Trichostatin A inhibits inflammation in phorbol myristate acetate-induced macrophages by regulating the acetylation of histone and/or non-histone proteins

QIAN ZHANG[1], FAN YANG[1], XUN LI[1], LUWEN WANG[1], XIAOGANG CHU[1], HONG ZHANG[2] and ZUOJIONG GONG[1]

[1]Department of Infectious Diseases; [2]Pharmaceutical Department, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, P.R. China

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Abstract. Histone deacetylase inhibitors (HDACi) are currently used in the routine clinical treatment of cancer. Alongside the antitumor activity of HDACi, increased attention has been paid to their anti-inflammatory effects. The present study aimed to analyze the inhibitory effects of the HDACi Trichostatin A (TSA), on the release of inflammatory mediators from macrophages differentiated from U-937 cells. A low dose of TSA (50 nM) was able to effectively decrease the levels of inflammatory cytokines in the cell supernatants, independent of apoptosis. In addition, the potential underlying mechanisms were explored, and TSA was shown to promote, rather than inhibit, the acetylation of histones. Furthermore, the inflammation-induced enhanced expression of class I HDACs was effectively inhibited by TSA. In addition, TSA enhanced the lipopolysaccharide (LPS)-induced expression of cyclooxygenase-2, but suppressed the LPS-induced expression of chemokine (C-C motif) ligand 7. The acetylation level of nuclear factor-κB p65 was decreased by LPS, but increased following treatment with TSA. In conclusion, TSA was able to inhibit inflammation in macrophages. However, whether the mechanism by which TSA inhibits inflammation is through significantly enhancing histone acetylation, in order to selectively suppress the expression of proinflammatory genes, and/or through regulating non-histone acetylation requires further research.

Correspondence to: Professor Zuojiong Gong, Department of Infectious Diseases, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuhan, Hubei 430060, P.R. China
E-mail: zjgong@163.com

Abbreviations: HDACi, histone deacetylase inhibitors; TSA, trichostatin A; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; MAPKs, mitogen-activated protein kinases; TLR4, toll like receptor 4; HDACs, histone deacetylases; HATs, histone acetyltransferases; PMA, phorbol myristate acetate; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorting; CXCL-2, chemokine (C-X-C motif) ligand 2; CCL-7, chemokine (C-C motif) ligand; COX-2, cyclooxygenase 2

Key words: trichostatin A, inflammation, histone deacetylase, macrophages

Introduction

Inflammation is induced in response to infectious diseases, and protects the body from invasion. Macrophages have a key role in the course of inflammation, which can be activated by various endogenous and/or exogenous factors. Once activated, macrophages may release a large amount of inflammatory cytokines into the extracellular matrix, which further aggravates the inflammatory response. It has previously been reported that the expression of numerous genes is induced or suppressed in macrophages, in response to the activating agent lipopolysaccharide (LPS) (1). LPS is an outer membrane glycolipid of gram-negative bacteria, which is recognized by Toll-like receptor 4 (TLR4) thereby initiating an inflammatory response (2,3).

TLR4 signaling triggers the activation of various transcription factors, including nuclear factor (NF)-κB and mitogen-activated protein kinases, such as extracellular signal-regulated kinases (4). NF-κB has a key role in various biological processes, including immunity, inflammation, wound healing, proliferation and apoptosis (5). Numerous inflammatory diseases, including rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease, are associated with the activation of NF-κB (6,7).

Acetylation of histones is a type of post-translational modification, which is regulated by the opposing activities of histone deacetylases (HDACs) and histone acetyltransferases (HATs). Histone acetylation is regulated by HATs, which activate gene transcription by relaxing the structure of chromatin, whereas deacetylation is controlled by HDACs, which induce the condensation or inactivation of chromatin, leading to gene suppression (8,9). In addition to histones, proteins without histones may also undergo acetylation or deacetylation by HATs and HDACs (10,11).

Histone deacetylase inhibitors (HDACi) are currently used in the routine clinical treatment of cancer (12,13). Alongside

the antitumor activity of HDACi, increased attention has been paid to their anti-inflammatory effects. Theoretically, HDACs are associated with gene repression via histone inactivation, whereas HDACi should promote gene transcription and enhance inflammatory responses. However, it has previously been reported that HDACi, such as valproic acid, suberoylanilide hydroxamic acid (SAHA) and Trichostatin A (TSA), are able to suppress the expression of proinflammatory cytokines and increase the survival rate of mice following septic shock (14-16). Therefore, the anti-inflammatory effects of HDACi, and the underlying mechanisms require further clarification.

TSA is a hydroxamic acid-based compound, which was originally developed as an antifungal agent (17). TSA selectively inhibits class I and II mammalian HDACs, but not class III HDACs (18). The U-937 monocyte-like cell line is widely used in research regarding inflammation, and it can be differentiated by phorbol myristate acetate (PMA) and activated by LPS (19). The present study aimed to analyze the inhibitory effects and potential mechanism of the HDACi TSA, on the release of inflammatory factors in macrophages differentiated from U-937 cells.

Materials and methods

Cell culture. U-937 cells (purchased from American Type Culture Collection, Manassas, VA, USA and preserved in our laboratory) were cultured and maintained at a cell density between $5x10^5$ and $1x10^6$ cells/ml in RPMI-1640 media (Gibco; Thermo Fisher Scientific, Inc., Villebon-sur-Yvette, France) supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France), 100 U/ml penicillin (Genom Co., Ltd., Hangzhou, China), and 100 μg/ml streptomycin (Genom Co., Ltd.). The cells were incubated at 37˚C in a humidified atmosphere containing 95% air and 5% CO_2. For macrophage-like cell differentiation, the serum-starved U-937 cells were plated in 6-well plates (Corning, Inc., Corning, NY, USA) at a density of $5x10^5$ cells/ml and treated with 60 ng/ml PMA (Sigma-Aldrich, St. Louis, MO, USA) for 24 h. The non-adherent cells were removed by washing twice with phosphate-buffered saline (PBS).

Experimental design and cell treatment. The macrophages were divided into three groups: The control group, the LPS-treated group and the TSA-treated group. For the TSA-treated group, TSA (Sigma-Aldrich) was added to the medium 2 h prior to treatment with LPS (1 μg/ml; Sigma-Aldrich). The final concentrations of TSA were 50, 100, 500 and 1,000 nM [TSA was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich), and the final DMSO concentration was <0.4%]. For the LPS-treated group, an equal dosage of DMSO was added to the medium 2 h prior to treatment with LPS (1 μg/ml). For the control group, the same doses of DMSO and normal saline were added to the medium at the same time point. The supernatants and cells were harvested 24 h after the addition of LPS.

Cell viability test. Cell viability was assessed using the Cell Counting kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan). The cells ($2x10^5$ cells/well) were plated in 96-well plates for the viability assay. Experimental procedures were performed according to the manufacturer's protocol.

Fluorescence-activated cell sorting (FACS). The apoptotic rate of the macrophages was measured by flow cytometry (Cell Lab Quanta SC; Beckman Coulter, Inc., Brea, CA, USA) using an Annexin V-Fluorescein Isothiocyanate (FITC) Apoptosis Detection kit (Lianke Biotech Co., Ltd., Hangzhou, China). Briefly, non-adherent and adherent cells were collected. The cells were suspended in 500 μl binding buffer and stained with Annexin V-FITC and propidium iodide (PI) at room temperature for 5 min in the dark. After removing the unbound Annexin V-FITC and PI by centrifugation at 2,000 x g at 4˚C for 5 min, the cells were resuspended in excess binding buffer. For each assay, ≥10,000 cells were analyzed by FACS. Data were expressed as the percentage of non-viable (Annexin V^++PI^+) cells.

Analysis of cell supernatants. The levels of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-10 and IL-18 in the cell supernatants were measured using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, Inc., San Diego, CA, USA), according to the manufacturer's protocols.

Reverse transcription-polymerase chain reaction (RT-PCR) assay. Total RNA was extracted from the isolated macrophages using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. RNA samples were quantified by measuring the absorbance (A) at 260 and 280 nm using a spectrophotometer (UV762; Shanghai Analysis Instrument Co., Ltd., Shanghai, China). The concentrations of RNA were calculated according to A260. Aliquots of total RNA (1 μg) from each sample were reverse transcribed into cDNA, according to the instructions of the First Strand cDNA Synthesis kit (Takara Bio, Inc., Otsu, Japan).PCR amplification was performed in a volume of 20 μl containing 2 μl cDNA, 0.25 μmol/l of each primer, 0.25 mmol/l deoxyribonucleotide triphosphates, and 1 U Takara TaqHS polymerase (Takara Bio, Inc.) using SYBR Green (Takara Bio, Inc.) as the fluorescence indicator on a Bio-Rad Cycler system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). In the PCR amplification reaction, the thermocycling conditions were as follows: Initial activation at 95˚C for 15 min, followed by 40 cycles of denaturation at 94˚C for 15 sec, annealing for 30 sec at 55˚C and extension for 30 sec at 72˚C. The primers used were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and the sequences were as follows. HDAC1, forward TAATAAGCAGCAGAC GGACATCG, reverse CATAATACTTGCCTTTGCCAGC; HDAC2, forward TGATGGAGATGTATCAACCTAGTGC, reverse TTGAAACAACCCAGTCTATCACCAG; HDAC3, forward TGG TTATACTGTCCGAAATGTTGC, reverse CTG TCA TAGGTCAGGAGGTCTGC; cyclooxygenase (COX)-2, forward AAATCCTTGCTGTTCCCACC, reverse TTTCTC CATAGAATCCTGTCCG; chemokine (C-X-C motif) ligand 2 (CXCL-2), forward AAAGTGTGAAGGTGAAGTCCCCC, reverse GTG ATGCTCAAACACATTAGGCG; chemokine (C-C motif) ligand 7 (CCL-7), forward ACAGAAGGACCA CCAGTAGCCAC, reverse GCTTTGGAGTTTGGGTTTTCT TGT; and GAPDH, forward CCACATCGCTCAGACACCAT, reverse CCAGGCGCCCAATACG. The quantification cycle (Cq) value was analyzed from the amplification plots, and gene expression was normalized against the Ct of the GAPDH housekeeping gene using the $2^{-\Delta\Delta Ct}$ method (20).

Western blot assay. The U-937 cells were washed twice with cold PBS, and lysed with lysis buffer (Beyotime Institute of Biotechnology, Beijing, China) on ice for 1 h. Following centrifugation at 12,000 x g at 4˚C for 5 min, the supernatants were collected. The concentrations of protein were measured with a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). Briefly, 50 μg protein extracts were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk for 1 h and then incubated with the following polyclonal primary antibodies at 4˚C overnight: Anti-HDAC1 (cat. no. 2062; 1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-HDAC2 (cat. no. 2540; 1:1,000; Cell Signaling Technology, Inc.), anti-HADC3 (cat. no. 2632; 1:1,000; Cell Signaling Technology, Inc.), anti-H2A (cat. no. 2578; 1:1,000; Cell Signaling Technology, Inc.), anti-AH2A (cat. no. 2576; 1:1,000; Cell Signaling Technology, Inc.), anti-H2B (cat. no. 2934; 1:1,000; Cell Signaling Technology, Inc.), anti-AH2B (cat. no. 2571; 1:1,000; Cell Signaling Technology, Inc.), anti-H3 (cat. no. sc-8654; 1:500; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-AH3 (cat. no. 8172; 1:1,000; Cell Signaling Technology, Inc.), anti-H4 (cat. no. 2935; 1:1,000; Cell Signaling Technology, Inc.), anti-AH4 (cat. no. 2591; 1:1,000; Cell Signaling Technology, Inc.), anti-p65 (cat. no. 6956; 1:1,000; Cell Signaling Technology, Inc.), anti-phosphorylated-p65 (cat. no. 3031; 1:1,000; Cell Signaling Technology, Inc.), anti-Ac-p65 (cat. no. 3045; 1:1,000; Cell signaling Technology, Inc.), anti-TLR4 (cat. no. sc-10741; 1:500; Santa Cruz Biotechnology, Inc.) and anti-β-actin (cat. no. 4967; 1:1,000; Cell Signaling Technology, Inc.). This was followed by incubation with a fluorescent secondary antibody (LI-COR Biosciences, Lincoln, NE, USA) at 37˚C for 2 h. The blot was analyzed using the Odyssey Infrared Imaging system (LI-COR Biosciences). Membranes were also probed for β-actin (Cell Signaling Technology, Inc.) and histone 3 (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) as additional loading controls.

Statistical analysis. Statistical analysis was performed using SPSS version 17.0 software for Windows (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation, and comparisons were made using Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

TSA decreases the viability of PMA-induced macrophages in a dose-dependent manner. In order to determine a suitable treatment dosage of TSA for the present study, the cytotoxic effects of TSA on the PMA-induced macrophages were detected by a CCK-8 assay. As shown in Fig. 1, the viability of macrophages decreased in a dose-dependent manner when exposed to various doses of TSA (50, 100, 500 and 1,000 nM). The cell viability was 93% when treated with 50 nM TSA, whereas the viability decreased to 42% when the cells were treated with 1,000 nM TSA. Cell viability was >80% when the dose of TSA was below 500 nM; therefore, the present study used 50, 100 and 500 nM TSA to explore the effects of TSA on macrophages.

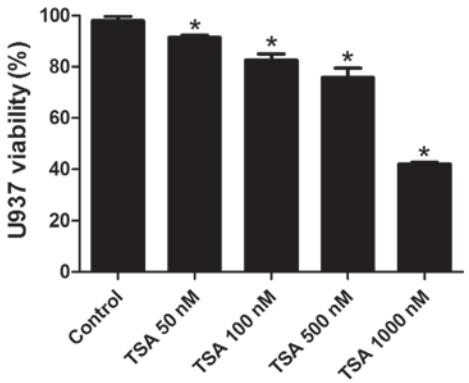

Figure 1. TSA decreased the viability of PMA-induced macrophages in a dose-dependent manner. The viability of macrophages decreased in a dose-dependent manner when exposed to various doses of TSA (50, 100, 500 and 1,000 nM). The cell viability was 93% when treated with 50 nM TSA, whereas the viability decreased to 42% when treated with 1,000 nM TSA. Cell viability was >80% when the dose of TSA was below 500 nM. Cell viability was assessed using the Cell Counting kit-8 assay. Data are presented as the mean ± standard deviation. *$P<0.05$, compared with the control. TSA, Trichostatin A; PMA, phorbol myristate acetate.

TSA decreases the levels of TNF-α, IFN-γ, IL-10 and IL-18 in macrophages. The expression levels of TNF-α, IFN-γ, IL-10 and IL-18 were measured in the cell supernatants by ELISA. As shown in Fig. 2, TNF-α, IFN-γ, IL-10 and IL-18 expression levels were significantly increased in the LPS-treated group, as compared with the control group ($P<0.05$). The expression levels of TNF-α, IFN-γ, IL-10 and IL-18 were markedly decreased in the TSA-treated group, as compared with the LPS-treated group ($P<0.05$), in a dose-dependent manner.

TSA suppresses the expression of class I HDACs in macrophages. To detect changes in the expression of the substrates of TSA, the mRNA and protein expression levels of class I HDACs (HDAC1, HDAC2 and HDAC3) were detected by RT-PCR and western blotting, respectively. As shown in Fig. 3, the expression levels of HDAC1, HDAC2 and HDAC3 were markedly increased in the LPS-treated group, as compared with the normal group; however, the expression levels were all decreased in the TSA-treated group. These results suggest that the expression of class I HDACs may be enhanced in the process of inflammation, and may be decreased by TSA alongside the inhibition of inflammation.

Low dose treatment with TSA (50 nM) does not induce apoptosis of macrophages. As shown in Fig. 4, as compared with the PMA-induced macrophages in normal conditions (Fig. 4A), a low dose of TSA (50 nM) had little effect on the apoptosis of macrophages (Fig. 4B). However, there was obvious apoptosis in the macrophages that were treated with higher doses of TSA (100 or 500 nM, respectively) (Fig. 4C and D). When LPS was added (Fig. 4E-H), as compared with the normal condition, the survival rate of the macrophages was significantly decreased (79.1 vs. 92.4%) (Fig. 4E). However, when treated with 50 nM TSA, the survival rate of the macrophages was increased to 88.2% (Fig. 4F). In addition, when treated with higher doses of TSA (100 or 500 nM respectively), the survival rate of the macrophages decreased to 67.3 and 52.4%, respectively (Fig. 4G and H). These results suggest that the inhibitory effects

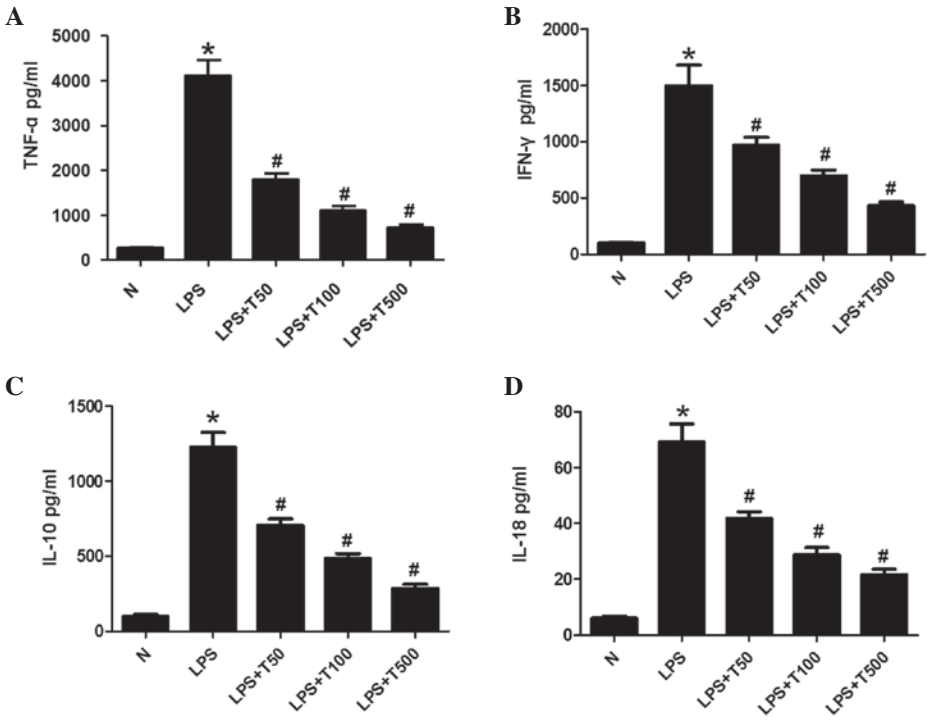

Figure 2. TSA decreased the expression levels of TNF-α, IFN-γ, IL-10 and IL-18 in macrophages. (A) TNF-α, (B) IFN-γ, (C) IL-10 and (D) IL-18 expression levels were significantly increased in the supernatants of the LPS-treated cells, as compared with the control group. The expression levels of TNF-α, IFN-γ, IL-10 and IL-18 were markedly decreased in the supernatants of the TSA-treated cells, as compared with the LPS-treated group, in a dose-dependent manner. Data are presented as the mean ± standard deviation. *P<0.05, vs. the control group; #P<0.05, vs. the LPS-treated group. N, control group; LPS, lipopolysaccharide; TSA, Trichostatin A; TNF, tumor necrosis factor; IFN, interferon; IL, interleukin.

of low dose TSA (50 nM) on the release of inflammatory cytokines are not due to TSA-induced apoptosis of macrophages.

TSA selectively suppresses the expression of proinflammatory genes in macrophages. The present study detected the mRNA expression levels of COX-2, CXCL-2 and CCL-7, in order to explore whether LPS-induced gene expression could be suppressed by a low dose of TSA (50 nM). As shown in Fig. 5, the mRNA expression levels of COX-2, CXCL-2 and CCL-7 were significantly increased in the LPS-treated group, as compared with the normal group (P<0.05). In addition, COX-2 and CXCL-2 mRNA expression levels were significantly increased in the TSA-treated group, whereas CCL-7 mRNA expression levels were decreased, as compared with the LPS-treated group (P<0.05). These results suggest that TSA may differentially regulate the expression levels of specific proinflammatory genes in macrophages.

TSA promotes histone acetylation in macrophages. To investigate whether the inhibitory effects of TSA on inflammation were due to the acetylation of histones, the acetylation levels of histones was determined. As shown in Fig. 6, the acetylation levels of histone (H)2A, H2B, H3 and H4 were significantly increased in the LPS-treated group, as compared with the normal group (P<0.05). Furthermore, the acetylation levels of H2A, H2B, H3 and H4 were all significantly increased in the TSA-treated group (P<0.05). These results suggest that the inhibitory effects of TSA on inflammation may be caused by enhancement of histone acetylation, in order to selectively suppress the expression of proinflammatory genes.

TSA enhances the acetylation of NF-κB p65 in macrophages. The present study determined whether the inhibitory effects of TSA on inflammation were via regulation of the TLR4/NF-κB pathway. As shown in Fig. 7, the protein levels of TLR4 in the LPS-treated group were significantly higher, as compared with the normal group (P<0.05), whereas they were decreased in the TSA-treated group. In addition, there were alterations in the acetylation and phosphorylation of NF-κB p65. The acetylation of NF-κB p65 was significantly decreased in the LPS-treated group, as compared with the normal group, but was significantly increased in the TSA-treated group (P<0.05). Conversely, the phosphorylation of NF-κB p65 was increased in the LPS-treated group, but was decreased in the TSA-treated group (P<0.05). These results suggest that TSA may affect the TLR4/NF-κB p65 signaling pathway by regulating the balance of acetylation and phosphorylation of NF-κB p65. Therefore, the TSA-induced inhibition of inflammation may be due to regulation of the acetylation of non-histone proteins.

Discussion

Epigenetic mechanisms have been identified as a major determinant of gene expression and have been implicated in the regulation of complex physiological and pathological processes. In addition to methylation, histone acetylation is considered a key principle of epigenetic regulation. The present study explored the inhibitory effects of the HDACi TSA, on the release of inflammatory mediators from macrophages differentiated from U-937 cells.

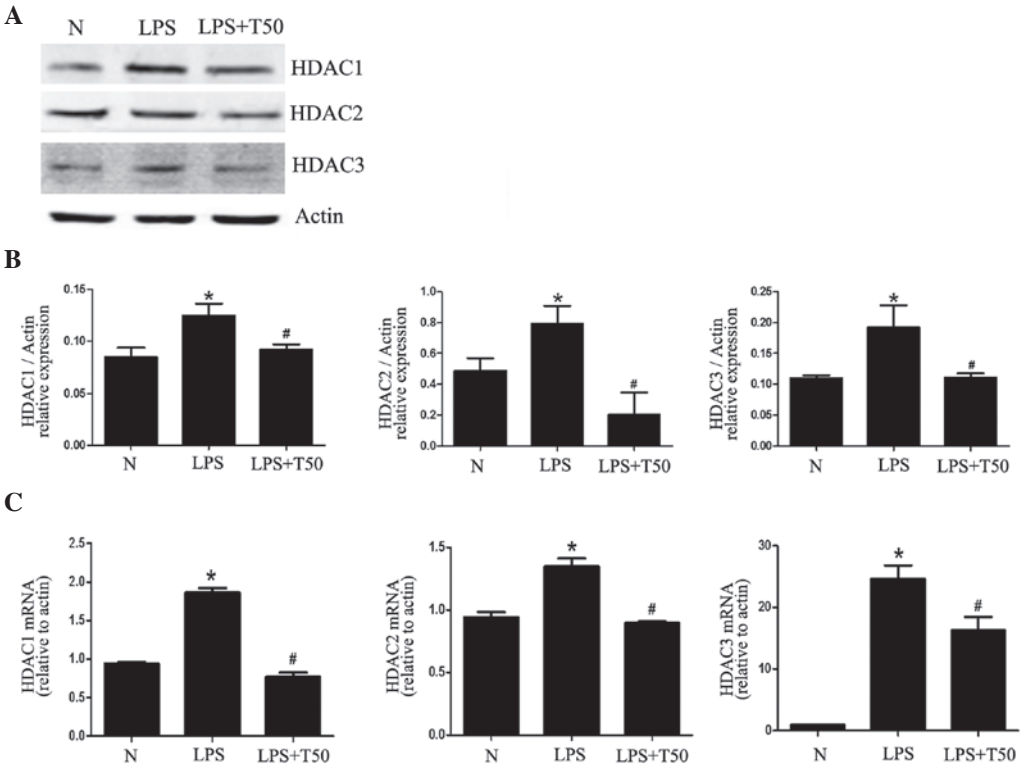

Figure 3. TSA suppressed the expression levels of class I HDACs in macrophages. (A and B) Protein expression and (C) mRNA expression levels of HDAC1, HDAC2 and HDAC3 were markedly increased in the LPS-treated group, as compared with the normal group, whereas the expression levels were all decreased in the TSA-treated group. Data are presented as the mean ± standard deviation. *P<0.05, vs. the control group; #P<0.05, vs. the LPS-treated group. N, control group; LPS, lipopolysaccharide; TSA, Trichostatin A; HDAC, histone deacetylase.

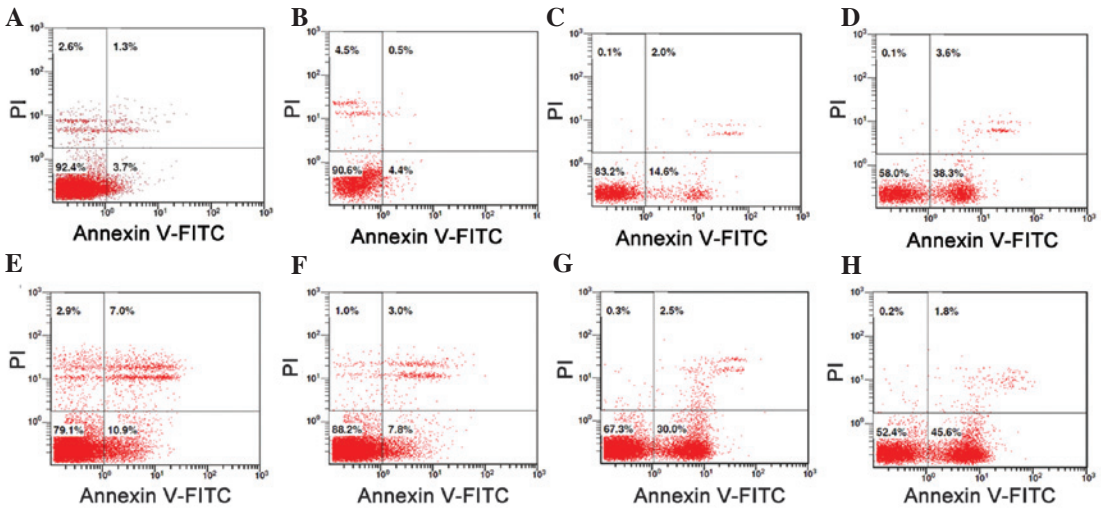

Figure 4. Low dose of TSA (50 nM) did not induce apoptosis of the macrophages. (A) Compared with the PMA-induced macrophages in normal conditions (control), (B) the low dose of TSA (50 nM) had little effect on apoptosis. However, there was marked apoptosis in the macrophages that were treated with (C) 100 nM or (D) 500 nM of TSA. (E) Compared with the control, the survival rate of the macrophages was significantly decreased (79.1 vs. 92.4%) without TSA treatment. (F) When treated with 50 nM TSA, the survival rate of the macrophages was increased to 88.2%. However, when treated with (G) 100 nM TSA or (H) 500 nM TSA, the survival rate of the macrophages was decreased to 67.3 and 52.4%, respectively. TSA, Trichostatin A; PMA, phorbol myristate acetate; FITC, fluorescein isothiocyanate; PI, propidium iodide.

Since TSA is currently considered an antitumor agent, the cytotoxicity of TSA was initially detected in the present study. Cell viability was >80% when treated with <500 nM TSA; therefore, the present study used 50, 100 and 500 nM TSA to explore the effects of TSA on macrophages. The inhibitory effects of TSA on the release of inflammatory cytokines were also investigated. The results of the present study demonstrated that TSA was able to reduce the cell supernatant expression levels of TNF-α, IFN-γ, IL-10 and IL-18 in a dose-dependent manner. FACS assay further demonstrated that the TSA-induced suppression of inflammatory factors was not due to the apoptosis of macrophages. These results

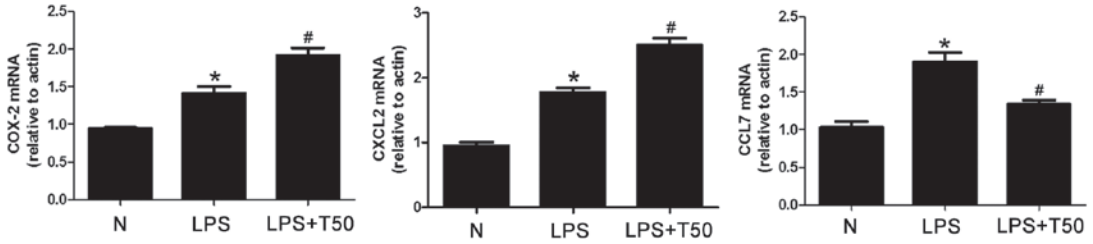

Figure 5. TSA selectively suppressed the expression levels of proinflammatory genes in macrophages. The mRNA expression levels of COX-2, CXCL-2 and CCL-7 were significantly increased in the LPS-treated group, as compared with the normal group. COX-2 and CXCL-2 mRNA expression levels were significantly increased in the TSA-treated group, whereas CCL-7 mRNA expression levels were decreased, as compared with the LPS-treated group. Data are presented as the mean ± standard deviation. *P<0.05, vs. the control group; #P<0.05, vs. the LPS-treated group. N, control group; LPS, lipopolysaccharide; TSA, Trichostatin A; COX, cyclooxygenase; CXCL2, chemokine (C-X-C motif) ligand 2; CCL6, chemokine (C-C motif) ligand 7.

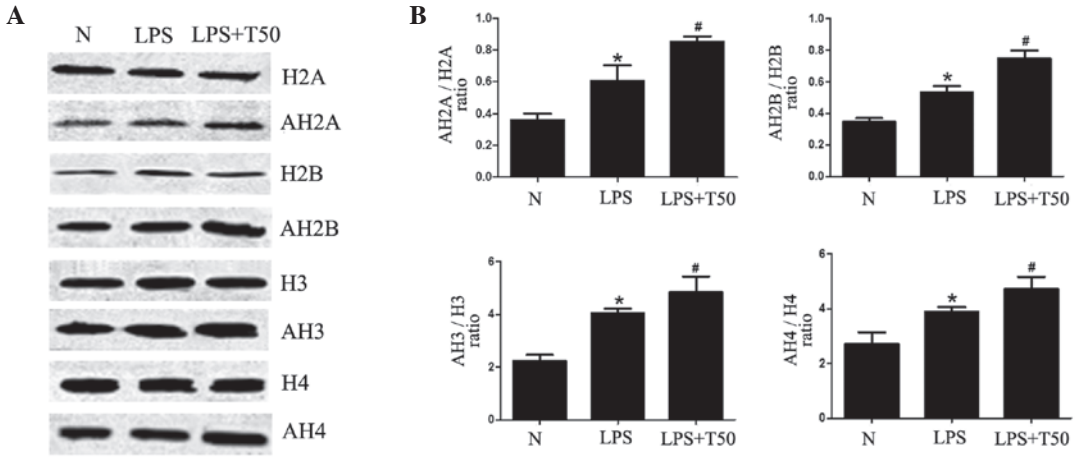

Figure 6. (A) TSA promoted histone acetylation in macrophages. (B) Acetylation levels of H2A, H2B, H3 and H4 were all significantly increased in the LPS-treated group, as compared with the normal group. Furthermore, the acetylation levels of H2A, H2B, H3 and H4 were all significantly increased in the TSA-treated group. N, control group; LPS, LPS-treated group; TSA, TSA-treated group. Data are presented as the mean ± standard deviation. *P<0.05, vs. the control group; #P<0.05, vs. the LPS-treated group. N, control group; LPS, lipopolysaccharide; TSA, Trichostatin A; H, histone; AH, acetylated histone.

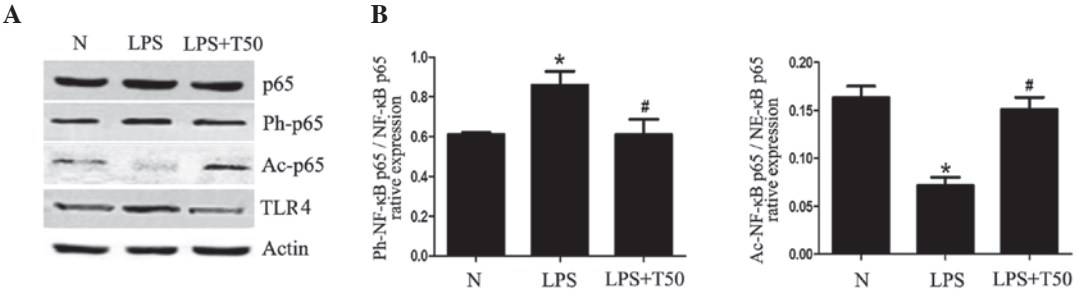

Figure 7. (A) TSA enhanced the acetylation of NF-κB p65 in macrophages. (B) Expression levels of TLR4 were significantly higher in the LPS-treated group, as compared with the normal group, and were decreased in the TSA-treated group. There were alterations in the acetylation and phosphorylation of NF-κB p65. The acetylation of NF-κB p65 was significantly decreased in the LPS-treated group, as compared with the normal group, but was significantly increased in the TSA-treated group. Conversely, the phosphorylation of NF-κB p65 was increased in the LPS-treated group, but was decreased in the TSA-treated group. Data are presented as the mean ± standard deviation. *P<0.05, vs. the control group; #P<0.05, vs. the LPS-treated group. N, control group; LPS, lipopolysaccharide; TSA, Trichostatin A; NF, nuclear factor; Ph, phosphorylated; Ac, acetylated; TLR4, Toll-like receptor 4.

indicated that the inhibitory effects of TSA on inflammation were independent of macrophage cell death.

Histone acetylation is controlled by HATs and HDACs. At present, 18 members of the HDAC family have been identified (21). The class I (HDAC1, 2, 3 and 8) and class II (HDAC4, 5, 6, 7, 9, 10 and 11) isoforms are Zn-dependent, whereas the class III HDACs (Sirtuins1, 2 and 7) are NAD^+-dependent. TSA and SAHA have a hydroxamate structure, and are able to inhibit Zn-dependent HDAC isoforms (21,22). In the present study, the expression levels of TSA substrates, HDAC1, HDAC2 and HDAC3, were detected. The results demonstrated that the expression levels of HDAC1, HDAC2 and HDAC3 were increased in the macrophages undergoing LPS-induced inflammation, and were decreased following TSA treatment. These

results suggested that the expression levels of class I HDACs were enhanced during the process of inflammation, and could be weakened by TSA alongside the inhibition of inflammation.

It has previously been reported that the expression levels of COX-2, CXCL-2 and CCL-7 are increased in LPS-induced inflammation (23). In the present study, the expression levels of COX-2, CXCL-2 and CCL-7 were detected; the mRNA expression levels of COX-2, CXCL-2 and CCL-7 were all increased in the LPS-treated group. However, only the expression levels of CCL-7 could be reduced by TSA. These results suggested that TSA may selectively suppress the expression of proinflammatory genes.

The nucleosome comprises an octamer of four core histones, an H3/H4 tetramer and two H2A/H2B dimers, which are surrounded by DNA (146 bp) (24). This architecture of chromatin is effectively regulated by histone acetylation. In the present study, histone acetylation was detected. The results demonstrated that the acetylation of H2A, H2B, H3 and H4 were all increased following the induction of inflammation by LPS. Furthermore, when treated with TSA, the acetylation levels of H2A, H2B, H3 and H4 were further increased. These results indicated that TSA promoted the acetylation, instead of inhibiting the acetylation, of histones. Previous studies have demonstrated that the acetylation of histones is closely associated with the activation of gene transcription (25-28), which may promote inflammation by enhancing the expression of proinflammatory genes (29,30). However, the results of the present study suggested that if the levels of histone acetylation were increased following treatment with the HDACi TSA, the expression of proinflammatory genes could be selectively suppressed.

In addition to histones, non-histone proteins may also be regulated by HATs and HDACs. In the present study, the acetylation levels of NF-κB p65 were measured. The results demonstrated that the acetylation of NF-κB p65 was decreased in the LPS-treated group and increased in the TSA-treated group. In addition to acetylated-NF-κB p65, the levels of TLR4 and phosphorylated-NF-κB p65 were detected. The results demonstrated that the expression levels of TLR4 and phosphorylated-NF-κB p65 were decreased following TSA treatment. These results suggested that TSA may predominantly inhibit the inflammatory response by regulating the acetylation of non-histone proteins. The specific mechanism underlying this effect requires further investigation.

The exact mechanism underlying the anti-inflammatory effects of TSA, and whether histone or non-histone-mediated effects are the most important remains unclear. Research regarding the development of HDAC-selective inhibitors and/or knockout mice may aid in resolving uncertainties.

In conclusion, the present study demonstrated that TSA may inhibit inflammation in macrophages. However, whether the mechanism by which TSA inhibits inflammation is through significantly enhancing histone acetylation to selectively suppress the expression of proinflammatory genes, and/or through regulating non-histone acetylation, requires further research.

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