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ScienceDirect
Veterinary Immunology and Immunopathology 124 (2008) 50–62
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Parturition invokes changes in peripheral blood mononuclear cell populations in Holstein dairy cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*

E.L. Karcher [a], D.C. Beitz [a], J.R. Stabel [b,*]

[a] *Department of Animal Science, Iowa State University, Ames, IA 50010, United States*
[b] *USDA-ARS, National Animal Disease Center, 2300 Dayton Road, Ames, IA 50010, United States*

Received 6 September 2007; received in revised form 7 January 2008; accepted 11 January 2008

Abstract

Johne's disease (JD) is characterized by a protracted period of subclinical infection. Infected cows may remain in the subclinical state until stressors such as parturition and lactation invoke more clinical signs of disease. The objective of this study was to evaluate changes in the percentages of $CD4^+$, $CD8^+$, and γδ T-cells, B-cells, monocytes, as well as the expression of the activation marker, CD5, on these cell subpopulations in the peripheral blood of dairy cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) during the periparturient period. Peripheral blood mononuclear cells (PBMCs) were collected from 3 wk pre- to 4 wk post-calving and freshly isolated or cultured for 7 d. Day 7 cultures were infected with live MAP at a 10:1 MOI (bacteria to adherent PBMC), and cultures were incubated for an additional 24 h. Fluorescent antibody labeling of lymphocyte subsets and monocytes was conducted and analyzed with flow cytometry. Freshly isolated PBMCs from subclinical cows expressed a greater ($P < 0.05$) percentage of $CD8^+$ and γδ T-cells compared with clinical cows. The percentage of $CD4^+$ T-cells increased ($P < 0.08$) in clinical cows as parturition approached. During the postpartum period, clinical cows had greater ($P < 0.05$) CD4:CD8 ratios compared with subclinical and control cows. After 8 d, uninfected PBMCs from clinical cows had greater ($P < 0.05$) percentages of $CD14^+$ cells compared with subclinical cows. When infected with live MAP, there was no effect of infection group or parturition on cell subpopulations. In fresh PBMCs, clinical cows expressed lower percentages of $CD4^+CD5^{bright}$ and $CD8^+CD5^{bright}$ compared with control cows, but greater percentages of $CD5^{dim}$ cells for all lymphocyte subsets. These results suggest changes in the percentages of lymphocyte subsets, monocytes, and CD5 markers are modulated by both infection status and the periparturient period.

Keywords: *Mycobacterium avium* subsp. *paratuberculosis*; Lymphocytes; Periparturient; Peripheral blood mononuclear cells

1. Introduction

Johne's disease (JD), caused by the intracellular pathogen *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is estimated to infect more than 22% of US dairy herds and cost the US dairy industry up to $250 million annually (Ott et al., 1999). In general, dairy cows will become infected with MAP as neonates through fecal–oral transmission. Once infected, cows may remain in the subclinical, or asymptomatic, stage of the disease for several years (Larsen et al., 1975). Stressors, such as parturition, may induce the transition from subclinical to clinical stage of the disease. Clinical

* Corresponding author. Tel.: +1 515 663 7304; fax: +1 515 663 7458.
E-mail address: judy.stabel@ars.usda.gov (J.R. Stabel).

0165-2427/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2008.01.006

animals are characterized by fecal shedding of the bacteria, intermittent but persistent diarrhea, progressive weight loss, and eventual death. The exact mechanism that triggers the progression of the disease remains unknown.

On-farm observations suggest that dairy cows infected with MAP may demonstrate increased signs of clinical disease during the weeks following parturition. Research on what prompts the progression of disease from the asymptomatic subclinical state to a more clinical state during this time period is lacking. The periparturient period, defined as 3 wk prior to and the 3 wk following parturition, represents a time of physiological stress for the dairy cow. Metabolic changes such as the rapid increase in non-esterified fatty acid concentrations (Radcliff et al., 2003; Karcher et al., 2007), accompanied by a postpartum decline in both blood glucose (Radcliff et al., 2003) and calcium concentrations (Kimura et al., 2006), present challenges to the dairy cow. Rapid fluctuations in both serum progesterone and estradiol during the periparturient period contribute additional stress (Weber et al., 2001; Radcliff et al., 2003). In addition, the cow is forced to deal with immunosuppression characterized by decreased lymphocyte function (Kehrli et al., 1989a; Meglia et al., 2005) and the decreased ability of neutrophils to migrate and phagocytize (Kehrli et al., 1989b; Lee and Kehrli, 1998). Parturition has a major impact on the number of T- and B-cells, both components of the adaptive immune system, and the number of monocyte/macrophages, effectors of the innate immune system, in the peripheral blood of healthy dairy cows. Studies have noted dramatic decreases in the percentage of peripheral blood $CD4^+$ T-cells and γδ T-cells at parturition (Van Kampen and Mallard, 1997; Kimura et al., 1999). In contrast, increased activity of $CD8^+$ lymphocytes has been observed in cows at calving compared with mid to late lactating cows (Shafer-Weaver and Sordillo, 1997). The percentage of B-cells in peripheral blood was highest immediately prior to and lowest immediately following parturition (Van Kampen and Mallard, 1997). In addition, the number of monocytes and monocyte-derived macrophages were increased at calving (Kimura et al., 2002). The total number and percentages of $CD4^+$ and $CD8^+$ (α/β T-cells), γδ T-cells, and B-cells, plays a significant role in the ability of the animal to respond to an infection.

In paratuberculosis, the progression from a subclinical to a clinical stage of disease is characterized by a shift from cell-mediated (Th1) immunity to an antibody-mediated (Th2) humoral response. This shift in Th1 to Th2 immunity is characterized by a decreased percentage of peripheral blood T-cells and an increase in the percentage of B-cells for clinically infected cows (Waters et al., 1999; Koets et al., 2002). More specifically, the percentages of γδ and $CD4^+$ T-cells in peripheral blood are remarkably decreased in clinical cows compared with healthy controls (Koets et al., 2002). The CD4:CD8 ratio is also decreased in chronically infected animals as the number of $CD8^+$ T-cells does not seem overtly affected by the transition to a clinical disease state (Koets et al., 2002). The decline in $CD4^+$ T-cells observed in clinical cows further illustrates the compromised nature of the immune system as these cells are key effectors of Th1-mediated immunity through the secretion of IFN-γ, a cytokine that is credited for controlling mycobacterial infections (Cooper et al., 1993; Flynn et al., 1993). Immunosuppressive T regulatory cells ($CD4^+CD25^+$) are upregulated in the ileum of MAP-infected dairy cows (Weiss et al., 2006) and have been shown to secrete an abundance of IL-10 and reduced IFN-γ compared with $CD4^+CD25^-$ (Belkaid et al., 2002).

To date, limited research is 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 cows with paratuberculosis. Therefore, the objective of this study was to determine the percentages of $CD4^+$, $CD8^+$, and γδ T-cells, B-cells, and monocytes in the peripheral blood of dairy cows naturally infected with MAP during the periparturient period as compared with healthy control cows. In addition, cell populations were further delineated by staining for CD5, a marker for T- and B-cell activation.

2. Materials and methods

2.1. Animals

Twenty-one multiparous Holstein cows and two primiparous Holstein cows (age range from 3 yr to 6 yr) were grouped according to infection status. These groups consisted of (1) noninfected healthy control cows ($n = 5$), (2) cows naturally infected with MAP, but asymptomatic ($n = 14$) and (3) naturally infected cows with clinical Johne's disease ($n = 4$). The two primiparous cows were both in the subclinically infected group. The stage of infection was determined by fecal shedding of MAP and IFN-γ. Infection was monitored bacteriologically for the fecal shedding of MAP by standard culture methods (Stabel, 1997). By definition,