XT22, BD Pharmingen). Flow cytometry data were acquired and analyzed as described above.

ELISA for TNF-α detection

The amount of TNF-α in the culture supernatant of LPS-stimulated macrophages was assayed with mouse TNF-α ELISA MAX^{TM} Deluxe kit purchased from BioLegend, Inc. The manufacturer's instructions were strictly followed.

Western blots

Whole cell lysates were prepared with RIPA lysis buffer supplemented with protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail 2 and 3 (Sigma). Nuclear and cytoplasmic extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific). The protein concentration was determined with a BCA protein assay kit (Beijing CellChip Biotechnology). Samples containing equal amounts of protein were resolved on 10 % SDS-PAGE. The proteins were transferred onto Immobilon P polyvinylidene fluoride membranes (Millipore). Immunoblots were developed as described previously [10]. The following primary antibodies were used: anti-GAPDH (Proteintech No. 60004-1-Ig), anti-p-JNK (Cell Signaling Technology No. 4668), anti-p-IKKα/β (Cell Signaling Technology No. 2078), anti-NF-κB/p65 (Cell Signaling Technology No. 3987), anti-HSF1 (Cell Signaling Technology No. 4356), and anti-HSF2 (Santa Cruz Biotechnology No. sc-13056).

Confocal microscopy

Cells collected from different culture conditions were made to adhere to coverslips by precoating the coverslips with poly-L-lysine, and fixed in 4 % paraformaldehyde (10 min on ice) [11]. Cells were washed with PBS containing 0.05 % Tween20 and permeabilized with 0.2 % Triton X-100 in PBS (10 min on ice). After washing, cells were blocked with 1 % BSA in PBS (1 h, room temperature) and incubated with primary antibody (diluted in the blocking buffer) overnight at 4 °C. Subsequently, cells were incubated with secondary antibody at room temperature for 1 h, and then with Hoechst 33342 (2 μg/ml, Sigma) for 10 min. After washing, the coverslips were mounted on glass slides with Aqua-Poly/Mount mounting medium (Polysciences, Inc.). Photomicrographs were taken on a Zeiss LSM 510 Meta confocal microscope (Zeiss). The primary and secondary antibodies used in this study were anti-NF-κB/p65 (Cell Signaling Technology No. 3987) and DyLight 488-conjugated goat anti-rabbit IgG antibody (ZSGB-BIO).

mRNA stability assay

TNF-α mRNA stability in RAW264.7 cells cultured under different gravity conditions was assayed as previously reported with slight modifications [12]. In brief, RAW264.7 cells were cultured under normal gravity and simulated microgravity, and then stimulated with LPS for 4 h. To block the transcription, actinomycin D was added to the cell culture at a final concentration of 10 μg/ml. The TNF-α mRNA levels before and 1 h after the actinomycin D treatment were assayed using the real-time PCR method described above. The half-life time ($T_{1/2}$) of TNF-α mRNA was calculated based on the equation: $T_{1/2} = \log(0.5 \times T)/\log(R_1/R_0)$, in which R_0 and R_1 are the RNA levels before and after actinomycin D treatment, and T is the duration of the actinomycin D treatment.

Statistical analysis

All data are presented as the mean ± SD. Student's unpaired t test for comparison of means was used to compare groups. A P-value <0.05 was considered to be statistically significant.

Results

LPS-induced TNF-α expression in macrophages was significantly impaired under simulated microgravity

To study the effects of simulated microgravity on LPS-induced cytokine expression in macrophages, we firstly cultured RAW264.7 cells, a mouse macrophage cell line [13], with LPS stimulation under normal gravity and simulated microgravity. A RCCS was used to create a simulated microgravity condition as reported previously [14]. As the macrophages are adherent cells, microcarriers (Cytodex 3) were supplemented in the rotary cell culture for the RAW264.7 cells to adhere to. After 24 h culture in RCCS, most of the RAW264.7 cells were found to adhere to the microcarriers (Fig. 1). The RAW264.7 cells cultured under normal gravity and simulated microgravity were both stimulated with 100 ng/ml LPS for 4 h. The total RNAs were extracted and reverse-transcribed into cDNAs. The expression of cytokine genes was assayed by real time-PCR. In both culture conditions, LPS stimulation increased the expression of proinflammatory cytokines (TNF-α and IL-1β) and interferon-β (Fig. 2a). Compared with the

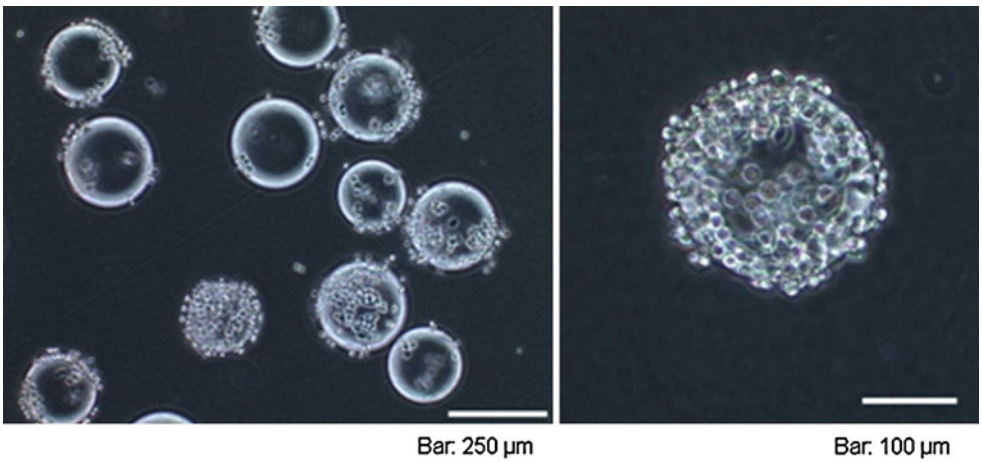

Fig. 1 RAW264.7 cells adhering to microcarriers in RCCS. RAW264.7 cells were cultured with Cytodex 3 microcarriers in a rotary cell culture system (RCCS-1) for 24 h. The cell culture was transferred to a petri dish and observed through a microscope (Olympus IX71). Original magnification: ×400

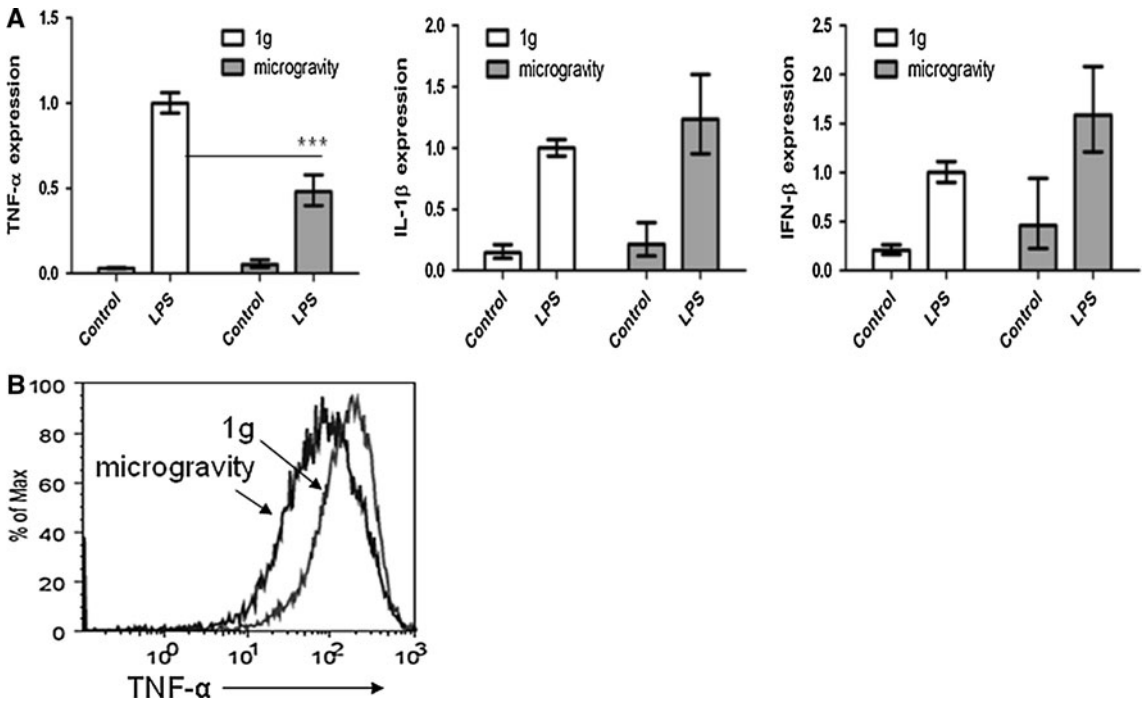

Fig. 2 LPS-induced TNF-α expression in RAW264.7 cells was significantly suppressed under simulated microgravity. **a** The significantly decreased TNF-α mRNA expression in RAW264.7 cells under simulated microgravity. RAW264.7 cells were cultured under normal gravity (1*g*) and simulated microgravity for 24 h and were then stimulated with 100 ng/ml LPS for an additional 4 h. The total RNAs in unstimulated (control) and LPS-stimulated cells were extracted and assayed for cytokine expression. Data represent one of two independent experiments. $***P < 0.001$ versus the LPS-stimulated group under 1*g*. **b** RAW264.7 cells were cultured under normal gravity (1*g*) and simulated microgravity for 24 h and were then stimulated with LPS for an additional 4 h in the presence of BD GolgiPlug (BD Biosciences) and stained intracellularly with PE-anti-mTNF-α. Flow cytometry data were acquired and analyzed. Data represent one of two independent experiments

RAW264.7 cells under normal gravity, the RAW264.7 cells under simulated microgravity expressed lower TNF-α ($P < 0.001$, Fig. 2a) and similar levels of IL-1β and interferon-β after LPS stimulation (Fig. 2a). TNF-α synthesis was further assayed by intracellular staining flow cytometry. Less TNF-α was detected in the LPS-stimulated RAW264.7 cells under simulated microgravity compared with under normal gravity (Fig. 2b), which is consistent with the LPS-induced TNF-α mRNA expression under simulated microgravity.

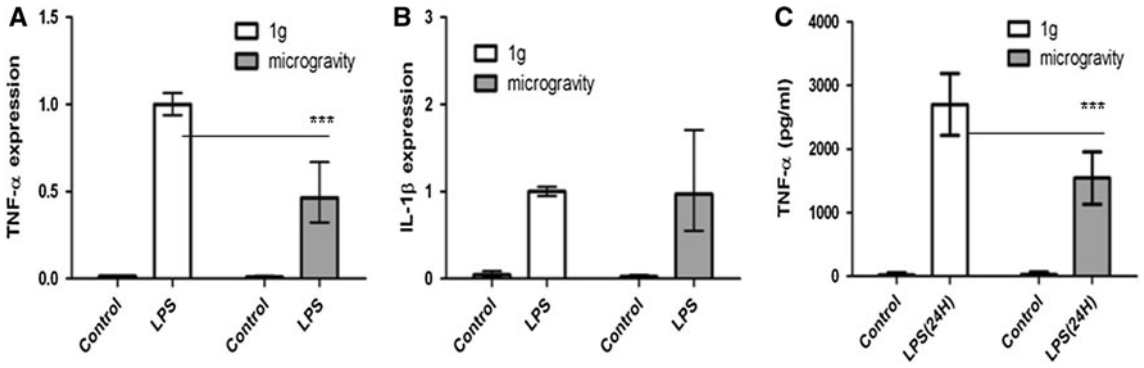

Fig. 3 LPS-induced TNF-α expression in primary macrophages was deficient under simulated microgravity. **a** The significantly decreased TNF-α mRNA expression in macrophages under simulated microgravity. Mouse peritoneal macrophages were cultured under normal gravity (1*g*) and simulated microgravity for 24 h and were then stimulated with 100 ng/ml LPS for an additional 4 h. The total RNAs in unstimulated (control) and LPS-stimulated cells were extracted and assayed for cytokine expression. **b** The unchanged IL-1β mRNA expression in primary macrophages under simulated microgravity. **c** Mouse peritoneal macrophages were cultured under normal gravity (1*g*) and simulated microgravity for 24 h and were then stimulated with LPS for an additional 24 h. The amounts of TNF-α in the culture supernatants of unstimulated (control) and LPS-stimulated cells were assayed by ELISA. Data are shown as mean + SD ($N = 3$), representing one of three independent experiments. ***$P < 0.001$ versus the LPS-stimulated group under 1*g*

We then tested the effects of simulated microgravity on LPS-induced cytokine expression in freshly isolated primary mouse macrophages. Mouse peritoneal macrophages were cultured and stimulated with LPS under normal gravity and simulated microgravity. In both culture conditions, LPS stimulation increased the expression of proinflammatory cytokines (TNF-α and IL-1β) (Fig. 3a, b). Compared with the macrophages under normal gravity, the macrophages under simulated microgravity expressed lower TNF-α and similar level of IL-1β after LPS stimulation (Fig. 3a, b). TNF-α secretion by macrophages in the culture supernatant was further determined by ELISA. Less TNF-α was secreted by the LPS-stimulated macrophages under simulated microgravity, compared with those macrophages under normal gravity (Fig. 3c). These results in primary macrophages are consistent with those found in the RAW264.7 cell line.

Simulated microgravity did not impact the mRNA stability of TNF-α in macrophages

mRNA levels are controlled by their stability in addition to the transcriptional regulation [15]. To address whether TNF-α mRNA is more unstable under simulated microgravity, we detected TNF-α mRNA stability in macrophages. TNF-α mRNA stability was assayed with the use of actinomycin D to block the transcription. RAW264.7 cells cultured under normal gravity and simulated microgravity were stimulated with LPS (100 ng/ml) for 4 h, and then treated with actinomycin D (10 μg/ml) for 1 h. The TNF-α expression before and after the actinomycin D treatment was assayed by real-time PCR. As shown in Fig. 4a, actinomycin D treatment decreased TNF-α expression in both culture conditions. The half-life time of TNF-α mRNA under simulated microgravity was identical to that under normal gravity (Fig. 4b). This indicates that TNF-α mRNA stability was not affected by simulated microgravity.

LPS-induced NF-κB signal transduction in macrophages was intact under simulated microgravity

To investigate the mechanisms underlying the deficiency of LPS-induced TNF-α expression in macrophages under simulated microgravity, we first assayed the surface expression of TLR4, which is the receptor for LPS. TLR4 expression on RAW264.7 cells under normal gravity and simulated microgravity was similar (data not shown), as was that on freshly isolated mouse peritoneal macrophages (data not shown).

We then assayed the LPS-induced activation of signaling pathways downstream of TLR4. Activation of both NF-κB and MAPK pathways contribute to the induction of proinflammatory cytokines [16]. The LPS-induced phosphorylation (activation) of IKK and JNK, which are signaling molecules of the NF-κB and MAPK pathways, respectively [17], was therefore analyzed by Western blotting. The amounts of phosphorylated IKK in LPS-stimulated RAW264.7 cells under normal gravity and simulated microgravity were similar, as were the amounts of phosphorylated JNK (Fig. 5a). These results suggest that the signal transduction from TLR4 to IKK and JNK in macrophages after LPS stimulation was intact under simulated microgravity. Activation of the NF-κB pathway leads to nuclear translocation of NF-κB, which in the nucleus acts as a transcriptional activator to induce the

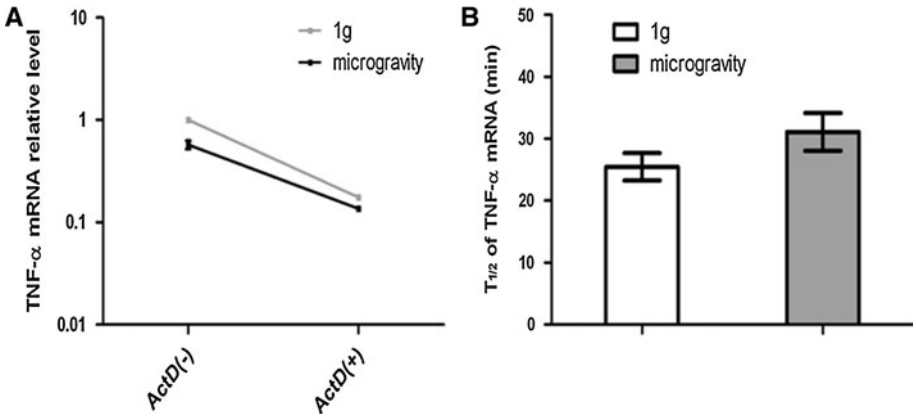

Fig. 4 TNF-α mRNA stability in macrophages under normal gravity and simulated microgravity. **a** RAW264.7 cells were cultured under normal gravity (1*g*) and simulated microgravity for 24 h and were then stimulated with 100 ng/ml LPS for an additional 4 h. To block the transcription, actinomycin D was added to the cell culture at a final concentration of 10 μg/ml. The TNF-α mRNA levels before actinomycin D treatment [ActD(−)] and 1 h after actinomycin D treatment [ActD(+)] were assayed. **b** The half-life time ($T_{1/2}$) of TNF-α mRNA was calculated based on the equation: $T_{1/2} = \log(0.5\times T)/\log(R_1/R_0)$, in which R_0 and R_1 are the RNA levels before and after actinomycin D treatment, and T is the duration of the actinomycin D treatment (60 min)

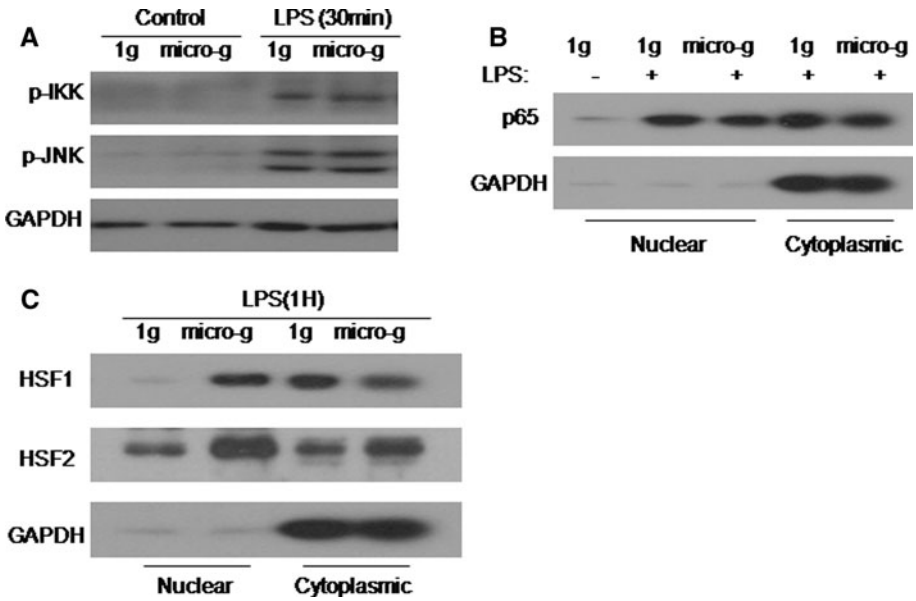

Fig. 5 Normal NF-kB activation and induced HSF1 and HSF2 activation in macrophages under simulated microgravity. **a** Immunoblots for p-IKK and p-JNK in macrophages. RAW264.7 cells were cultured under normal gravity (1*g*) and simulated microgravity (*micro-g*) for 24 h and were then stimulated with 100 ng/ml LPS for an additional 30 min. Whole cell lysates were prepared for detection of p-IKK, p-JNK and GAPDH by Western blotting. GAPDH served as internal control. **b** LPS-induced nuclear translocation of NF-κB under normal gravity and simulated microgravity. RAW264.7 cells were cultured under normal gravity (1*g*) and simulated microgravity (*micro-g*) for 24 h and were then stimulated with 100 ng/ml LPS or control medium for an additional 1 h. Nuclear and cytoplasmic extracts were prepared from unstimulated and LPS-stimulated cells. Western blotting for p65 and GAPDH was performed. **c** Immunoblot for nuclear HSF1. RAW264.7 cells were cultured under normal gravity (1*g*) and simulated microgravity (*micro-g*) for 24 h and were then stimulated with 100 ng/ml LPS for an additional 1 h. Nuclear and cytoplasmic extracts were prepared from the collected cells. Western blotting for HSF1, HSF2, and GAPDH was performed. Results are one representative of two independent experiments showing identical results

expression of proinflammatory cytokines [18]. The LPS-induced nuclear translocation of NF-κB in RAW264.7 cells under normal gravity and simulated microgravity was compared. Based on Western blots of nuclear extracts, the amounts of nuclear p65 after LPS stimulation under the two culture conditions were similar (Fig. 5b). Furthermore, the nuclear translocation of p65 in macrophages after LPS stimulation was also determined by immunofluorescence confocal microscopy. LPS stimulation led to similar nuclear translocation of NF-κB in the two culture

conditions (data not shown). Collectively, no significant difference in LPS-stimulated NF-κB signal transduction in macrophages between the normal gravity condition and the simulated microgravity condition was determined.

Heat shock factor-1 (HSF1) and HSF2 was activated in macrophages under simulated microgravity

After observing the unimpaired NF-κB activation in macrophages under the simulated microgravity condition, we explored the possibility that the suppressed LPS-induced TNF-α mRNA expression under simulated microgravity may be caused by the elevated transcriptional repressor of TNF-α promoter induced by simulated microgravity. HSF1 may represent a good candidate for such a transcriptional repressor. Firstly, HSF1 is the master transcriptional factor in the heat shock response [19]. It locates in the cytoplasm when the cells are in an unstressed state. Upon heat stress, HSF1 quickly translocates into the cell nuclei to activate the transcription of heat shock proteins, which then exert protective roles countering the damaging effects of heat [20]. It was reported that the heat shock protein Hsp70 was up-regulated in cells cultured under simulated microgravity [21]. This suggests that HSF1 might be activated under simulated microgravity. Secondly, HSF1 was previously shown to be a transcriptional repressor of TNF-α promoter [22–24]. We therefore hypothesized that the suppression of LPS-induced TNF-α expression under simulated microgravity may be caused by activation of HSF1. To assess the activity of HSF1 during LPS stimulation under normal gravity and simulated microgravity, we detected nuclear HSF1 by Western blotting. In the nuclear extracts of the LPS-stimulated RAW264.7 cells under normal gravity, HSF1 was almost undetectable (Fig. 5c). In contrast, a large quantity of HSF1 was detected in nuclear extracts of LPS-stimulated RAW264.7 cells under simulated microgravity (Fig. 5c). In addition, nuclear HSF2 was also increased under simulated microgravity (Fig. 5c). These results indicate that the simulated microgravity-induced nuclear translocation of HSF1 may contribute to the deficiency of LPS-induced TNF-α expression in macrophages under microgravity conditions.

Discussion

In the present study, we demonstrated that LPS-induced TNF-α mRNA and protein expression in mouse macrophages was significantly reduced under simulated microgravity. This is consistent with the report showing that LPS- plus IFN-γ-stimulated RAW264.7 cells secreted less TNF-α, IL-6, and IL-12 under simulated microgravity [5]. In addition, LPS-stimulated splenocytes of space-flown mice were found to secrete less TNF-α than the ground controls [25]. Our observation using primary macrophages in the in vitro culture system indicates that direct effects of microgravity on macrophages may contribute to decreased macrophage functions in mice experiencing microgravity. However, it is reported that LPS-stimulated bone marrow-derived macrophages in space were found to secrete more TNF-α than the ground controls [26]. In the previous study, both the flight cells and the ground controls were kept at 20–25 °C, instead of 37 °C which was the cell culture temperature in our experiments. The inconsistency may be due to the different cell culture temperature.

In addition, LPS-induced IL-1β expression in mouse macrophages was unchanged under simulated microgravity, while the LPS-induced TNF-α expression was suppressed. The differential effects of simulated microgravity on IL-1β and TNF-α expression suggest the different sensitivity to microgravity of signaling pathways regulating inflammatory cytokines IL-1β and TNF-α in macrophages. Nevertheless, the decreased inflammatory response of macrophages caused by microgravity may contribute to the increased susceptibility to infections of astronauts [1, 27].

To investigate the mechanisms underlying reduced LPS-induced TNF-α expression under simulated microgravity, we compared LPS-stimulated signal transduction and TNF-α mRNA stability between the cells cultured under normal gravity and simulated microgravity, but found no significant difference. As far as we know, no study on the mRNA stability of a specific gene under space flight or simulated microgravity has previously been published. The unchanged NF-κB activation and TNF-α mRNA stability indicate the insensitivity of these processes to microgravity. Instead, we found that the master transcription factor of the heat-shock response, HSF1, was activated under simulated microgravity. The activated HSF1 may bind to the heat shock response element-like sequences present in TNF-α promoter and repress TNF-α transcription [22–24]. In the widely accepted model for HSF1 activation, increasing numbers of unfolded proteins release HSF1 from chaperone proteins upon heat shock, leading to nuclear translocation of HSF1 [20]. The activation of HSF1 and HSF2 in cells cultured under simulated microgravity suggests that there might be massive unfolded proteins in these cells. In any case, the microgravity-induced HSF1 and HSF2 activity will inhibit TNF-α expression in mouse macrophages.

In summary, we found that LPS-induced TNF-α expression in mouse macrophages was significantly suppressed under simulated microgravity. NF-κB activation and TNF-α mRNA stability were resistant to microgravity. The repressor of TNF-α promoter, HSF1, which was activated under simulated microgravity, may be one of the key

mechanisms for the reduced TNF-α expression in macrophages under microgravity.

Acknowledgments We thank Dr. Chenming Sun for his review of the manuscript, Mrs Jianxia Peng and Mrs. Xiaoqiu Liu for expert technical assistance, Mrs. Yanli Hao for excellent laboratory management, and Mr. Hongfei Wu for outstanding animal husbandry. This work was supported by grants from the National Basic Research Program of China (2011CB710903, 2010CB945301, YZ) and the National Natural Science Foundation for general and Key Programs (C81130055, U0832003, YZ).

Conflict of interest The authors declare no conflict of interests.

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