A Novel Strategy for the Prevention of *Staphylococcus aureus*-Induced Mastitis in Dairy Cows

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**Introduction**

Mastitis is an inflammation of the mammary gland that most frequently develops in cows in response to intramammary bacterial infection. Mastitis remains one of the most prevalent and costly production diseases in dairy herds worldwide. In the U.S., economic losses associated with mastitis are estimated to approach $2 billion annually. These losses are primarily attributable to decreased milk production, however, other contributing factors include veterinary costs, replacement costs for culled animals, and loss of premium payments due to increased milk somatic cell counts. Although management practices can reduce the incidence of mastitis, the ubiquitous nature of mastitis pathogens in the cow’s environment precludes complete prevention through good management alone. Selective breeding, which has the potential to enhance certain traits associated with production, is of limited use in preventing mastitis due to the low heritability of the disease. Thus, under current conditions intramammary infections and the development of mastitis remain inevitable. Antimicrobial therapeutics for treating mastitis remain limited and are often sub-optimal. The development of new strategies for the prevention and/or treatment of mastitis should continue to be a high priority among animal health initiatives.

*Staphylococcus aureus*-induced mastitis

*Staphylococcus aureus* are one of the most prevalent bacteria that cause mastitis and are responsible for ~25-30% of all intramammary infections. Mastitis caused by *S. aureus* is most often subclinical, however, a substantial incidence rate of clinical mastitis is associated with this pathogen. *S. aureus* is regarded as a contagious mastitis pathogen because it is commonly spread from infected to non-infected cows at milking. Although the main reservoir of these bacteria is infected udders, *S. aureus* have been recovered from surfaces all over the cow and can be readily disseminated from animal caretakers who are carriers of this pathogen. Following penetration of the teat canal, these bacteria release a variety of toxins and products that are injurious to the milk-producing cells of the mammary gland and impair the gland’s immune defense mechanisms. The intramammary formation of abscesses around these bacteria and their capacity to reside intracellularly contribute to the ability of *S. aureus* to establish a chronic infection that can persist for the life of the animal. The result of establishment of a subclinical, chronic intramammary infection is long-term decreased milk production that often goes undetected.

Effective strategies for the prevention and treatment of mastitis caused by *S. aureus* remain elusive. Vaccines to prevent the establishment of intramammary infection by this pathogen have been around for decades, however, their efficacy has been limited. Their limited effectiveness may be due, in part, to improper immunization schedules, ineffective adjuvant formulation, and their inability to cross-protect against various strains. Since multiple strains can be present within any one herd or even within an individual cow, vaccines or other strategies with less restrictive strain specificity will be required to decrease the incidence of mastitis caused by *S. aureus*.

Once established, *S. aureus* infections are difficult to treat. These bacteria are able to reside intracellularly and are shed periodically into the milk. Their residence inside of the cells within the gland and the formation of abscesses around foci of infection restrict their contact with administered antibiotics. The percentage of *S. aureus*-infected animals that can be cured during lactation with currently approved antibiotics is only between 10 – 30%. In a recent study of the efficacy of pirlimycin, a common antibiotic used in the treatment of these infections, only 13% of *S. aureus* intramammary infections were cured following the recommended two-day therapy. Extending the therapy to five consecutive days increased the cure rate to only 31%, a level that is below the break-even point at which costs incurred through treatment are balanced by increased production and the premium pay associated with milk containing lower numbers of milk somatic cells. Thus, treatment options for lactating cows infected with *S. aureus* remain suboptimal.

**Lysostaphin treatment of *S. aureus* intramammary infections**

Lysostaphin is a proteolytic enzyme produced by *Staphylococcus simulans* that cleaves specific bonds found in the peptidoglycan cell wall component of *S. aureus*. Due to its potent lytic activity towards this pathogen, lysostaphin’s ability to kill *S. aureus* has been evaluated in several animal disease models. In a model of endocarditis in rabbits, lysostaphin was
more effective than vancomycin at reducing valve vegetation bacterial counts. Further, lysostaphin has been reported to be effective in the treatment of eye infections caused by *S. aureus*. With the advent of antibiotic-resistant bacteria, another key finding of lysostaphin’s ability to kill *S. aureus* is its equivalent lytic activity toward both methicillin-resistant and sensitive strains.

The efficacy of lysostaphin for the treatment of *S. aureus*-induced mastitis has also been evaluated in a variety of animal models, including mice, goats, and cows. Infusion of lysostaphin into the *S. aureus*-infected glands of mice was shown to significantly reduce the number of viable bacteria. Lysostaphin has also been demonstrated to have a cure rate of ~20% when used to treat *S. aureus* intramammary infections in lactating cows. Although this rate is comparable to commonly used antibiotics, lysostaphin’s targeted specificity and low toxicity may make its use more advantageous. Further studies evaluating formulation, dosing, and treatment durations will be needed to determine whether its efficacy as an intramammary infusate can be enhanced.

**A novel strategy for prevention of *S. aureus*-induced mastitis**

Novel research with transgenic animals has revealed an efficacious strategy for the use of lysostaphin in the prevention of mastitis caused by *S. aureus*. Initial research in this area was conducted using a mouse model of mastitis. Researchers established that the engineering of mice to express and secrete lysostaphin into milk conferred resistance against *S. aureus* intramammary infection. The protection conferred was dependent upon the level of expression with the highest expressing line demonstrating complete resistance. After establishing the potential of this strategy in a mouse model of mastitis, the next logical step was to test this proof of concept in the actual economically relevant animal, the dairy cow.

In the first study of its kind to explore the potential of genetic engineering to enhance disease resistance in cattle, researchers from the USDA’s Agricultural Research Service developed transgenic cattle that expressed lysostaphin in the mammary gland. Using a system that restricted expression to the mammary secretory epithelium, the scientists were able to develop cows that secreted lysostaphin directly into the milk of the gland. Milk levels of lysostaphin varied between the transgenic animals and ranged from 0.9 to 14 µg/ml. Of the mammary glands of three transgenic cows that were infused with *S. aureus*, only 14% became infected. In comparison, 71% of the quarters challenged in the control animals became infected. Even the transgenic cow expressing the least amount of lysostaphin (*i.e.*, 0.9 µg/ml) had an infection rate of only 33%. The highest expressing cow of the three transgenic cows infused with *S. aureus*, who expressed 11 µg/ml of lysostaphin in her milk, was completely resistant to infection. The concentration of lysostaphin in the milk of the transgenic cows remained fairly consistent throughout lactation. The consistent level of expression conferred resistance throughout lactation as the highest expressing cow maintained complete resistance to multiple challenges throughout this period.

The data presented by these researchers suggest that lysostaphin expression in the gland prevents the establishment of infection. The authors monitored cows for both a febrile response and for the induction of acute phase protein synthesis, the latter of which is a sensitive marker of infection. In addition to these systemic indicators, individual quarters were monitored for changes in milk somatic cell counts, which during mastitis are primarily composed of white blood cells that play a role in combating infection. As expected, control cows that developed established *S. aureus* infections had increased body temperatures, elevated levels of circulating acute phase proteins, and increased milk somatic cells counts. In contrast, the transgenic animals demonstrated none of these signs of inflammation. These data suggest that lysostaphin prevents infection through its bactericidal properties and, thus, prevents the onset of inflammation.

Transgenic expression of antibiotic appears to have great promise when compared with traditional antibiotic therapy or intramammary infusion of lysostaphin. The key difference between these approaches is that one prevents the establishment of infection, whereas, the other is used to cure already established infections. It may be that once an *S. aureus* infection becomes established, it may be too late to intervene. Thus, prevention of infection, perhaps using a transgenic approach as demonstrated by these researchers, may be the more effective way to address this mastitis problem.

Clearly there are public concerns regarding the use of transgenic animals for food production. However, the approach demonstrated by the authors had little effect on milk composition. Further, others have demonstrated that lysostaphin has a low immunogenicity, thus, it remains unlikely to generate an allergic response if consumed. Finally, in an age of
considerable concern regarding the development of antibiotic resistance, alternatives that can minimize their use in food-borne animals, such as in the treatment of mastitis, require further investigation.

References

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