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Original Article

JAK2/STAT5 signaling in the lipopolysaccharide (LPS)-induced inhibition of osteoblast differentiation

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Abstract: Bacterium infection commonly develops in bone fracture or in the followed open conduction and internal fixation, and Lipopolysaccharide (LPS) from bacteria has been recognized to be an important pathogenic factor in bone fracture healing. Therefore, the effects of LPS on bone metabolism are therefore relevant to clinical challenge of bone healing. In the present study, we investigated the role of JAK2/STAT5 signaling in the lipopolysaccharide (LPS)-induced inhibition of osteoblast differentiation. We investigated the influence of LPS treatment on the expression of JAK2/STAT5 signaling, with real-time quantitative PCR and western blotting. Then we determined the regulation of LPS on the osteoblast differentiation via measuring the Alkaline phosphatase (ALP) activity, matrix mineralization, and the expression of ALP, Procollagen type I N-terminal propeptide (PINP), Runt-related transcription factor 2 (RUNX2) AND Bone morphogenetic protein 2 (BMP-2) in the LPS-treated MC3T3-E1 cells. In addition, we evaluated the JAK2/STAT5 signaling-dependence of the influence by LPS on the osteoblast differencitaion with RNAi technology. It was demonstrated that LPS promoted JAK2/STAT5 signaling in both mRNA and protein levels in mouse osteoblast MC3T3-E1 cells, whereas inhibited the osteoblast differentiation via reducing the matrix mineralization, decreasing the ALP activity and suppressing the expression of such markers as ALP, PINP, RUNX2 and BMP-2 in mRNA levels in MC3T3-E1 cells. On the other side, RNAi-mediated JAK2/STAT5 signaling knockdown abrogates the inhibition by LPS to osteoblast differentiation. In summary, this study has shown that LPS inhibits osteoblast differentiation by suppressing the expression of ALP, PINP and RUNX2, all of which are essential for the osteoblast differentiation, in a JAK2/STAT5 signaling-dependent manner. This study may provide insights into the signal pathway of LPS-induced bone loss or delayed bone fracture healing.

Keywords: JAK2/STAT5 signaling, lipopolysaccharide (LPS), osteoblast, differentiation

Introduction

Bacterium infection usually occurs in bone fracture or in the followed open conduction and internal fixation, it often cause the delay of healing, even the nonunion [1, 2]. Physiological bone remodeling is balanced between bone formation and resorption by interaction between osteoblasts and osteoclasts [3], which is mediated by systematic factors such as calcitropic hormones, growth factors, and cytokines [4, 5].

Lipopolysaccharide (LPS), a major pathogenic component of the bacterial outer membrane, has multiple inflammatory actions and is the first identified bacterial component capable of inducing bone resorption by increasing the release of inflammatory osteolytic factors from osteoblastic cells [1, 6]. Although the mechanism of LPS on osteoclasts and bone resorption has been widely studied in vitro and in vivo [7-9], the mechanisms underlying the effect of LPS on osteoblasts and bone formation are not well known. Making these mechanisms clear has important implications for clinical practice.

In this research, we investigated the influence of LPS treatment on the expression of Janus kinase 2 (JAK2)/Signal Transducer and Activator of Transcription 5 (STAT5) signaling and determined the regulation of LPS on the osteoblast differentiation. Our results demonstrated LPS inhibits osteoblast differentiation via suppressing the expression of Alkaline phosphatase (ALP), Procollagen type I N-terminal propeptide

(PINP), Runt-related transcription factor 2 (RUNX2) AND Bone morphogenetic protein 2 (BMP-2), which are key factors for the osteoblast differentiation, in a JAK2/STAT5 signaling-dependent manner. Our research will provide valuable insights into the signal pathway of LPS-induced bone loss or delayed bone fracture healing.

Materials and methods

Cell culture

MC3T3-E1 cells were cultured in α-minimum essential medium (α-MEM; containing 50 μg/ml of ascorbic acid and 10 mM of β-glycerophosphat) (Life tech, USA) supplemented with 10% FBS and 1% penicillin-streptomycin. The medium was changed twice a week. Cells were discarded beyond 15th passage. For all experiments, cells were seeded at a density of 50,000 cells/cm^2. Treatments were performed 24 h later when cells had become confluent [10, 11].

Assay of mineralization

The mineralization of MC3T3-E1 cells was determined in 24-well plates using von Kossa staining [12-14]. Briefly, the cells were fixed with 95% ethanol and stained with $AgNO_3$ by the von Kossa method to detect phosphate deposits in bone nodules after confluent cells were grown in α-MEM for 2 weeks. The mineralized dots of each well were calculated under the inverted microscope.

Assay of relative activity of ALP assay and expression levels of ALP, BMP-2, RUNX-2, and PINP

After treatment with LPS, cells in 24-well plates were rinsed three times with PBS. ALP activity was assayed as previously described [15]. In brief, the assay mixtures contained 0.1 mM Mof 2-amino-2-methyl-1-propanol, 1 mM of $MgCl_2$, 8 mM of *p*-nitrophenyl phosphate disodium (Sigma, USA), and cell homogenates were added into the wells. After 3 minutes of incubation, the reaction was stopped with 0.1 N NaOH and the absorbance of each well was read at 405 nm wave length. A standard curve was mapped with *p*-nitrophenol as well. Each value was normalized for total protein concentration.

The expression levels of ALP, BMP-2, RUNX2, and PINP were quantified by ELISA method with BMP-2 quantification kit (Abnova, USA), ALP quantification kit (eBioscience, USA), RUNX-2 quantification kit (WKSUBIO, China), and PINP quantification kit (Beijing Baiao Laibo Technology CO., LTD, China), respectively.

MTT cell viability assay

Cells viability was measured with MTT method in 96-well plates [16]. Briefly, after cells were treated by indicated dosages of LPS, 20 μl of MTT in the final concentration of 5 mg/ml was added and the cells were incubated for 4 h at 37°C. Then the supernatant was carefully removed, 200 μl of DMSO was added to each well and mixed. The plate was put into a 37°C incubator to dissolve air bubbles and OD450 value of each well was measured at 570 nm wavelength using a microplate reader (Thermo scientific, USA). The results were expressed as (A570 of control wells-A570 of treated wells)/(A570 of control wells-A570 of blank wells) × 100%.

Western blot analysis

Cells were collected and were lysed with lysis buffer (Invitrogen, USA) on ice for 20 min. The cell lysates were centrifugated at 15,000×g at 4°C for 30 min. The supernatant was collected as the total cellular protein extract. Protein concentration was determined using the Pierce™ BCA Protein Assay Kit (Thermofisher scientific, USA). The samples of total cellular protein were loaded onto 10% SDS polyacrylamide gel. The separated proteins were electrophoretically transferred to PVDF membranes (Bio-rad, USA). The membrane was blocked overnight in blocking buffer containing PBS-T and 5% non-faty milk. Then the membrane was incubated with primary mouse antibody against β-actin, toll-like receptor (TLR)-2, TLR-4, STAT5b, and JAK2 (Santa Cruz Biotechnology, CA) separately for 1 hour and was washed with PBST for 4 times subsequently. Following incubating with the secondary HRP-conjugated goat anti-mouse IgG for 1 hour, the PVDF membrane was washed for 4 times and was treated with ECL reagent (Pierce, USA) and exposed to X-ray film. Each band was quantified using Image software.

Evaluation of mRNA level using quantitative real-time PCR (qRT-PCR)

mRNA level of each detected gene was determined by qRT-PCR as described previously [17-

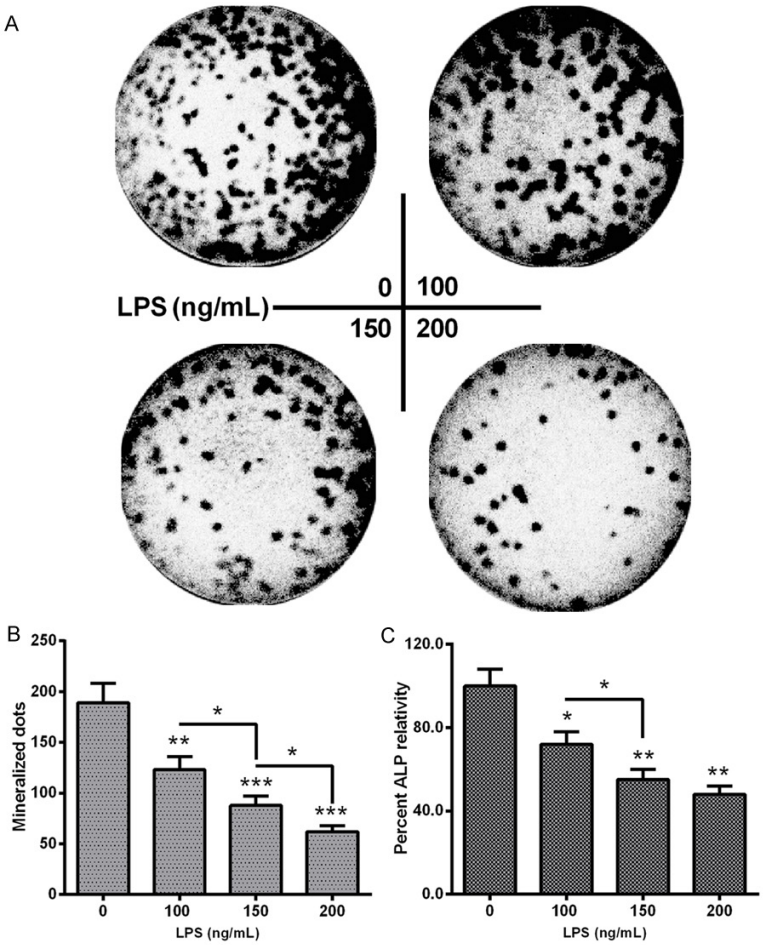

Figure 1. Reduced by LPS treatment matrix mineralization and alkaline phosphatase (ALP) activity in MC3T3-E1 cells. A: Matrix mineralization of MC3T3-E1 cells was stained by Von Kossa staining, post the treatment with 0, 100, 150 or 200 ng/mL LPS for 48 hours; B: Mineralized dots formed by the MC3T3-E1 cells, post the treatment with LPS. C: ALP activity in the MC3T3-E1 cells, post the treatment with 0, 100, 150 or 200 ng/mL LPS for 12 hours; Each result was averaged for quartic independent experiments. And statistical significance was presented as *P<0.05, **P<0.01, or ***P<0.001.

19]. Briefly, total RNA was isolated from cells using Trizol reagent (Life Technologies, USA) according to the manufacturer's instructions. Then cDNA was synthesized by RT using the Reverse Transcription kit (Life tech, China). Then qPCR was performed using the corresponding primers. The cycle threshold (Ct) value of the target gene was normalized to β-actin from the same sample as relative mRNA levels. Each sample was assessed in triplicate.

Small interference RNA (siRNA) assay

MC3T3-E1 cells were grown to 50% confluence and transfected with indiacted dosage of *siRNA* targeting JAK2 or Non-targeting *siRNA* (Dharmacon, Chicago) using FuGene 6, according to the manufacturer's instructions. 12 hours after transfection, JAK2 expression level was detected using western blotting assay. Osteogenic differentiation related genes (TLR2, TLR4, and JAK2) were analyzed by real-time PCR, and matrix mineralization was also determined. As well, 24 hours later, the protein levels of ALP, RUNX2), BMP-2 and PINP were assayed by ELISA method.

Statistical analysis

Data are depicted as the mean ± standard deviation that is calculated from three independent experiments. For a comparison between two groups, Student's t-test was used. For multiple comparisons among three or more groups, one-way analysis of variance was used. P<0.05 was considered as a statistically significant difference.

Results

LPS treatment reduced matrix mineralization and alkaline phosphatase (ALP) activity in MC3T3-E1 cells

In order to discriminate the impact of LPS treatment on the matrix mineralization and the ALP activity, matrix mineralization of MC3T3-E1 cells was stained by Von Kossa staining, post the treatment with 0, 100, 150 or 200 ng/mL LPS for 48 hours. As shown in **Figure 1A**, the mineralized dots formed by the MC3T3-E1 cells were obviously decreased as the increase of LPS dose. A dose-inhibition effect relationship was observed between the LPS dose and numbers of mineralized dots, statistic differences were also found between each two groups (**Figure 1B**). The ALP activities of LPS-treated MC3T3-E1 cells were also measured post the treatment with 0, 100, 150 or 200 ng/mL LPS for 12 hours, three LPS-treated groups all displayed

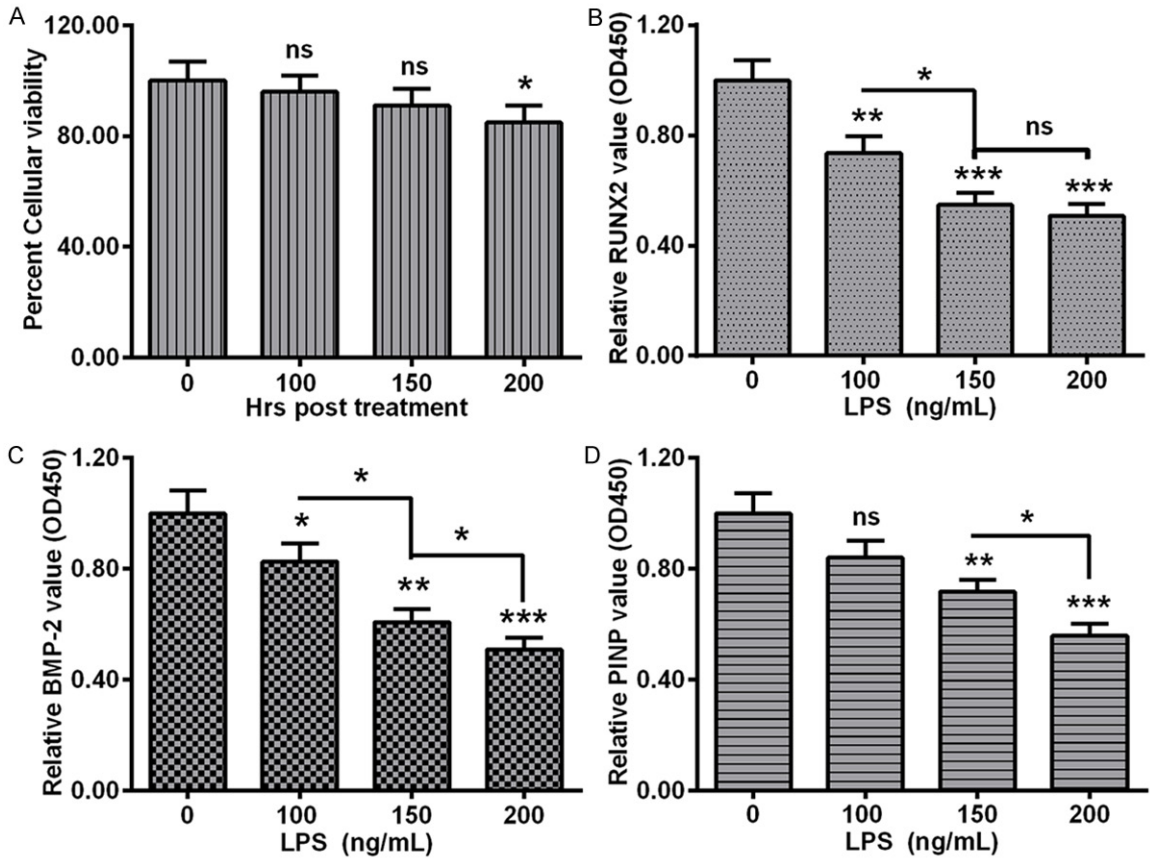

Figure 2. LPS-mediated reductions of osteogenic differention-associated markers in MC3T3-E1 cells. MC3T3-E1 were treated with 0, 100, 150 or 200 ng/mL LPS for 24 hours, then the cellular viability (A) was assayed with MTT kit, and the protein levels of Runt-related transcription factor 2 (RUNX2) (B), bone morphogenetic protein 2 (BMP-2) (C) and propeptide of type I procollagen I (PINP) (D) were assayed with ELISA method. Each result was averaged for quartic independent experiments. And statistical significance was presented as *P<0.05, **P<0.01, ***P<0.001 or (ns) no significance.

the lower activities compared with the control group with statistic differences (**Figure 1C**); specially, the relative ALP activity of 150 ng/ml LPS-treated group was suppressed by 20% compared with the 100 ng/ml LPS-treated group (P<0.05).

LPS treatment reduced osteogenic differention-associated markers in MC3T3-E1 cells

We next checked the MC3T3-E1 cellular viability and the levels of osteogenic differention-associated molecules such as RUNX2, BMP-2, and PINP. As shown in **Figure 2A**, 200 ng/ml LPS treatment could down-regulate the cellular viability by approximately 15% (P<0.05) in comparison with control group. The relative RUNX2 level was decreased by approximately 25%, 40%, and 50% respectively, when compared with the control group. In contrast to the control group, the relative RUNX2 level was down-regulated with statistic difference in LPS-treated group at each time point; however, no statistic difference was observed between two groups of 150 ng/ml LPS and 200 ng/ml LPS.

Regarding the relative BMP-2 level after treatment of different doses of LPS, similar results were observed. LPS down-regulated the BMP-2 level efficiently in each group, a dose-inhibition effect relationship was found between the BMP-2 level and LPS dose, 200 ng/ml LPS-treated cells presented the lowest BMP-2 level among groups (**Figure 2C**). With respect to the PINP level, it was suppressed by 25% at 150 ng/ml LPS treatment with a statistic difference (P<0.01), and cells treated by 200 ng/ml LPS showed the decreased BMP-2 level by 20% with a statistic difference (P<0.01) (**Figure 2D**).

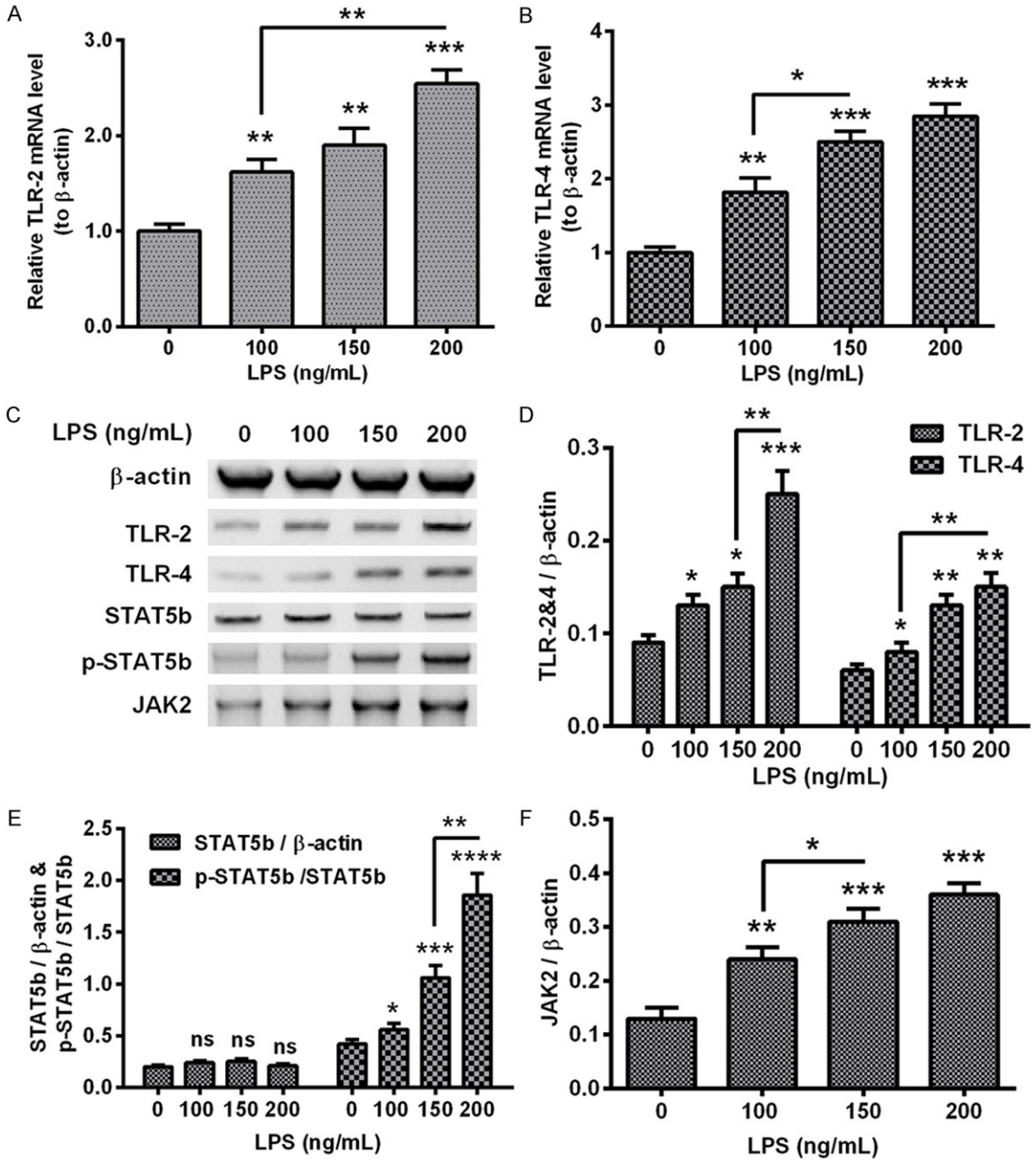

Figure 3. Promotion by LPS to Toll-like receptor (TLR)-2/4 epxression and STAT5/JAK2 signaling in MC3T3-E1 cells. (A and B) mRNA levels of TLR-2 (A) -4 (B) in the MC3T3-E1 cells, which were treated with 0, 100, 150 or 200 ng/mL LPS for 12 hours; (C) Western blot analysis of TLR-2, TLR-4, STAT5b, phosphorylated STAT5b and JAK2 in the LPS-treated MC3T3-E1 cells for 24 hours, with β-actin as internal control; (D) TLR-2 and -4 in protein level in the LPS-treated MC3T3-E1 cells; (E) Relative level of STAT5b to β-actin or relative level of phosphorylated STAT5b to non-phosphorylated STAT5b in the LPS-treated MC3T3-E1 cells; (F) Relative level of JAK2 to β-actin in the LPS-treated MC3T3-E1 cells. All data were presented as mean ± SD for triple independent results, and statistical significance was presented as *P<0.05, **P<0.01, ***P<0.001 or (ns) no significance.

Regulation by LPS on TLR-2/4 epxression and on STAT5/JAK2 signaling in MC3T3-E1 cells

We determined the transcription levels of TLR-2 and TLR-4 in MC3T3-E1 cells, as indicated in **Figure 3A**, treatment by 100 ng/ml, 150 ng/ml, and 200 ng/ml improved the TLR-2 transcription levels by 60%, 70%, and 150% with statistic differences, respectively. Specially, TLR-2 transcription levels of cells treated by 200 ng/

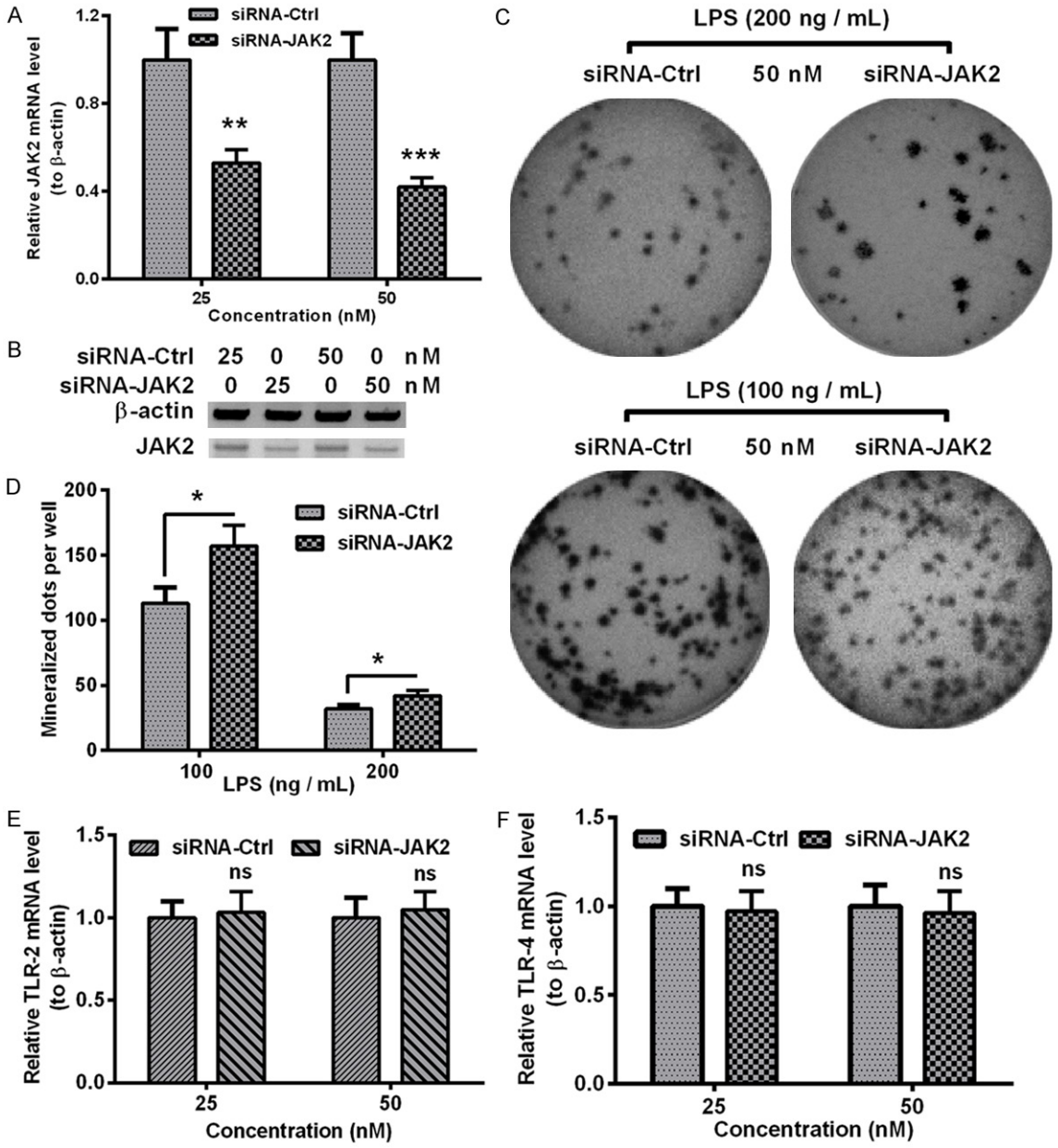

Figure 4. RNAi-mediated JAK2 knockdown ameliorates the LPS-mediated inhibition in matrix mineralization in MC3T3-E1 cells. MC3T3-E1 cells were transfected with 25 or 50 nM JAK2-specific siRNA (siRNA-JAK2) or control siRNA (siRNA-Ctrl) for 12 hours, and then the mRNA (A) or protein (B) level of JAK2 was examined by quantitative real-time RT-PCR or western blotting assay; (C and D) Matrix mineralization (C) and counting of the mineralized dots (D) of MC3T3-E1 cells, post the treatment with 100 or 200 ng/mL LPS and with the transfection with 25 or 50 nM siRNA-JAK2 or siRNA-Ctrl for 48 hours; (E and F) the mRNA level of TLR-2 (E) or TLR-4 (F) in the MC3T3-E1 cells, which were transfected with 25 or 50 nM siRNA-JAK2 or siRNA-Ctrl for 12 hours. Experiments were performed independently in triplicate. Statistical significance was shown as *P<0.05, **P<0.01, ***P<0.001 or (ns) no significance.

ml LPS were improved by about 50% (P<0.01). Similar to TLR-2, TLR-4 transciption levels also showed a LPS dose-dependent increase, statistic difference also presented between the groups treated by 100 ng/ml and 150 ng/ml LPS (P<0.05).

We analyze the TLR-2, TLR-4, STAT5b, phosphorylated STAT5b and JAK2 in the LPS-treated MC3T3-E1 cells, with β-actin as internal control. As shown in **Figure 3C**, intuitively, all five proteins presented a LPS dose-dependent increase except for STAT5b. The amount of

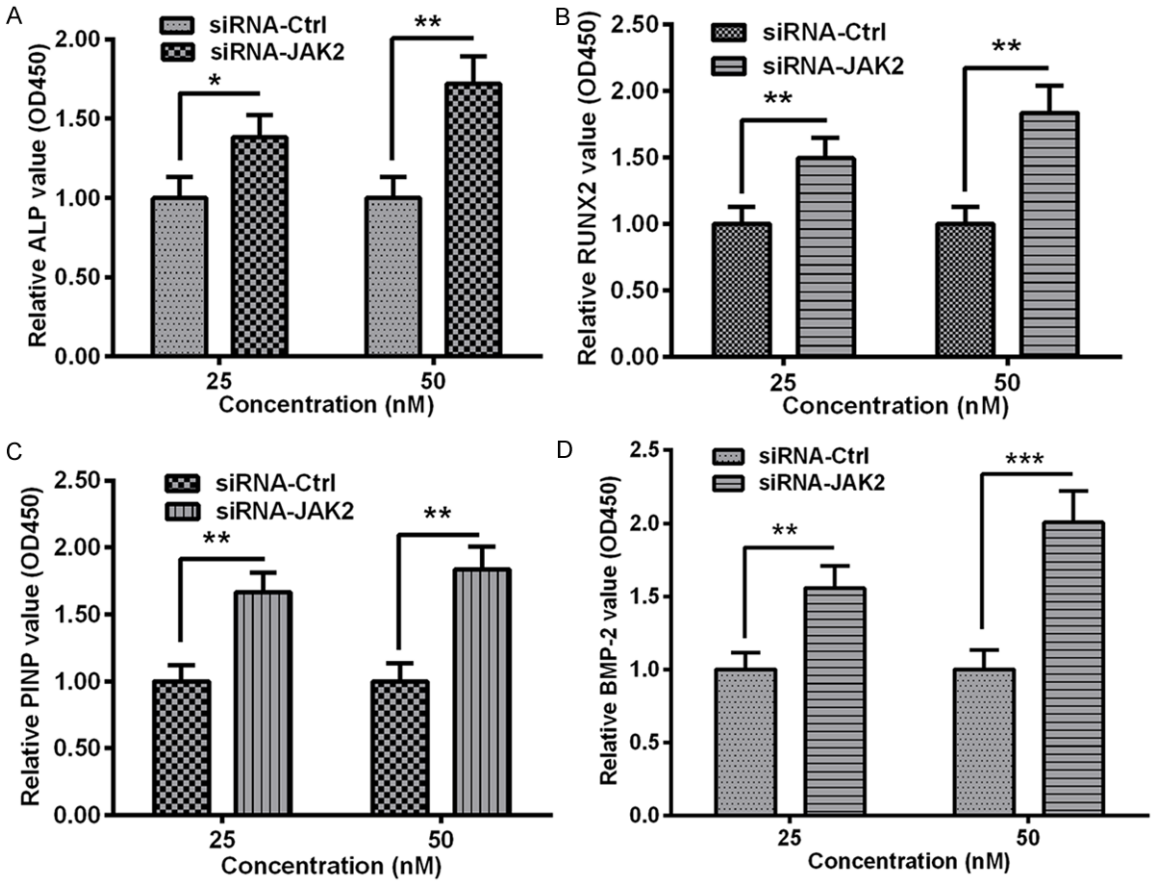

Figure 5. RNAi-mediated JAK2 knockdown ameliorated the LPS-mediated reduction of osteogenic differention-associated markers in the MC3T3-E1 cells. MC3T3-E1 cells were transfected with 25 or 50 nM siRNA-JAK2 or siRNA-Ctrl for 24 hours, and then the protein levels of ALP (A), RUNX2 (B), BMP-2 (C) and PINP (D) were assayed. Each result was averaged for triple independent experiments. Statistical significance was shown as *P<0.05, **P<0.01 or ***P<0.001.

each band was quantified and analyzed, TLR-2 and TLR-4 both presented dose-dependent up-regulation. When the LPS reached 200 ng/ml, the TLR-2 level was improved by 66% (P<0.01), compared with that at 150 ng/ml LPS; TLR-4 level at 200 ng/ml was up-regulated by 80% (P<0.01) in comparison with that at 100 ng/ml (**Figure 3D**). Interestingly, no statistic differences were found in the relative STAT5b level between groups (**Figure 3E**). However, the relative level of phosphorylated STAT5b (pSTAT5b) to non-phosphorylated STAT5b (STAT5b) rose as the LPS dosage increased with statistic differences, and the pSTAT5b/STAT5b level at 200 ng/ml was elevated by 50% compared with that at 500 ng/ml (**Figure 3E**). Similar to the above description, the JAK2 level was gradually elevated accompanied by the increased dosage of LPS (**Figure 3F**); particularly, cells treated by 150 ng/ml LPS present a lower LAK2 level than that treated by 200 ng/ml LPS (P<0.05).

RNAi-mediated JAK2 knockdown ameliorated the LPS-mediated inhibition in matrix mineralization and osteogenic differention-associated markers in MC3T3-E1 cells

To confirm the JAK2 function in the LPS-mediated inhibition in matrix mineralization, MC3T3-E1 cells were transfected with 25 or 50 nM JAK2-specific siRNA (siRNA-JAK2) or control siRNA (siRNA-Ctrl). Twelve hours post-infection, the mRNA level and protein level were quantified by quantify Real time PCR and western blotting. As shown in **Figure 4A**, relative JAK2 mRNA level was reduced by 50% for 25 nM Si RNA-JAK2 treatment, and by 60% for 50 nM siRNA-JAK2 treatment. The expression of JAK2 was also decreased greatly by siRNA-JAK2 transfection (**Figure 4B**).

To explore the function of LPS on the Matrix mineralization, 100 ng/ml or 200 ng/ml LPS was added in the siRNA transfected MC3T3-E1 cells, as presented in **Figure 4C** and **4D**, the mineralized dot count in 200 ng/ml LPS-treated group was clearly decreased when compared with that of 100 ng/ml LPS-treated group.

We next checked the TLR-2/TLR4 mRNA level in the siRNA transfected MC3T3-E1 cells. Interestingly, no statistic difference for the TLR-2/TLR-4 was found between siRNA-control group and siRNA-JAK2 group, whether 25 nM or 50 nM of siRNA was used (**Figure 4E** and **4F**).

To investigate the role of JAK2 in the LPS-mediated osteogenic differention, MC3T3-E1 cells were transfected with 25 or 50 nM siRNA-JAK2 or siRNA-Ctrl for 24 hours, and then the protein levels of ALP, RUNX2, BMP-2, and PINP were analyzed by ELISA. As shown in **Figure 5A-D**, all four proteins were apparently improved with significant statistic differences, when cells were treated by 25 or 50 nM siRNA-JAK2.

Discussions

In this research, we investigated the influence of LPS treatment on the expression of JAK2/STAT5 signaling, determined the regulation of LPS on the osteoblast differentiation and the expression of ALP, PINP and RUNX2 in the LPS-treated MC3T3-E1 cells. We also evaluated the JAK2/STAT5 signaling-dependence of the influence of LPS on the osteoblast differentiation using RNAi technology. Our Results confirmed that LPS promoted JAK2/STAT5 signaling in both mRNA and protein levels in vitro, whereas inhibited the osteoblast differentiation by reducing the matrix mineralization, decreasing the ALP activity, and down-regulating the ALP, PINP and RUNX2 in mRNA levels in MC3T3-E1 cells. Also, JAK2/STAT5 signaling knockdown abrogates the LPS inhibition to osteoblast differentiation.

Although LPS has been identified as a major bacterial bone-resorbing factor, little is known of its mechanism [20-22]. LPS is a well-known stimulator of cytokine and has been reported to stimulate osteoblasts to secrete the osteolytic factors: IL-1, IL-6, granulocyte-macrophage colony stimulating factor, prostaglandin E2, and nitric oxide [23-25]. However, its role in bone remodeling is still unclear. In our research, we found that LPS promoted JAK2/STAT5 signaling in mouse osteoblast MC3T3-E1 cells, and inhibited the osteoblast differentiation via reducing the matrix mineralization, decreasing the ALP activity and suppressing the expression of ALP, PINP and RUNX2 in mRNA in LPS-treated cells. On the other hand, siRNA against JAK2 could improve the mineralized dot count in the LPS-treated cells, the expression of RUNX2, PINP, and BMP-2 was greatly improved. The result clearly disclosed the JAK2/STAT5 signal pathway is a vital element in controlling the osteoblast differentiation.

The other interesting point in this research is to interrogate the function of pSTAT5b in the LPS-treated cells. STAT5B is expressed in many cell types, including osteoblasts [26, 27]. As shown in **Figure 3E**, the results indicated that LPS treatment did not alter the ratio of STAT5b/β-actin, whereas improved the ratio of p-STAT5b/β-actin. This observation clearly clarified the importance of the p-STAT5b in the JAK2-STAT5B pathway in the micro-environment of osteoblastic cells. Also, JAK2-STAT5B pathway played a direct or indirect role in this crucial transcription factor BMP families for osteoblastogenesis [28-30], and our results disclosed the LPS treatment could down-regulated BMP-2 expression in a dose -dependent manner, and this mechanism has to be clarified in further research.

Conclusion

In conclusion, this study has shown that LPS inhibits osteoblast differentiation by suppressing the expression of ALP, PINP and RUNX2 in a JAK2/STAT5 signaling -dependent manner, and results will provide profound insights into the signal pathway of LPS-induced bone loss or delayed bone fracture healing.

Disclosure of conflict of interest

None.

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References

[1] Hausmann E, Raisz LG, Miller WA. Endotoxin: stimulation of bone resorption in tissue culture. Science 1970; 168: 862-864.

[2] Millar SJ, Goldstein EG, Levine MJ, Hausmann E. Modulation of bone metabolism by two chemically distinct lipopolysaccharide fractions from Bacteroides gingivalis. Infect Immun 1986; 51: 302-306.
[3] Martin TJ, Ng KW. Mechanisms by which cells of the osteoblast lineage control osteoclast formation and activity. J Cell Biochem 1994; 56: 357-366.
[4] Kadono H, Kido J, Kataoka M, Yamauchi N, Nagata T. Inhibition of osteoblastic cell differentiation by lipopolysaccharide extract from Porphyromonas gingivalis. Infect Immun 1999; 67: 2841-2846.
[5] Nair SP, Meghji S, Wilson M, Reddi K, White P, Henderson B. Bacterially induced bone destruction: mechanisms and misconceptions. Infect Immun 1996; 64: 2371-2380.
[6] Gopalsami C, Yotis W, Corrigan K, Schade S, Keene J, Simonson L. Effect of outer membrane of Treponema denticola on bone resorption. Oral Microbiol Immunol 1993; 8: 121-124.
[7] Amano S, Kawakami K, Iwahashi H, Kitano S, Hanazawa S. Functional role of endogenous CD14 in lipopolysaccharide-stimulated bone resorption. J Cell Physiol 1997; 173: 301-309.
[8] Miyata Y, Takeda H, Kitano S, Hanazawa S. Porphyromonas gingivalis lipopolysaccharide-stimulated bone resorption via CD14 is inhibited by broad-spectrum antibiotics. Infect Immun 1997; 65: 3513-3519.
[9] Sveen K. Effects of a chemotactic factor and Bacteroides fragilis lipopolysaccharide on bone resorption in tissue culture. Acta Pathol Microbiol Scand A 1980; 88: 263-268.
[10] Wang D, Christensen K, Chawla K, Xiao G, Krebsbach PH, Franceschi RT. Isolation and characterization of MC3T3-E1 preosteoblast subclones with distinct in vitro and in vivo differentiation/mineralization potential. J Bone Miner Res 1999; 14: 893-903.
[11] Xiao G, Gopalakrishnan R, Jiang D, Reith E, Benson MD, Franceschi RT. Bone morphogenetic proteins, extracellular matrix, and mitogen-activated protein kinase signaling pathways are required for osteoblast-specific gene expression and differentiation in MC3T3-E1 cells. J Bone Miner Res 2002; 17: 101-110.
[12] Cheng SL, Lai CF, Blystone SD, Avioli LV. Bone mineralization and osteoblast differentiation are negatively modulated by integrin alpha (v) beta3. J Bone Miner Res 2001; 16: 277-288.
[13] Joung YH, Lim EJ, Kim MS, Lim SD, Yoon SY, Lim YC, Yoo YB, Ye SK, Park T, Chung IM, Bae KY, Yang YM. Enhancement of hypoxia-induced apoptosis of human breast cancer cells via STAT5b by momilactone B. Int J Oncol 2008; 33: 477-484.
[14] Nohutcu RM, McCauley LK, Koh AJ, Somerman MJ. Expression of extracellular matrix proteins in human periodontal ligament cells during mineralization in vitro. J Periodontol 1997; 68: 320-327.
[15] Lowry OH, Roberts NR, Wu ML, Hixon WS, Crawford EJ. The quantitative histochemistry of brain. II. Enzyme measurements. J Cell Biochem 1954; 207: 19-37.
[16] Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K, Nakagawa N, Yasuda H, Goto M, Tsuda E, Higashio K, Gillespie MT, Martin TJ, Suda T. Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. Bone 1999; 25: 517-523.
[17] Gao A, Kantarci A, Herrera BS, Gao H, Van Dyke TE. A critical role for suppressors of cytokine signaling 3 in regulating LPS-induced transcriptional activation of matrix metalloproteinase-13 in osteoblasts. Peer J 2013; 1: e51.
[18] Joung YH, Lim EJ, Darvin P, Chung SC, Jang JW, Do Park K, Lee HK, Kim HS, Park T, Yang YM. MSM enhances GH signaling via the Jak2/STAT5b pathway in osteoblast-like cells and osteoblast differentiation through the activation of STAT5b in MSCs. PLoS One 2012; 7: e47477.
[19] Kikuchi T, Matsuguchi T, Tsuboi N, Mitani A, Tanaka S, Matsuoka M, Yamamoto G, Hishikawa T, Noguchi T, Yoshikai Y. Gene expression of osteoclast differentiation factor is induced by lipopolysaccharide in mouse osteoblasts via Toll-like receptors. J Immunol 2001; 166: 3574-3579.
[20] Bandow K, Maeda A, Kakimoto K, Kusuyama J, Shamoto M, Ohnishi T, Matsuguchi T. Molecular mechanisms of the inhibitory effect of lipopolysaccharide (LPS) on osteoblast differentiation. Biochem Biophys Res Commun 2010; 402: 755-761.
[21] Reddi K, Meghji S, Wilson M, Henderson B. Comparison of the osteolytic activity of surface-associated proteins of bacteria implicated in periodontal disease. Oral Dis 1995; 1: 26-31.
[22] Thammasitboon K, Goldring SR, Boch JA. Role of macrophages in LPS-induced osteoblast and PDL cell apoptosis. Bone 2006; 38: 845-852.
[23] Hanazawa S, Amano S, Nakada K, Ohmori Y, Miyoshi T, Hirose K, Kitano S. Biological characterization of interleukin-1-like cytokine produced by cultured bone cells from newborn mouse calvaria. Calcif Tissue Int 1987; 41: 31-37.
[24] Keeting PE, Rifas L, Harris SA, Colvard DS, Spelsberg TC, Peck WA, Riggs BL. Evidence for

interleukin-1 beta production by cultured normal human osteoblast-like cells. J Bone Miner Res 1991; 6: 827-833.
[25] Littlewood AJ, Russell J, Harvey GR, Hughes DE, Russell RG, Gowen M. The modulation of the expression of IL-6 and its receptor in human osteoblasts in vitro. Endocrinology 1991; 129: 1513-1520.
[26] Darvin P, Joung YH, Yang YM. JAK2-STAT5B pathway and osteoblast differentiation. JAK-STAT 2013; 2: e24931.
[27] Hennighausen L, Robinson GW. Interpretation of cytokine signaling through the transcription factors STAT5A and STAT5B. Genes Dev 2008; 22: 711-721.
[28] Jove R. Preface: STAT signaling. Oncogene 2000; 19: 2466-2467.
[29] Lavery K, Hawley S, Swain P, Rooney R, Falb D, Alaoui-Ismaili MH. New insights into BMP-7 mediated osteoblastic differentiation of primary human mesenchymal stem cells. Bone 2009; 45: 27-41.
[30] Lin GL, Hankenson KD. Integration of BMP, Wnt, and notch signaling pathways in osteoblast differentiation. J Cell Biochem 2011; 112: 3491-3501.