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 D_3 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 D_3 hormone,1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is a known modulator of immune responses. The enzyme 1α-hydroxylase (1α-OHase) synthesizes $1,25(OH)_2D_3$ from 25-hydroxyvitamin D_3 ($25(OH)D_3$), the predominant circulating form of vitamin D_3. In the classical endocrine pathway of vitamin D metabolism, $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $25(OH)D_3$ 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.*

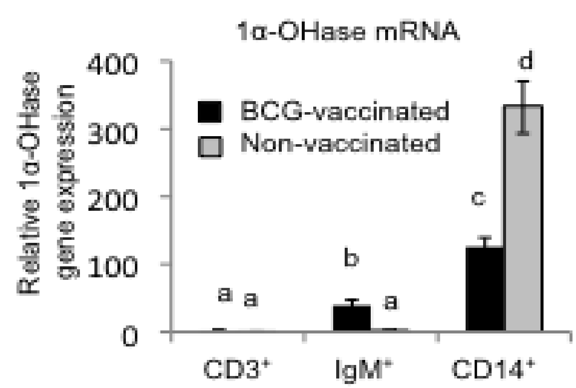

Figure 1. PBMCs from bull calves either vaccinated with *M. bovis* BCG (n = 7) or not vaccinated (n = 4) were stimulated for 24 hrs with *M. bovis* purified protein derivative. Stimulated PBMCs were sorted by using FACS according to surface expression of CD3 (T-cells), IgM (B-cells), or CD14 (monocytes). 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.

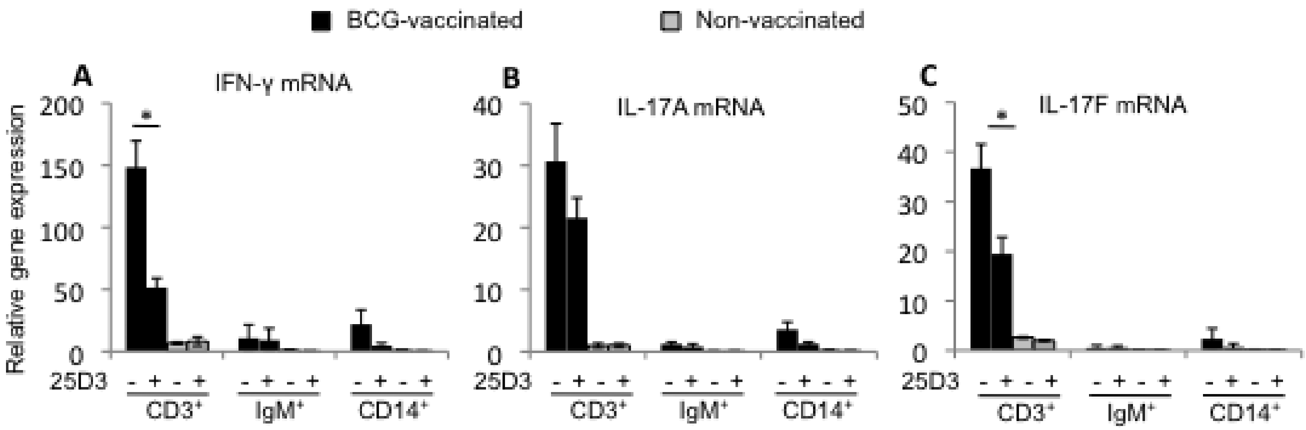

Figure 2. PBMCs from bull calves either vaccinated with *M. bovis* BCG (n = 8) or not vaccinated (n = 4) were stimulated for 24 hrs with *M. bovis* purified protein derivative. Stimulated PBMCs were sorted by using FACS according to surface expression of CD3 (T-cells), IgM (B-cells), or CD14 (monocytes). Gene expression was measured by using RT-qPCR. The amount of each gene in each sample was normalized to ribosomal protein S9 mRNA. The expression of each gene is relative to the expression of that gene in un-stimulated PBMCs. * Means are different; *P* < 0.05.

Addition of 25(OH)D_3 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$(OH)_2D_3$ has been shown to suppress IFN-γ production by antigen-stimulated PBMCs. The ability of 25(OH)D_3 treatment to suppress antigen-specific T-cell responses indicates that 1,25$(OH)_2D_3$ 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$(OH)_2D_3$ 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$(OH)_2D_3$ 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$(OH)_2D_3$ by 1α-OHase in immune cells, as demonstrated here, is dependent on the 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. Current recommendations for supplementation of vitamin D_3 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)D_3 at 20 - 50 ng/mL. Recent human epidemiology studies indicate that circulating 25(OH)D_3 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.