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Macrophage-activating lipopeptide-2 induces cyclooxygenase-2 and prostaglandin E_2 via toll-like receptor 2 in human placental trophoblast cells

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Abstract

We have examined whether toll-like receptor (TLR)2-mediated stimulation by macrophage-activating lipopeptide-2 (MALP-2), originally purified from *Mycoplasma fermentans*, induces cyclooxygenase (COX)-2 and prostaglandin (PG)E_2 in human placental trophoblast cells. The signaling mechanism by which MALP-2 exerts its effect has also been examined. Human placental trophoblast cells isolated from term placenta were used. TLR expression in trophoblast cells was confirmed by multiplex PCR and immunocytochemistry, and examined whether MALP-2 induces COX-2 and PGE_2 by Northern blotting, RT-PCR, Western blotting and ELISA, respectively. The activation of NF-κB and MAP kinases (ERK1/2 and p38) was examined by Western blotting. The effects of inhibitors of NF-κB, MEK1/2 and p38 on MALP-2-induced PGE_2 production were also evaluated. TLR2, TLR6 and TLR4 were expressed in human placental trophoblast cells. MALP-2 significantly induced COX-2 expression and enhanced PGE_2 production in a dose-dependent manner. MALP-2 induced the activation of NF-κB, ERK1/2 and p38 MAPK. Inhibitors of NF-κB, MEK1/2 and p38 blocked MALP-2-inducible PGE_2 production. TLR2-mediated stimulation by MALP-2 induces COX-2 and PGE_2 in human placental trophoblast cells via NF-κB and MAP kinases pathways.

Keywords: Toll-like receptor; MALP-2; COX-2; NF-κB; MAP kinases

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1. Introduction

Microbial infection participates in induction of preterm labor and delivery through prostaglandin (PG) synthesis. In studies of the mechanisms of preterm labor caused by microbial infection, lipopolysaccharide (LPS) had been often used as a bacterial pathogen model (Anteby et al., 1998; Svinarich et al., 1996; Fortunato et al., 1996; Shimoya et al., 1999). LPS is a component from the Gram-negative bacterial cell wall, and is well known to activate monocytes and macrophages. However, infection is caused not only by Gram-negative bacteria, including LPS, but also by a variety of microbes such as Gram-positive bacteria or *Mycoplasmataceae*. Several bacterial cell components other than LPS have been shown to possess the potential to activate monocytes and macrophages, but have been seldom used in studies of the mechanisms of preterm labor. These bacterial components are termed pathogen-associated molecular patterns (PAMPs), including lipopeptides, peptidoglycan and bacterial DNA containing an unmethylated CpG motif. *Mycoplasmataceae* possess lipopeptides in their cell membrane as PAMPs.

The toll-like receptor (TLR) family is involved in the recognition of microbial components, and each TLR recognizes and discriminates specific PAMPs (Medzhitov et al., 1997; Takeda and Akira, 2004). The TLR family is important to innate host defense against pathogens, and now consists of 11 members (TLR1–TLR11). TLR2 is essential for the recognition of microbial lipopeptide or peptidoglycan (Takeda and Akira, 2004). TLR1 and TLR6 are associated with TLR2, and discriminate subtle differences between triacyl- and diacyl-lipopeptides, respectively (Takeuchi et al., 2001). The innate immune system plays important roles during pregnancy (Guleria and Pollard, 2000), but little is known about the detailed roles of TLR in human placenta.

In this study, we used macrophage-activating lipopeptide-2 (MALP-2) as a model of bacterial pathogen to study the mechanism of preterm labor caused by *Mycoplasmataceae* infection. MALP-2, diacylated lipopeptide originally purified from *Mycoplasma fermentans*, is known to require the cooperation of TLR2 and TLR6 for stimulation, and to induce proinflammatory cytokines in macrophages (Mühlradt et al., 1997; Takeuchi et al., 2001). In this cooperation of TLR2 and TLR6, TLR6 can augment, but is not essential for, the TLR2 response to MALP-2 (Into et al., 2004). We have examined whether MALP-2 induces cyclooxygenase (COX)-2 and PGE_2 in human placental trophoblast cells. In addition, the involvement of NF-κB and MAP kinases in MALP-2-inducible PGE_2 production has also been addressed.

2. Materials and methods

2.1. Reagents

LPS from the *Escherichia coli* serotype 0111:B4 was purchased from Sigma–Aldrich, St. Louis, MO. Synthetic MALP-2 was purchased from Alexis, Tokyo, Japan. TL2.1, a mouse monoclonal anti-human TLR2 antibody, was purchased from Hycult Biotechnology, Uden, Netherlands, and used as a neutralizing antibody against TLR2. The NF-κB inhibitor, *N*-