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Signaling through interleukin-1 type 1 receptor diminishes *Haemophilus somnus* lipooligosaccharide-mediated apoptosis of endothelial cells

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Abstract

During sepsis, endothelial cells are both a source and target of pro-inflammatory cytokines (e.g. IL-1α, IL-1β, TNFα and others), which may be detrimental to vascular homeostasis. Our laboratory has demonstrated that *Haemophilus somnus*, a gram-negative pathogen of cattle that causes sepsis and vasculitis, and its lipooligosaccharide (LOS) induce caspases-3, -8 and -9 activation, and apoptosis of endothelial cells in vitro. In this study, we provide evidence that *H. somnus* LOS increases IL-1α and IL-1β mRNA expression, and caspase-1 activation in endothelial cells. Addition of a caspase-1 inhibitor (YVAD), or incubation in a high extracellular potassium buffer (150 mM), reduced caspase-1 activation and significantly enhanced *H. somnus* LOS-mediated caspase-3 activation. Likewise, blocking the IL-1 type 1 receptor by addition of IL-receptor antagonist (IL-1ra) significantly enhanced LOS-mediated caspase-3 activation. Conversely, addition of exogenous recombinant bovine IL-1β (100 ng/mL) to endothelial cells diminished LOS-mediated apoptosis. IL-1β has been reported previously to protect numerous cell types from apoptosis by activating PI3 kinase/p-Akt signaling pathways. Addition of selective PI3 kinase inhibitors (e.g. wortmannin and LY294002) significantly enhanced LOS-mediated caspase-3 activation. Exposure of endothelial cells to IL-1β or LOS increased pAkt protein as assessed by western blot. Overall, these results suggest that signaling through the IL-1 type 1 receptor diminishes *H. somnus* LOS-mediated apoptosis.

Keywords: Haemophilus somnus; Endothelial cell; Apoptosis; Interlukin-1 beta; Lipooligosaccharide; Phosphatidylinositol-3-kinase

1. Introduction

Gram-negative sepsis is a life-threatening condition that accounts for an estimated 2–11% of all admission to critical care units in the USA and Europe, with an incidence of 175.9 per 100,000 patients admitted to US hospitals [1]. The pathogenesis of gram-negative sepsis is characterized by a rapid and robust release of pro-inflammatory mediators such as cytokines, reactive nitrogen and oxygen intermediates, and eicosanoids in response to bacterial endotoxin [2]. As a result, endothelial cells are exposed to high levels of pro-inflammatory molecules, such as interleukin (IL)-1 beta (IL-1β), IL-1 alpha (IL-1α) and tumor necrosis factor alpha (TNFα), which may cause endothelial cell dysfunction [1].

IL-1β is a potent activator of endothelial cells, causing an increase in adhesion molecule expression [3] and chemokine release [4], and inducing apoptosis of many cell types [5–10]. The expression of pro-IL-1β (31–33 kDa) in endothelial cells is triggered by various inflammatory stimuli including bacterial endotoxin [11]. Post-translational processing of pro-IL-1β by activated caspase-1, which is also known as IL-1 converting enzyme (ICE), facilitates release of the mature 17.5 kDa cytokine [12,5,13]. Likewise, IL-1α is produced by endothelial cells [14], and shares similar biological activities with IL-1β (e.g. binding to IL-1 type 1 receptor). However, IL-1α differs in the mechanism of secretion and activation. For example, secretion of mature IL-1α (17 kDa) occurs independent of

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caspase-1 [15], and may involve calpain proteases [16]. Targeted expression of proIL-1α (33 kDa) to cell membranes occurs by conjugation with myristoleic acid, which may result in a pool of membrane bound cytokine capable of yielding mature IL-1α [17,18].

Some studies have demonstrated that caspase-1 activation and IL-1β is associated with apoptotic death of some cell types [19,6,7,9]. However, other investigators have demonstrated that exogenous IL-1β protects cells from various apoptotic stimuli (e.g. pro-inflammatory cytokines, serum deprivation and others) [20–23], and that this protection may involve activation of phosphatidylinositol 3-kinase (PI3 kinase), which in turn leads to phosphorylation and activation of Akt (also known as protein kinase B) [24–27]. Phosphorylated Akt (pAkt) can prevent or delay apoptosis by phosphorylation-dependent inhibition of several pro-apoptotic signaling molecules. For example p-Akt phosphorylates the pro-apoptotic protein Bad, which prevents its binding to Bcl-2, and limits cytochrome C release from mitochondria [28]. Likewise, pAkt was shown to directly phosphorylate caspase-9, and inhibit its activation [29]. In addition, pAkt has been shown to upregulate expression of vascular endothelial cell growth factor [30], which diminished LPS-induced apoptosis of endothelial cells [31].

Haemophilus somnus is a gram-negative pathogen of cattle that causes a variety of disease problems including pneumonia [32], abortion [33], thrombotic meningoencephalitis [34], and infertility [35,36]. Vasculitis and thrombosis are commonly detected in *H. somnus* infected tissues [32], suggesting that *H. somnus* targets endothelial cells in vivo. It has been reported that *H. somnus* and its lipooligosaccharide (LOS) induce apoptosis of endothelial cells in vitro [37] in a caspase-3 and caspase-8 dependent manner [38]. In the present study, we investigated whether caspase-1 activation and stimulation of the IL-1 type 1 receptor affects LOS-induced endothelial cell apoptosis. We provide evidence that IL-1α and IL-1β mRNA expression, and caspase-1 activity, are increased in endothelial cells incubated with LOS, and that stimulation of the IL-1 type 1 receptor diminishes LOS-induced apoptosis via a mechanism that appears to involve, at least in part, activation of PI3 kinase.

2. Results

2.1. LOS exposure increases IL-1α and IL-1β mRNA expression and caspase-1 activation in endothelial cells

During gram-negative sepsis, endothelial cells are both a source and a target of pro-inflammatory cytokines, such as IL-1α and IL-1β [2]. We sought to determine if IL-1α and IL-1β mRNA expression were increased in response to *H. somnus* LOS. As shown in Fig. 1, IL-1β mRNA was significantly elevated ($P<0.05$) by LOS at 1 h, and IL-1α mRNA was significantly increased ($P<0.05$) at 1 and 2 h. Because the release of mature IL-1β requires proteolytic

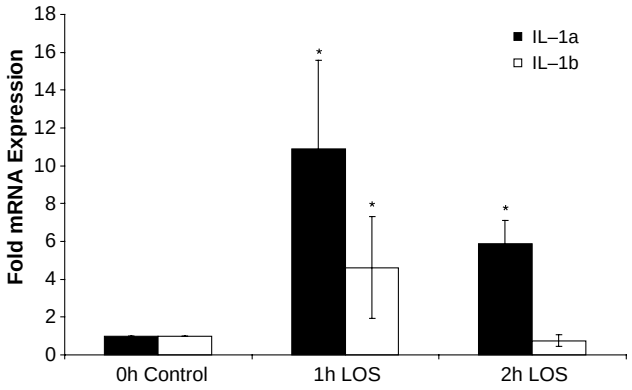

Fig. 1. LOS induces IL-1α and IL-1β mRNA expression. Endothelial cells (1×10^6) were incubated with LOS (500 ng/mL) for 0–2 h. Levels of mRNA expression were determined using real-time PCR with cytokine specific primers. These data represent the mean ± SEM fold change in IL-1α or IL-1β mRNA expression of three independent experiments. (*$P<0.05$).

cleavage by caspase-1, we sought to determine whether exposure to LOS induces caspase-1 activation in bovine endothelial cells. We observed a significant increase ($P<0.01$) in caspase-1 activity in LOS treated endothelial cells (Fig. 2), that was blocked by addition of a caspase-1 tetrapeptide inhibitor (YVAD; 40 μM). Likewise, when endothelial cells were incubated with a high potassium buffer (150 mM KCl supplemented with 20% FBS), which has been reported previously to prevent caspase-1 activation [39], LOS-mediated caspase-1 activity was significantly reduced ($P<0.01$) (Fig. 3). Overall, these data indicate that LOS exposure activates IL-1α and IL-1β mRNA, and the IL-1β processing enzyme caspase-1, in bovine endothelial cells. We attempted to quantify bovine IL-1β levels by

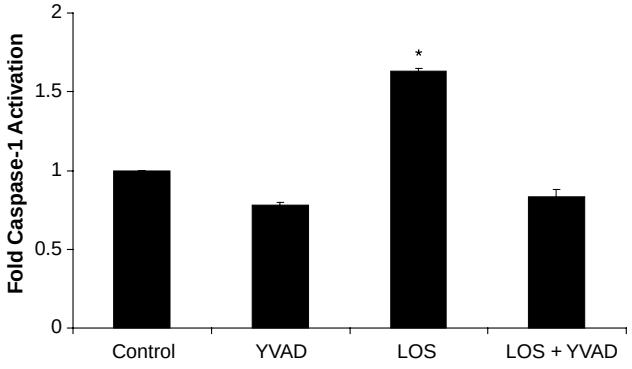

Fig. 2. LOS induces caspase-1 activation. Endothelial cells (1×10^6) were incubated with LOS (500 ng/mL) for 6 h. Some cultures of endothelial cells were also treated with a caspase-1 inhibitor (YVAD; 40 μM) at the time LOS was added. EC incubated with YVAD or medium alone (control) served as negative controls. Cell lysates were assayed for caspase-1 activation, as described in the methods section. These data represent the mean ± SEM fold increase in caspase-1 activation of three independent experiments. (*$P<0.01$). Addition of DMSO, the vehicle for caspase-1 inhibitor, had no effect on caspase-1 activation (0.92 fold ± 0.05).