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1,25-Dihydroxyvitamin D_3 Enhances Bovine Mammary Epithelial Innate Immune Responses

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Summary and Implications

Bovine mammary epithelial cells that were treated with bacterial lipopolysaccharide and 1,25-dihydroxyvitamin D_3 showed an increase in the expression of the genes for inducible nitric oxide synthase (iNOS) and S100 calcium binding protein A12 (S100 A12). iNOS and S100 A12 are part of the innate immune response and expressed in the mammary gland during mastitis. Production of 1,25-dihydroxyvitamin D_3 in the mammary gland during mastitis, then, may be an important component of the innate immune response.

Introduction

The active form of vitamin D_3 is 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$). 1,25$(OH)_2D_3$ binds and activates the vitamin D receptor (VDR), a nuclear hormone receptor that regulates the expression of genes that contain a vitamin D response element in their promoter. 1,25$(OH)_2D_3$ is produced from 25-hydroxyvitamin D_3 (25$(OH)D_3$), the predominant circulating form of vitamin D_3, by the enzyme 1α-hydroxylase (1α-OHase). We have recently shown that bovine monocytes express 1α-OHase in response to toll-like receptor (TLR) recognition of bacteria. Production of 1,25$(OH)_2D_3$ by 1α-OHase in activated bovine monocytes increased production of nitric oxide and expression of the chemokine RANTES in monocytes. We also have observed that 1α-OHase is expressed in inflamed mammary tissue during mastitis. Mammary epithelial cells are involved in protecting the mammary gland from bacterial infection and production of 1,25$(OH)_2D_3$ by 1α-OHase in the mammary gland may affect their innate immune function. The objective of this experiment was to determine the effects of 1,25$(OH)_2D_3$ and lipopolysaccharide (LPS) on iNOS, RANTES, and S100 calcium binding protein A12 (S100 A12) gene expression in bovine mammary epithelial cells (MEC).

Materials and Methods

MEC were derived from mammary tissue biopsies of three Holstein cows in mid-lactation. The cells were cultured in RPMI 1640 plus 10% fetal bovine serum and mammary epithelial growth supplement (Invitrogen). Prior to treatment, MEC were transferred to 96-well tissue culture treated plates and cultured until they were confluent. MEC were treated with 0 or 1 μg/mL LPS along with 0, 0.1, 1 or 10 nM 1,25$(OH)_2D_3$ for 16 hours. After treatment, total RNA was isolated from MEC and reverse transcribed to cDNA. Quantitative real-time PCR using the $2^{-\Delta\Delta Ct}$ method was used to measure relative abundance of 24-hydroxylase (24-OHase), iNOS, RANTES and S100 A12. Ribosomal protein S9 (RPS9) was used as the reference gene.

Results and Discussion

Treatment of MEC with 1,25$(OH)_2D_3$ caused an increase in 24-OHase gene expression in a dose dependent manner in both the presence and absence of LPS (Figure 1A). The 24-OHase enzyme adds a hydroxyl group to the 24 position of vitamin D metabolites, which inactivates 1,25$(OH)_2D_3$. In bovine monocytes, induction of 24-OHase expression by 1,25$(OH)_2D_3$ was repressed by LPS treatment, so regulation of 24-OHase expression differs between monocytes and MEC.

In MEC treated with LPS, both iNOS and S100 A12 gene expression increased with 1,25$(OH)_2D_3$ dose (Figure 1B and C). Increased expression of iNOS resulted in increased nitric oxide production by MEC (not shown). Nitric oxide may increase antimicrobial activity and blood flow in the mammary gland during mastitis, but it also may lead to MEC apoptosis. The function of S100 A12 is not completely understood, but it has been shown to have chemotactic and antimicrobial properties.

RANTES expression in MEC did not increase with 1,25$(OH)_2D_3$ treatment (Figure 1D). Treatment with LPS, however, caused an increase in MEC RANTES expression. In contrast, RANTES expression was up-regulated by 1,25$(OH)_2D_3$ treatment in LPS-stimulated bovine monocytes, but was not affected by LPS treatment alone.

In conclusion, 1,25$(OH)_2D_3$ acts in bovine MEC to enhance expression of iNOS and S100 A12 that is induced by TLR recognition of LPS. Therefore, production of 1,25$(OH)_2D_3$ by 1α-OHase in the mammary gland during mastitis may affect the host defense capabilities of MEC. Production of 1,25$(OH)_2D_3$ by 1α-OHase is dependent on availability of 25$(OH)D_3$. The circulating concentration of 25$(OH)D_3$ is dependent on acquisition of vitamin D_3 in the diet or in skin exposed to sunlight. So, dietary intake of vitamin D_3 and exposure to sun may affect incidence and severity of mastitis in cattle.

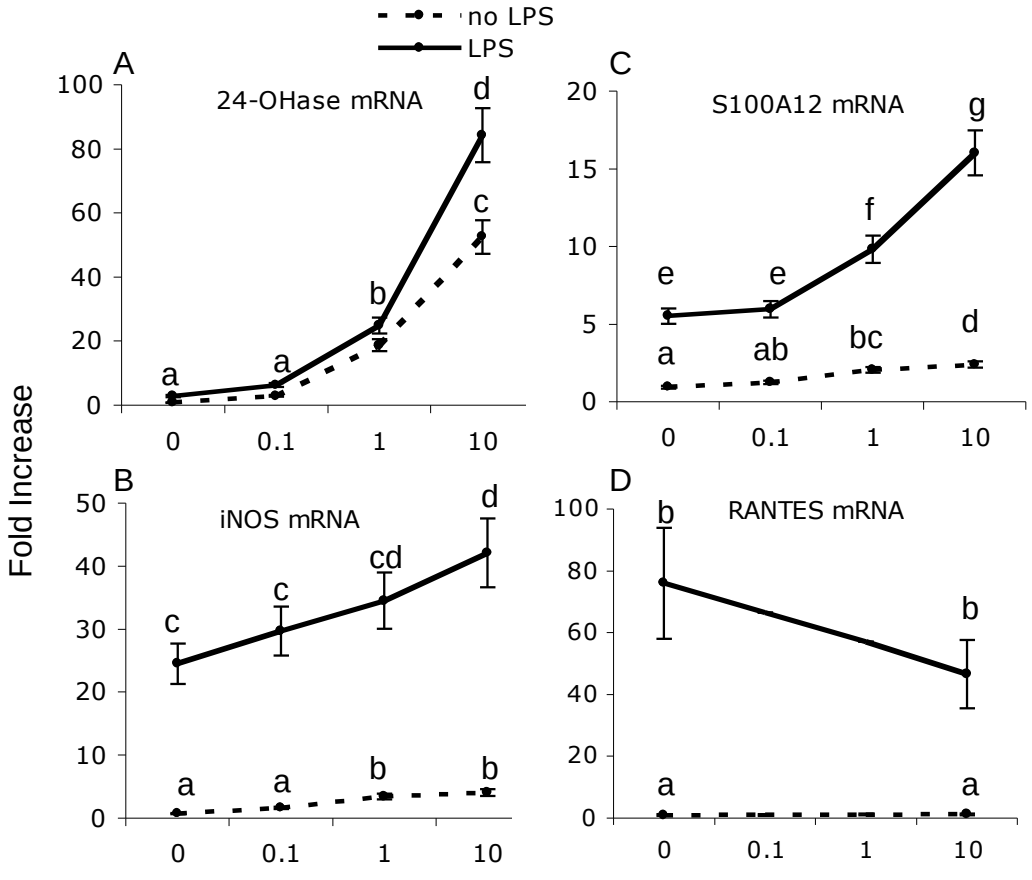

Figure 1. Mammary epithelial cell cultures were treated with 0 or 1 μg/mL LPS and 0 or 10 nM 1,25-dihydroxyvitamin D_3 for 16 hours. The amount of 24-hydroxylase (24-OHase; **A**), inducible nitric oxide synthase (iNOS; **B**), S100 calcium binding protein A12 (S100 A12; **C**), and RANTES (**D**) mRNA in MEC was measured by quantitative PCR. The expression of each gene was normalized to ribosomal protein S9 expression. The fold increase is relative to the control treatment. Results are the average of three separate experiments done in duplicate. Cells were from different cows for each experiment. Error bars represent standard error. Means with different letters are different ($P < 0.05$).