Regulation of Immune Responses to *Mycobacteria bovis* by a Paracrine Mechanism of Vitamin D Signaling in Cattle

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**Summary and Implications**

We provide evidence that T-cell responses to *Mycobacteria bovis* are suppressed by the production of 1,25-dihydroxyvitamin D₃ in monocytes and B-cells from cattle. Current vitamin D requirements for cattle are solely based on the classical endocrine mechanism of vitamin D signaling that regulates calcium homeostasis and should be re-evaluated to account for vitamin D signaling mechanisms in the immune system.

**Introduction**

The vitamin D₃ hormone, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) is a known modulator of immune responses. The enzyme 1α-hydroxylase (1α-OHase) synthesizes 1,25(OH)₂D₃ from 25-hydroxyvitamin D₃ (25(OH)D₃), the predominant circulating form of vitamin D₃. In the classical endocrine pathway of vitamin D metabolism, 1,25(OH)₂D₃ production by 1α-OHase is regulated in the kidneys in response to calcium homeostasis. In contrast, 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)₂D₃ by 1α-OHase in activated bovine monocytes increases production of the immune modulator, nitric oxide, and expression of the chemokine RANTES in monocytes. Previous studies also have shown that exogenous 1,25(OH)₂D₃ suppresses pro-inflammatory interferon-γ (IFN-γ) and interleukin 17 (IL-17) responses of helper T-cells. The objectives of this experiment were to evaluate the expression of 1α-OHase in monocytes, T-cells, and B-cells, and determine if immune cell 1,25(OH)₂D₃ synthesis could regulate T-cell IFN-γ, IL-17A and IL-17F responses to *M. bovis*.

**Materials and Methods**

Peripheral blood mononuclear cells (PBMCs) were collected from 12 Holstein bull calves. Eight of the calves were previously vaccinated with *M. bovis* Bacillus Calmette-Guerin (BCG) and the other four served as the non-vaccinated controls. The PBMCs were treated with 10 μg of *M. bovis* purified protein derivative (*M. bovis* PPD) and 0 or 100 ng/mL of 25(OH)D₃ in cell culture media for 24 hrs. The stimulated PBMCs were sorted by using fluorescence activated cell sorting (FACS) according to surface expression for CD3 (T-cells), CD14 (monocytes), and IgM (B-cells). Relative expression of 1α-OHase, IFN-γ, IL-17A, and IL-17F mRNA was determined by using RT-qPCR. Ribosomal protein S9 was used as the reference gene to normalize the content of mRNA in each sample. The reported expression of each gene is relative to non-stimulated PBMCs. ANOVA was performed using SAS with a model that included effects for treatment and vaccination status. Multiple comparison tests of the means were made using the Tukey-Kramer adjustment.

**Results**

Stimulation of PBMCs from BCG-vaccinated calves with *M. bovis* PPD induced 1α-OHase expression in monocytes (CD14⁺ cells) and B-cells (IgM⁺ cells) but not in T-cells (CD3⁺ cells) (Figure 1). However, in the stimulated PBMCs from the non-vaccinated calves 1α-OHase was only induced in the monocytes, indicating that induction of 1α-OHase in monocytes occurs by innate recognition of *M. bovis* but induction in B-cells occurs by antigen-specific recognition of *M. bovis*.

![Figure 1](image-url)  
**Figure 1.** PBMCs from bull calves either vaccinated with *M. bovis* BCG (*n* = 7) or not vaccinated (*n* = 4) were stimulated for 24 hrs with *M. bovis* purified protein derivative. Stimulated PBMCs were sorted by using FACS according to surface expression of CD3 (T-cells), IgM (B-cells), or CD14 (monocytes). Gene expression was measured by using RT-qPCR. The amount of 1α-OHase mRNA in each sample was normalized to ribosomal protein S9 mRNA. The expression of 1α-OHase is relative to un-stimulated PBMCs. Means with different letters are different; *P* < 0.001.
Addition of 25(OH)D3 to *M. bovis* PPD-stimulated PBMCs from the vaccinated calves suppressed IFN-γ and IL-17F responses in T-cells. Antigen-specificity of the IFN-γ and IL-17F responses by T-cells from the vaccinated calves was indicated by the lack of response in T-cells from non-vaccinated calves.

**Discussion**

Previously, 1,25(OH)2D3 has been shown to suppress IFN-γ production by antigen-stimulated PBMCs. The ability of 25(OH)D3 treatment to suppress antigen-specific T-cell responses indicates that 1,25(OH)2D3 was being produced in the PBMC cultures. Because monocytes and B-cells were the cells that expressed 1α-OHase, we propose that synthesis of 1,25(OH)2D3 in monocytes and B-cells suppress T-cell responses through a paracrine mechanism of vitamin D signaling. The paracrine mechanism of vitamin D signaling, in contrast to the classical endocrine mechanism that regulates 1,25(OH)2D3 synthesis in the kidneys, enables local control of vitamin D responsive genes.

The existence of intracrine and paracrine vitamin D signaling mechanisms employed by the bovine immune systems has implications for vitamin D nutrition. Production of 1,25(OH)2D3 by 1α-OHase in immune cells, as demonstrated here, is dependent on the availability of 25(OH)D3. The circulating concentration of 25(OH)D3 is dependent on acquisition of vitamin D3 in the diet or in skin exposed to sunlight. Current recommendations for supplementation of vitamin D3 in the diets of cattle are based on vitamin D metabolism by the endocrine system. Those recommendations are aimed to maintain circulating concentrations of 25(OH)D3 at 20 - 50 ng/mL. Recent human epidemiology studies indicate that circulating 25(OH)D3 concentrations below 30 ng/mL may result in increased risk for certain infectious diseases such as tuberculosis. Therefore, vitamin D requirements for cattle may need to be re-examined to account for vitamin D signaling mechanisms in the immune system.