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Effect of recombinant bovine granulocyte colony-stimulating factor covalently bound to polyethylene glycol injection on neutrophil number and function in periparturient dairy cows

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ABSTRACT

Dairy cows often experience decreased immune function around the time of calving, typified by impaired polymorphonuclear neutrophil (PMN) function and a transient neutropenia. This is associated with increased disease incidence, including mastitis, retained placenta, and metritis. In an attempt to improve PMN functional capacity during the periparturient period, we injected cows with recombinant bovine granulocyte colony-stimulating factor covalently bound to polyethylene glycol (PEG rbG-CSF) twice subcutaneously, about 6 d before calving and within 24 h after calving. Twenty-one cows in their second pregnancy were enrolled in this study and divided into 2 groups: PEG rbG-CSF treated (n = 11) and saline-treated controls (n = 10). The PMN numbers quickly and dramatically increased after PEG rbG-CSF administration and remained elevated through the end of the experiment (13 d after calving). Exocytosis of myeloperoxidase by stimulated PMN, which is generally decreased in periparturient cows, was markedly increased by PEG rbG-CSF after injection. Higher myeloperoxidase exocytosis persisted for at least 10 d after calving. The PMN superoxide anion release and phagocytosis activity did not differ between groups. Injection of PEG rbG-CSF was safe for cows, with no significant negative effects observed. The greatest single effect of PEG rbG-CSF administration was a dramatic increase in circulating numbers of PMN. The increased numbers of PMN ready to move to a site of infection early in the course of an infection may improve the ability of the cow to ward off clinical disease in the periparturient period.

Key words: granulocyte-colony stimulating factor, neutrophil, periparturient immunosuppression

INTRODUCTION

Dairy cows suffer from decreased immune cell function around the time of calving. Many indices of polymorphonuclear neutrophil (**PMN**) and lymphocyte functions decrease gradually starting about 2 or 3 wk before calving, with most indices reaching a nadir at the time of calving (0 to 2 d after calving) and then gradually recovering in 2 to 4 wk (Kehrli et al., 1989; Kimura et al., 1999; Kimura et al., 2002). Almost all cows experience decreased immune function during the periparturient period, but in some cows the immune suppression is more pronounced. This periparturient immunosuppression is associated with a high incidence of diseases (both metabolic and infectious; Burton et al., 2001; Kimura et al., 2006). Metritis, retained placenta, and mastitis are diseases known to be associated with decreased PMN function (Waller, 2000; Kimura et al., 2002; Hammon et al., 2006). The reason for decreased PMN function during the periparturient period has been attributed to increased nutrient demand for fetal growth and colostrum production at the end of pregnancy. At the onset of lactation, most cows experience negative energy and protein balance, as well as alterations in mineral and vitamin status (Waller, 2000; Goff et al., 2002; Martinez et al., 2012), which are suspected to contribute to decreased PMN function (Kimura et al., 1999; Kimura et al., 2002; Hammon et al., 2006). Supplementing vitamin E (Politis et al., 1995, 2001), selenium (Smith et al., 1984; Cebra et al., 2003), and copper (Scaletti and Harmon, 2012) may improve PMN function and reduce disease incidence, particularly when diets are marginal in these substances.

To directly improve PMN function, one group of researchers subcutaneously injected recombinant bovine granulocyte colony-stimulating factor (**rbG-CSF**) daily and examined PMN number and function in cows with and without *Staphylococcus aureus* infection of 1 mammary quarter (Kehrli et al., 1991). They demonstrated a large increase in PMN number by rbG-CSF injection, as well as improved ingestion of bacteria and

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improved cytotoxicity by PMN. However, not every PMN function improved. For example, the ability of PMN to move randomly or toward a chemotactic target through agarose was reduced by rbG-CSF treatment.

The granulocyte colony-stimulating factor injection has been used in human medicine to increase PMN numbers in cancer patients who undergo myelosuppressive chemotherapy to improve their ability to fight infections (Hata et al., 2011; Inaba et al., 2011). A recent report compared daily dosing of rhG-CSF (average 4.8 d of treatment) with a single dose of a polyethylene glycolated form (covalently bound) of rhG-CSF (**PEG rhG-CSF**) and demonstrated better efficacy of PEG rhG-CSF in increasing PMN numbers in patients undergoing chemotherapy (Almenar Cubells et al., 2013). Administering human granulocyte colony-stimulating factor to patients with human immunodeficiency virus reduces the incidence of bacterial infections and the number of days spent in a hospital (Kuritzkes, 2000). The rhG-CSF was approved in the United States in 1991 for use in decreasing the incidence of infection, as manifested by febrile neutropenia in patients undergoing myelosuppressive chemotherapy (Crawford, 2003; Hartung et al., 2003), especially when a risk for infection was anticipated (before surgery, before chemotherapy).

We hypothesized that subcutaneous injection of PEG rbG-CSF at approximately 7 d before parturition and on the day of calving would increase PMN numbers and reverse the periparturient suppression of PMN function in dairy cows. The objective was to evaluate PMN number and function from 2 wk before expected parturition to 2 wk after calving in periparturient Holstein cows treated or not treated with PEG rbG-CSF.

MATERIALS AND METHODS

Animal Treatment and Sampling

Twenty-one Holstein cows in their second pregnancy were selected and housed at the Iowa State University Dairy Farm. Cows were selected from the herd only if their BCS was at least 3.0 and not more than 3.75 and they showed no signs of lameness. Previous milk production was not factored into this decision to enroll the cows. Once enrolled in the study, the cows were alternately assigned into 2 groups based on expected calving date: control (**CONT**) cows received saline (n = 10) and granulocyte colony-stimulating factor (**GCSF**) cows received PEG rbG-CSF (n = 11). Treatments were administered via subcutaneous injection using an 18 gauge × 2.5-cm needle. All cows were fed and managed following standard procedures of the Iowa State University Dairy Farm. A high-straw, low-DCAD close-up diet was fed the last 3 wk of gestation, followed by a corn silage-based lactation diet balanced for 35 kg of milk production. Blood samples were obtained Monday, Wednesday, and Friday from May 16 to August 17, 2012. Blood was collected from cattle by jugular venipuncture. Blood intended for PMN function assays was collected using acid citrate dextrose as an anticoagulant (Roth and Kaeberle, 1981b). Blood was also collected into separate tubes with EDTA.2K (for complete blood count) and heparin Na (for plasma isolation) as anticoagulants. Animals were handled using procedures approved by the Animal Care and Use Committee of Iowa State University.

The PEG rbG-CSF [approximately 15 mg (as an active ingredient)/3 mL per cow] or saline (3 mL/cow) treatments were administered subcutaneously in the lumbar region twice. The first dose was administered approximately 7 d before expected parturition, depending on expected calving date and judgment of investigators based on physical changes including swelling of vulva and filling of udder. The second dose was administered within 24 h after calving. Neutrophil function, plasma BHBA, NEFA, and Ca, and complete blood count were evaluated 3 times a week (Monday, Wednesday, and Friday) beginning 2 to 3 wk before expected parturition and continuing until 2 wk (13 to 16 d) after parturition.

Reagents

The PEG rbG-CSF and sterile saline were provided by Elanco Animal Health in individual, prefilled 3-mL syringes with rubber cap for single use. They were kept in a refrigerator at 4°C until use.

All reagents for immunologic assays were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified. A solution of 3,3′,5,5′-tetramethylbenzidine hydrochloride (2.5 m*M* in water) was made fresh each day immediately before use. A solution of H_2O_2 (5.3 m*M* in water) was prepared fresh daily. The detergent cetyltrimethylammonium bromide (0.02% in water) was used as the lysing agent for determining total myeloperoxidase (**MPO**) content of PMN. Cytochalasin B (1 mg/mL), phorbol 12-myristate 13-acetate (**PMA**; 1 mg/mL), and calcium ionophore A23187 (1 mg/mL) were dissolved in dimethyl sulfoxide as stock solutions and stored at −80°C. Cytochrome C from equine heart was dissolved in Hanks' balanced salt solution (**HBSS**; Mediatech, Herndon, VA) at 37.5 mg/mL and stored at −20 C as a stock solution.

Total and Differential White Blood Cell Count

Enumerations of total PMN, monocytes, eosinophils, basophils, total white blood cells, and platelets in the