

II-9. Nitric oxide production and inducible nitric oxide synthase expression induced by lipid A-associated proteins from *Porphyromonas gingivalis*

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Objectives

The purpose of this study was to examine the effects of lipid A-associated proteins (LAP) from *Porphyromonas gingivalis*, a major cause of inflammatory periodontal disease, on the production of nitric oxide (NO) and expression of inducible nitric oxide synthase (iNOS) in the murine macrophage cell line RAW264.7. We also attempted to throw light on the signaling mechanisms involved in *P. gingivalis* LAP-induced NO production.

Materials and Methods

The LAP from *P. gingivalis* 381 were prepared by standard hot phenol-water extraction of endotoxin isolated by the butanol method. NO production was assayed by measuring the accumulation of nitrite in culture supernatants. Western blot analysis of iNOS and analysis of reverse transcription (RT)-PCR products were carried out.

Results

We found that *P. gingivalis* LAP can induce iNOS expression and stimulate the release of NO without additional stimuli and demonstrated that multiple signaling pathways such as NF- κ B, microtubule polymerization, protein tyrosine kinase, and protein kinase C are involved in *P. gingivalis* LAP-stimulated NO production. The production of NO required L-arginine.

Conclusions

The present study clearly shows that *P. gingivalis* LAP fully induced iNOS expression and NO production in RAW264.7 cells in the absence of other stimuli. The ability of *P. gingivalis* LAP to promote the production of NO may be important in the pathogenesis of inflammatory periodontal disease.