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Application of a mouse-mastitis model for evaluation of antigen vaccines against *Staphylococcus aureus*-induced mastitis

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Introduction

Mastitis is the most common infectious disease of dairy cattle. Bacteria such as *Staphylococcus aureus*, streptococci, *Escherichia coli* and some others cause mastitis. *S. aureus* is the most frequent cause (*Hodges et al, 1984*), but the mechanisms by which *S. aureus* causes mastitis are not well known. However, increased virulence of *S. aureus* has been associated with increased development of the disease (*Jonsson et al, 1985*; *Bramley et al. 1989*). Specific components (e.g. proteins or carbohydrates) present on the *S. aureus* surface endow the organism with specific virulence properties. For instance, the polysaccharide capsule may protect the organism from phagocytosis (*Wilkinson 1958*) and immunity directed towards polysaccharide capsular antigens has been shown to be protective to the host (*Lee et al. 1989; Fattom et al. 1990*). Fibronectin-binding proteins (FnBPs) aid adhesion of the organisms to the mammary gland epithelium and thereby, promote initiation of the disease (*Mamo et al. 1988, Mamo et al. 1991*). Furthermore, antibodies against FnBPs were also found to be protective (*Luk et al. 1989; Schennings et al. 1993; Mamo et al. 1995*).

Antibiotic therapy has contributed to the reduction of mastitis, but often ineffective, particularly against *S. aureus*. The reason for this is still unclear, although resistance to phagocytosis or, in contrast, intracellular survival of the bacteria in phagocytic cells are postulated as important factors (*Anderson 1978; Paape et al. 1981*). Despite a great deal of research, improvement of the resistance of the host to mastitis remains elusive. Application of specific immune strategies has been necessary and several researchers have been engaged in developing vaccines against staphylococcal mastitis (*Watson 1988*; *Foster 1991; Rainard et al. 1991*). Animal models have been used both for studying the pathogenesis of mastitis and for the evaluation of therapeutic drugs or prophylactic vaccines against mastitis. The aim of this study is to demonstrate that BSVS mouse strain used as a model for mastitis is also a relevant model for evaluation of antigen vaccines against *S. aureus*-induced mastitis.

Materials and Methods

Animals

Female BSVS (Bacterial sensitive virus sensitive) mice, inbred strain (*Webster 1937*), maintained by random mating at the National Veterinary Institute, Uppsala, Sweden, 10-12 weeks of age, weighing 25-30gms were used for both vaccination and induction of experimental mastitis. Mice were kept grouped (5-6) in respective type of Macrolone cages in a conventional environment 22-24°C room temperature, fed with commercial rodent diet and tap water *ad libitum*.

Lactating BSVS mice have relatively large teats which enables the administration of bacteria *via* the teat canal (intramammary) into the mammary glands (*Chandler 1970*). Moreover, gross and microscopic changes appearing in the mammary glands post-challenge could be examined easily.

Vaccines

Bacterial surface antigens (proteins) isolated from *S. aureus* cell surface purified, characterised and prepared as recombinants were used to vaccinate the mice. The proteins were fibronectin-binding protein (FnBP), (*Fröman et al. 1987*), fibrinogen-binding protein (FgBP), (*Bodén et al. 1989*); collagen-binding protein (CnBP), (*Switalski et al. 1989*) and α-toxoid (*Adlam et al. 1977*). Methods of purification, characterisation and preparation as recombinants have been described elsewhere (*Adlam et al. 1977; Fröman et al. 1987; Switalski et al. 1989; Jönsson et al. 1991; Bodén et al. 1992; Patti et al. 1993; Mamo et al. 1994a; Mamo et al. 1994b).*

Vaccination procedures

Two days after mating mice were vaccinated subcutaneously in the neck region with 20-22mg of the antigen emulsified with complete Freund's adjuvant (primary vaccination). Control mice were injected with only adjuvants. Booster doses were given after 14 days with the same amount of immunogen, but emulsified with incomplete Freund's adjuvant. Injection sites were observed for any abscess formation due to adjuvants.

Induction of mastitis in mice/Challenge

S. aureus strains have been used to induce mastitis in mice (*Jonsson et al. 1985*). Bacteria were cultured at 37°C for 18hr in tryptic soy broth (Difco). The bacteria were washed in PBS, pH 7.4. The size of the inoculum varied from 100-250µl bacterial suspension containing 10^5 c.f.u./ml in a single dose. Animals in control groups received 100-250µl sterile saline. Bacteria were administered following the first pregnancy at days 10-15 of lactation when the teats were at their largest. The offspring were removed from their mothers and caged separately 2h before inoculation. Prior to inoculation mice were anaesthetised and thereafter the teat tips (1-2mm) aseptically removed with a scissors in order to allow an easy insertion of the inoculating needle into the teat canal. Bacteria were inoculated into the left 4th (L-4) and the right 4th (R-4) mammary glands of mice. The clinical condition of challenged mice was frequently observed for 48 hours before euthanasia.

Detection of antibodies in the sera

Mice were bled (1ml) by tail vein puncture on the day of the first vaccination and after 4 weeks. Serum was prepared and the production of antibodies in the serum was quantified using a conventional ELISA.

Bacterial recovery

Forty-eight hours post-challenge, mice were killed by cervical dislocation, dissected and challenged mammary glands (L-4 and R-4) were removed to evaluate the histological changes and for quantification of bacteria. The L-4 glands were homogenised in physiological saline and serially diluted suspensions were plated on semi-dried blood agar. Following incubation at 37°C for 18hrs colonies identified as *S. aureus* are counted and the number of colony forming unit (c.f.u.) per gland was determined.

Histopathological examination of mammary glands

Hardness, colour changes of the mammary glands tissue, severe necrosis, etc. were visualised under a stereo-microscope (40X magnification). Type of lesions, vacuolisation, infiltration of inflammatory cells, etc. were determined on the thin-sectioned and Haematoxylin-Eosin (HE) stained tissues under a light microscope (40X magnification) according to Haraldsson et al. (1984). A 0-4 grade scoring system, which is based on the development of the lesions and infiltration of the inflammatory cells in the glands was applied. Grade 4 = non-reactive total necrosis; grade 3 = advanced regressive changes combined with mild inflammatory reaction; grade 2 = disseminated inflammatory reaction; grade 1 = disseminated inflammatory reaction combined with focal necrosis and grade 0 = pathological reaction.

Results

Results from hopathological eamination showed that most of mammary gland tissues from mice

vaccinated with FnBP, FgBP or CnBP + α-toxoid and challenged with *S. aureus* showed a lower grade lesion (grade 0-1) with most tissues histologically normal (Fig. 2). Tissues from control mice showed in most cases severe lesions/necrosis with severe inflammatory reaction (grade 4) (Fig. 1). A remarkably lower number of bacteria (10^4 - 10^5 c.f.u.) were recovered from mammary glands of mice vaccinated with FnBP, CnBP + α-toxoid or FgBP compared to number of bacteria recovered from control animals mammary glands (10^8-10^9 c.f.u.) (Fig. 3).

The antibody responses in the serum of mice vaccinated with FnBPs were much more raised compared to controls (Fig. 4).

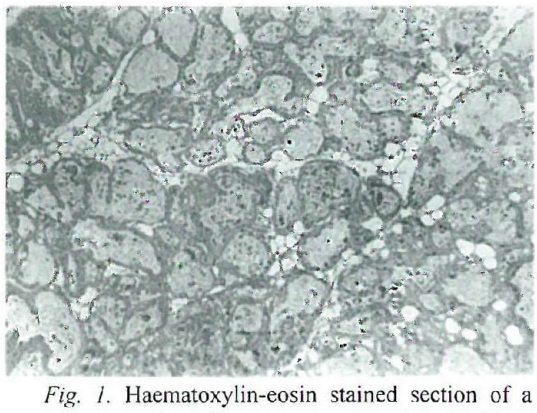

Fig. 1. Haematoxylin-eosin stained section of a mammary gland from a mouse inoculated with S. aureus (105 c.f.u./ml), 24 hr post-inoculation. It shows a total necrosis (grade 4). Note: vacuolation and damage of the secretory epithelium.

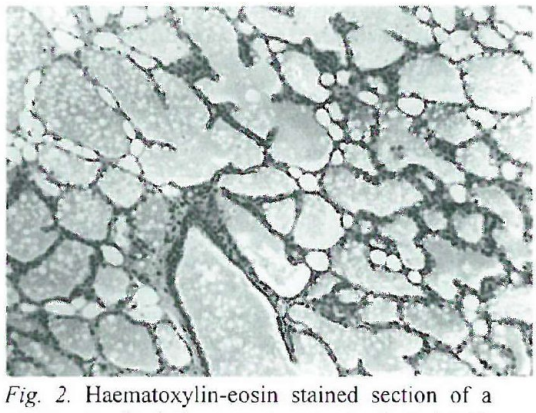

Fig. 2. Haematoxylin-eosin stained section of a mammary gland from a mouse vaccinated with FnBP and inoculated with *S. aureus* (10^5 c.f.u./ml), 24 hr post-inoculation. It shows a normal appearance (grade 0).

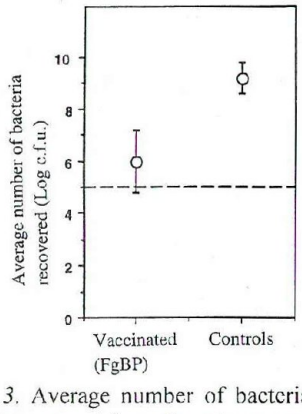

Fig. 3. Average number of bacteria (log CFU ± S.D.) recovered from the mammary glands of mice vaccinated with FgBP (n=10) and controls (n=5). The horizontal line indicates the number of bacteria with which the mice were challenged (*Mamo, et al. 1994b*).

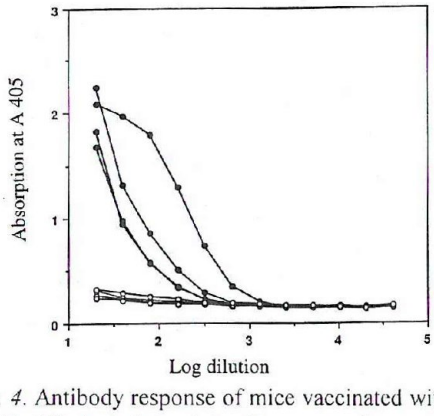

Fig. 4. Antibody response of mice vaccinated with FnBP (filled circles, •) (n=4) compared to non-vaccinated (empty circles, o) (n=4) compared by ELISA (*Mamo, et al. 1994a*). Absorption values at 405nm are plotted as a function of serum dilution.

Discussion

Infection by *Staphylococcus aureus* is the most frequent cause of intramammary infection (masitis) in ruminants. Despite extensive research, improvement of the resistance of the host to mastitis remains elusive. Application of specific immune strategies has been necessary and several research projects have been aimed at developing vaccines against staphylococcal mastitis. Therefore, animal models are needed both for studying the pathogenesis of mastitis and for the evaluation of therapeutic drugs.

In this study protection has been defined as the lack of mastitis among vaccinated and challenged groups of mice compared with the control group. The number of typical and atypical lesions developed in the glands of vaccinated versus control groups of mice were compared. In most cases mice vaccinated with FnBPs and FgBPs were found to be clinically healthy. Bacterial recovery from the mammary gland tissues of the vaccinated mice was much lower compared to controls (Fig. 3). Mammary gland tissue from vaccinated mice were found to be relatively normal (Fig. 2). Lesions developed in the glands of vaccinated mice were insignificant compared to the severe lesions developed in the glands of control mice (Fig. 1). A serum antibody response was detected in the sera of mice vaccinated with FnBP compared to controls (Fig. 4). According to the above-described characteristics, BSVS mice vaccinated with FnBp, FgBP or CnBP + α-toxoid (*Mamo et al. 1998, unpublished*) were relavely protected against *S. aureus* infection and the efficacy of these different vaccines could be also compared.

This study demonstrates the relevance of the BSVS mouse mastitis model for evaluation of the protective potentials of antigen vaccines against *S. aureus*-induced mastitis.

Summary

The BSVS-mouse mastitis model has been shown to largely mimic boyine intramammary infection (mastitis), where bacteria penetrate through the teat canal, teat cisterns and establish in the mammary glands. The model enables study of the onset of infection and histopathological changes occurring during the development of infection in the mammary glands. Mice were immunised with recombinant antigen vaccines and thereafter were challenged with *S. aureus*. Vaccine-evoked serum-antibody responses and protection were determined. This study demonstrates the relevance of the BSVS-mouse mastitis model for evaluation of recombinant surface-antigen vaccines against *S. aureus*-induced mastitis.

Acknowledgement

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