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Effects of muramyl dipeptide on osteoclast formation induced by LPS, IL-1 and TNF- α in mouse culture system

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Abstract. Muramyl dipeptide (MDP), the essential structure responsible for the immunoadjuvant activity of peptidoglycan, exists in Gram-positive and -negative bacterial walls. MDP synergistically enhances osteoclast formation induced by LPS, IL-1 and TNF- α through RANKL expression in osteoblasts. Nod2-mediated signals appear to be involved in the MDP-induced RANKL expression in osteoblasts. © 2005 Published by Elsevier B.V.

Keywords: MDP; Nod2; RANKL; LPS; TNF- α ; IL-1

1. Introduction

Muramyl dipeptide (MDP), the essential structure for the immunoadjuvant activity of peptidoglycan, exists in Gram-positive and -negative bacterial walls. We previously reported that MDP enhanced LPS-induced proinflammatory cytokine production in human monocytic cells [1]. Nod2 is proposed to be an intracellular sensor of MDP. A frameshift mutation of Nod2 is shown to be involved in the susceptibility to Crohn's disease. We explored effects of MDP on osteoclast formation in murine culture systems.

2. Materials and methods

Osteoblasts obtained from ddY (normal) were cocultured with bone marrow cells for 7 days in the presence or absence of MDP, LPS, IL-1, TNF- α , $1\alpha,25(\text{OH})_2\text{D}_3$ or PGE_2 [2].

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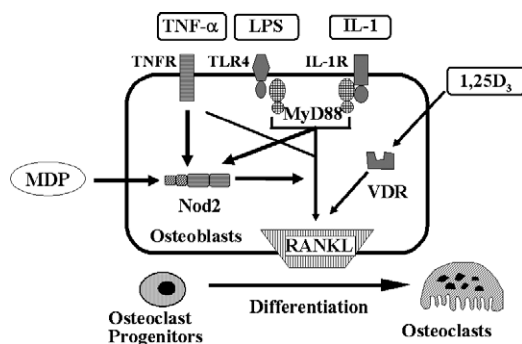


Fig. 1. Possible role of MDP in osteoclastogenesis induced by LPS, IL-1 and TNF- α .

Cells were then fixed and stained for TRAP (a marker enzyme of osteoclasts). Osteoblasts were also cultured in the presence or absence of those factors, and the expression of RANKL was examined. To repress Nod2 expression, small interference RNA (siRNA) for Nod2 was expressed in primary osteoblasts.

3. Results and discussion

MDP alone could not induce osteoclast formation in the coculture, but enhanced it as induced by LPS and IL-1 but not by $1\alpha,25(\text{OH})_2\text{D}_3$ or PGE_2 . MDP failed to enhance osteoclast formation from osteoclast progenitors induced by RANKL and M-CSF. MDP upregulated RANKL expression in osteoblasts treated with LPS but not $1\alpha,25(\text{OH})_2\text{D}_3$. Nod2 mRNA was undetectable in untreated osteoblasts, but was strongly expressed in osteoblasts treated with LPS and IL-1 but not with $1\alpha,25(\text{OH})_2\text{D}_3$. TNF- α also stimulated expression of Nod2 mRNA in osteoblasts. Indeed, MDP enhanced TNF- α -induced osteoclast formation in the coculture and RANKL mRNA expression in osteoblasts. Osteoclasts rapidly died due to apoptosis. LPS and RANKL stimulated the survival of osteoclasts, which was not enhanced by MDP. The depletion of intracellular Nod2 by siRNA blocked MDP-induced upregulation of RANKL mRNA in osteoblasts. These results suggest that MDP enhances osteoclast formation induced by LPS, IL-1 and TNF- α through RANKL expression in osteoblasts, but not the survival of osteoclasts supported by LPS and RANKL (Fig. 1) [2]. Nod2-mediated signals appear to be involved in the MDP-induced RANKL expression in osteoblasts.

References

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