Immunity to Mastitis. A Review

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Introduction

Several reviews of early studies of immunity to mastitis have been compiled, but lack of controlled experiments and adequate identification of antigens used in vaccination tend to make these first reports of only historical interest. To comprehend the problems and difficulties involved in attaining an immune state in the bovine against the various forms of mastitis, the following factors should be understood:

1. Mastitis is caused by several groups of pathogens—staphylococci, streptococci, coliforms, and several others of lesser importance. In addition, several of these groups have a very large number of species and types within species. For example, the streptococci that cause mastitis are largely of Lancefield Group B and to a lesser extent Groups C and E. Group B contains only Streptococcus agalactiae; however, there are at least five types (41) of this species. The staphylococci are considerably more complicated, having many more strains and types. None of these appear to give cross immunity; thus, we have a very large number of antigens involved in this problem.

2. The mammary gland in a lactating cow contains a warm, rich nutrient medium for the growth of bacteria and it is evacuated twice a day directly to the outside. In effect, there is direct contact with the outside environment.

3. The ability of the bovine to produce antibodies in response to stimulation with many antigens is not as great as with most animals. Even when the level of antibody in the circulation is high, an adequate or effective level is not necessarily found in the milk. Immunoglobins (antibodies) are a family of proteins in the blood serum. They vary from a molecular weight of 160,000 (sedimentation coefficient of 7S) to over 1,000,000 (19S). Milk secreted during normal lactation contains a low level of immunoglobins. The same types of immunoglobulins in bovine serum (IgG, IgM, and IgA) have also been found in mammary secretions; however, the proportional amount varied with the character of the secretion (23). Dixon (10) and MacKenzie among others (20, 21) have shown with radioactively labelled serum immunoglobulins that these proteins pass into the milk directly from the blood serum; however, their level in the milk is very low. The concentration of immunoglobulin in the milk increases appreciably with infection or in the otherwise irritated udder, indicating that permeability of the glandular epithelium changes under these conditions. The infection is often well established at this time and the increased amount of antibody is probably ineffective.

While these factors point out some of the special difficulties involved in immunization of the bovine against mastitis, it must not be interpreted that progress has not been made in this approach to mastitis control. Most progress has been limited to research studies; however, general field application is probably to follow.

Immunity in the Bovine as It Applies to the Lactating Mammary Gland

To effectively immunize against mastitis, the cow must have antibodies at or near the site of infection, i.e., the udder, and probably effective antibodies can only be those in the milk. Immunoglobins (antibodies) are a family of proteins in the blood serum. They vary from a molecular weight of 160,000 (sedimentation coefficient of 7S) to over 1,000,000 (19S). Milk secreted during normal lactation contains a low level of immunoglobins. The same types of immunoglobulins in bovine serum (IgG, IgM, and IgA) have also been found in mammary secretions; however, the proportional amount varied with the character of the secretion (23). Dixon (10) and MacKenzie among others (20, 21) have shown with radioactively labelled serum immunoglobulins that these proteins pass into the milk directly from the blood serum; however, their level in the milk is very low. The concentration of immunoglobulin in the milk increases appreciably with infection or in the otherwise irritated udder, indicating that permeability of the glandular epithelium changes under these conditions. The infection is often well established at this time and the increased amount of antibody is probably ineffective.

The work of MacKenzie et al. (21) with radioactively labeled immunoglobulins used human serum proteins. The relevance of their results of the selective transfer of proteins into the milk is clouded with heterologous species of immunoglobulins (human) and their use of the lactating ewe rather than the bovine.

Many studies of local immunity in the bovine mammary gland have been reported in the literature. Most of them, if not all, are concerned with measuring antibody in the milk or colostrum after infusion of various antigenic preparations into the udder. Such a method of
stimulation is without exception extremely irritating to delicate tissues of the udder and for this reason is impractical and useless for field application, although it has demonstrated that local antibody production does result from intrammary infusion of antigens. Outteridge and Lasselles (27) have shown that the infusion of staphylococcal toxoid or toxoided α-hemolysin into the quarters of ewes during the dry period resulted in a higher colostrum and milk titer in the infused quarters than in normal quarters. This local production of antibody did not continue after the first week postpartum. Numerous other workers have published reports indicating that local stimulation of antibodies in the mammary gland is possible. Kerr (16) infused *Brucella abortus*, *Salmonella pullorum*, and *Trichomonas foetus* and concluded that specific antibodies were produced within the gland. In addition, Porterfield et al. (30), Mitchell (22), and Smith (35) as well as others have demonstrated intramammary production of antibodies to various antigens, including several viruses; however, these studies were for purposes other than developing immunity to mastitis and are relevant only in that they establish that the udder is capable of immune activity. Additional evidence for the ability of the mammary gland to function immunologically comes in the form of anatomical studies. Hampl (14, 15) has described both the macro and micro anatomy of the udder, demonstrating the size, shape, number and position of intramammary lymph nodes and their histological appearance. Thus, there is evidence for the presence of cells presumably capable of immune activity in the tissues in the udder and surrounding tissues.

Several workers (32) have indicated that mastitis caused by the staphylococci is probably our major problem in control of this disease. Their reasoning has been that the other group of important mastitis pathogens, i.e., the streptococci, can be largely controlled using antibiotics. While it is certainly true that many streptococci are susceptible to antibiotics, the essentially unchanged incidence of streptococcal mastitis over the past ten years is indicative of the value of continuing research on each of the major genera of bovine pathogens. Table 1 contains data extracted from the annual reports of the New York State Mastitis Control Program (24). These data indicate that by bacteriological culture of each quarter-milk sample nearly half the cow population is infected in at least one quarter. The same data for the last three years, on a per-cow basis, are shown in Table 2. While these figures indicate that little has been accomplished by the use of antibiotics and improved management, they are biased, in that given the opportunity the New York State Control Program can and does free many herds completely of *S. agalactiae*. In 1968 (31), 855 herds, representing 41,407 cows, were freed of this pathogen. The percentage of mastitis-free cows then rose to 53.4%; the incidence of hemolytic staphylococci was 17.9%, nonagalactiae streptococci 34.9%, and miscellaneous pathogens 4.5%. Most improvement by antibiotic treatment of infective udders is probably better demonstrated by using clinical

<table>
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<tr>
<th>Year</th>
<th>No. of cows</th>
<th>Negatives a</th>
<th><em>Streptococcus agalactiae</em></th>
<th>Hemolytic staphylococci</th>
<th>Nonagalactiae streptococci</th>
<th>Misc.</th>
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</tr>
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</table>

a Per cent of total number of cows.

b Per cent of quarters infected by each pathogen.

c Nonagalactiae streptococci include all streptococci other than *Streptococcus agalactiae*.  

**Table 1.** Relative incidence of the various mastitis pathogens in New York State during the last ten years.
TABLE 2. Relative incidence of the various mastitis pathogens in New York State during the three years beginning 1966.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cows</th>
<th>Negativesa</th>
<th>Streptococcus agalactiae</th>
<th>Hemolytic staphylococci</th>
<th>Non-agalactiae streptococci</th>
<th>Misc.</th>
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</tbody>
</table>

*a Based on total number of infected cows. Results are given as percentage of total number of cows infected with each group of pathogen.

Initial surveys

Staphylococcal Mastitis

The literature contains numerous reports of immunization studies of cattle against staphylococcal mastitis. Few have reported complete failure and none indicates complete success. Some of the difficulties in assessing the value of these preparations are:

1. The antigenic dissimilarity of the many strains of staphylococci that cause mastitis. Bacteriophage typing of bovine staphylococci has been extensively studied by several workers, and among them Davidson (7) found 70 different phage patterns among the cultures from the Weybridge herd at the Ministry of Agriculture Central Veterinary Laboratory, Weybridge, England. However, only 13 were commonly occurring mastitis strains and the remaining 57 strains accounted for only 8.6% of the isolations. It has also been reported that although the total number of phage-typed strains of staphylococci is large and the isolates from different geographic locations have different phage types, perhaps within a more local area the number of types would be more restricted. Pargaonker et al. (28) found only one of 210 cultures from the United States with the same phage pattern as the predominant type from Canada. The relationship between phage types and immunological specificity has not been clarified. No attempt at typing with serological methods has been reported. Norcross and Stark (26) have listed the antigens from the staphylococci studied, to determine their immunizing capacity. Various workers have reported that preparations of the whole bacteria, cell-wall teichoic acids, toxoids and, possibly, coagulase have been useful for the stimulation of protective antibodies. If some of these antibodies are type-specific, as established by Derbyshire (8) and others, and if there are many types of staphylococci that cause mastitis, it is obvious that the problem of immunity against staphylococcal mastitis is a complex one. Brown (3) concluded after reviewing the literature that antitoxin (or antibody against staphylococcal a toxin) is active in neutralizing the toxin produced during mastitis. While this does not eliminate the infection, it is useful in lessening the severity of the tissue irritation. Slanetz et al. (34) have published extensive studies of cell-toxoid vaccines that have proved useful in preventing staphylococcal mastitis. Again, it appears that immunity is conferred against only a very limited number of the wide spectrum of staphylococci types. It becomes clear, then, that effective immunity can be achieved only if autogenous bacterin-toxoid preparations are used. This entails the determination of the strain or strains involved in each herd, the culture and inactivation of these strains, and preparation of a bacterin. This cell suspension when added to toxoid and adjuvant and inoculated into cows has been reported to produce increased resistance to udder infection (5, 29, 34). The protection extends only to the strains used in preparation of the vaccine and perhaps closely related strains.

Basic studies designed to determine what components of the staphylococcal cell are active in stimulating antibodies that will eliminate infection are essential to the eventual production of a suitable immunogen. With this informa-
tion the immunologist may determine how many different types of this antigen are present in large numbers of infections and perhaps produce a polyvalent antigen preparation. With a preparation such as this it is probably unnecessary to include antigens to stimulate antibodies against extracellular toxic products, since the infection could be eliminated before they are produced in high concentration.

Immunity to Streptococcal Mastitis

Mastitis caused by streptococci generally falls into the second most important group of mastitis pathogens. Three species are involved: *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. *S. agalactiae* is by far the most prevalent.

*Streptococcus agalactiae* belongs to Lancefield Group B on the basis of a group antigen composed of rhamnose, N-acetylgalactosamine, and galactose. It is a very common mastitis pathogen and has been found to be increasingly prevalent in humans, notably the female urinogenital tract and in newborn infants (2, 13). There are at least five types of *S. agalactiae*, differentiated serologically by a carbohydrate antigen (41). Early work was hampered by lack of knowledge of these antigens and their immunological importance. Currently, there is no preparation effective in producing immunity to this pathogen. Lancefield (18) in 1966 discussed two type-specific antigens that stimulated protective antibodies and Dodd (11) reported another; the latter was not determined to be type-specific and was a mucoprotein. Considerable progress has been made recently in immunity in streptococcal mastitis by the adaptation of a passive mouse protection test to the assay of protective antibodies in bovine milk and serum. With this test Norcross et al. (25) were able to compare in the bovine the results of immunization with certain antigenic preparations, as well as the effect of the route of inoculation, and the duration of immunity. Using a strain of *S. agalactiae* designated Cornell, Strain 50 (Type II) they reported that immunization of cows with inactivated bacterin resulted in protective antibodies in the milk and blood. They inoculated the bacterin in the area of the supramammary lymph nodes and found if only the right node was immunized, the protective antibodies will appear in the milk of the right mammary quarters first, followed somewhat later in the left side. In addition, challenge of immunized cows with the same strain of *S. agalactiae* demonstrated heightened resistance to this type. There was no resistance to heterologous types of *S. agalactiae*; however, if it is substantiated that there are not many more than five types of this organism it would not be unreasonable to prepare a bacterin that included all the type antigens. In later studies Norcross (25) immunized a cow with a polyvalent bacterin containing all five type-specific antigens and successfully demonstrated that there were protective antibodies to each type in the serum by the passive mouse protection test. The duration of heightened resistance has not been established.

*Streptococcus dysgalactiae*

An extensive amount of research has been reported throughout the past 30 years concerning the type of mastitis caused by *S. dysgalactiae*. These studies have been initiated despite a rather low incidence of herd infections, because of the extensive pathology associated with this microbe within the mammary gland (4, 12, 19). The natural habitat of *S. dysgalactiae*, unlike *S. agalactiae* which is essentially an exclusively mammary gland resident, includes not only the bovine udder but also the female reproductive tract and tonsils of cattle, where it has some pathogenic activity, and also includes several other domestic animals (19). Control programs have generally not been effective in reducing the incidence of infection with *S. dysgalactiae*.

*Streptococcus dysgalactiae* falls into Lancefield Group C on the basis of a carbohydrate antigen having a structure of rhamnose units with N-acetylgalactosamine terminals. The latter seem to play some role in determining the serological specificity (17, 33); however, the immunogenic properties have not been established. In addition, three type-specific antigens have been identified by bacterial agglutination and precipitation reactions. These were called IIa, IIb, and IIc, but some other strains have been reported to have antigens in common with all of these and were called group strains (1, 19). A carbohydrate antigen has been identified as capable of stimulating the production of protective antibodies (37, 39). Stark et al. (37–39) have reported a study with this antigen and correlated the presence of protective antibody as determined by the passive mouse protection test with complement fixation and hemagglutination tests. The latter was considered a sensitive method of assay of the immune state. It has not been established, as with *S. agalactiae*, that the antigen that determines group or type specificity also stimulates the production of protective antibodies. Both the IgG and IgM fractions of bovine anti-*S. dysgalactiae* sera have been shown to contain pro-
protective antibody when tested by the passive mouse protective test and direct challenger of immunized cows (38, 39).

**Streptococcus uberis**

The incidence of mastitis caused by *Streptococcus uberis* is greater than that of *S. dysgalactiae*; however, much less research into the immunity of the former has been reported. *S. uberis* is probably an obligatory parasite of the udder surface and this site serves as a source for secondary infections of the mammary parenchyma (40). It is often found inhabiting the duct and sinus of the mammary gland as a nonpathogen. As with the other streptococci, animals often carry *S. uberis* within the udder without showing clinical mastitis.

Although it is generally agreed that *S. uberis* is a member of Lancefield Group E, several workers have shown that many bacteria which culturally and chemically resemble this microbe do not contain a typical Group E antigen. One survey indicated that of 496 isolates identified by cultural means as *S. uberis* only 83 reacted with Group E antiserum (6). The Group E antigen has been identified biochemically as a carbohydrate composed of several sugars, mainly rhamnose and glucose and sometimes mannose (33). At least three types have been identified on the basis of serological typing, IIIa, IIIb, and IIIc, but more than 11 types are likely (19, 36).

Agglutinins have been detected against *S. uberis* in serum, milk, and dry udder secretions, but to date no extensive immunological or immunochemical studies have been performed with this organism.

**Discussion**

The literature contains many reports of the successful stimulation of local antibody production by intramammary injections of antigenic substances. However, the parenchyma of the mammary gland is extremely sensitive to infusion with any foreign substance and severe inflammation is a frequent sequel to infusion; even then, only after a number of such infusions is a significant immune response actually attained (38). A significant response is difficult to assess; somewhat better response results are obtained from infusion during the dry period.

On the other hand, as mentioned earlier, a certain degree of irritation within the udder has been found to increase this organ’s permeability to serum proteins, including immune globulins. Thus, the instillation of sterile salt solution will, in a cow with humoral antibodies, result in an increase in milk antibodies. It has reported that the mild irritation often caused by infection of the mammary gland by *S. uberis* may be an advantage for immune cattle, in that increased antibody content in milk has been shown to result from such stimulation (9).

The problem of control by immunological means will also be influenced by the ecology of the causative microbes. In *S. agalactiae*, for all practical purposes, if it is eliminated from the mammary gland and is not reintroduced from another gland infected with this same species, the animal should be free forevermore. On the other hand, the staphylococci and other streptococci are present and multiply in many other places on the bovine and other animals, as well as in the soil, and reintroduction is therefore much more likely.

Two areas of study are basic to the determination of whether an immune state can be induced in the bovine gland:

1. Are antibodies, even if they have been demonstrated to be protective, effective in nature and quantity to eliminate udder infection?
2. If antibodies in the udder are effective, can they be induced over an extended period?

These two questions can be answered only by extensive field trials using well-characterized immunogenic preparations.

Finally, it should be emphasized that immunogens must be evaluated as prophylactic agents and not on the basis of their therapeutic value (29). It should not be expected that immunization will eliminate established udder infections; at best, the immune state may prevent future invasion by the pathogens represented in the bacterin preparation.

**References**


