Development of Mastitis in a Mouse Infection Model from Four Bovine Isolates: A Nutrigenomics Study

A. Rowson[1], *DVM;* **E. Aalseth**[2], *DVM, PhD;* **Y. Wang**[1], *PhD;* **N.E. Forsberg**[1], *PhD;* **S.B. Puntenney**[1], *PhD*
[1]*OmniGen Research, Immunology, Corvallis, OR*
[2]*Dairy Health Consulting, Lake Stevens, WA*

Introduction

The goal of this study was to evaluate the ability of a commercially-available feed supplement (OmniGen-AF) to reduce development of mastitis in a mouse mastitis model. The rationale for completing this study was two-fold. First, dairy producers have reported that OmniGen-AF reduced somatic cell counts and reduced mastitis after the introduction of the product into herds. Second, recent studies have shown that the product increased markers of both innate and adaptive immunity. Our hypothesis, therefore, was that feeding the product would reduce the development and severity of mastitis in a mouse model. To assess this hypothesis, five experiments were completed with three bovine mastitis isolates. The isolates included *Streptococcus uberis (S. uberis)*, *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)*.

Materials and Methods

Each of the five studies was completed in a similar manner. Timed pregnant CD1 mice (n=24) were received from Charles River laboratories on Day 15 of gestation. Mice were assigned (two per cage) to one of three treatments: 1) control fed with sterile PBS infusion, 2) control-fed with pathogen infusion and 3) OmniGen-AF-fed with pathogen infusion. Control feeding consisted of *ad libitum* Teklad 8604. OmniGen-AF feeding consisted of this same diet supplemented with 0.5% (w/w) OmniGen-AF. Pathogen infusion consisted of infusion of 100 cfu of pathogen into the L4 and R4 teat canals. To accomplish infusion, baby mice were euthanized on Day 10 of lactation after which mothers were anesthetized with ketamine and xylazine. Their abdomen was sterilized and the terminal 0.5 mm of the L4 and R4 teats were removed. Pathogen was infused 3 mm into the teat canal with a blunted 33 gauge needle. Infection was allowed to progress until signs of inflammation were obvious (24-48 hr) after which mice were anesthetized, blood was drawn via cardiac puncture and mammary tissue was taken for analysis of pathogen DNA concentration. Concentrations of pathogen DNA were assessed using quantitative PCR and a standard curve was developed which enabled the calculation of CFU-equivalents of pathogen within mammary tissue and blood. *S. uberis* infection was assessed in Experiment 1. *E. coli* infection was assessed in Experiments 2, 3 and 5. *S. aureus* infection was assessed in Experiment 4. Data from individual studies and from the combined studies 2, 3 and 5 were assessed by ANOVA. Where differences ($P<0.05$) were revealed, a subsequent LSD multiple range test was used to evaluate differences ($P<0.05$) between individual treatment means.

Results

Infusion of mouse mammary glands with bovine isolates of *S. uberis, E. coli* and *S. aureus* caused mammary infections to develop over the next 24-48 hours. Signs of infection included swelling and reddening of the glands and behavioral changes in the mice (lethargy and depression). With all pathogens, high concentrations of pathogen DNA were detected in mammary tissues at these times. Lower concentrations of pathogen DNA were detected in blood. For *S. uberis* and *S. aureus*, feeding of 0.5% OmniGen-AF reduced infection of the gland by over 90% ($P<0.05$). OmniGen-AF did not cause a significant reduction ($P>0.05$) in E. coli DNA levels in mammary tissue in any of the three studies; however, in each, mammary *E. coli* DNA was numerically reduced by approximately 60-90% ($P>0.05$). However, analysis of Experiments 2, 3 and 5 with individual experiments serving as blocks revealed that OmniGen-AF reduced ($P<0.05$) *E. coli* infection of the mouse mammary gland.

Significance

This research provides support for the hypothesis that the feeding of OmniGen-AF reduces mastitis in a murine model. These observations support field reports that indicate that the product reduces somatic cells and mastitis on commercial dairies and provides evidence that molecular changes in markers of immunity (reported elsewhere) bring about meaningful changes in the immune function. Further research is needed using the primary species (dairy cows) to substantiate these results.