NOVEL CONCEPTS OF MASTITIS CONTROL

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INTRODUCTION

Implementation of mastitis control measures based on proper milking practices and hygiene, reducing exposure to environmental pathogens, and traditional dry cow antibiotic therapy have lowered the prevalence of this disease in dairy cows over the past 25 years. But 30 to 40% of dairy cattle remain infected, and in order to further reduce incidence of infection dairymen may need to include more novel approaches to mastitis control. The purpose of this paper is to present a compilation of the more recent novel developments in mastitis control, and how this new knowledge may be implemented in the future by practitioners and dairymen to control this disease.

ROLE OF CYTOKINES IN MAMMARY IMMUNITY

Resistance to disease is mediated, in part, by leukocytes that are directed against microorganisms that enter the body. However, dairy cows become immunocompromised at certain times during the lactation cycle, i.e., leukocyte activity is insufficient to prevent new cases of mastitis and cure existing infections. Cytokines are proteins that are produced naturally in the dairy cow, and function by regulating the activity of leukocytes involved in protecting the udder against mastitis-causing bacteria. Interferon-gamma (INF-γ) is a cytokine that modulates phagocytic leukocyte populations which include neutrophils and macrophages. Because INF-γ has been shown to greatly enhance the ability of leukocytes to destroy bacteria, as well as regulate acute inflammatory responses induced by bacterial toxins in humans, studies have been conducted to determine if this cytokine is effective in controlling mastitis in dairy cows.

Many cases of acute toxic mastitis occur just before or after calving and are often caused by *Escherichia coli*. Such infections occur because circulating leukocytes do not respond rapidly enough or in sufficient numbers to prevent establishment of these bacteria in the udder. In one investigation, dairy cows were infused intramammarily with INF-γ and challenged 24 hours later with *E. coli*. Compared with untreated controls, those treated with the cytokine had fewer infected quarters, exhibited milder clinical symptoms, and experienced infections of shorter duration. In addition, all INF-γ-treated cows survived the *E. coli* challenge, whereas the mortality rate in controls was 42%. Success of treatment was attributed to the ability of this cytokine to enhance leukocyte activity and minimize the deleterious effects of endotoxin.

Colony stimulating factors (CSF) are another group of cytokines required for the proliferation and differentiation of stem cells into functional mature leukocytes (3). Granulocyte/macrophage colony stimulating factor (GMCSF) induces maturation of bone marrow cells into granulocytes (neutrophils) and macrophages. Subcutaneous administration of recombinant bovine GMCSF prior to drying off caused an increase in total milk somatic cell count (SCC), mostly neutrophils, by day 2 of treatment (12). The enhanced antimicrobial activity of these cells remained elevated throughout the early nonlactating period, and these leukocytes may be more competent in defending the udder during periods when cellular activity is normally compromised.

Administration of granulocyte colony-stimulating factor (GCSF) to lactating dairy cows was also found to markedly increase neutrophil numbers in both blood and milk compared with placebo controls (5). The GCSF-treated cows challenged with *Staphylococcus aureus* exhibited a 47% reduction in new intramammary infection compared with controls. Results suggested that the reduced rate of

infection was due to the recruitment of neutrophils into mammary quarters via GCSF injections prior to challenge, providing a phagocytic line of defense.

In addition to enhancing phagocyte activity, other cytokines regulate the activity of lymphocytes. Interleukin-2 (IL-2) is produced by certain lymphoid cells and modulates lymphocyte activity by stimulating cell proliferation and maturation, as well as antibody production. Antibodies in milk promote phagocytosis of pathogenic bacteria and neutralize toxins. The local administration of IL-2 to cows at the beginning of the dry period was found to expand lymphocyte populations in both mammary tissues and secretions during involution, stimulate the local production of antibodies and and accelerate the involution process (4), all of which promote resistance to invading bacteria during the dry period.

NEW DEVELOPMENTS IN VACCINATION ATTEMPTS

Vaccination has been attempted in efforts to increase antibody concentrations blood and in milk to specific microorganisms, providing immunity by inhibiting bacterial growth and toxin production. Because chronic *S. aureus* infections respond poorly to therapy and are responsible for reduced milk yield, mastitis vaccines against this microorganism have been studied extensively. *Staphylococcus aureus* produces a pseudocapsule when grown in the udder that interferes with mammary defenses by inhibiting the ability of leukocytes to destroy bacteria by blocking 3Cb receptors. Recent success has been realized with a novel *S. aureus* bacterin (14) that promotes the production of antibodies directed against the pseudocapsule, thereby stimulating phagocytosis. In addition, the vaccine contains staphylococcal toxoids and the adjuvant dextran sulfate. In a field trial using five commercial dairies, cows were injected intramuscularly at 8 and at 4 weeks prior to calving with either the vaccine or a placebo. Results showed that vaccination reduced new subclinical cases of *S. aureus* mastitis by 25% and reduced clinical cases of mastitis by about 50%. Subsequent research has shown that administering this bacterin at drying off, followed by a booster injection 6 weeks later significantly reduced new infections after experimental challenge with *S. aureus*. Whereas 92% of the quarters of unvaccinated control cows challenged with this organism became infected, only 36% of the challenged quarters of cows vaccinated intramuscularly, and 60% of the challenged quarters of cows vaccinated in the area of the supramammary lymph node became infected. Vaccinated cows showed significantly higher antistaphylococcal IgG_1 and IgG_2 antibody titers compared with unvaccinated control cows.

In a New York trial using a similar vaccine (9), heifers were vaccinated in the area of the supramammary lymph node with a similar vaccine 2 months before calving or left as untreated controls. At 4 and 2 weeks prior to calving, vaccinated animals were given booster injections in the area of the supramammary lymph node. After calving, all cows were challenged with *S. aureus* by infusing live organisms into the udder. Among vaccinates, 33% of quarters became infected, whereas infection rate in controls was 71%. Only 12% of the infections became chronic in vaccinates, but 64% became chronic in the controls. Thus, vaccination with the *S. aureus* bacterin reduced new quarter infections by 52%.

Progress has also been achieved with vaccines against coliform mastitis. The *E. coli* J-5 mutant vaccine is a heat-killed bacterin that is administered subcutaneously at drying off, 30 days later, and again within 14 days of calving. A field study with this vaccine using approximately 500 cows showed that prevalence of coliform mastitis over the first 100 days of lactation was only 2.6% in vaccinated cows, whereas prevalence in unvaccinated controls was 12.8% (1). Results to date indicate a very safe and effective product. Current testing shows that vaccinated cows exhibit over 70% fewer cases of clinical coliform mastitis than unvaccinated controls during lactation. It is stressed that the practice of immunization against mastitis should play a supplemental role to other effective traditional management and nutritional practices to control intramammary infections in dairy cows.

DIETARY SUPPLEMENTATION TO REDUCE MASTITIS

Diet plays a role in the cow's ability to resist infection, and it is now possible to improve the disease-fighting ability of an animal that has poor resistance due to dietary deficiencies. There are certain essential micronutrients (i.e. selenium and vitamin E) needed in a dairy cow's diet to maintain udder health. For example, neutrophils isolated from cows supplemented with vitamin E and selenium exhibit increased capacity to kill mastitis-causing bacteria (2). In an initial study, (10) selenium and vitamin E were provided in the diet beginning 60 days before calving, continuing throughout lactation; in addition, selenium was injected into cows 21 days prior to freshening. Results proved that the elevated blood levels of these nutrients reduced the prevalence of mastitis as well as SCC during the subsequent lactation. In addition, the number of clinical cases was reduced, duration of infection was lower, and new mastitis cases at calving were not as prevalent compared with unsupplemented cows.

To reach adequate blood levels, it is necessary to supplement the diet with 3 mg selenium per cow per day in the dry period and 7 mg per cow per day during lactation. In addition, an injection of 50 mg selenium 21 days before calving is recommended. For vitamin E, it is necessary to supplement the diet with 400 to 600 IU of vitamin E per cow each day during lactation and about 1000 IU during the dry period. Dietary supplementation is not a replacement for proper management practices, but where deficiencies exist, evidence indicates that proper selenium and vitamin E levels will reduce both the incidence and severity of bovine mastitis.

MASTITIS CONTROL IN REPLACEMENT HEIFERS

The greatest development of milk-producing tissue in heifers occurs during the first pregnancy. Thus, mammary glands must be protected from the harmful effects of mastitis-causing bacteria to insure maximum milk production. An initial study designed to determine prevalence of mastitis in breeding age and pregnant heifers demonstrated that bacteria were isolated from 97% of heifers (13). The most common isolates were *Staphylococcus chromogenes, Staphylococcus hyicus*, and *S. aureus*. *Staphylococcus aureus* was isolated from mammary secretions of 37% of heifers and 15% of quarters. Infections caused by this species in heifers are of great concern because of the contagious nature of this species and possible harmful effect on future milk production.

Antibiotic therapy in heifers is advantageous over treatment of lactating cows because treatment can be performed before calving and the risk of antibiotic residues at calving is minimal. Recent studies with pregnant heifers using cephalosporin-based antibiotics have been very successful. In one trial, a group of heifers, either naturally or experimentally infected with *S. aureus*, were infused intramammarily at 8 to 12 weeks prepartum with one dose of a commercially prepared product containing 300 mg cephapirin benzathine (7). Another group of animals with *S. aureus*-infected quarters served as untreated controls. Results demonstrated that 90% of naturally occurring infections and 100% of experimentally induced *S. aureus* infections were eliminated in treated animals by the time of calving, and cured quarters remained infection-free for at least 2 months into lactation. These treated quarters were free from antibiotic residues at freshening. After antibiotic infusion, SCC in infected quarters decreased from 15,000 x 10^3/ml to 4,000 x 10^3/ml 1 week later, and decreased to 700 x 10^3/ml at calving. In contrast, none of the untreated *S. aureus*-infected quarters had spontaneously cured by the time heifers calved. Treated heifers in which *S. aureus* infections were cured produced over 10% more milk than controls during the first 2 months of lactation. Quarters remaining infected at calving were treated with a lactating cow product containing 200 mg cephapirin benzathine; cure rate was 50%.

Studies to determine if prepartum infusion of lactating cow antibiotic preparations into heifer mammary glands shortly before calving influences rate of infection during early lactation have also been performed (6). Primigravid Jersey heifers received either no intramammary antibiotic infusion, infusions with 200 mg of sodium cloxacillin, or 200 mg of cephapirin sodium 7 days before expected parturition. Approximately 90% of heifers were infected 7 days prior to expected parturition. During early lactation, 78% of untreated control heifers were infected. In contrast, only 17.6% of heifers were

infected following infusion of antibiotics 7 days before parturition. Of the treated heifers, 2.1% of glands infused with cephapirin sodium and 8.6% of glands infused with sodium cloxacillin were infected during early lactation.

One disadvantage of prepartum antibiotic infusions in heifers is the potential for antibiotic residues in milk, especially if heifers calve sooner than expected. The majority of positive samples were from cloxacillin-treated heifers that calved within 5 days of treatment. All samples obtained 3 days after parturition (when milk is likely marketed for human consumption) were negative for antibiotic residues.

NEW STRATEGIES FOR ANTIBIOTIC THERAPY

Antibiotic preparations that are infused intramammarily do not penetrate to all areas of an infected gland due to blockage from scarring and inflammation. Systemic therapy offers an additional route for antibiotics to reach deep tissue sites. Combination of multiple intramuscular injections together with intramammary infusions has resulted in much higher tissue antibiotic concentrations. It was demonstrated that this combination therapy was more effective in curing chronic *S. aureus* infections than intramammary infusion alone during lactation (8). In one study, 49 cows with 78 subclinically infected quarters were tested. One group of cows received intramammary infusion at each milking for six milkings with a lactating cow product containing 62.5 mg amoxicillin. Another group of cows received the same intramammary infusion regimen plus one intramuscular injection daily of 9 million units procaine penicillin G for 3 days. The combination of intramammary and intramuscular treatment cured 51% of quarters (48% of cows) compared with 25% of quarters (30% of cows) for intramammary infusion alone. Thus, combination therapy was twice as effective as conventional infusion alone by allowing more antibiotic to penetrate deep areas of infection, increasing the cure rate.

Therapy at drying off has become common practice as a standard component of recommended mastitis control programs. However, insertion of conventional mastitis tube cannulas can result in temporary dilation of the teat sphincter muscle, and the keratin plug that normally occludes the teat duct is sometimes removed. This may allow the entrance of bacteria, causing either a new infection or an additional one to the infection being treated. Novel infusion cannulas have been developed to reduce the introduction of organisms via local treatment procedures. Alternatively, systemic therapy at drying off does not risk infection with organisms introduced via the teat duct during infusion. Antibiotic classes such as fluoroquinolones are well distributed in body fluids, have a long half-life, and kill phagocytosed bacteria. Soback *et al.* (11) compared the subcutaneous injection of the fluoroquinolone norfloxacin nicotinate (10 mg/kg), the intramuscular injection of oxytetracycline-HCl (20 mg/kg), the infusion of 500 mg cephapirin benzathine, and an untreated control at drying off. The numbers of existing *S. aureus* infections were reduced only in the norfloxacin nicotinate group, and new infection rate appeared lower using systemic treatment. Overall, results indicated that use of the fluoroquinolone was more effective than the other treatments. Systemic use of macrolides is also being tested as a dry cow therapeutic because of the affinity of this class of antibiotic for mammary tissues.

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SUMMARY

Several approaches for increasing the specific and nonspecific defenses of the udder appear to have merit in the area of mastitis control for further reducing the prevalence of infection in dairy cows. Mastitis vaccines have been available, but generally have not been recommended; however, recent formulations have improved efficacy, especially against clinical coliform mastitis. Use of cytokines for enhancing udder immunity is purely experimental, but evidence suggests a role for these proteins during critical stages of the lactation cycle. Dietary supplementation with various micronutrients has also proved beneficial, and additional nutrients may be found to augment udder immunity in the future. Because mastitis in young dairy animals is now being recognized, future herd mastitis control measures should include breeding age and pregnant heifers. Although antibiotics have played an important role in dry cow therapy, use during lactation has yielded less than positive results. Future treatment regimens should be geared toward maintaining higher drug levels in milk and mammary tissue for a sufficient period of time to eliminate infection without risk of residues.