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Toll-like receptor-4-dependence of the lipopolysaccharide-mediated inhibition of osteoblast differentiation

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ABSTRACT. Bone fractures or bones subjected to open conduction and internal fixation are easily infected by bacteria; bacterial lipopolysaccharide (LPS) has been recognized as an important

pathogenic factor affecting bone fracture healing. Therefore, the effect of LPS on bone metabolism is relevant for bone healing. In this study, we investigated the effect of LPS on the expression of Toll-like receptor (TLR)-4 (an LPS receptor) by using real-time quantitative PCR and western blotting. We also examined the regulatory role of LPS in osteoblast differentiation by measuring the ALP activity, matrix mineralization, and *ALP*, *OCN*, and *Runx2* mRNA (essential factors affecting osteoblast differentiation) expression in LPS-treated mouse osteoblast MC3T3-E1 cells. We also evaluated the effect of TLR-4 on LPS-mediated inhibition of osteoblast differentiation using RNA interference. LPS promotes TLR-4 mRNA and protein expression in MC3T3-E1 cells ($P < 0.05$, $P < 0.01$ or $P < 0.001$), and inhibits osteoblast differentiation by downregulating matrix mineralization and ALP activity ($P < 0.05$, $P < 0.01$ or $P < 0.001$), and suppressing the expression *ALP*, *OCN*, and *Runx2* mRNA in MC3T3-E1 cells ($P < 0.05$ or $P < 0.01$). Conversely, RNAi-mediated TLR-4 knockdown abrogates the LPS-mediated inhibition of osteoblast differentiation ($P < 0.05$ or $P < 0.01$). In summary, LPS was shown to inhibit osteoblast differentiation by suppressing the expression of *ALP*, *OCN*, and *Runx2* in a TLR-4-dependent manner. The results of this study may provide insights into the signal pathway of LPS-induced bone loss or delayed bone fracture healing.

Key words: Toll-like receptor (TLR)-4; Lipopolysaccharide; Osteoblast differentiation

