

Vaccination with *Streptococcus uberis* Adhesion Molecule Induces Isotypic Antibody Responses in Bovine Serum and Colostrum

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Introduction

Despite implementation of mastitis control practices that have significantly reduced the incidence of contagious mastitis, recent studies have shown that as the prevalence of contagious mastitis pathogens decreased, the incidence of environmental mastitis pathogens increased. *Streptococcus uberis* is one of the most commonly isolated environmental pathogens, infecting mammary glands when conditions are favorable. During the last decade, our research efforts have concentrated on understanding the mechanisms utilized by *S. uberis* to invade the mammary gland. Using in vitro models we have identified and partially characterized *S. uberis* adhesion molecule (SUAM). We demonstrated that SUAM was involved in adherence to, internalization into, and persistence of *S. uberis* in bovine mammary epithelial cells and may be an excellent target for vaccine development. The objective of this study was to evaluate isotypic antibody responses of cattle vaccinated with recombinant SUAM (rSUAM) in serum and colostrum.

Materials and Methods

Antigen preparation and vaccination trial: The SUA gene was amplified by PCR from genomic DNA of *S. uberis* UT888, and the product was sub-cloned into the expression vector pBAD. Following expression, rSUAM was affinity purified using a His-tag affinity column and the concentration determined by BCA assay. Thirty uninfected primiparous dairy cows in late lactation were divided randomly into three groups of 10 cows each: A = control, B = 200 ug rSUAM, and C = 400 ug rSUAM. Cows in groups B and C received an emulsion containing adjuvant, phosphate-buffered saline (PBS) and affinity purified rSUAM at 200 or 400 ug per injection. Cows in group A received an emulsion containing adjuvant and PBS. Cows were vaccinated subcutaneously in the neck region at drying off (day-0), 28 d after drying off (day+28) and within 7 days after calving. Serum samples were collected at D-0, D+28, calving (C-0), calving vaccination (CV), during early lactation and CV+14. Colostrum was collected at calving. ELISA: Flat bottom plates were coated with rSUAM at

a concentration of 50 ng/well in PBS at 4°C overnight. Plates were blocked with PBST+Gelatin (PBSTG). Primary antibodies were diluted 1:8000 for serum and 1:800 for colostrum and incubated for one hour at 37°C. The secondary antibody consisted of peroxidase-conjugated goat anti-bovine IgG for total IgG or sheep anti-bovine IgG1 or IgG2 for isotypes. Western blotting: rSUAM was subjected to electrophoresis and transferred onto nitrocellulose membranes. Membranes were blocked with Tris Saline-Tween20-gelatin (TSGT) for one hour and probed with primary antibodies (diluted 1:800 for colostrum and 1:50 for serum). The secondary antibody consisted of horseradish peroxidase-conjugated goat anti-bovine IgG (H+L specific) diluted 1:5000 in Tris-saline-tween-gelatin. The color-reaction was developed in fresh HRP-color developer (Biorad) containing 4-chloro-1-naphthol and 30% hydrogen peroxide. Following development, blots were washed in double distilled water and air-dried. No blocking step or gelatin was used for colostrum WB's.

Results

We utilized ELISA and western blotting to measure total IgG, and/or IgG1 and IgG2 responses following vaccination with three doses of rSUAM or a placebo. We detected significant increases in serum IgG1 and IgG2 antibodies of vaccinated cattle against rSUAM when compared to the control group over time. In addition, we found antibodies against rSUAM in the colostrum of vaccinated cattle but not in the control group. Results from these experiments suggest that: 1) rSUAM effectively induced an immune response in vaccinated cows, 2) anti-rSUAM antibodies were detected in serum and colostrum of vaccinated cows, and 3) rSUAM induced IgG1 and IgG2 isotypes in the serum of vaccinated cows.

Significance

In conclusion, rSUAM appears to be a good immunogen and should be considered further as a potential vaccine candidate for the prevention and control of *S. uberis* mastitis.