Toll-Like Receptor Signaling in Bovine Macrophages Increases 1,25-Dihydroxyvitamin D_3 Production

A.S. Leaflet R2276

Corwin Nelson, graduate research assistant, BBMB;
Donald Beitz, distinguished professor of Animal Science,
Tim Reinhardt, research animal scientist, National Animal Disease Center USDA/ARS;
John Lippolis, research molecular biologist, National Animal Disease Center USDA/ARS

Summary and Implications

Recognition of pathogen associated molecular patterns by Toll-like receptors (TLR) increases 1α-hydroxylase expression in bovine macrophages. This results in increased conversion of 25-hydroxyvitamin D_3 ($25(OH)D_3$) to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) in bovine macrophages. Production of $1,25(OH)_2D_3$ in bovine macrophages indicates that $1,25(OH)_2D_3$ may be involved in modulating the bovine immune response.

Introduction

Toll-like Receptor Signaling

Macrophages are members of the innate immune system and are able to recognize invading pathogens and initiate an immune response. Recognition of pathogen associated molecular patterns can occur by TLR present on macrophages and can activate transcription of numerous genes that are involved in host defense.

Vitamin D

Vitamin D_3 can be acquired in the skin by radiation from UVB light or in the diet and is readily converted to $25(OH)D_3$ in the liver. The substrate for 1α-hydroxylase is $25(OH)D_3$, which is converted to the active steroid hormone, $1,25(OH)_2D_3$. Meanwhile, the vitamin D receptor (VDR) is activated upon binding of the active hormone. Activated VDR functions as a transcription factor by binding vitamin D response elements (VDRE) in promoters of vitamin D responsive genes.

Materials and Methods

Macrophage Cultures

Bovine mononuclear cells were isolated from peripheral blood from four lactating Holstein cows. The mononuclear cell fraction was cultured for two hours in tissue culture flasks. After two hours non-adherent cells were removed. Adherent cells were cultured in RPMI 1640 with 10% fetal bovine serum for seven days to obtain bovine monocyte derived macrophages (BMDM). BMDM were stimulated with 100 ng/ml tripalmitoylated lipopeptide (Pam3CSK4), a TLR2/1 ligand, or 100 ng/ml lipopolysaccharide (LPS), a TLR4 ligand.

mRNA Quantification

RNA from stimulated BMDM was isolated and genomic DNA was removed with DNase. DNA free RNA was reverse transcribed to cDNA and quantitative real-time PCR using the $2^{-\Delta\Delta Ct}$ method was used to measure relative abundance of 1α-hydroxylase and 24-hydroxylase cDNA. Ribosomal Protein S9 (RPS9) was used as the reference gene.

Conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$

BMDM cultures were supplemented with 50 ng/ml $25(OH)D_3$ and stimulated with 100 ng/ml LPS and 30 ng/ml bovine interferon gamma (IFNγ) for 36 hours. Expression of 24-hydroxylase mRNA was used as a measure of $1,25(OH)_2D_3$ production in BMDM. The 24-hydroxylase promoter has a VDRE and transcription of 24-hydroxylase is up-regulated in the presence of $1,25(OH)_2D_3$.

Results and Discussion

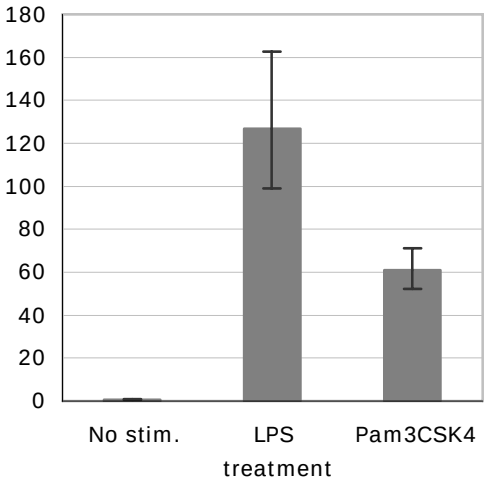

Figure 1. Expression of 1α-hydroxylase mRNA relative to RPS9 in BMDM after 8 hours of stimulation.

Stimulation of BMDM with Pam3CSK4 or LPS for eight hours increased expression of 1α-hydroxylase (Figure 1). Subsequent conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ in BMDM stimulated with LPS and supplemented with $25(OH)D_3$ was demonstrated by an increase in 24-hydroxylase expression (Figure 2).

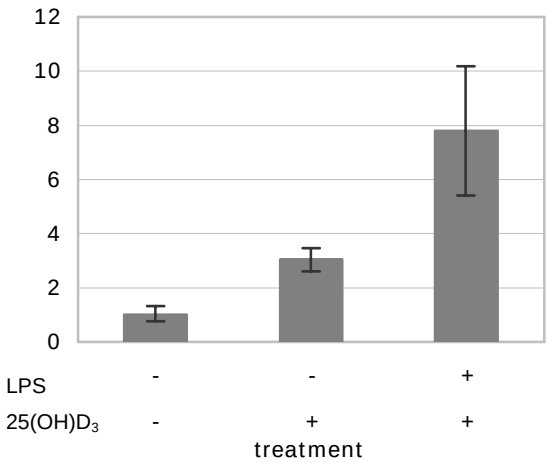

Figure 2. Expression of 24-hydroxylase mRNA relative to RPS9 in BMDM after 36 hours of stimulation.

Production of $1,25(OH)_2D_3$ upon TLR signaling demonstrates that an autocrine signaling pathway of vitamin D occurs in bovine macrophages. This observation is in contrast to the well known endocrine pathway where $1,25(OH)_2D_3$ is produced in the kidney and is involved in regulating plasma calcium homeostasis. However, $1,25(OH)_2D_3$ that is produced in bovine macrophages may be responsible for up-regulating factors involved in host defense mechanisms. TLR signaling in human macrophages and epithelial cells causes a similar increase in 1α-hydroxylase expression and production of $1,25(OH)_2D_3$. In humans $1,25(OH)_2D_3$ has been shown to increase expression of cathelicidin antimicrobial peptide (CAMP). The increased expression of CAMP was shown to enhance the antimicrobial activity of human macrophages. However, none of the bovine cathelicidin genes seem to be regulated in the same manner. Therefore, microarray and proteomic analysis will need to be done to determine which genes are regulated by $1,25(OH)_2D_3$ in bovine macrophages. Futhermore, studies will be done to determine if $1,25(OH)_2D_3$ enhances the antimicrobial activity of bovine macrophages.