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Mycoplasma bovis-derived lipid-associated membrane proteins activate IL-1β production through the NF-κB pathway via toll-like receptor 2 and MyD88

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ABSTRACT

Mycoplasma bovis causes pneumonia, otitis media, and arthritis in young calves, resulting in economic losses to the cattle industry worldwide. *M. bovis* pathogenesis results in part from excessive immune responses. Lipid-associated membrane proteins (LAMPs) can potently induce host innate immunity. However, interactions between *M. bovis*-derived LAMPs and Toll-like receptors (TLRs), or signaling pathways eliciting active inflammation and NF-κB activation, are incompletely understood. Here, we found that IL-1β expression was induced in embryonic bovine lung (EBL) cells stimulated with *M. bovis*-derived LAMPs. Subcellular-localization analysis revealed nuclear p65 translocation following EBL cell stimulation with *M. bovis*-derived LAMPs. An NF-κB inhibitor reversed *M. bovis*-derived LAMP-induced IL-1β expression. TLR2 and myeloid differentiation primary response gene 88 (MyD88) overexpression increased LAMP-dependent IL-1β induction. TLR2-neutralizing antibodies reduced IL-1β expression during LAMP stimulation. LAMPs also inhibited IL-1β expression following overexpression of a dominant-negative MyD88 protein. These results suggested that *M. bovis*-derived LAMPs activate IL-1β production through the NF-κB pathway via TLR2 and MyD88.

1. Introduction

Mycoplasma bovis causes arthritis, otitis media, and pneumonia in young calves, causing enormous financial losses to the cattle industry and morbidity and mortality in calves worldwide (Bush and Rosenbusch, 2003; Song et al., 2012). We reported the first isolation of *M. bovis* in China in 2008 (Xin et al., 2008). Although great efforts have been made to understand the molecular basis of *M. bovis* infection, the cellular responses to *M. bovis* are still largely unknown (Bush and Rosenbusch, 2002).

Mycoplasma lipid-associated membrane proteins (LAMPs) are cell-surface lipoproteins that mediate interactions with host cells (Rottem, 2003). LAMPs induce potent of inflammatory responses during mycoplasma infection (Jun et al., 2009). Shimizu et al. reported that *Mycoplasma pneumoniae*-derived LAMPs can induce NF-κB activation in human acute monocytic leukemia cell lines (Shimizu et al., 2008). Jun et al. also reported that *Mycoplasma genitalium*-derived LAMPs can activate NF-κB through Toll-like receptors (TLRs) 1, 2 and 6 and CD14 via an MyD88-dependent pathway (Jun et al., 2009).

NF-κBs are inducible transcription factors that mediate not only cell proliferation and survival, but also help elicit cytokine- and pathogen-induced immune and inflammatory responses (Li and Verma, 2002). In heterodimeric form, NF-κB is comprised of a 50-kDa subunit (p50) and a 65-kDa subunit (p65) (Ghosh et al., 1998). NF-κB is normally maintained in the inactive form in the

Abbreviations: LAMPs, lipid-associated membrane proteins; TLRs, Toll-like receptors; EBL, embryonic bovine lung; MyD88, myeloid differentiation primary response gene 88; CCU, color change unit; DMSO, dimethyl sulfoxide; DAPI, 4′,6′-diamidino-2-phenylindole-dihydrochloride.

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Table 1
Sequences of primers used for subcloning TLR2, MyD88, and DN-MyD88.

Gene name	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
TLR2	GGAATTCGGATGCCACGTGCTTTGTGGAC	CCGCTCGAGCTAGGACCTTATTGCAGCTC
MyD88	GCGTCGACCATGGCTGAAGGAGTAC	GAAGATCTTCAGGGCATGGACAGGGC
DN-MyD88	GCGTCGACCGACCCCCTAGGGCAAAA	GAAGATCTTCAGGGCATGGACAGGGC
P65	AGACTCGAGCTATGGACGACCTCTTCCC	GGGGTACCTTAGGAGCTGATCTGACTCAG

cytoplasm through binding with a member of the inhibitory kappa B (IκB) family. Following pro-inflammatory stimulation, IκB can become phosphorylated by IκB kinase (IKK) and be proteolytically degraded. Subsequent phosphorylation of the NF-κB p65 subunit unmasks its nuclear localization signal sequence (NLS). This, in turn promotes nuclear NF-κB translocation and NF-κB binding to the regulatory elements of target genes, which regulate various biological functions (May and Ghosh, 1998; Yang et al., 1999). IL-1β, which is regulated by the NF-κB signaling pathway, is an important inflammation-associated gene that is upregulated during many types of pathogenic infection (Arthur and Ley, 2013; Kawai and Akira, 2007).

Embryonic bovine lung (EBL) cells can be especially useful for studying infectious disease processes in cattle, including those caused by *M. bovis* (Dewals et al., 2008; Song et al., 2012). In our previous study, we used EBL cells to identify 2 adhesion factors of *M. bovis* (Song et al., 2012; Zou et al., 2013). In the present study, we used EBL cells to investigate the molecular mechanism by which *M. bovis*-derived LAMPs induce IL-1β expression in EBL cells and discovered that *M. bovis*-derived LAMPs induce NF-κB activation through TLR2 in a MyD88-dependent pathway.

2. Materials and methods

2.1. Mycoplasma strain, LAMP preparation, and cell culture

Mycoplasma was cultured in modified pleuropneumonia-like organism (PPLO) medium supplemented with 20% inactivated horse serum (HyClone), 10% yeast extract, thallium acetate (0.125 mg/ml) and penicillin (200 IU/ml). The origin and growth

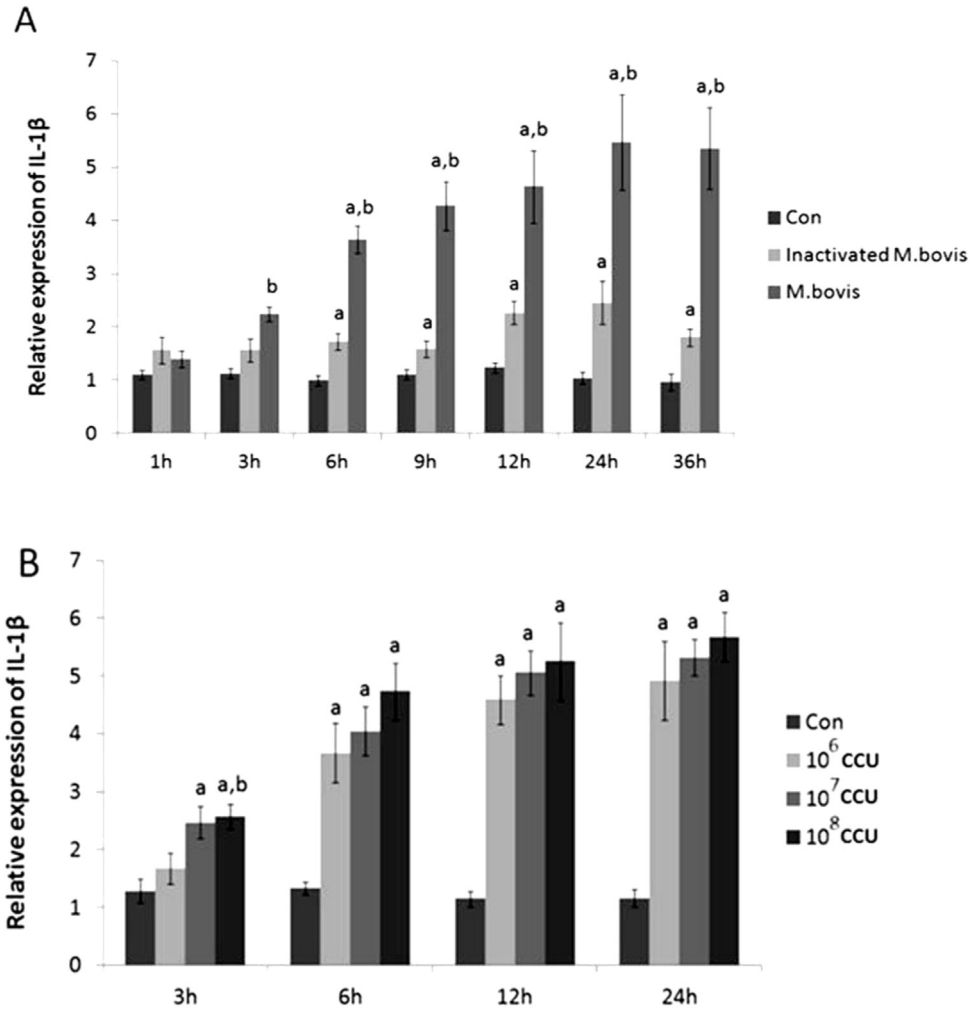

Fig. 1. *M. bovis* enhanced IL-1β expression in EBL cells. (A) EBL cells were control-inoculated, inoculated with *M. bovis*, or inoculated with inactivated *M. bovis* (10^7 CCU). (a) $p < 0.05$, compared with the control group; (b) $p < 0.05$, compared with the inactivated group. (B) EBL cells were inoculated with *M. bovis* at 10^6, 10^7, or 10^8 CCU. (a) $p < 0.05$, compared with the control group; (b) $p < 0.05$, compared with the *M. bovis* (10^6 CCU) group. The values are presented as the means ± the SEMs of 3 independent tests.