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Intramammary immunization with ultraviolet-killed *Escherichia coli* shows partial protection against late gestation intramammary challenge with a homologous strain

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ABSTRACT

The objective of this study was to evaluate the efficacy of intramammary immunization with UV-killed *Escherichia coli* ECC-Z on prevention of intramammary colonization after a challenge with a dose of the homologous *E. coli* ECC-Z live bacteria. A total of 10 cows were included in a study to evaluate the efficacy of intramammary immunization. All 10 cows received an intramammary immunization of 100 cfu of UV-killed *E. coli* ECC-Z bacteria into one hind quarter at the time of dry off. Approximately 2 wk before the anticipated calving date, both hind quarters of all cows were challenged with 100 cfu of live *E. coli* ECC-Z bacteria. Five of the cows were vaccinated parenterally with a commercial J5 bacterin, and 5 cows served as controls with no parenteral vaccination. The cows were then followed over time and infection risk, clinical scores, somatic cell count, and milk production were observed over time. The results of these 10 cows showed partial protection of intramammary immunization on the outcome of a subsequent homologous intramammary challenge. Immunization resulted in a lower probability of infection, a lower bacteria count, lower somatic cell counts and milk conductivity, a lower clinical mastitis score, and increased milk production compared with unimmunized control quarters. Once the analysis was corrected for immunization, parenteral J5 vaccination had no significant effect on any of the measured parameters. These results provide the first evidence that intramammary immunization may improve the outcome of an intramammary *E. coli* infection in late gestation and onset of mastitis immediately following parturition. Unlike systemic vaccination, which generally does not reduce the intramammary infection risk, the intramammary immunization did show a 5-times reduced odds of an established intramammary infection after challenge. Cytokine profiles indicated a local return of proinflammatory response after challenge as the data showed a more pronounced increase in in IFN-γ with a subsequent negative feedback due to a spike in the level of IL-10 in immunized quarters relative to nonimmunized quarters. Although these results are preliminary and obtained on only 10 cows, the results provide insight into the biological benefits of triggering mucosal immunity in the mammary gland.

Key words: *Escherichia coli*, vaccination, mastitis, late gestation

INTRODUCTION

Clinical mastitis is one of the most common and costly challenges to the dairy industry (Gröhn et al., 2004). Approximately 40% of clinical mastitis cases are attributed to gram-negative, mostly coliform, bacterial infection (Barkema et al., 1998; Gröhn et al., 2004; Oliveira et al., 2012). Bovine coliform mastitis is generally considered to be caused by opportunistic infections (Bradley and Green, 2001), although evidence for a host-adapted subpopulation is growing (Bradley and Green, 2001; Dogan et al., 2006; Shpigel et al., 2008; Lippolis et al., 2014). The incidence of clinical mastitis peaks on most farms immediately following parturition (Barkema et al., 1998), although several observational and experimental challenge studies indicate the presence of the mastitis-causing bacteria isolated from the dry gland before parturition without clear inflammatory indicators of mastitis (Green et al., 2007; Quesnell et al. 2012). The late gestation challenge study using *Escherichia coli* ECC-Z by Quesnell et al. (2012) showed that cows in late gestation respond to challenge with an IL-10 dominated response and a minimal response

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of proinflammatory cytokines, such as IFN-γ and IL-1β. This suggested shift toward a predominantly anti-inflammatory, highly regulated response during the dry period likely reflects an adaptation in maternal immune signaling during late gestation to protect the semiallogenic fetus (Quesnell et al., 2012; Pomeroy et al., 2015).

Dairy cows may be vaccinated parentally against coliform mastitis using a core J5 bacterin that has been repeatedly reported to reduce clinical severity of response to these infections (Hogan et al., 1992, 1995; Wilson et al., 2008). However, these vaccination studies have not shown a reduction in incidence of clinical coliform cases. Local immune responses are important for protection against pathogens that predominately infect and cause disease at mucosal sites. Mucosal vaccines have shown to be effective both in the case of viral (i.e., oral polio vaccine, intranasal infectious bovine rhinotracheitis vaccine, oral rabies vaccine) and bacterial (i.e., oral cholera vaccine, oral *E. coli* vaccine, *Staphylococcus aureus* intramammary vaccine) pathogens (Levine et al., 2000; Pavot et al., 2012; Vilte et al., 2012; Gogoi-Tiwari et al., 2015). Hogan et al. (1997) and Smith et al. (1999) studied the effect of a systemic vaccination in combination with an intramammary *E. coli* J5 bacterin immunization schedule on responses of antibody titers. The results showed that intramammary immunization enhanced IgG titers in serum in the dry period and in lactation and in whey in early lactation compared with subcutaneous immunizations (Hogan et al., 1997; Smith et al., 1999). However, the immunization schedule had minimal effect on systemic and local signs of clinical mastitis following challenge. Smith et al. (1999) concluded that the reason for this lack of protection was due to the 4-h boiling of the bacteria in the creation of the bacterin vaccine.

More recent studies have used so-called ghost bacteria as vaccine organisms. These ghost organisms are killed bacteria where the internal structures of the bacteria are destroyed but the bacterin have the same cell surface composition as their living counterparts (Mayr et al., 2005). Such ghost organisms display all surface components in a natural nondenatured form, even highly sensitive and fragile structures such as pili are well protected. It has been shown that ghost organisms are able to induce a strong mucosal immune response (Jalava et al., 2003). The potency, safety, and relatively low production cost of bacterial ghosts also offer a significant technical advantage (Yuki and Kiyono, 2003; Mayr et al., 2005; Lubitz et al., 2009).

Therefore, the objective of the current study was to evaluate the efficacy of intramammary immunization with UV-killed *E. coli* ECC-Z on prevention of intramammary colonization after a challenge with a dose of the homologous *E. coli* ECC-Z bacterium prepartum, a period of heightened susceptibility to IMI. The *E. coli* ECC-Z strain used for our study has been used in previous challenge trials in both the dry period and lactation, is well characterized, and known to cause mild clinical mastitis (Dogan et al., 2006; Quesnell et al., 2012; Sipka et al., 2013; Lippolis et al., 2014). The effect of immunization on clinical signs, milk production, SCC, and cytokine profiles was also evaluated.

MATERIALS AND METHODS

Cows

Ten adult Holstein cows were selected from the Cornell University Teaching and Research Dairy herd. Selection was based on the following criteria: cows had completed at least 1 previous lactation and were expected to have an approximate 45- to 48-d dry period. Also, cows with any signs of detectable illness, including but not limited to clinical mastitis at time of dry off or with any of the last 6 monthly individual cow SCC before dry off greater than 250,000 cells/mL, were excluded. Cows were culture-negative at time of enrollment, and culture-negative before challenge with *E. coli* ECC-Z as determined by bacteriology described within the section Mammary Gland Secretion and Blood Sample Collection. Finally, cows with any major traumatic injury to the teat ends were excluded.

Housing

Cows in both groups were housed, fed, and managed identically throughout the dry period and postpartum. Three weeks before the anticipated calving date, cows were transferred from freestalls to the Cornell University Large Animal Research and Training Unit facility. Here the cows were housed in individual maternity pens and had unlimited access to water and feed. Cows remained in the study throughout the nonlactating period, calving, and first 7 d of the ensuing lactation. No adverse reactions to the vaccine before the intramammary *E. coli* challenge were observed in any of the cows. The study protocol was approved by the Cornell University committee on Animal Use and Care (protocol 2006–0158).

Parenteral Vaccination and Intramammary Immunization

The selected cows were paired by expected calving date so they were approximately the same and then were randomized to receive parenteral J5 vaccine or serve as control. The J5 vaccine (*Escherichia coli* Bacterin, J5 strain; Zoetis, Kalamazoo, MI) was administered by