

Parturition Invokes Changes in Peripheral Blood Mononuclear Cell Populations in Holstein Dairy Cows Naturally Infected with *Mycobacterium Avium* Subsp. *Paratuberculosis*

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Summary and Implications

Twenty-one 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 (JD). The effect of parturition and infection on the percentages of CD4⁺, CD8⁺, and T-cells, B-cells, and monocytes in the peripheral blood were monitored. The data suggest that changes in the percentages of lymphocyte subsets and monocytes are modulated by both infection status and the periparturient period.

Introduction

Johne's disease, 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. In general, dairy cows will become infected with MAP as neonates through the fecal-oral transmission. Once infected, cows may remain in the subclinical, or asymptomatic, stage of the disease for several years. Stressors, such as parturition, may induce the transition from subclinical to clinical stage of the disease. Parturition has a major impact on the numbers of T- and B- cells, both components of the adaptive immune system, and the number of monocytes/macrophages, effectors of the innate immune system, in the peripheral blood of healthy dairy cows. To date, limited data are available characterizing detailed aspects of periparturient immunosuppression in the dairy cow. Further, it is not clear what impact the periparturient period and its associated stressors may have on host immunity in dairy cows with paratuberculosis.

Materials and Methods

Twenty-one multiparous and two primiparous Holstein cows were grouped according to infection status. The three groups consisted of 5 noninfected healthy controls; 14 cows naturally infected with MAP, but asymptomatic; and 4 naturally infected cows with clinical JD. Animals were categorized by historical monitoring for fecal shedding of bacteria, gamma interferon (IFN- γ) expression, and MAP 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 50 μ l of cell suspension was added to 50 μ l of primary monoclonal antibody to CD4⁺, CD8⁺, $\gamma\delta$ T-cells, B-cells, and monocytes. After a 30 min incubation at 4°C, plates were centrifuged at 400 x g for 2 min and the supernatant decanted. Secondary antibody cocktail was added and the cells were evaluated using flow cytometry. Data was analyzed using 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

CD4⁺ T-cells, also known as T-helper cells, play an active role in initiating both the humoral and cell-mediated immune responses. The percentage of CD4⁺ T cells in PBMCs from healthy adult cattle is approximately 25-35%. In the current study, the number of CD4⁺ T-cells in the peripheral blood averaged $21.6\% \pm 2.1$, $24.3\% \pm 1.3$, and $25.7\% \pm 2.4$ for control, subclinical, and clinical cows, respectively, with no differences noted between infection groups (Figure 1). Compared with the prepartum period, the percentages of CD4⁺ T-cells increased at parturition for clinically infected cows ($P < 0.08$), whereas healthy control cows showed a gradual decline in the number of CD4⁺ T-cells from -21 to -7 d.

CD8⁺, or cytotoxic T-cells are important for their ability to recognize viral and bacterial antigens and to target T-cells displaying these antigens for apoptosis. In the current study, the percentage of CD8⁺ T-cells ranged from 10-22%, which is equivalent to published values for adult dairy cows (Figure 2). On average, the percentage of CD8⁺ T-cells was greater ($P < 0.05$) in subclinically infected cows ($20.3\% \pm 2.1$) compared with clinically infected ($10.3\% \pm 3.7$) and control ($10.3\% \pm 3.3$) cows. Subclinically infected cows expressed a 2-fold higher percentage of CD8⁺ cells in the postpartum period.

The CD4 to CD8 ratio is often used as an indicator of immune status. For example, in HIV-infected populations, a diminished CD4:CD8 ratio may be used to accurately predict the occurrence of an AIDS-related complication. On the first sampling day of the current study (d -21), the CD4:CD8 ratios were significantly lower for infected cows compared with control (1.9 vs. 4.0) (data not shown). Subclinically infected cows had lower CD4:CD8 ratios, whereas the clinically infected cows had higher CD4:CD8 during the immediate postpartum period.

$\gamma\delta$ T-cells initiate immune responses and may regulate host inflammatory response to infection although their specific roles in host immunity are still relatively undefined.

The percentage of $\gamma\delta$ T-cells is greatest in the calf (40%) and gradually declines to approximately 5% in adult PBMCs. In the current study, the overall percentage of $\gamma\delta$ T-cells was lower ($P < 0.01$) in clinical cows ($1.7\% \pm 0.9$) compared with subclinical cows ($4.4\% \pm 0.5$), with a tendency ($P < 0.06$) to be lower than control cows ($3.9\% \pm 0.8$) (Figure 3). Both control and subclinical cows tended to have increased ($P < 0.12$) numbers of $\gamma\delta$ T-cells as parturition approached, with further increase noted for subclinically infected cows in the postpartum period. The exact function of $\gamma\delta$ T-cells remains to be found, although there is evidence to suggest that these cells play a significant role in the innate immune response to initial mycobacterial infection.

In this study, the percentage of B-cells across infection groups ranged from 28-35% (Figure 4). Neither infection status of the cows nor parturition had an effect on the overall percentages of B-cells. However, the percentage of B-cells from subclinical cows declined as parturition approached ($P < 0.03$). An increase in the percentage of B-cells from clinical cows was noted from -14 d to +7 d ($P < 0.06$). In the advanced stages of JD, antibody production by B-cells does little to protect the host from the progressive MAP-infection.

There was no overall effect of infection or parturition on percentages of CD14⁺ cells, but a trend for an interaction of infection group and parturition ($P < 0.12$) (data not shown). Subclinical cows expressed a greater percentage of CD14⁺ cells on +7, +14, and +21 d compared with control and clinical cows.

Results of this study indicate that in dairy cows the percentage of lymphocyte subsets are modulated by natural infection with MAP and by the periparturient period. These results contribute to the limited available information that focuses on the impact periparturient immunosuppression may have on the ability of the host to respond to progressing MAP infection. The data presented are important because they highlight changes in the immune response of infected cattle at parturition that may be an attempt to limit the progression of Johne's disease during the highly stressful time of parturition. Altering changes in lymphocyte immune responses may be one mechanism to assist the infected dairy cow in attempting to manage the highly stressful periparturient period.

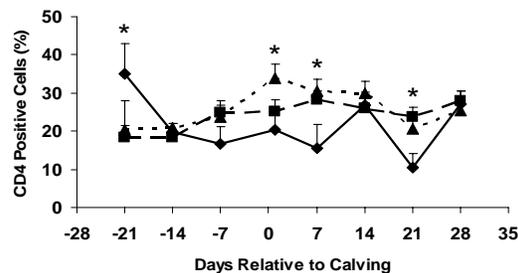


Figure 1. Percentage of CD4⁺ T-cells from PBMCs isolated from control (◆), subclinical (■), and clinical (▲) periparturient dairy cows. There was an effect of parturition ($P < 0.05$) and an interaction of infection group and parturition ($P < 0.01$). Data are means \pm SE. Significant differences between infection groups on a given day are represented by asterisks ($P < 0.05$).

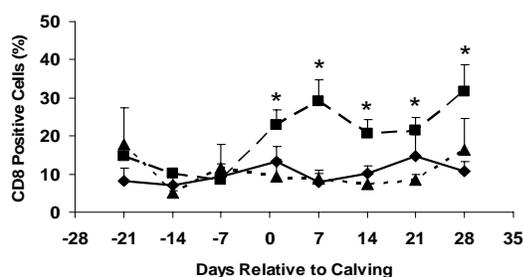


Figure 2. Percentage of CD8⁺ T-cells from PBMCs isolated from control (◆), subclinical (■), and clinical (▲) periparturient dairy cows. Subclinical cows expressed a greater percentage compared with control and clinical cows ($P < 0.05$). There was an effect of parturition and an interaction of infection group and parturition ($P < 0.01$). Data are means \pm SE. Significant differences between infection groups on a given day are represented by asterisks ($P < 0.05$).

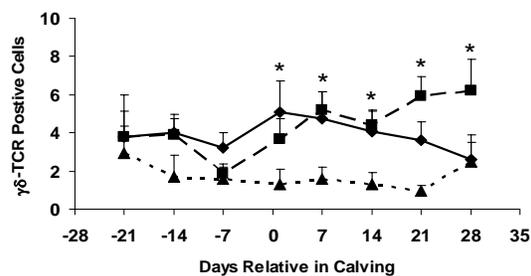


Figure 3. Percentage of $\gamma\delta$ T-cells from PBMCs isolated from control (◆), subclinical (■), and clinical (▲) periparturient dairy cows. Clinical cows expressed a lower percentage compared with the control ($P < 0.06$) and subclinical cows ($P < 0.01$). Data are means \pm SE. Significant differences between infection groups on a given day are represented by asterisks ($P < 0.05$).

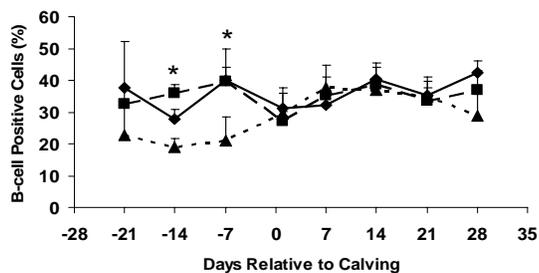


Figure 4. Percentage of B-cells from PBMCs isolated from control (♦), subclinical (■), and clinical (▲) periparturient dairy cows. Data are means \pm SE. Significant differences between infection groups on a given day are represented by asterisks ($P < 0.05$).

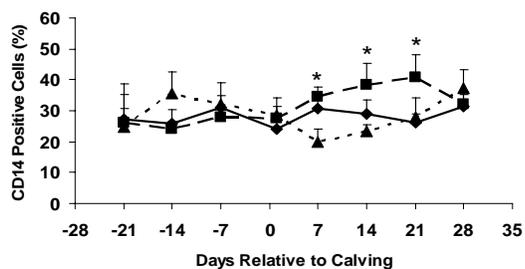


Figure 5. Percentage of CD14⁺-cells from PBMCs isolated from control (♦), subclinical (■), and clinical (▲) periparturient dairy cows. There was a tendency for interaction of infection group and parturition ($P < 0.12$). Data are means \pm SE. Significant differences between infection groups on a given day are represented by asterisks ($P < 0.05$).