Sequential changes in NF-κB and STAT-3 mRNA in polymorphonuclear leukocytes and liver samples in endotoxin-challenged calves

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Introduction

Inflammatory diseases, such as bovine respiratory disease complex (BRD), are the most frequently observed diseases in dairy and feedlot cows, and most common cause of economic loss. Calves are highly sensitive to the component of the gram-negative bacterial envelope known as LPS or endotoxin. The acute-phase response to endotoxin may regulate transcription factors, such as nuclear factor kappa B (NF-κB) or STAT-3, and the release of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), from neutrophils and monocytes/macrophages. Many studies have reported NF-κB transcription factors and signaling pathways to be the central coordinators in immune and inflammatory responses, and STAT-3 was found to regulate the expression of numerous genes in response to cellular stimuli. The activation of and interaction between NF-κB and STAT-3 are vital for inflammatory control, but it is unknown how endotoxin affects the NF-κB family and STAT-3 in calves. In this study, we examined sequential changes in mRNA levels of TLR-4, NF-κB1, NF-κB2, IL-6, and STAT-3 in polymorphonuclear leukocytes (PMNs) and the liver using an endotoxin challenge model.

Materials and Methods

Twelve Holstein and Jersey calves [BW 379.9 ± 81.1 lb (172.3 ± 36.8 kg)] were enrolled in this study. The health status of the animals was based on physical, biochemical, thoracic ultrasound, and radiological examinations. These calves had no clinical signs such as coughing, nasal discharge, fever, or pulmonary wheezing sounds, and were divided into 2 groups (n=6 each group). The calves in the LPS group received 2.5 µg/kg of BW ultrapure O111:B4 LPS in 10 mL of each autologous serum via a catheter in the jugular vein, whereas the control calves received a similar volume of saline. Blood samples were collected from the contralateral jugular vein before the endotoxin challenge, and at 0.5, 1, 2, 4, 8, 12, and 24 hours after administration, and were stored in tubes containing EDTA or heparinized tubes. Liver samples were simultaneously collected by ultrasound-guided liver biopsy using a true-cut needle. Immediately prior to testing, plasma samples were diluted 20-fold in endotoxin-free water and agitated by vortex for 10 sec. Specimens were then heated for 10 min at 176°F (80°C) in order to inactivate interfering substances such as protease and beta-glycan. Plasma endotoxin activity was measured by the limulus amebocyte lysate kinetic turbidimetric assay (LAL-KTA). The mRNA expression level of GAPDH, TLR-4, NF-kB1, NF-kB2, IL-6, and STAT-3 in PMNs and the liver was measured by real-time PCR. GAPDH was used as an endogenous control. Data were expressed as the mean ± standard deviation. In diarrheic calves within the same group, mean values for each dependent variable were compared with the base value using one-way ANOVA followed by Dunnett’s test. The significance level was P<0.05.

Results

The endotoxin activity in the plasma was significantly increased, peaking (0.952 ± 1.00 EU/ml) at 30 min after LPS administration (P<0.001). Then, it decreased toward baseline levels at 4 hr after administration. Endotoxin activity and the expression levels of mRNA in the controls were not significantly altered from the baseline values. The expression of TLR-4 (2-4 hr), NF-kB2 (1-4 hr), and STAT-3 (2 hr) in PMNs, and TLR-4 (2-8 hr), NF-kB2 (2-4 hr), IL-6 (2 hr), and STAT-3 (4-8 hr) in the liver was markedly increased in the LPS-treated group (P<0.05).

Significance

We found that NF-kB2 and STAT-3 mRNA increased in PMNs and liver samples from calves that received LPS, but no significant changes in NF-kB1 in the PMNs or liver samples were observed. Therefore, in calves, the systemic inflammatory responses related to LPS induce NF-kB2 instead of NF-kB1. NF-kB and STAT-3 may act as transcriptional factors that lead to inflammation by LPS in calves; however, the manner in which NF-kB and STAT-3 interact and communicate with each other remains unclear.