Anti-lipopolysaccharide antibody mitigates ruminal lipopolysaccharide release without acute-phase inflammation or liver transcriptomic responses in Holstein bulls

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ABSTRACT

Anti-lipopolysaccharide (LPS) antibody administration has the potential benefits of neutralizing and consequently controlling rumen-derived LPS during subacute ruminal acidosis. Four Holstein bulls were used in this crossover study with a 2-week wash-out period. Anti-LPS antibody (0 or 4 g) was administered once daily for 14 days. Significantly lower ruminal LPS and higher 1-h mean ruminal pH were identified in the 4 g group. However, blood metabolites, acute-phase proteins, cytokines, and hepatic transcriptomes were not different between the two groups. Therefore, anti-LPS antibody administration mitigated ruminal LPS release and pH depression without accompanying responses in acute-phase inflammation or hepatic transcriptomic expression.

Keywords: Anti-lipopolysaccharide antibody; cattle; liver transcriptome; rumen fermentation; subacute ruminal acidosis

INTRODUCTION

The occurrence of ruminal acidosis or subacute ruminal acidosis (SARA) in cattle fed a high-grain diet causes higher acidity, resulting in higher lipopolysaccharide (LPS) activity in the rumen [1]. The increased ruminal LPS translocates to the bloodstream and can provoke inflammatory and acute-phase protein (APP) responses in cattle [2]. Furthermore, transcriptomic analysis of liver tissue revealed that the metabolic consequences of uncontrolled inflammation induced by LPS challenge can be particularly harmful during the early stages of lactation, when there is a marked degree of body fat mobilization [3]. Therefore, LPS neutralization and related roles of liver cells are important in cattle fed a high-grain diet.
Previously, in vitro (peptide-bound bead method) [4] and in vivo (mouse model) [5] studies using LPS-binding peptides were performed to neutralize LPS. However, there has been little research regarding anti-LPS antibody administration in cattle despite the potential benefits of neutralizing and consequently controlling rumen-derived LPS. Therefore, we investigated the effects of ruminal anti-LPS antibody administration on rumen fermentation and LPS activity, as well as hepatic transcriptomic adaptation during SARA challenge.

MATERIALS AND METHODS

Anti-LPS antibody preparation

Anti-LPS antibody was produced under patented and proprietary procedures (EW Nutrition Japan, Japan) as described previously [6]. Briefly, 1 mL antigen (1 × 10^9 CFU/g inactivated whole Escherichia coli O139) was injected intramuscularly into egg-laying hens (Hy-Line W36). Then, the egg yolk was separated to prepare the product, yielding 1 g of the product bound to 0.25 g purified LPS from E. coli O111. We determined the amount of anti-LPS antibody based on previously reported ruminal LPS concentrations (up to 5 μg/mL) in growing Holstein steers (body weight 330–380 kg) with a rumen volume of approximately 100 L [7].

Animals and experimental design

The experimental protocol was approved by the Iwate University Laboratory Animal Care and Use Committee (A2014534; Japan). Four rumen-fistulated Holstein bulls (5–6 months of age; 162 ± 9 kg) were used in a crossover study with a 2-week washout period. Cattle were fed a roughage diet (orchard and timothy mixed hay: 5.6–7.0 kg/day) during the first 11 days (days −11 to −1; pre-challenge), followed by a high-grain diet (50% concentrate and 50% soybean flakes: 3.0–3.6 and 3.0–3.8 kg/day, respectively) for 2 days (days 0 and 1; SARA challenge), and then a roughage diet for 1 day (day 2; post-challenge). The high-grain diet contained 80.5% total digestible nutrients, 15.7% crude protein, 12.8% acid detergent fiber, and 25.7% neutral detergent fiber.

The cattle were administered 0 (control group) or 4 g anti-LPS antibody per head once daily via the rumen fistula for 14 consecutive days. The diets were supplied daily at 800 and 1,630 h in 2 equal portions. The feed composition and amounts were based on the requirements of the Japanese Feeding Standard for Dairy Cattle.

Sampling and measurements

Ruminal pH was measured using a radio transmission system (YCOW-S; DKK-TOA, Japan) as described previously [8]. Rumen fluid and blood samples were collected at 800 and 1,400 h on days −1, 0, and 1 and at 800 h on day 2. Then, fluid and blood samples were prepared for further analysis as described previously [9].

The concentrations of total volatile fatty acids (VFAs), NH₃-N, lactic acid, and individual VFAs (acetic, propionic, and butyric acids) were determined [9]. Rumen LPS activity was measured by kinetic limulus amebocyte lysate assay (Pyrochrome with Glucashield; Seikagaku, Japan) [9]. For APP analyses, the plasma concentrations of LPS binding protein (LBP), haptoglobin (HP), and serum amyloid A (SAA) were measured using commercial kits [9]. Concentrations of plasma tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-6 (IL-6) were measured by sandwich enzyme-linked immunosorbent assay [9].
Transcriptome analysis of liver tissue
Liver tissue was biopsied at 800 h on day 2 in the 0 and 4 g groups. Preparation of RNA samples for microarray analysis and data processing were performed as described previously [10]. The entire microarray data set has been deposited in the Gene Expression Omnibus database with the following accession numbers: platform, GPL22091; samples, GSM 3901089 to GSM 3901115; series, GSE133152.

Statistical analysis
The normality of the distributions of variables was assessed using the Shapiro-Wilk test. The significance of differences among groups was evaluated using the unpaired t-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables (Prism ver. 8.10; GraphPad Software, USA). Mixed-model repeated-measures analysis of variance, using time as a fixed effect, followed by Dunnett’s multiple comparison method was performed to determine the significance of within-group differences. The microarray data were analyzed using the unpaired t-test with Benjamini-Hochberg false discovery rate (FDR) multiple testing correction (FDR corrected \( p < 0.10 \)) (GeneSpring 12.0; Agilent Technologies, USA). In all analyses, \( p < 0.05 \) was taken to indicate statistical significance.

RESULTS
Ruminal LPS, pH, VFAs, and blood metabolites
The ruminal LPS activity (0 g anti-LPS antibody) changed significantly \( (p < 0.05) \) during the SARA challenge. The ruminal LPS activity was significantly \( (p < 0.05) \) increased on day 2 (800 h) compared with day −1 (800 h). Significantly \( (p < 0.05) \) lower LPS activity was identified on day 2 (800 h) in the 4 g group compared with the 0 g group (Fig. 1).

![Fig. 1. Changes in ruminal LPS activity in Holstein bulls. Anti-LPS antibody was administered once daily at a dose of 0 or 4 g. Days −1, 0, 1, and 2 denote observations during the pre-challenge (day −1), subacute ruminal acidosis challenge (days 0 and 1; gray squares), and post-challenge (day 2) periods. Sampling times of day are shown as 8 (800 h) and 14 (1,400 h). Values represent means ± SE. *Significant difference between the 0 and 4 g groups at that time point \( (p < 0.05) \); †Significant changes during the SARA challenge period \( (p < 0.05) \). LPS, lipopolysaccharide.](https://vetsci.org)
The 1-h mean ruminal pH (0 and 4 g anti-LPS antibody) changed significantly \((p < 0.05)\) during the SARA challenge. The 1-h mean ruminal pH was significantly \((p < 0.05)\) higher on days 0 (1,300 h) and 1 (300–700 h and 900–1,100 h) in the 4 g compared with 0 g group (Fig. 2).

During the SARA challenge, the total VFA concentration (4 g group) was significantly \((p < 0.05)\) increased on days 1 (1,400 h) and 2 (800 h) compared with day −1 (800 h) (Fig. 3). However, no significant change in the \(\text{NH}_3\)-N or lactic acid concentration was detected during the SARA challenge period.

The levels of peripheral blood APPs (LBP, HP, and SAA) were increased in the 0 g group, compared with the 4 g group, during the later part of the SARA challenge period (Fig. 3). The LBP and SAA concentrations in the 0 g group increased significantly \((p < 0.05)\) during the SARA challenge. However, no significant changes in the levels of HP or cytokines (TNF-\(\alpha\), IFN-\(\gamma\), and IL-6) were detected during the SARA challenge period.

**Microarray analysis of liver tissue**

There were no significantly differentially expressed genes (FDR corrected \(p < 0.10\)), including genes encoding APPs and cytokines, between the 0 and 4 g groups.

**DISCUSSION**

In the present study, ruminal LPS release and pH depression were alleviated by administration of anti-LPS antibody during SARA challenge. It is plausible that the anti-LPS antibody,
showing high binding affinity to LPS, may affect living gram-negative bacteria, consistent with other studies using avian-derived polyclonal antibodies against *Fusobacterium necrophorum* and *Streptococcus bovis* in crossbred steers [11] and anti-LPS-enriched colostrum in a mouse model [5]. However, the present study verified the selective suppression of ruminal LPS activity, without significant changes in the rumen fermentation or blood metabolite profiles.

![Graphs showing changes in rumen fermentation parameters, peripheral blood APPs, and cytokines.](https://vetsci.org)

Fig. 3. Changes in the levels of rumen fermentation parameters, peripheral blood APPs, and cytokines. The levels of rumen fermentation parameters (total VFAs, NH₃-N, and lactic acid), peripheral blood APPs (LBP, HP, SAA), and cytokines (TNF-α, IFN-γ, IL-6) were determined in the groups administered 0 and 4 g of anti-lipopolysaccharide antibody. Days −1, 0, 1, and 2 denote observations during the pre-challenge (day −1), subacute ruminal acidosis challenge (days 0 and 1; gray squares), and post-challenge (day 2) periods. Sampling times of day are shown as 8 (800 h) and 14 (1,400 h). Values represent means ± SE.

VFA, volatile fatty acid; LBP, lipopolysaccharide binding protein; HP, haptoglobin; SAA, serum amyloid A; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; IL-6, interleukin-6; APP, acute-phase protein.

*Significant changes during the SARA challenge period (p < 0.05).
Once rumen-derived LPS enters the circulation, it activates Kupffer cells to release proinflammatory cytokines, such as TNF-α, IFN-γ, and IL-6, into the systemic circulation and triggers the secretion of APPs, such as LBP, HP, and SAA [12,13]. However, oral administration of anti-LPS-enriched colostrum alleviated immune-mediated colitis by lessening bowel inflammation in a mouse model, suggesting that the gut microbiome may serve as a target for regulatory T-cell-based immunotherapy [5]. In the present study, we found significant changes in the LBP and SAA concentrations only in the 0 g group. Furthermore, gradual increases in LBP, HP, and SAA concentrations during the later parts of the SARA challenge period were observed in the 0 g compared with 4 g group, suggesting the need for longer-term observations. Therefore, we postulated that the significantly lower ruminal LPS levels on day 2 in the administration groups may play a suppressive role in the acute-phase inflammatory responses of the peripheral blood in comparison with the 0 g group during SARA challenge.

In the present study, no differences in hepatic gene expression were detected between the 0 and 4 g groups although the ruminal LPS activity was significantly lower in the 4 g group. This was consistent with previous reports showing no significant effects of a single and mild episode of SARA on the ruminal epithelial barrier function immediately after the episode [14] and with the lack of significant differences in peripheral blood hepatic enzyme levels (aspartate aminotransferase and γ-glutamyltransferase) in the present study. That is, ruminal LPS release by SARA challenge may have a limited effect on hepatic responsiveness, likely due to relatively low LPS activity compared with a previous study (36.0 endotoxin units [EU] × 10^3/mL on day 2 in the present study vs. 47.17 and 79.04 EU × 10^3/mL in low- and high-grain-fed dairy cows, respectively [15]). Furthermore, the present study was performed over a short period to evaluate liver adaptation to both SARA challenge and anti-LPS antibody administration. Therefore, further studies are required to verify the effects of anti-LPS antibody administration on alterations in the hepatic transcriptome using longer-term challenge models.

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