Modulation of Cytokine Gene Expression and Secretion during the Periparturient Period in Dairy Cows Naturally Infected with *Mycobacterium avium* subsp. *paratuberculosis*

A.S. Leaflet R2198

Elizabeth Karcher, graduate research assistant;
Donald C. Beitz, professor of animal science and biochemistry;
Judy Stabel, lead scientist Johne's project, NADC-USDA

Summary and Implications

Twenty multiparous and two primiparous Holstein cows were grouped according to infection status with *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative microorganism for Johne's disease. The effect of parturition and infection on the progression of Johne's disease was monitored by determining the expression and secretion of key cytokines. Despite the ability of MAP and parturition to modulate cytokine gene expression, the transition from subclinical to clinical disease state did not occur during the immediate postpartum period in this study.

Introduction

Johne's disease (JD), caused by MAP, is estimated to infect more than 22% of US dairy herds and cost the US dairy industry in excess of $200 million annually. Although it is generally accepted that the stress of parturition may cause infected cows to advance to the clinical stage during the weeks following calving, research on parturition in the progression of the disease during this time period is lacking. The transition from a subclinical, or asymptomatic, to a clinical stage of infection is characterized by a shift from cell-mediated (Th_1) response to a non-protective antibody-mediated (Th_2) response. Data are needed to better understand the progression of JD during the physiologically stressful period surrounding parturition. Based on this need and the known ability of cytokines to regulate the pathogenesis of JD, the objective of this study was to characterize cytokine expression and secretion in periparturient dairy cows naturally infected with MAP. This is the first comprehensive study to evaluate cytokine expression during the periparturient period in dairy cows and to evaluate the effects of MAP infection on response during the periparturient period.

Materials and Methods

Twenty multiparous and 2 primiparous Holstein cows were grouped according to infection status. Three groups consisted of 5 noninfected healthy controls; 12 cows naturally infected with MAP, but asymptomatic; and 5 naturally infected cows with clinical JD. Animals were categorized by historical monitoring for fecal shedding of bacteria, gamma interferon (IFN-γ) expression, and antibody titer. Blood was collected from the jugular vein at -21, -14, -7, +1, +7, +14, +21, and +28 days relative to calving. Peripheral blood mononuclear cells (PBMC) were isolated and cultured in RPMI for 24 hours at 39°C, under 5% CO_2, and in 75-cm^2 flasks. Cells were not stimulated (NS) or stimulated with concanavalin A (ConA). RNA was extracted using TriZol reagent, purified using the RNeasy® Mini Kit protocol for RNA cleanup, and converted into first strand cDNA. Real time-PCR then was performed using the Applied Systems 7500 DNA sequence detection system to evaluate the expression of IFN-γ, tumor necrosis factor alpha (TNF-α), interleukin (IL)-12, transforming growth factor beta (TGF-β), IL-4, and IL-10. Cytokine secretion of IFN-γ, IL-10, and TGF-β was determined by enzyme-linked immunosorbent assay. Data were analyzed using the PROC Mixed analysis of SAS. Means differed if $P < 0.05$ and tended to differ if $0.05 \leq P \leq 0.15$.

Results and Discussion

We first examined the Th_1 cytokines IFN-γ, TNF-α, and IL-12. These cytokines are responsible for the initial response to invading MAP and have been shown to enhance the granulomas associated with the disease. Non-stimulated PBMCs isolated from subclinical animals expressed greater IFN-γ mRNA compared with control ($P < 0.05$) and clinical ($P < 0.12$) animals. Subclinical cows also tended to have decreased IFN-γ at calving (Figure 1). Expression of TNF-α was not affected by MAP infection or by parturition (data not shown). Interleukin-12 mRNA expression was greater in infected animals (subclinical and clinical combined) compared to the controls ($P < 0.05$) for both NS- and ConA-stimulated PBMCs. Infected cows tended to have decreased IL-12 at calving and during the post-partum period (Figure 2).

We were also interested in the Th_2 cytokines, IL-4 and IL-10, and TGF-β, a T_{reg} cytokine. These cytokines are considered to be suppressive and anti-inflammatory. They are capable of inhibiting Th_1 cytokines and suppressing T cell functions. When PBMCs were stimulated with ConA, subclinical cows expressed higher amounts of IL-4 compared with controls ($P < 0.01$) and clinical ($P < 0.08$) cows (Figure 3). An overall effect of calving was not observed; however, IL-4 expression in infected animals declined as parturition approached. Stimulating the PBMCs with ConA resulted in IL-10 being expressed at greater amounts in infected animals compared with that in healthy controls (Figure 4). Finally, TGF-β mRNA expression was not affected by MAP infection. However, for all animals,

there was a trend for increased expression at calving and then a decline in the first 3 weeks following calving (Figure 5).

Finally, we wanted to observe the amount of cytokines secreted by the PBMCs in a 24-hour period. When stimulated with ConA, IFN-γ secretion tended to be higher in subclinical cows compared to control (P <0.15) and clinical (P <0.10) cows (Figure 6). Secretion of IL-10 for both NS- and ConA-stimulated PBMCs did not seem to be affected by MAP infection or parturition (data not shown). Although not statistically significant, TGF-β secretion was numerically higher for clinical cows compared with control and subclinical animals from parturition to +14 days after calving (Figure 7). By the end of the study, not one of the 12 subclinically infected cows transitioned to the clinical stages of JD.

In conclusion, infection with MAP resulted in increased mRNA expression of IFN-γ, IL-12, IL-10, and IL-4 in infected cows. The expression of these four cytokines declined at parturition. TGF-β was the only cytokine observed that had increased mRNA expression at calving. These results indicate an ability of MAP and parturition to modulate cytokine expression in the dairy cow. However, the transition from subclinical to clinical state did not occur in any animals in this study Future studies are needed to evaluate animals that transition from subclinical to clinical near or at parturition as well as characterize cytokine gene expression as lactation progresses.

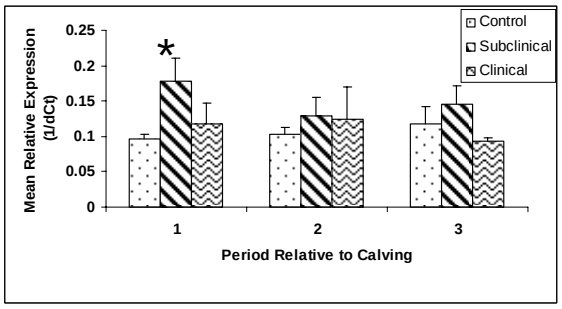

Figure 1. Interferon gamma mRNA expression of non-stimulated PBMCs isolated from the whole blood of control and infected periparturient cows naturally infected with MAP. Data are least square means and standard errors. Period 1: -21 and -14 days; Period 2: -7, +1, and +7 days; Period 3: +14, +21, and +28 days. Subclinical cows expressed IFN-γ compared with control (P <0.05) and clinical (P <0.12) cows. There was not an effect of parturition.

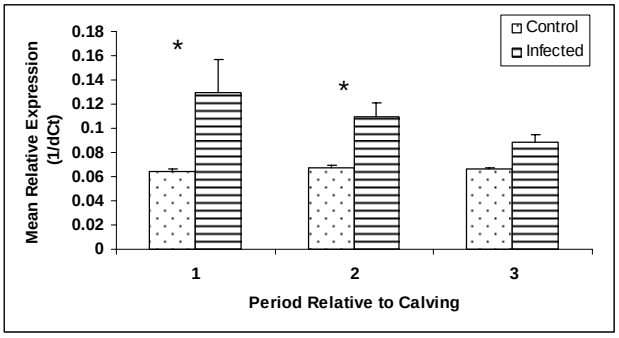

Figure 2. Interleukin 12 mRNA expression of ConA-stimulated PBMCs isolated from the whole blood of control and infected periparturient cows naturally infected with MAP. Data are least square means and standard errors. Period 1: -21 and -14 days; Period 2: -7, +1, and +7 days; Period 3: +14, +21, and +28 days. Infected cows expressed greater amounts than did controls (P <0.02). There was not an effect of parturition.

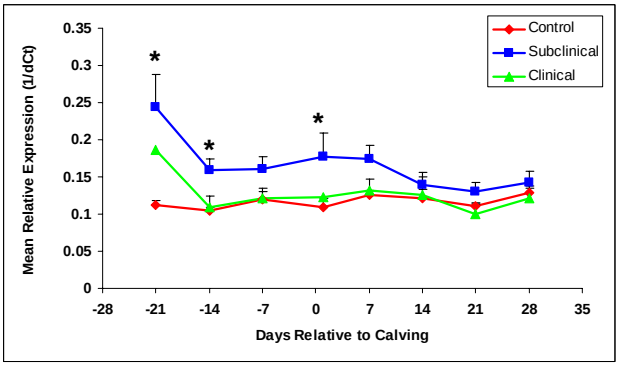

Figure 3. Interleukin 4 mRNA expression of ConA-stimulated PBMCs isolated from the whole blood of control and infected periparturient cows naturally infected with MAP. Data are least square means and standard errors. There was an overall effect of infection status (P <0.01). Subclinical cows expressed greater IL-4 compared with that in control (P <0.01) and clinical (P <0.08) cows. There was not an effect of parturition.

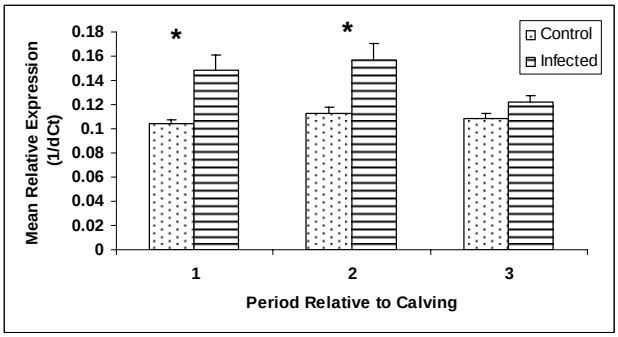

Figure 4. Interleukin 10 mRNA expression of ConA-stimulated PBMCs isolated from the whole blood of control and infected periparturient cows naturally infected with MAP. Data are least square means and standard errors. Period 1: -21 and -14 days; Period 2: -7, +1, and +7 days; Period 3: +14, +21, and +28 days. Infected cows had greater IL-10 expression compared with controls (P <0.02). There was not an effect of parturition.

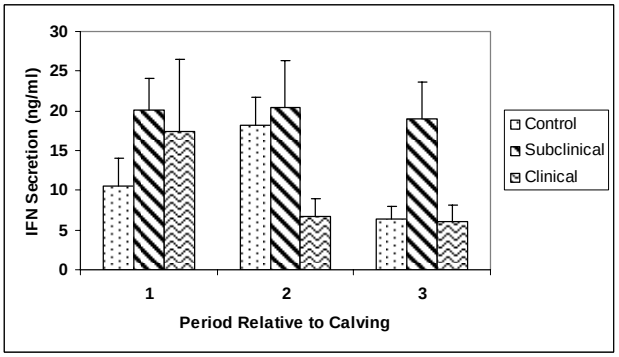

Figure 6. Secretion of gamma interferon from ConA-stimulated PBMCs isolated from the whole blood of control and infected periparturient cows naturally infected with MAP. Data are least square means and standard errors. Period 1: -21 and -14 days; Period 2: -7, +1, and +7 days; Period 3: +14, +21, and +28 days. Subclinical cows tended to have increased secretion compared with control (P <0.15) and clinical (P <0.10) cows. There was not an effect of parturition.

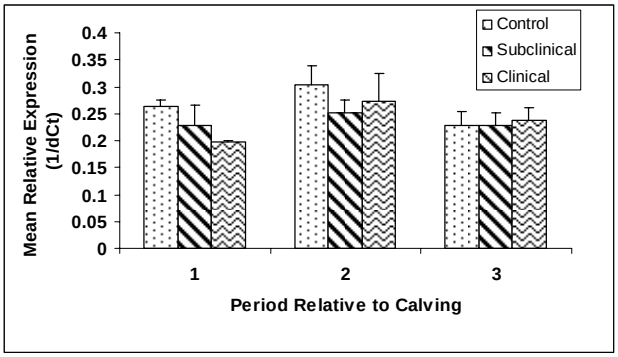

Figure 5. Transforming growth factor beta mRNA expression of ConA-stimulated PBMCs isolated from the whole blood of control and infected periparturient cows naturally infected with MAP. Data are least square means and standard errors. Period 1: -21 and -14 days; Period 2: -7, +1, and +7 days; Period 3: +14, +21, and +28 days. There was no effect of MAP infection on expression. Overall, cows had greater expression during period 2 compared with period 1 (P <0.08) and period 3 (P <0.07).

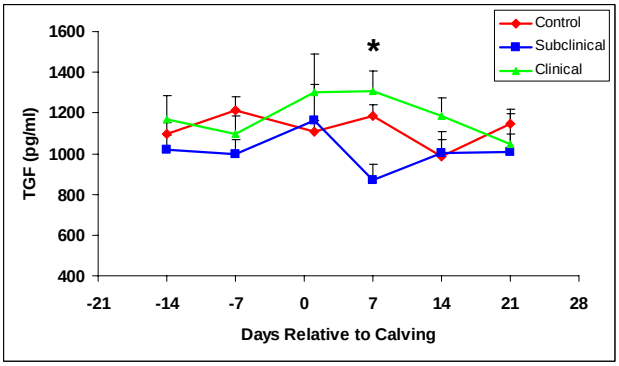

Figure 7. Secretion of transforming growth factor beta from non-stimulated PBMCs isolated from the whole blood of control and infected periparturient cows naturally infected with MAP. Data are least square means and standard errors. There was no effect of MAP infection or parturition. However, clinical cows secreted more TGF-β numerically compared with control and subclinical cows.