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The effect of pegylated granulocyte colony-stimulating factor treatment prior to experimental mastitis in lactating Holsteins

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ABSTRACT

Neutrophils are the first-acting and most prominent cellular defense against mastitis-causing pathogens. This makes neutrophil activation and expansion obvious candidates for targeted therapeutics. The granulocyte colony-stimulating factor (G-CSF) cytokine stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream, which results in neutrophilia as well as increasing the presence of other progenitor cells in the bloodstream. A pegylated form of G-CSF (PEG-gCSF) has been shown to significantly decrease naturally occurring mastitis rates in cows postpartum. The use of PEG-gCSF had not been evaluated in response to an experimental mastitis challenge. In an effort to examine the effect and mechanism of PEG-gCSF treatment, we challenged 11 mid-lactation Holsteins with ~400 cfu *Escherichia coli* P4 by intramammary infusion. Five cows received 2 PEG-gCSF injections, one at 14 d and the other at 7 d before disease challenge, and 6 cows remained untreated. To evaluate the response of cows to the PEG-gCSF treatment, we measured complete blood counts, somatic cell counts, bacterial counts, milk yield, and feed intake data. The PEG-gCSF-treated cows had significantly increased circulating levels of neutrophils and lymphocytes after each PEG-gCSF injection, as well as following mastitis challenge. The PEG-gCSF-treated cows had significantly lower bacterial counts and lower milk BSA levels at the peak of infection. In addition, control cows had significant decreases in milk yield postinfection and significantly reduced feed intake postinfection compared with PEG-gCSF-treated cows. Collectively, PEG-gCSF treatment resulted in reduced disease severity when administered before a bacterial challenge. Mechanistically, we show that G-CSF treatment increases cell surface expression of an E-selectin ligand before infection on neutrophils and monocytes found in the blood. These cells quickly disappear from the blood shortly after infection, suggesting a mechanism for the reduced mastitis severity by priming immune cells for quick targeting to the site of infection.
Key words: mastitis, cytokine, granulocyte colony-stimulating factor, immunology

INTRODUCTION

Mastitis is the primary health and economic concern for the dairy industry. In dairy cows, mastitis can result in culling, milk loss, reduced milk quality, increased treatment costs (Ryman et al., 2015; Ceniti et al., 2017) and is responsible for an estimated $2 billion/yr in loss to the industry in the United States, generally varying costs between $95 to $211 per case per cow (USDA-APHIS, 2007; Cha et al., 2011; Ryman et al., 2015; Ceniti et al., 2017). Mastitis affects all dairy livestock and can be caused by a plethora of microorganism pathogens, including most commonly in bovine cases *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus uberis* (Ryman et al., 2015; Ismail, 2017). Symptoms of mastitis can include udder swelling, heat, hardness, or redness; animals may be lethargic or have changes in milk composition and quality, and show losses in milk production and feed intake (Yeiser et al., 2012). Mastitis can vary in levels of severity that can be influenced by environmental and genetic components unrelated to disease challenge (Mallard et al., 1998; Burvenich et al., 2007).

Neutrophils, as well as additional innate effector cells such as monocytes, are activated during normal inflammation responses to infections, and are considered to be the primary innate responders to mastitis infection (Kehrli et al., 1991a). Neutrophils have various killing mechanisms to destroy pathogens (Paape et al., 2003; Brinkmann et al., 2004; Lippolis et al., 2006). Upon encountering invading bacteria, neutrophils can ingest the bacteria into phagosomes that fuse with lysosomes. This process stimulates neutrophils to produce large amounts of oxidizing agents in a process referred to as respiratory burst, which generates various antimicro-

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bial oxidants that kill pathogens, and is also negatively correlated with mastitis disease severity (Heyneman et al., 1990). Last, neutrophils can secrete genomic DNA bound with antimicrobial proteins to physically trap and destroy pathogens (Brinkmann et al., 2004).

Myeloperoxidase (**MPO**) has long been known to be important to neutrophil function because of its role in the generation of antimicrobial oxidants (Paape et al., 2003; Silvescu and Sackstein, 2014). Periparturient immune suppression and mastitis susceptibility is associated with decreased neutrophil function and is speculated to be a contributor to reduced immune health especially in the first few weeks postpartum (Kehrli et al., 1991b; Mallard et al., 1998; Revelo et al., 2011). Enzymatic function and expression of MPO have been shown to be reduced during the periparturient period (Cai et al., 1994; Lippolis et al., 2006). Recently, MPO has been shown to be a cell surface activation marker on both neutrophils and monocytes in a uniquely glycosylated form that can act as a ligand for E-selectin, which is important for leukocyte migration to areas of inflammation (Silvescu and Sackstein, 2014).

Immune cell regulation for the treatment and prevention of mastitis is a developing field for dairy health therapy. Although a variety of commercial and herd-specific vaccines exist, they are not cost efficient or adequately effective to control all mastitis (Ismail, 2017). While mastitis preventative vaccines remain limited in their efficacy, the most common course of mastitis treatment is antibiotics. Concerns over selecting antibiotic resistant strains of bacteria is the major reason for the search for antibiotic alternatives. Nonantibiotic alternative therapies are under development that include immune stimulators designed to regulate immune cells important to mastitis defense, with an emphasis on neutrophils (Canning et al., 2017; McDougall et al., 2017). One such way to mediate the immune response is the administration of cytokines, such as granulocyte colony-stimulating factor (**G-CSF**).

The G-CSF is well established as a regulator of myeloid cells. Although evidence indicates that G-CSF is produced by several immune cell sources, it is well established to be produced by monocytes and macrophages in response to inflammatory signaling (Sieff, 1987; Kehrli et al., 1991a; Xu et al., 2000). Granulocyte colony-stimulating factor induces proliferation of myeloid progenitor cells from the bone marrow but also has been shown to stimulate granulocyte maturation and activate circulating mature myeloid cells such as neutrophils (Sieff, 1987; Kehrli et al., 1991a; Xu et al., 2000). As a therapeutic, G-CSF is used in human medicine to recover depleted immune cells (Xu et al., 2000; Silvescu and Sackstein, 2014) and in veterinary health practices to initiate neutrophilia (Kimura et al., 2014; Silvescu and Sackstein, 2014). Recombinant bovine G-CSF supplementation has been shown to significantly increase circulating leukocytes including increased numbers of mature and immature band neutrophils (Harp et al., 1991; Kehrli et al., 1991b; Stabel et al., 1991). A pegylated G-CSF (**PEG-gCSF**) bovine treatment is commercially available (Imrestor/pegbovigrastim, Elanco, Greenfield, IN) with a utilization emphasis on mastitis prevention during the periparturient critical period. In previously reported work, a 2-injection treatment of PEG-gCSF prepartum (first injection approximately 1 wk before calving and second injection day of calving) has been shown to significantly decrease the incidence of naturally occurring mastitis up to 30 d postpartum compared with untreated control cows (Ruiz et al., 2017). This 2-step PEG-gCSF treatment induced increased circulating numbers of monocytes, lymphocytes, and targeted expansion of neutrophils (McDougall et al., 2017).

Exploring the mechanism of neutrophil differentiation, activation, and migration will continue to be important in development of mastitis therapeutics. The objective of this study was to assess for the first time the effect of PEG-gCSF on experimental mastitis and provide early insight into some of the mechanisms of a G-CSF immune response.

MATERIALS AND METHODS

Experimental Design

Eleven mid-lactation Holsteins averaging 141.3 ± 20.6 DIM at the start of the experiment were used for this study. Five cows received subcutaneous injections of PEG-gCSF 14 and 7 d before disease challenge (2 injections total); each injection consisted of 15 mg of PEG-gCSF in 2.7 mL in a single-dose syringe (Imrestor, Elanco). Six cows remained as untreated controls. Previous experience with our experimental mastitis model demonstrated that a group size of 5 cows could yield significant differences by treatment (Lippolis et al., 2011). Of the experimental animals, 9 were multiparous and 2 were primiparous. The 2 primiparous cows were randomly balanced between control and treatment groups. All 11 lactating Holsteins were challenged with ~400 cfu *E. coli* P4 by intramammary infusion in a single quarter, which were clean before the trial start as determined by bacterial culture (data not shown). Feed intake, milk yield, bacterial counts, BSA levels in milk, and DNA in milk will be used as metrics to determine infection severity. Feed intake and total milk yield were recorded daily in addition to relevant blood