3-1988

Effects of Staphylococcus Aureus infection on bovine mammary gland plasma cell populations and immunoglobulin concentrations in milk

Mehmet Z. Doymaz

To the Graduate Council:

I am submitting herewith a thesis written by Mehmet Z. Doymaz entitled "Effects of Staphylococcus Aureus infection on bovine mammary gland plasma cell populations and immunoglobulin concentrations in milk." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

Stephen P. Oliver, Major Professor

We have read this thesis and recommend its acceptance:

J.P. Chen, F.M. Hopkins

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Mehmet Z. Doymaz entitled "Effects of Staphylococcus aureus Infection on Bovine Mammary Gland Plasma Cell Populations and Immunoglobulin Concentrations in Milk." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

Stephen P. Oliver, Major Professor

We have read this thesis
and recommend its acceptance

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's Degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of the source is made.

Permission for extensive quotations from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Mehmet Z. Dagnaz

Date Dec 10. 1987

EFFECTS OF *STAPHYLOCOCCUS AUREUS* INFECTION ON BOVINE MAMMARY GLAND PLASMA CELL POPULATIONS AND IMMUNOGLOBULIN CONCENTRATIONS IN MILK

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Mehmet Z. Doymaz

March 1988

ACKNOWLEDGEMENTS

I would like to express my most sincere thanks and gratitude to Dr. S. P. Oliver whose guidance, ecouragement, and patience made this study possible.

I would also like to thank Drs. J. P. Chen and F. M. Hopkins for serving as committee members and for their helpful advice.

I thank Dr. L. M. Sordillo-Gandy for her assistance in all aspects of this study.

And, to each and every taxpayer of my country.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

LITERATURE REVIEW

1. INTRODUCTION

Mastitis is defined as an inflammation of the mammary gland. Bovine mastitis is a complex disease because of its multifarious causes, and pathogenesis (Jain, 1979). Mastitis usually results from udder infection with one or more microorganisms (Bramley and Dodd, 1984). In the past, Staphylococcus aureus and Streptococcus agalactiae were responsible for the overwhelming majority of clinical cases of mastitis (Kowalski, 1977). Studies have shown that elimination of Strep. agalactiae from the herd is possible (McDonald, 1977). However, Staph. aureus infections are difficult to control (Buddle and Cooper, 1978). Eradication of Strep. agalactiae does not eliminate mastitis as a formidable problem. In the absence of these bacteria, others such as coagulase-positive and coagulase-negative staphylococci, Corynebacterium bovis, streptococci other than Strep. agalactiae, coliforms, mycoplasmas, and pseudomonads are isolated more frequently (Oliver, 1987; Oliver and Mitchell, 1983; Smith et al., 1985). Thus, control measures that are effective against contagious

mastitis pathogens do not appear to be as effective against environmental pathogens (Smith et al., 1985).

Milking hygiene and use of antibiotics are the most utilized procedures to control mastitis (Buddle and Cooper, 1978). Antibiotic therapy at cessation of milking is more effective than during lactation (Stang, 1977). Objectives of nonlactating cow antibiotic therapy are to eliminate existing infections and to provide an effective level of antibiotics in the mammary gland to prevent new intramammary infection (IMI) (Woods, 1977). High concentrations of antibiotics in the mammary gland during the early nonlactating period are important because mammary glands are highly susceptible to new IMI during this period (Oliver, 1987; Oliver and Mitchell, 1983).

Apart from hygiene and antibiotics, another potential method of prevention of mastitis is to increase natural defenses of the mammary gland during both nonlactating and lactation period. Heightening resistance to mastitis was one of the earliest approaches studied by mastitis research workers (Slanetz, 1963). However, results, for the most part, were discouraging because of lack of precise information concerning the bovine immune system and effective immunogens (Norcross, 1977). Limited information on mammary gland immune mechanisms such as immunoglobulin (Ig) synthesis and transfer, plasma cell migration and proliferation, and immunocompetent cell interactions in the

mammary gland have hindered also progress for increasing resistance to mastitis.

2. NATURAL DEFENSE MECHANISMS OF THE BOVINE MAMMARY GLAND

Increasing the defensive ability of the host is one of the most effective prevention methods utilized against many human and animal diseases (Tizard, 1982a). However, the complexity of mastitis and the mammary gland immune system has slowed progress related to heightening natural resistance of the mammary gland to new intramammary infections (Norcross, 1977). Constant exposure to mastitis pathogens and the large epithelial surface of the mammary gland are inherent difficulties for mammary gland defense mechanisms (Bramley and Dodd, 1984; Sheldrake and Husband, 1985).

Bovine mammary gland defense systems are comprised of two main effector mechanisms; specific and nonspecific defenses (Opdebeeck, 1982). Nonspecific defense mechanisms are teat end tissues, phagocytic cells, and antibacterial substances such as lactoferrin, lysozyme, complement proteins, and lactoperoxidase-thiocyanate-hydrogen peroxide system (Craven and Williams, 1985). Specific defense mechanisms are cell mediated immunity (CMI) and antibody mediated immunity (Opdebeeck, 1982).

Nonspecific Defense Mechanisms of the Mammary Gland

Teat End Tissues

Teat end tissues are important barriers because the portal of entry for mastitis pathogens is the teat canal (Nickerson, 1985). Milk provides a favorable environment for bacterial growth once bacteria gain entrance into the mammary gland (Ward and Sebunya, 1981). Thus, structures such as the streak canal (ductus papillaris), teat sphincter muscle, and Furstenberg's rosette play a critical role in the first line of defense against mastitis pathogens (Comalli et al., 1984). The streak canal is the area between Furstenberg's rosette and the distal teat end (Heald, 1985) and consists of stratified squamous epithelium and a keratin layer (Nickerson and Pankey, 1983). Keratin is derived from the teat canal lining (Nickerson, 1985). Keratin has antibacterial properties and functions as a plug for the teat orifice (Chandler et al., 1969; Nickerson, 1985). Furstenberg's rosette is the distal termination of the teat cistern and consists of double layered epithelium (Nickerson and Pankey, 1983).

Increased numbers of intra- and subepithelial leucocytes in Furstenberg's rosette and the streak canal support the hypothesis that these anatomical structures are more than a physical barrier against invading organisms (Nickerson and Pankey, 1983; Nickerson et al. 1984).

Morphometric studies of uninfected bovine mammary gland tissue have shown that plasma cells are the most prevalent cell type in Furstenberg's rosette (Nickerson and Pankey, 1983). Plasma cell populations at this site of the mammary gland could be important since Ig secreted by plasma cells might have easier access to the teat cistern and subsequently to invading pathogens (Nickerson and Pankey, 1983).

Complement Proteins in Milk

Complement proteins have important functions in bacterial lysis and enhancement of phagocytosis (Craven and Williams, 1985). Activation of the complement system by either the classical pathway which is a result of specific antigen-antibody reaction or by alternative pathway causes lysis of foreign cells or facilitates attachment of foreign particles to phagocytic cells bearing C3b receptors (Craven and Williams, 1985). The concentration of complement proteins in milk is low compared to serum (Poutrel and Rainard, 1986). However, passive transfer of complement from serum to mammary secretions increased after altered epithelial permeability during early involution and inflammation. During this period, complement proteins in conjunction with Ig might function as effective opsonins for phagocytes and mediate lysis of bacteria (Poutrel and Rainard, 1986).

Phagocytosis

Bovine mammary gland phagocytes are a major antibacterial force during the establishment and elimination of IMI (Paape and Wergin, 1977). Differential cell counts in mammary secretions from uninfected quarters showed that phagocytes comprised 80-90 % of the total cells (Paape et al., 1981). Macrophages were the predominant cell type (MacDonald and Anderson, 1981; Harmon and Adams, 1987). However, after irritation, neutrophil concentrations increased and constituted 90 % of the total cell population (Paape et al., 1981). The dramatic increase in total cell numbers to several million per ml milk during inflammation demonstrates the potential phagocytic and antibacterial ability of mammary gland leucocytes, particularly of neutrophils (Paape et al., 1979).

New IMI still occur in spite of the enormous phagocytic potential of the mammary gland. Although the mechanism is not entirely understood, there are several reports explaining why mammary gland phagocytes are unable to prevent and eliminate IMI effectively (Paape and Wergin, 1977; Mullan et al., 1985; Targowski and Niemialtowski, 1986). One of the prominent explanations is decreased glycogen stores of mammary gland PMN (Paape, 1979). Also, indiscriminate engulfment and degradation of casein micelles and milk fat globules interfere with phagocytosis by mammary gland PMN (Paape et al., 1981). During indiscriminate

particle ingestion, PMN lose an extensive amount of plasma membrane associated with pseudopods and release lytic contents of granules into phagosomes (Paape et al., 1981). Thus, lytic enzymes and energy stores of phagocytes are exhausted during engulfment and degradation of casein micelles and fat globules (Paape et al., 1981).

Reports related to effects of lacteal secretions on phagocytic abilities of mammary leucocytes are conflicting. After showing inhibition of macrophage phagocytosis by dry secretions, Mullan et al. (1985) concluded that dry secretions interferred with particle-uptake mechanisms of macrophages. Targowski and Niemialtowski (1986) confirmed the inhibition of lacteal leucocyte phagocytosis by dry secretions, colostrum, and mastitic milk. They (Targowski and Niemialtowski, 1986) suggested also the presence of a blocking factor for Fc receptors of leucocytes in mammary secretions. However, Jain and Lasmanis (1978) showed increased phagocytosis in the presense of mastitic milk and concluded that mastitic milk was almost as favorable an environment for phagocytosis by PMN as serum. Demonstrating the same effect with milk whey, Hill et al. (1983) stated that mammary secretions were favorable for phagocytosis and killing of nonencapsulated Escherichia coli by bovine neutrophils. Using a chemiluminescense assay, Harmon and Adams (1987) studied bovine mammary gland macrophage oxidative burst activity and concluded that lacteal

secretions from infected quarters increased macrophage chemiluminescense response indicating increased phagocytosis in the presence of secretions.

Specific Defense Mechanisms of the Bovine Mammary Gland

Specific defense of the bovine mammary gland is one function of immunocompetent lymphocytes (Opdebeeck, 1982). The proportions of T- and B- lymphocytes in normal milk are reported as 47 and 20 % of total lymphocytes, respectively (Concha et al., 1978). Null cells comprise the remainder of lymphocytes. This ratio is very close to that of blood lymphocytes (Concha et al., 1978). Cellular interactions of bovine mammary gland specific immunity are largely unknown. However, the presence of both CMI and humoral immunity have been shown (Opdebeeck, 1982).

Cell Mediated Immunity

The term CMI is used to describe immunity mediated by lymphocytes and phagocytes rather than antibody (Roitt et al., 1985a). However, it is not entirely possible to separate CMI from humoral immunity (Roitt et al. 1985a). Cell mediated immmunity of the bovine mammary gland has not been studied extensively, although there are reports indicating blastogenic response of T- cells to various mitogens (Outteridge and Lee, 1981; Nonnecke and Kehrli,

1985; Collins and Oldham, 1986, Torre and Oliver, 1987a; 1987b; 1987c) and induced delayed type hypersensitivity (Nickerson and Nonnecke, 1986).

Humoral Immunity

Immunoglobulins are produced by plasma cells which are large ovoid cells with a basophilic cytoplasm and eccentrically located nucleus (Junqueiria and Carneiro, 1983). The cytoplasm contains abundant rough endoplasmic reticulum and juxtanuclear golgi apparatus (Bessis, 1961). The abundance of endoplasmic reticulum is explained by extensive synthesis of Ig (Roitt et al., 1985 b). The spherical nucleus has a clock-faced appearence because of the location of heterochromatin (Junqueiria and Carneiro, 1983).

Plasma cells develop from B- lymphocytes (Tizard, 1982b). Immature B- lymphocytes produced by the bone marrow migrate from the bursa equivalent organs to secondary lymphoid organs such as the spleen and lymph nodes. B-lymphocytes in secondary lymphoid tissues are mature enough to respond to antigenic stimulation (Tizard, 1982b). After encountering an antigen in the presence of accesory cells such as macrophages and helper T- lymphocytes, B-lymphocytes proliferate into two distinct cell types; large, short lived, pyroninophilic, Ig-producing plasma

cells and smaller longer lived memory cells (Roitt et al., 1985 b).

The origin of plasma cells in the bovine mammary gland is not completely understood. Since antigen recognition through T-lymphocyte cooperation is not expected to occur in the mammary gland (Butler, 1981), the draining lymph nodes might be the site for initial antigen processing and plasma cell proliferation. Willoughby (1966) demonstrated that specific antibody producing plasma cells first appeared at the supramammary lymph nodes in Staph. aureus infected quarters. As the infection progressed, plasma cells could be detected even at remote lymph nodes (Willoughby, 1961). The presence of specific Ig producing cells in the mammary gland to intestinal antigens has led researchers to hypothesize a common mucosal immune system (Roux et al,. 1977; Lascelles et al., 1981; Kortbeek-Jacobs et al., 1984). According to this theory, plasma cells migrate to the mammary gland and to other mucosal surfaces from gut-associated lymphoid tissue after stimulation by antigens present on intestinal surfaces (Lascelles et al., 1981).

Mechanisms associated with homing of plasma cells specifically to mucosal surfaces are not documented well. In rodents, one of the factors affecting migration of IgA producing plasma cells to mucosal surfaces is thought to be antigenic attraction (reviewed by Sheldrake and Husband, 1985).

Plasma cells after leaving Peyer's patches might migrate to all tissues equally. However, antigens at mucosal surfaces allow plasma cells to proliferate extensively. Other factors which might influence homing mechanisms of plasma cells are thought to be hormonal effects and regional blood flow patterns (Sheldrake and Husband, 1985).

Humoral immunity of ruminant mammary glands is a unique system (Morgan et al., 1981). Immunoglobulins are found in milk, colostrum, and dry secretions. The major Ig in lacteal secretions is IgG, whereas IgA is the major Ig in other secretory fluids (Mach and Pahud, 1971; Butler et al., 1972; Lascelles, 1979). However, in humans, mice, and rabbits, IgA constitutes the predominant Ig in milk (Butler, 1973). The bovine placenta is thought to be nonpermeable to IgG subclasses, whereas, in humans, maternal IgG is transferred selectively through the placenta to the fetus (Lascelles et al., 1981). Thus, the abundance of IgG in bovine colostrum and to a lesser extent in milk most likely provides passive immunity for the newborn calf during early life (Butler, 1973).

Immunoglobulin G_1 and IgG_2 constitute all IgG found in mammary secretions (Butler, 1973). Immunoglobulin G is involved in a variety of immunological functions in the bovine immune system such as opsonization, complement fixation, bacterial agglutination, and toxin neutralization

(Butler, 1973; Watson, 1980; Colditz and Watson, 1985). Receptors for the Fc portion of IgG_1 and IgG_2 on bovine macrophages have been reported (Rossi and Kiesel, 1977). Investigating the functional properties of bovine IgG_1 and IgG_2, McGuire et al. (1979) noted that fixation of bovine complement, precipitation of ovalbumin and mediation of passive cutaneous anaphylactic reactions in bovine skin were carried out by both IgG subclasses. Immunoglobulin G_2 also caused phagocytosis of equine erythrocytes by bovine neutrophils and peripheral blood monocytes, and precipitation of dinitrophenyl-ovalbumin, while IgG_1 did not appear to be involved in either phagocytosis or precipitation (McGuire et al., 1979). However, phagocytosis assays with cultured monocytes indicated that both IgG subclasses had opsonic activity (McGuire et al., 1979). Also, there are reports indicating the cytophilicity of IgG subclasses in ruminant species (Watson, 1975; 1976; Desiderio and Campbell, 1980). Immunoglobulin G_2 for neutrophils and IgG_1 for macrophages were reported to be cytophilic in sheep (Watson, 1975; 1976; Yasmeen, 1981). Studying the functional and morphological characteristics of bovine mammary gland macrophages, Desiderio and Campbell (1980) demonstrated the cytophilicity of bovine IgG_2 and IgG_1 for bovine macrophages using a membrane fluorescence assay. Cytophilic Ig bearing leucocytes provided not only a high level of passive transfer of IgG into mammary

secretions during inflammation but also had an enhanced phagocytic ability (Colditz and Watson, 1985).

Opsonic activity of bovine IgM for neutrophils has been shown also (Williams and Hill, 1982). After systematic separation of serum proteins, Williams and Hill (1982) found that IgM was responsible for opsonization of E. coli and Staph. aureus by bovine neutrophils. Fc receptors for IgM on bovine phagocytic cells have not been identified. However, Paape et al. (1987) demonstrated that IgM binds to bovine peripheral blood neutrophils and suggested that binding was mediated by receptors.

Specific functions of IgA in bovine mammary gland secretions are not known. Recently, however, Mackie et el. (1986) noted loss of opsonic activity of bovine milk whey following depletion of IgA and attributed at least some of the opsonic activity of milk to secretory IgA (sIgA). In humans and mice, sIgA was shown to be bacteriostatic for E. coli (Rogers and Synge, 1978), inhibitory for bacterial attachment to epithelial cells (Williams and Gibbons, 1972), and toxin neutralizing for enteropathogenic bacteria (Roux et al.,1977).

The origin of lacteal Ig is not always associated with plasma cells present in lymphoid tissues and circulation (Butler, 1971). Some lacteal Ig are synthesized by plasma cells located in mammary gland tissue (Butler et al., 1971; Yurhack et al., 1971). In lactating mammary glands, plasma

cells are located generally in subepithelial stroma although occasional intraepithelial plasma cells have been observed (Nickerson et al., 1984; Leary et al., 1986).

Most IgG_1 and IgG_2 in mammary secretions are thought to be derived from serum, whereas IgM and IgA are thought to be synthesized by plasma cells located in the gland (Lascelles, 1979; Lascelles et al., 1981; Nickerson, 1985). By tracing radioactively labelled Ig in serum and secretions, Newby and Bourne (1977) showed that in lactating cows no apparent local IgG_1 synthesis occurred. However, equal amounts of IgG_2 were produced by mammary gland plasma cells and derived from serum (Newby and Bourne, 1977). Much of IgM was derived from serum during colostrum formation although as much as 50 % of total IgM could be produced locally (Newby and Bourne, 1977). Results on IgA synthesis were similar to that of IgM, however; greater proportions of IgA in colostrum were synthesized by mammary gland plasma cells (Newby and Bourne, 1977).

Immunoglobulins, whether serum derived or locally produced, are tranported from intercellular spaces to the alveolar lumen by several mechanisms (Lascelles, 1979). Selective transport of IgG is widely accepted. This mechanism is based on the fact that proportions of IgG_1 and IgG_2 in blood are similar. However, in milk the ratio is greatly increased in favor of IgG_1 (Lascelles, 1979). Studying kinetic analysis of the binding of IgG_1 and IgG_2 to

bovine mammary cells, Sasaki et al. (1977) showed IgG_1 binding sites on epithelial cells and concluded that a highly selective transport mechanism exists in the bovine mammary gland for IgG_1. Immunoglobulin G_1 binds to receptors on epithelial cells and a transport vesicle is formed at the basal portion of the plasma membrane. Immunoglobulins in this vesicle are ferried across the cytoplasm and contents of the vesicle are discharged into the alveolar lumen (Lascelles, 1979).

The current concept for sIgA transfer is that free secretory component synthesized by epithelial cells functions as the receptor for the dimeric IgA molecule (Watson, 1980). However, Butler et al. (1986) reported that the dimeric form of the molecule can be transported across epithelial cells as well as the IgA molecule with secretory piece.

The transfer mechanism of IgM from intercellular spaces into secretions is not well documented. However, in the human secretory immune system, a possible competition between IgM and IgA for free secretory component has been suggested, since IgM exhibited affinity for secretory component synthesized by hepatocytes (Hall, 1986).

Concentration gradients produced by plasma cells at the basal membrane of epithelial cells are also thought to facilitate transfer of locally synthesized IgM and IgA (Watson, 1980). Altered cellular tight junctions during

inflammation and involution might affect nonselective accumulation of all Ig isotypes into lacteal secretions (Lascelles, 1979; Watson, 1980).

Depending upon the stage of lactation and the type of antigenic stimulation, concentrations of plasma cells in ruminant mammary gland tissue and proportions of Ig produced by plasma cells or derived from serum can change in ruminants (Campbell et al., 1950; Willoughby, 1966; Lee and Lascelles, 1970; Norcross, 1971). As early as 1950, plasmacytosis in the bovine udder was shown to occur during parturition and experimental cessation of milking which were synchronous with heightened Ig formation (Campbell et al., 1950). Later, using immunofluorescense assays, Lee and Lascelles (1970) showed plasmacytosis in locally stimulated sheep mammary glands, but not normal mammary tissue during the periparturient period. In contrast to large numbers of IgA specific cells, considerably smaller numbers of IgG_1, IgM, and IgG_2 specific fluorescing cells were observed. Using similar techniques, Yurchak et al. (1971) noted that normal cow mammary gland tissue contained primarily IgG-producing cells.

Quantitative cytological analysis of uninfected lactating bovine mammary gland tissue showed a marked increase in subepithelial plasma cells from milk secreting parenchyma to the distal teat end mucosa (Nickerson et al., 1984). After demonstrating predominantly IgG and IgA

producing plasma cells in lacteal secretions by an indirect Jerne plaque assay, Chang et al. (1980) stated that antibody forming cells accumulated in the bovine mammary gland as a result of local stimulation. Studying the cellular response of the bovine mammary gland to experimental Staph. aureus infection, Nickerson and Heald (1982) reported similar results. Plasma cells accumulated in tissue stroma and most contained IgA and IgG.

Recently, Sordillo and Nickerson (1987) reported that Ig-producing cell numbers in uninfected nonlactating bovine mammary glands increased gradually from drying off, reached peak concentrations 2 week prepartum, and decreased significantly at the time of parturition. Immunoglobulin G_1- and IgG_2-producing cells were most numerous followed by IgM- and IgA-producing cells. Immunoglobulin M-producing plasma cells were more numerous in coagulase-negative staphylococci and Corynebacterium bovis infected quarters during the nonlactating period.

In addition to affecting plasma cell migration to the mammary gland, systemic and local antigenic stimulation altered total and isotypic Ig concentrations in secretions (Chang et al., 1981). Thus, the appropriate antigenic preparation, dose, time, and route of administration for an optimal immune response have been important aspects of mastitis research (Slanetz et al., 1963; Norcross and Stark, 1969; Brock et al., 1975; Adlam et al., 1981;

Watson and Kennedy, 1981; Opdebeeck and Norcross, 1985). Most studies to date have evaluated the immunogenicity of Staph. aureus, since increasing resistance to Staph. aureus infection is considered more beneficial than treatment of clinical cases (Mellenberger, 1977).

After adherence to the epithelium of the mammary gland (Frost, 1975; Frost et al., 1977; Wanasinghe, 1981a; 1981b), Staph. aureus penetrates through the epithelium and creates an inflammatory foci (Gudding et al., 1984). Pathogenicity factors such as alpha and beta toxins, leucocidin, coagulase, and hyaluronidase enhance penetration of Staph. aureus and compromise natural defense systems of the host (Anderson, 1982). Cellular and inflammatory debris produced during this process facilitates the formation of larger foci. Staphylococcus aureus is also able to survive within phagocytic cells (Anderson, 1982). Since antibiotics can not effectively reach the center of inflammatory foci, effectiveness of antibiotics to eliminate chronic Staph. aureus IMI is reduced greatly. Also, intraphagocytic staphylococci are not susceptible to antibiotics (Anderson, 1982).

Results obtained from immunization studies are conflicting. Several reports indicated that local or systemic antigenic stimulation increased specific antibody titers in serum and mammary secretions and prevented experimental IMI by challenge exposure (Derbyshire, 1961;

Slanetz et al., 1963; Derbyshire and Smith, 1969; Janovics and Armitage, 1977; Guidry et al., 1980; Watson and Kennedy, 1981; Watson, 1984; Opdebeeck and Norcross, 1984; Nonnecke et al., 1986). However, minimal specific antibody titer increase and little, if any, protection from experimental and natural infections have been reported also (Ohme and Coles, 1967; Brock et al., 1975; Adlam et al., 1981; Pankey et al., 1985).

In an early study, Slanetz et al. (1963) showed that cows vaccinated with staphylococcal cell-toxoid preparartions resulted in increased resistance to infection when challenged with Staph. aureus. Watson (1984) reported that intramammary infusion of an attenuated live staphylococcal vaccine in lactating heifers increased IgG_1 and IgG_2 antibodies in serum. Vaccinated animals had a greater resistance to challenge with the homologous bacterial strain than did controls. Derbyshire (1961; et al., 1969) found that intramammary vaccination of goats with cell-toxoid preparations of Staph. aureus increased resistance to staphylococcal mastitis.

After systemic and local immunization with a Staph. aureus cell-toxoid vaccine, Adlam et al. (1981) noted small and short-lived increases in antibody concentrations and concluded that the immune response was unlikely to alter the course of natural infection caused by staphylococci. Oehme et al. (1967) evaluated field use of a vaccine prepared from

whole bacterin and toxoids of Staph. aureus using an intramuscular vaccination procedure. The vaccination provided neither therapeutic nor prophylactic effects on Staph. aureus mastitis (Oehme et al., 1967). Pankey et al. (1985) used protein A and a commercial bacterin as vaccines against experimental Staph. aureus mastitis and reported that the rate of new IMI was similar for vaccinated and unvaccinated cows. Brock et al. (1975) reported no protective effect of intramammary or intramuscular vaccination against Staph. aureus and concluded that unless some very effective novel antigens were discovered, vaccination was unlikely to prevent Staph. aureus mastitis.

Opdebeeck and Norcross (1984) studied effects of adjuvants on the response of the bovine mammary gland to vaccination with staphylococcal and streptococcal antigens. They (Opdebeeck and Norcross, 1984) reported that high concentrations of antibody might be maintained throughout an average lactation if cows were vaccinated via the supramammary lymph node with an optimum dose of antigen emulsified in Freund's incomplete adjuvant. Opdebeeck and Norcross (1985) looked also at the immunogenic properties of a combined vaccine that contained killed Staph. aureus and Strep. agalactiae and demonstrated that no interaction occurred between staphylococcal and streptococcal components. Increased antibody titers and absence of antigenic competition between the two components of the

vaccine were interpreted as promising results for future vaccine studies.

Despite some encouraging laboratory studies on immunization, few studies have been successful using vaccines for practical applications. The rapidly expanding knowledge and understanding of the bovine mammary gland immune system has provided some explanations as to why previous immunization studies have been unsuccessful. Additional studies related to the underlying mechanisms of bovine mammary gland defense systems such as the homing mechanism of plasma cells, effector mechanisms of CMI, and phagocytic cell functions are needed before investigating new immunization techniques. Factors affecting plasma cell migration and proliferation of cells in the mammary gland have been overlooked. Also, the classification and quantification of Ig-producing plasma cells in different areas of mammary tissue have, for the most part, received little attention. A more thorough understanding of these factors might provide a basis for future manipulations of bovine mammary gland immunity.

3. SUMMARY

Bovine mammary gland defenses consist of two components; nonspecific and specific defense mechanisms. Nonspecific defenses are teat end tissues, phagocytic cells,

and antibacterial substances in secretions such as lactoferrin, complement proteins, and lysozyme. Specific defenses include CMI and humoral immunity. Immunoglobulins in lacteal secretions are derived from serum and/or synthesized by plasma cells located in sub- and intraepithelial mammary tissue. Stage of lactation and infection status of the animal can influence plasma cell concentrations in mammary tissue. Plasma cell numbers increase during the nonlactating period and with infection which coincides with increased antibody titers in mammary secretions (Nickerson and Heald, 1982; Sordillo and Nickerson, 1987). Results of immunization studies are conflicting. Generally, increased antibody concentrations have been reported after immunization. However, increased antibody titers did not necessarily correlate with enhanced resistance to IMI.

REFERENCES

1. Adlam, C., Keery, J. B., Edkins, S. E., and Ward, P. D., 1981. Local and systemic antibody responses in cows following immunization with staphylococcal antigens in the dry period. J. Comp. Path., 91: 105-113.
2. Anderson, J. C., 1982. Progressive pathology of staphylococcal mastitis with a note on control, immunisation and therapy. Vet. Rec., 110: 372-376.
3. Bessis, M. C., 1961. Ultrastructure of lymphoid and plasma cells in relation to globulin and antibody formation. Lab. Inv., 10: 1040-1067.
4. Bramley, A. J., and Dodd, F. H., 1984. Reviews of the progress of Dairy Science; Mastitis control-progress, prospectes. J. Dairy Sci., 51: 481-512.
5. Brock, J. H., Steel, E. D., and Reiter, B., 1975. The effect of intramuscular and intramammary vaccination of cows on antibody level and resistance to intramammray infection by Staphylococcus aureus. Res. Vet. Sci., 19: 152-158.
6. Buddle, B. M., and Cooper, M. G., 1978. Aspects of the epidemiology of bovine staphylococcal mastitis. New Zealand Vet. J., 26: 296-298.

7. Butler, J. E., 1971. A review of the bovine immunoglobulins. J. Dairy Sci., 54: 1315-1317.

8. Butler, J. E., 1973. Synthesis and distribution of immunoglobulins. J. Am. Vet. Med. Assoc., 163: 795-798.

9. Butler, J. E., 1981. A concept of humoral immunity among ruminants and an approach to its investigation. In: J. E. Butler (Editor). The Ruminant Immune System. Plenum, New York, London. pp. 3-55.

10. Butler, J. E., Frenyo, V. L., Whipp, S. C., Wilson, R. A., and Koertge T. E., 1986. Metabolism and transport of bovine serum sIgA. Comp. Immunol. Micro. Inf. Dis., 9: 303-315.

11. Butler, J. E., Kiddy, C. A., Maxwell, C., Hylton, M. B., and Asofsky, R., 1971. Sythesis of immunoglobulins by various tissues of the cow. J. Dairy Sci., 54: 1323-1324.

12. Butler, J. E., Maxwell, C. F., Pierce, C. S., Hylton, B., Asofski, R., and Kiddy, C. A., 1972. Studies on the relative synthesis and distribution of IgA and IgG_1 in various tissues and body fluids of the cow. J. Immunol., 109: 38-46.

13. Campbell, B., Porter, R. M., and Peterson, W. E., 1950. Plasmacytosis of the bovine udder during colostrum secretion and experimental cessation of milking. Nature, 166: 913.

14. Chang, C. C., Winter, A. J., and Norcross, N. L., 1980. Antibody producing cells in bovine lacteal secretions after local immunization. Am. J. Vet. Res., 41: 1416-1418.

15. Chang, C. C., Winter, A. J., and Norcross, N. L., 1981. Immune response in the bovine mammary gland after intestinal, local, and systemic immunization. Infec. Immun., 31: 650-659.

16. Chandler, R. L., Lepper, A. W. D., and Wilcox, J., 1969. Ultrastructural observations on the bovine teat duct. J. Comp. Pathol., 79: 315-319.

17. Colditz, I. G., and Watson, D. L., 1985. Immunophysiological basis for vaccinating ruminants against mastitis. Aust. Vet. J., 62: 145-153.

18. Comalli, M. P., Eberhart, R. J., Griel, Jr, L. C., and Rothenbacher, H., 1984. Changes in the microscopic anatomy of the bovine teat canal during mammary involution. Am. J. Vet. Res., 45: 2236-2242.

19. Collins, R. A., and Oldham, G., 1986. Proliferative responses and IL-2 production by mononuclear cells from bovine mammary secretions and the effect of mammary secretions on peripheral blood lymphocytes. Immunol., 58: 647-651.

20. Concha, C., Homberg, O., and Morein, B., 1978. Proportion of B- and T-lymphocytes in normal bovine milk. J. Dairy Res., 45: 287-290.

21. Craven, N., and Williams, M. R., 1985. Defenses of the mammary gland against infection and prospects for their enhancement. Vet. Immunol. Immunopathol., 10: 71-127.

22. Derbyshire, J. B., 1961. Further immunological studies in experimental staphylococcal mastitis. J. Comp. Pathol., 71: 146-158.

23. Derbyshire, J. B., and Smith, G. S., 1969. Immunization against experimental staphylococcal mastitis in the goat by intramammary infusion of cell-toxoid vaccine. Res. Vet. Sci., 10: 559-564.

24. Desiderio, J. W., and Campbell, S. G., 1980. Bovine mammary gland macrophages; isolation, morphologic features, and cytophilic immunoglobulins. Am. J. Vet. Res., 41: 1595-1599.

25. Frost, A. J., 1975. Selective adhesion of microorganisms to the ductular epithelium of the bovine mammary gland. Infec. Immun., 12: 1154-1156.

26. Frost, A. J., Wanasinghe, D. D., and Wilcock, J. B., 1977. Some factors effecting selective adherence of microorganisms in the bovine mammary gland. Infec. Immun., 15: 245-253.

27. Gudding, R., McDonald, J. S., and Cheville, N. F., 1984. Pathogenesis of Staphylococcus aureus mastitis; Bacteriologic, histologic, and ultrastructural pathologic findings. Am. J. Vet. Res., 45: 2525-2531.

28. Guidry, A. J., Paape, M. J., Pearson, R. A., and Williams, W. F., 1980. Effect of local immunization of the mammary gland on phagocytosis and intracellular kill of Staphylococcus aureus by polymorphonuclear neutrophils. Am. J. Vet. Res., 41: 1427-1431.

29. Hall, J. G., 1986. The physiology of secretory immunity. In: W. I. Morrison, (Editor). The Ruminant Immune System in Health and Disease. Cambridge Univ. Press, Cambridge, London, New York, New Rochelle, Melbourne, Sydney, pp. 409-428.

30. Harmon, B. G., and Adams, L. G., 1987. Assesment of bovine mammary gland macrophage oxidative burst activity in a chemiluminescence assay. Am. J. Vet. Res., 48: 119-125.

31. Heald, C. W., 1985. Milk collection. In: B. Larson (Editor). Lactation. The Iowa State Univ. Press, Ames, 200 pp.

32. Hill, A. W., Henegan, D. J. S., Field, T. R., and Williams, M. R., 1983. Increase in specific opsonic activity in bovine milk following experimental Escherichia coli mastitis. Res. Vet. Sci., 35: 222-226.

33. Jain, N. C., and Lasmanis, J., 1978. Phagocytosis of serum resistant and serum sensitive coliform bacteria (Klebsiella) by bovine neutrophils from blood and mastitic milk. Am. J. Vet. Res., 39: 425-427.

34. Jain, N. C., 1979. Common mammary pathogens and factors in infection and mastitis. J. Dairy Sci., 62: 128-134.

35. Junquiera, L. C., and Carneiro, J., 1983. Connective tissue. In: L. C. Junquiera, and J. Carneiro (Editors), Basic Histology. Lange Medical Pub., Los Altos, 107 pp.

36. Kortbeek-Lacobs, J. M. C., Van Kooten, P. J. S., Van Der Donk, J. A., Van Dijk, J. E., and Rutten, V. P., 1984. The effect of oral immunization on the population of lymphocytes migrating to the mammary gland of the sow. Vet. Micro., 9: 287-299.

37. Kowalski, J. J., 1977. Microbial agents and bovine mastitis. J. Am. Vet. Med. Assoc., 170: 1175-1177.

38. Lascelles, A. K., 1979. The immune system of the ruminant mammary gland and its role in the control of mastitis. J. Dairy Sci., 62: 154-160.

39. Lascelles, A. K., Beh, K. J., and Husband, A. J., 1981. Origin of antibody in mammary secretion with particular reference to the IgA system. In: J. E. Butler (Editor). The Ruminant Immune System, Plenum, New York, London, pp. 493-511.

40. Leary, Jr, H. L., Larson, B. L., and Nelson, D. R., 1982. Immunohistochemical localization of IgG_1 and IgG_2 in peripartum and lactating bovine mammary tissue. Vet. Immunol. Immunopathol., 3: 509-514.

41. Lee, C. S., and Lascelles, A. K., 1970. Antibody producing cells in antigenically stimulated mammary glands and in the gastrointestinal tract of the sheep. Aust. J. Exp. Med. Sci., 48: 525-535.

42. Mach, J. P., and Pahud, J. J., 1971. Secretory IgA; Major immunoglobulin in most bovine external secretions. J. Immunol., 106: 552-563.

43. Mackie, D. P., Meneely, D. J., Pullock, D. A., and Logan, E. F., 1986. The loss of opsonic activity of bovine milk whey following depletion of IgA. Vet. Immunol. Immunopath., 11: 193-198.

44. McDonald, J. S., 1977. Streptococcal and staphylococcal mastitis. J. Am. Vet. Med. Assoc., 170: 1157-1159.

45. McGuire, T. C., Musoke, A. J., and Kurtti, T., 1979. Functional properties of bovine IgG_1 and IgG_2; Interaction with complement, macrophages, neutrophils, and skin. Immunol., 38: 249-256.

46. Mellenberger, R. W., 1977. Vaccination against mastitis. J. Dairy Sci., 60: 1016-1021.

47. Morgan, K. C., Bourne, F. C., Newby, T. J., and Bradley, P. A., 1981. Humoral factors in the secretory immune systems of ruminants. In: J. E. Butler (Editor), The Ruminant Immune System. Plenum, New York, London, pp. 391-411.

48. Mullan, N. A., Carter, E. A., and Nguyen, K. A. T., 1985. Phagocytic and bactericidal properties of bovine macrophages from nonlactating mammary glands. Res. Vet. Sci., 38: 160-166.

49. Newby, T. J., and Bourne, J., 1977. The nature of local immune system of the bovine mammary gland. J. Immunol., 118: 461-465.

50. Nickerson, S. C., 1985. Immune mechanisms of the bovine udder; An overview. J. Am. Vet. Med. Assoc., 187: 41-45.

51. Nickerson, S. C., and Heald, C. W., 1982. Cells in local reaction to experimental Staphylococcus aureus infection on bovine mammary gland. J. Dairy Sci., 65: 105-116.

52. Nickerson, S. C., and Nonnecke, B. J., 1986. Tuberculin elicited cellular immune response in the lactating bovine mammary gland vaccinated intramammarily with Mycobacterium bovis. Vet. Immunol. Immunopathol., 13: 39-50.

53. Nickerson, S. C., and Pankey, J. W., 1983. Cytologic observations of the bovine teat end. Am. J. Vet. Res., 44: 1433-1441.

54. Nickerson, S. C., Pankey, J. W., and Boddie, N. T., 1984. Distribution, location, and ultrastructure of plasma cells in the uninfected, lactating bovine mammary gland. J. Dairy Res., 51: 209-217.

55. Nonnecke, B. J., and Kehrli, M. E., 1985. Isolation of mononuclear cells from bovine milk by continuous flow and density gradient centrifugation; Response of cells to mitogens. Am. J. Vet. Res., 46: 1259-1262.

56. Nonnecke, B. J., Elsken, L. A., and Kehrli, Jr, M. E., 1986. Local and systemic immune response in the cow after intramammary vaccination during lactation. Vet. Immunol. Immunopath., 11: 31-44.

57. Norcross, N. L., 1971. Immune response in the mammary gland. J. Dairy Sci., 54: 1880-1887.

58. Norcross, N. L., 1977. Immune response of mammary gland and the role of immunization in mastitis control. J. Am. Vet. Med. Assoc., 170: 1228-1231.

59. Oehme, F. W., and Coles, E. H., 1967. Field use and evaluation of a vaccine for bovine staphylococcic mastitis. J. Dairy Sci., 50: 1792-1797.

60. Oliver, S. P., 1987. Importance of the dry period in the control of intramammary infections by environmental mastitis pathogens. Natl. Mastitis Council Proc., pp. 81-92.

61. Oliver, S. P., and Mitchell, B. A., 1983. Susceptibility of bovine mammary gland to infections during the dry period. J. Dairy Sci., 66: 1162-1166.

62. Opdebeeck, J. P., 1982. Mammary gland immunity. J. Am. Vet. Med. Assoc., 181: 1061-1065.

63. Opdebeeck, J. P., and Norcross, N. L., 1984. Comparative effect of selected adjuvants on the response in the bovine mammary gland to staphylococcal and streptococcal antigens. Vet. Immunol. Immunopathol., 6: 341-351.

64. Opdebeeck, J. P., and Norcross, N. L., 1985. Immunogenic properties of Staphylococcus aureus and Steptococcus agalactiae administered separately and in combination to lactating cows. Aust. Vet. J., 62: 114-115.

65. Outteridge, P. M., and Lee, C. S., 1981. Cellular immunity in the mammary gland with particular reference to T- and B-lymphocytes and macrophages. In: J. E. Butler (editor), The Ruminant Immune System. Plenum, New York, London, pp. 513-534.

66. Paape, M. J., and Wergin, W. P., 1977. The leucocyte as a defense mechanism. J. Am. Vet. Med. Assoc., 170: 1214-1223.

67. Paape, A. J., Cheng, T., and Guidry, A. J., 1987. Binding of IgM to bovine polymorphonuclear neutrophil (PMN) leucocytes. J. Dairy Sci., Suppl., 70: 212.

68. Paape, M. J., Wergin, W. P., Guidry, A. J., and Pearson, R. E., 1979. Leucocytes: Second line of defense against invading mastitis pathogens. J. Dairy Sci., 62: 135-153.

69. Paape, M. J., Wergin, W. P., Guidry, A. J., and Schultze, W. D., 1981. Phagocytic defense of the ruminant mammary gland. In: J. E. Butler (Editor). The Ruminant Immune System. Plenum, New York, London, pp. 555-578.

70. Pankey, J. W., Boddie, N. T., Watts, J. L., and Nickerson, S. C., 1985. Evaluation of protein A and a commercial bacterin as vaccines aginst Staphylococcus aureus mastitis by experimental challenge. J. Dairy Sci., 68: 726-731.

71. Poutrel, B., and Rainard, P., 1986. Hemolytic and bactericidal activities of bovine complement in mammary secretions of cows during the early nonlactating (dry) period. Am. J. Vet. Res., 47: 1961-1962.

72. Rogers, H. J., and Synge, C., 1978. Bacteriostatic effect of human milk on Escherichia coli; The role of IgA. Immunol. 34: 19-28.

73. Roitt, I., Brostoff, J., and Male, D., 1985a. Cell mediated immunity. In: I. M. Roitt, J. Brostoff, and D. Male (Editors). Immunology. The C. V. Mosby Co., St Louis, Toronto, 11.1 pp.

74. Roitt, I., Brostoff, J., and Male, D., 1985b. Cell mediated immunity. In: I. M. Roitt, J. Brostoff, and D. Male (Editors). Immunology. The C. V. Mosby Co., St Louis, Toronto, pp. 2.4-2.5.

75. Rossi, C. R., and Kiesel, G. K., 1977. Bovine immunoglobulin G subclass receptor sites on bovine macrophages. Am. J. Vet. Res., 38: 1023-1025.

76. Roux, M. E., McWilliams, M., Phillips-Quagliata, J. M., Weisz-Carrington, P., and Lamm, M. E., 1977. Origin of IgA secreting plasma cells in the mammary gland. J. Exp. Med., 146: 1311-1322.

77. Sasaki, M., Larson, B. L., and Nelson, D. R., 1977. Kinetic analysis of the binding of immunoglobulins IgG_1 and IgG_2 to bovine mammary cells. Biochim. Biophys. Acta, 497: 160-170.

78. Sheldrake, R. F., and Husband, A. J., 1985. Immune defences at mucosal surfaces in ruminants. J. Dairy Res., 52: 599-613.

79. Slanetz, L. W., Bartley, C. H., and Allen, F. E., 1963. Vaccination of dairy cattle against staphylococcic mastitis. Am. J. Vet. Res., 24: 923-931.

80. Smith, K. L., Todhunter, D. A., and Schoenberger, P. S., 1985. Environmental mastitis; cause, prevalence, prevention. J. Dairy Sci., 68: 1531-1553.

81. Sordillo, L. M., and Nickerson, S. C., 1987. Quantification and immunoglobulin classification of plasma cells in nonlactating bovine mammary tissue. J. Dairy Sci., (Accepted for publication).

82. Stang, A. M., 1977. Pharmacological principles of systemic and intramammary mastitis therapy. J. Am. Vet. Med. Assoc., 170: 1180-1181.

83. Targowski, S. P., and Niemialtowski, M., 1986. Inhibition of lacteal leucocyte phagocytosis by colostrum, nonlactating secretion and mastitic milk. Am. J. Vet. Res., 47: 1940-1945.

84. Tizard, I., 1982a. Immunoprophilaxis; General principles of vaccination and vaccines. In: I. Tizard (Editor), An Introduction to Veterinary Immunology. W. B. Saunders Co., Philedelphia, London, Toronto, Mexico City, Rio De Jeneiro, Sydney, Tokyo, pp. 178-192.

85. Tizard, I., 1982b. Cells and tissues of the immune system. In: I. Tizard (Editor), An Introduction to Veterinary Immunology. W. B. Saunders Co., Philedelphia, London, Toronto, Mexico City, Rio De Jeneiro, Sydney, Tokyo, pp. 56-73.

86. Torre, P. M., and Oliver, S. P., 1987a. Inhibition of blood mononuclear cell blastogenesis by bovine mammary secretions. J. Dairy Sci., Suppl., 70: 165.

87. Torre, P. M., and Oliver, S. P., 1987b. Inhibition of mitogen-induced mononuclear cell blastogenesis following exposure to bovine dry secretion whey proteins. J. Dairy Sci., Suppl., 70: 243.

88. Torre, P. M., and Oliver, S. P., 1987c. Changes in blastogenic activity of bovine peripheral blood mononuclear cells throughout the nonlactating period. J. Dairy Sci., Suppl., 70: 257.

89. Wanasinghe, D. D., 1981a. Adherence as a prerequisite for infection of the bovine mammary gland by bacteria. Acta Vet. Scand., 22: 109-117.

90. Wanasinghe, D. D., 1981b. In vitro adherence of Staphylococcus aureus to bovine mammary gland epithelial cells. Acta Vet. Scand., 22: 99-108.

91. Ward, G. E., and Sebunya, T. K., 1981. In vitro phagocytosis and killing of coliforms by bovine mammary leucocytes. Am. J. Vet. Res., 42: 1941-1943.

92. Watson, D. L., 1975. Cytophilic attachment of ovine IgG_2 to autologous polymorphonuclear leucocytes. Aust. J. Exp. Biol. Med. Sci., 53: 527-529.

93. Watson, D. L., 1976. The effect of cytophilic IgG_2 on phagocytosis by ovine polymorphonuclear leucocytes. Immunol., 31: 159-165.

94. Watson, D. L., 1980. Immunologic functions of mammary gland and its secretion. Comparative review. Aust. J. Biol. Med. Sci., 33: 403-422.

95. Watson, D. L., 1984. Evaluation of attenuated, live staphylococcal mastitis vaccine in lactating heifers. J. Dairy Sci., 67: 2608-2613.

96. Watson, D. L., and Kennedy, J. W., 1981. Immunisation against experimental staphylococcal mastitis in sheep; Effect of challenge with a heterologous strain of Staphylococcus aureus. Aust. Vet. J., 57: 309-313.

97. Williams, M. R., and Hill, A. W., 1982. A role for IgM in the in vitro opsonization of Staphylococcus aureus and Escherichia coli by bovine polymorphonuclear leucocytes. Res. Vet. Sci., 33: 47-53.

98. Williams, R. C., and Gibbons, R. J., 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: A mechanism for antigen disposal. Science, 177: 697-699.

99. Willioughby, R. A., 1966. Bovine staphylococcic mastitis; An immunohistochemical study of the cellular sites of antibody formation. Am. J. Vet. Res., 27: 522-532.

100. Woods, J. M., 1977. Therapy of nonlactating cows. J. Am. Vet. Med. Assoc., 170: 1195.

101. Yasmeen, D., 1981. Antigen specific cytophilic activity of sheep IgG_1 and IgG_2 antibodies. Aus. J. Exp. Biol. Med. Sci., 59: 297-302.

102. Yurchak, A. M., Butler, J. E., and Thomasi, Jr, T. B., 1971. Fluorescent localization of immunoglobulins in the tissues of the cow. J. Dairy Sci., 54: 1324-1325.

CHAPTER II

EFFECTS OF *STAPHYLOCOCCUS AUREUS* INFECTION ON BOVINE MAMMARY GLAND PLASMA CELL POPULATIONS AND IMMUNOGLOBULIN CONCENTRATIONS IN MILK

1. ABSTRACT

Mammary tissues, and milk samples from 10 *Staphylococcus aureus* infected and 5 uninfected quarters were examined to determine numbers of immunoglobulin (Ig)-producing plasma cells in tissue and Ig concentrations in milk. Results of peroxidase-anti-peroxidase staining of tissues showed no significant differences in numbers of plasma cells between infected and uninfected quarters with the exception of increased numbers of IgG_1-producing cells at Furstenberg's rosette. Numbers of plasma cells were highest at Furstenberg's rosette followed by parenchymal and streak canal tissue. In parenchymal tissue from uninfected quarters, IgA-producing plasma cells were observed most frequently, whereas IgG_1-producing cells were more numerous in tissue from *Staph. aureus* infected quarters. No significant differences in concentrations of Ig isotypes in milk from *Staph. aureus* infected and uninfected quarters were observed. However, concentration of Ig isotypes tended to be higher in milk from infected quarters. Immunoglobulin

G_1 was highest in concentration in all quarters followed by IgA. High relative indices of occurrence of milk IgA, IgM, and IgG_1 were noted in milk from all quarters. A low index of IgG_2 in all quarters suggests that IgG_2 was nonselectively transferred from serum. Data suggests that the antigenic effect of Staph. aureus infection on the humoral immune response of the bovine mammary gland is minimal. Persistency of Staph. aureus infection may result, in part, from suboptimal stimulation of the immune system.

2. INTRODUCTION

Studies attempting to increase natural defense capabilities of the bovine mammary gland against mastitis causing pathogens have, for the most part, been disappointing (Oehme and Coles, 1967; Brock et al., 1975; Adlam et al., 1981; Pankey et al., 1985). This is related most likely to several factors particularly lack of insights about effector mechanisms of mammary gland immunity (Norcross, 1977; Colditz and Watson, 1985).

Plasma cells in mammary gland tissue are thought to be the source of antibodies synthesized locally (Butler et al., 1971; Yurchak et al., 1971; Lascelles et al., 1981). However, the pattern of plasma cell migration to the mammary gland is largely unknown. Willoughby (1966), in early

immunohistochemical studies with Staph. aureus infected bovine mammary glands, showed the presence of specific Ig-producing plasma cells in interalveolar stroma. In uninfected mammary glands, Yurchak et al. (1971) found primarily IgG-producing plasma cells. Nickerson and Heald (1982) noted, however, that IgA-producing plasma cells were the major plasma cell type in both Staph. aureus infected and uninfected secretory parenchyma, followed by IgG-producing cells. Morphometric analysis of uninfected lactating mammary glands demonstrated increased number of plasma cells from secretory parenchyma to teat end tissues Nickerson and Pankey, 1983; Nickerson et al., 1984; Collins et al., 1986). However, the isotype classification of Ig-producing plasma cell in teat end tissues has received little attention.

In this study, effects of Staph. aureus intramammary infection (IMI) on plasma cell numbers in different tissue areas of the bovine mammary gland and on Ig concentrations in milk were determined. Also, a possible relationship between plasma cells in tissue and Ig isotypes in milk was investigated.

3. MATERIALS AND METHODS

Experimental Design

Mammary tissue from 10 Staph. aureus infected quarters (n=4 cows), and from 5 uninfected quarters (n=4 cow) were examined in this study. Animals in mid- to late lactation from the University of Tennessee Dairy Research Herd were used. Quarter foremilk samples were collected 48 and 24 hours prior to slaughter to determine infection status. Milk samples were collected 24 hours prior to slaughter for compositional analysis. Tissue and blood samples were collected at slaughter. Tissue samples were processed for immunohistochemical and ultrastructural examination. Milk and blood samples were used for compositional analysis. Data were analysed by least square analysis of variance using the general linear model procedure (SAS User's Guide; Satatistics, 1982) to determine effects of infection on numbers of Ig-producing plasma cells in tissue, concentrations of Ig isotypes and bovine serum albumin (BSA) in mammary secretions and serum, and the relative occurrence of milk Ig isotypes in milk.

A relative index of occurrence for each milk Ig isotype was calculated as described previously (Guidry et al., 1980; Oliver and Smith, 1982).

$$\text{Index of relative occurrence} = \frac{[\text{Milk Ig}] \times [\text{Serum BSA}]}{[\text{Milk BSA}] \times [\text{Serum Ig}]}$$

Microbiological Procedures

Procedures for the isolation and identification of mastitis pathogens from milk were as described by the National Mastitis Council (Brown et al., 1981). Briefly, milk samples (10 ul) from each quarter were plated on blood agar containing 5% bovine red blood cells. After 24 hours incubation at 37^0C, colony morphology and hemolytic properties were evaluated in conjunction with biochemical tests. Gram-positive and coagulase-producing staphylococci were further identified by the API Staph Track System (Analytical Products, Plainview, NY).

The number of somatic cells in milk was determined electronically using a Coulter counter (Model ZBI, Coulter Electronics Inc., Hialeah, FL). Quarters were considered infected when both milk samples collected at 48 and 24 hour before slaughter contained the same bacterial species and numbers of somatic cells were >500,000/ml. Previous bacteriologic history of quarters showed that one half of Staph. aureus infected quarters (n=5) were in multiparous cows and the infection persisted from the previous lactation. The remaining Staph. aureus infected quarters were in primiparous cows.

Compositional Analysis

Quarter foremilk samples were collected 24 and 48 hours prior to slaughter and centrifuged at 48,000X g for 30 minutes to remove fat and cellular debris. Whey was prepared by decreasing the pH to 4.5 by dropwise addition of glacial acetic acid and samples were centrifuged at 48,000X g for 30 minutes. Blood was collected at slaughter and serum obtained by centrifugation at 2,000X g for 15 minutes. Samples were stored frozen until assayed.

Immunoglobulin content of skim milk and serum was determined by sandwich Enzyme Linked Immunosorbent Assay (ELISA) as described by Guidry and Miller (1985). Ninety-six well microtiter plates were coated with affinity purified guinea pig-anti-bovine Ig (200 ul antibody for each well), and incubated for 5 hours at 25^{0}C. After washing unbound antibody with 0.015 M NaCl containing 0.05% Tween-20, milk samples and standard Ig isotypes were added to wells, and incubated for 15 hours at 25^{O} C. Unbound antibody was removed with 0.015 M NaCl containing 0.05% Tween-20 and affinity purified rabbit-anti-bovine Ig was added to wells. After incubation for 2 hours at 25^{0}C, excess antibody was removed and affinity purified sheep-anti-rabbit gammaglobulin (200ul) conjugated to alkaline phosphatase was added to each well. After 2 hours incubation at 25^{0}C and removal of unbound antibody, p-nitrophenylphosphatase disodium (1mg/ml), in 0.5 M

carbonate buffer pH 9.8 with 0.001 M $MgCl_2$, was added to wells and optical density was measured at 405 nm in a Titertek Multiscan at 30, 60, and 90 minutes.

Bovine serum albumin of milk whey and serum was determined by electroimmunodiffusion on cellulose acetate plates as described by Schanbacher and Smith (1974). Rocket heights were measured in mm and concentrations were calculated by linear regression analysis. The concentration of BSA was used to determine the relative occurrence of Ig isotypes in milk (Guidry et al., 1980; Oliver and Smith, 1982).

Tissue Preparation

Parenchymal and teat end tissues were obtained at slaughter and prepared as described by Sordillo and Nickerson (1987). Parenchymal tissue was obtained from a 5 cm deep incision on the upper lateral part of the quarter, 15 cm above the gland cistern, and approximately 5 mm^3 piece of secretory tissue was excised from each quarter (Nickerson and Heald, 1982). Teat end tissues were removed and disected into two parts; streak canal and teat cistern. Tissues from the distal terminal end of the teat cistern and proximal end of the streak canal were considered Furstenberg's rosette and streak canal, respectively.

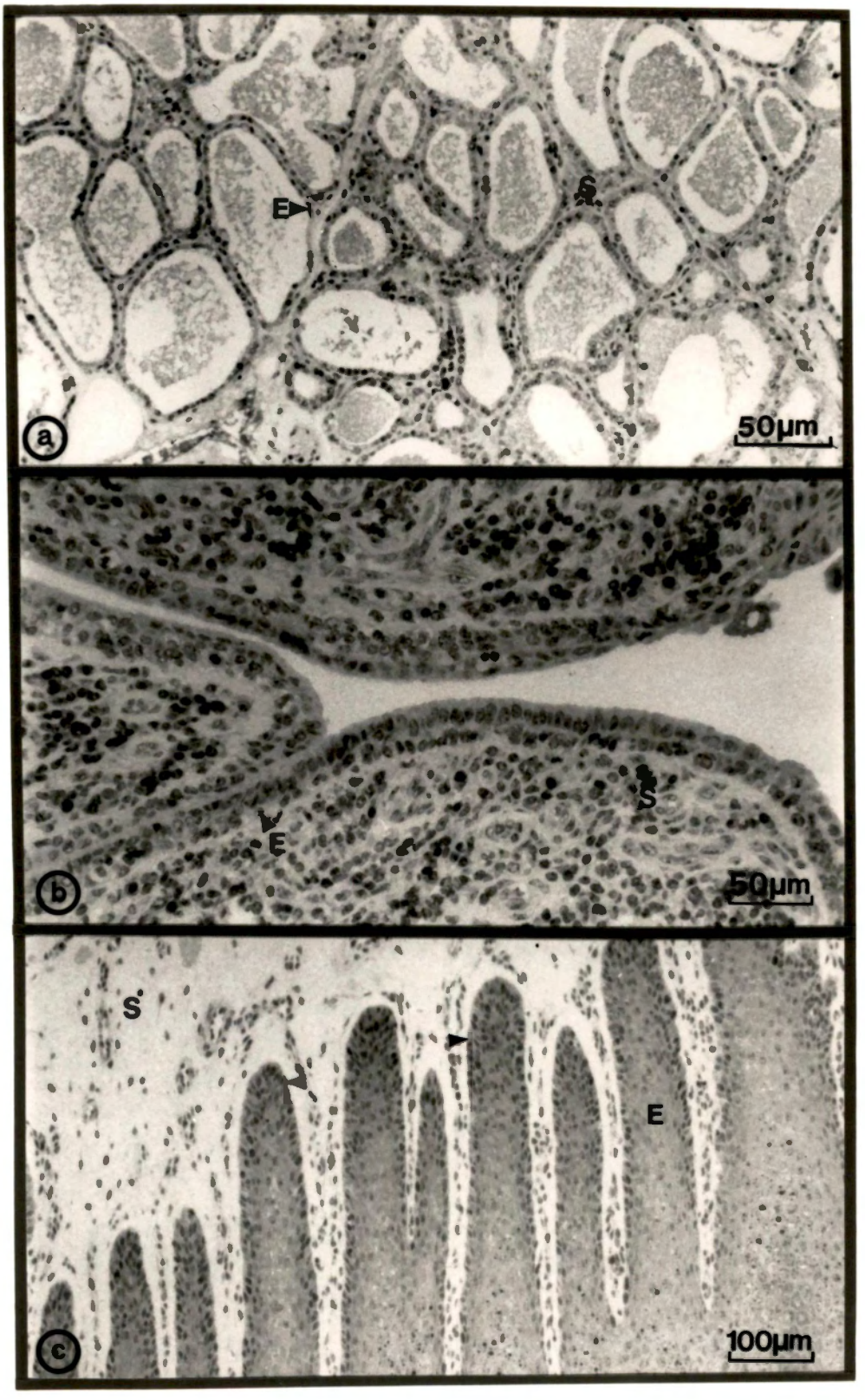
Tissues used for light microscopy were fixed for 4 hours in Bouins solution (4^0C), dehydrated in a series of

graded alcohol, cleared with xylene, and embedded in paraffin. Plasma cells were determined by morphometric analysis on 1-3 um thick serial sections. Sections were stained with peroxidase-anti-peroxidase (Miles Scientific, Naperville, IL). Tissue sections were deparaffinized in xylene and rehydrated through a graded series of alcohol. Endogenous peroxidase activity was eliminated by addition of H_2O_2 and normal goat serum was used to block non-specific binding. Affinity purified rabbit-anti-bovine-IgG_1,-IgG_2, -IgM, or -IgA, primary antibodies (obtained from Dr. A. J. Guidry, Milk Secretion and Mastitis Lab., USDA, Beltsville, MD) were diluted in saline (1:40) and incubated with tissue samples in a humid chamber at 25^0C for 30 minutes. Normal rabbit serum instead of primary antibody was incubated with tissue samples as a negative control. Excess antibody was removed with tris buffer (0.05 M, pH 7.6) and goat-anti-rabbit Ig serum was used as the secondary antibody. After 30 minutes incubation at 25^0C in a humid chamber, tissue was washed with tris buffer and incubated with rabbit peroxidase-anti-peroxidase labelled antibody at 25^0C for 30 minutes. Slides were washed with tris buffer and incubated with 0.3% H_2O_2 in water and amino-ethyl-carbosole at 25^0C for 30 minutes. After counterstaining with Mayer`s hematoxylin, tissue was mounted using glycerol gelatin and examined microscopically. Immunoglobulin-producing plasma cells were characterized as

positively stained large ovoid cells with a round, eccentrically located, clock-faced nucleus. Quantitative morphometric analysis was used to enumerate plasma cells from 20 randomly selected microscopic fields ($5.3X10^4$ um^2/section). In parenchymal tissue and Furstenberg's rosette, plasma cells were counted in epithelial and stromal tissue areas (Figure 1a, 1b). However, in tissue from the streak canal, an ocular reference grid was superimposed over the stromal area and the microscopic field was confined to the area from the basement membrane through stromal tissue (Figure 1c). Isotype specific Ig-producing plasma cells were counted at a magnification of 600X and recorded.

Ultrastructural examination of plasma cells was conducted from tissue samples prepared for electron microscopy (Sordillo and Nickerson, 1987). After fixation in 2.5% glutaraldehyde for 3-4 hours and in osmium-tetraoxide for 2 hours at 4^oC, tissue samples were stained with uranyl acetate for 16-18 hours at 4^0C. Tissue was dehydrated in a graded series of ethanol, cleared in propylene oxide, infiltrated with epoxy resin (Poly-Bed 812 Embedding Media, Polysciences Inc., Warrington, PA), and cured at 60^0C for 24 hours. Tissue was sectioned, and 60 nm thick sections were stained with uranyl acetate (5% uranyl acetate in 50% methanol) for 20 minutes and 0.4% lead citrate for 10 minutes. Plasma cells were observed with a Phillips 100 transmission electron microscope.

Figure 1. Immunoglobulin-producing plasma cells were counted in epithelial(E) and stromal (S) areas of secretory parenchyma (a, 290x magnification), and Furstenberg's rosette (b, 248x magnification). In tissue from the streak canal(c, 135x magnification), microscopic fields were confined to the area from basement membrane (arrow heads) through stromal tissue.


E
S
a
50µm
S
E
b
50µm
S
E
c
100µm

4. RESULTS

Plasma cell numbers in parenchymal tissue are shown in Table I. No significant differences were found in plasma cell numbers of Staph. aureus infected and uninfected quarters. However, slight increases were observed in infected quarters with the exception of IgA-producing plasma cells. Plasma cells were usually found in interalveolar stroma (Figure 2a), although some were located adjacent to epithelial cells. Immunoglobulin A-producing plasma cells were the most prevalent plasma cell type in uninfected quarters, followed by IgG_1- and IgM-producing cells. In Staph. aureus infected quarters, IgG_1-producing plasma cells were more numerous although numbers of IgG_1-, IgM-, and IgA-producing plasma cells were close. Occasionally, macrophage-like Ig positive cells (IgG_1, IgG_2) were observed (Appendix, Table VII), especially in tissues from infected quarters. These cells were not included in plasma cell data analysis since the possibility of the presence of cytophilic Ig-bearing macrophages has been reported in the bovine mammary gland (Desiderio and Campbell, 1980).

Of all tissues evaluated, Furstenberg's rosette contained the most plasma cells (Table I). The number of IgG_1 producing plasma cells was significantly higher ($P < 0.05$) in infected quarters. Plasma cell numbers of other Ig

TABLE I. Distribution of Plasma Cells in Tissues from *Staphylococcus aureus* Infected and Uninfected Bovine Mammary Glands.[1]

Tissue area	Ig isotypes	Uninfected quarters (n=5) $\bar{x}$	se	Infected quarters (n=10) $\bar{x}$	se
Secretory parenchyma	IgG_1	1.71	0.78	2.55	0.58
	IgG_2	0.63	0.62	1.26	0.46
	IgM	1.46	0.47	2.49	0.35
	IgA	2.46	0.56	2.40	0.41
Furstenberg's rosette	IgG_1	10.63	5.90	28.33*	4.57
	IgG_2	7.71	5.07	21.00	3.93
	IgM	1.75	0.51	2.50	0.40
	IgA	3.58	0.91	3.75	0.71
Streak canal	IgG_1	0.83	0.49	0.79	0.35
	IgG_2	0.17	0.18	0.21	0.13
	IgM	0.25	0.33	0.35	0.24
	IgA	0.33	0.45	0.81	0.32

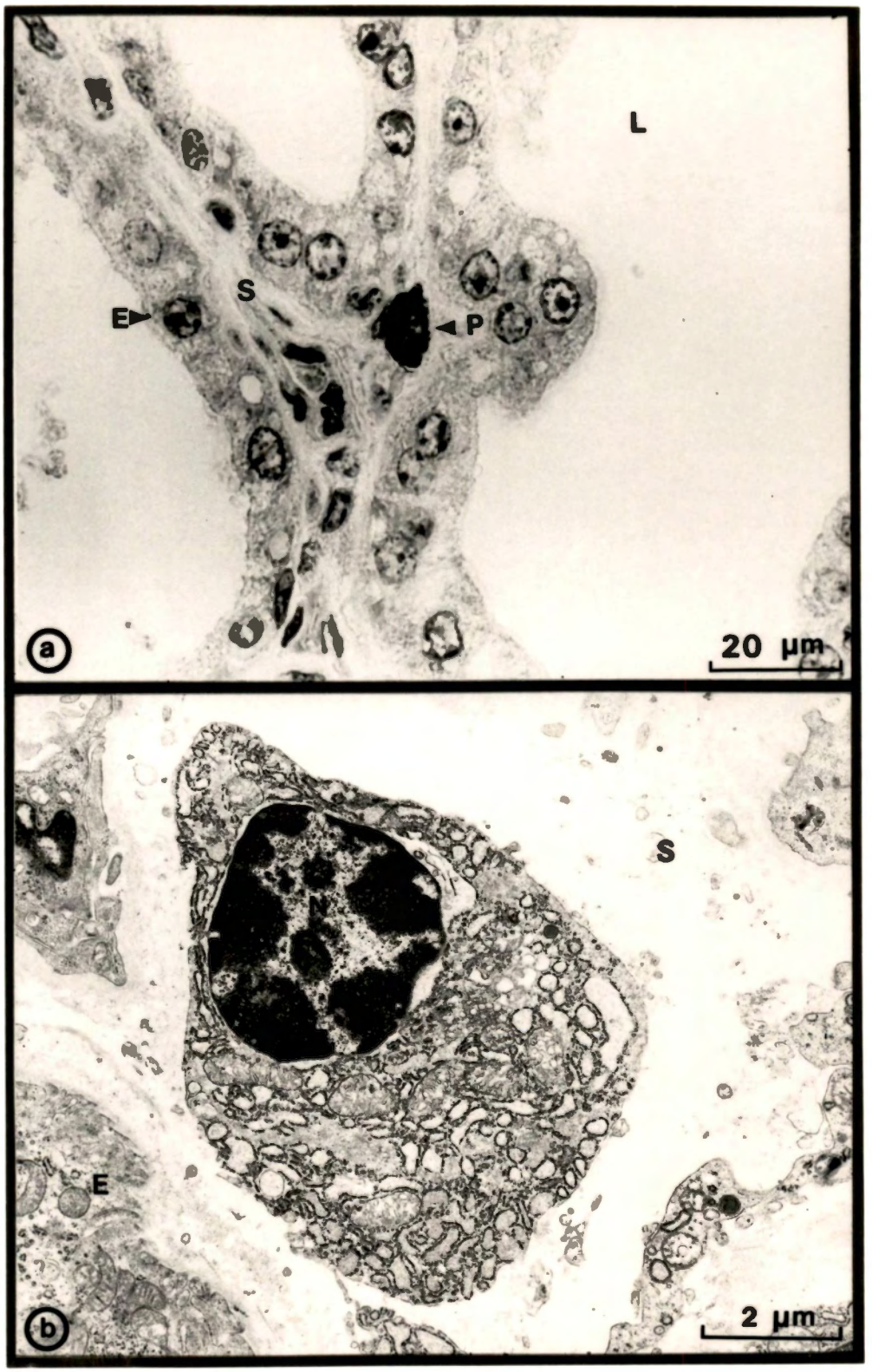
[1] Mean number of plasma cells/5.3×10^4 um^2

* $P < 0.05$ from uninfected.

Figure 2. Immunoglobulin-producing plasma cells in secretory parenchyma were generally observed in subepithelial stroma .

a. Light micrograph of a positively stained plasma cell (933x magnification).

b. Electron micrograph of a plasma cell exhibiting round eccentrically located nucleus with peripherally located heterochromatin (10780x magnification). E, epithelial cells; L, lumen; N, nucleus; P, plasma cell; S, stroma.


L
S
E
P
a
20 µm
S
E
b
2 µm

isotypes were slightly, but not significantly, higher in tissue from Staph. aureus infected quarters. Immunoglobulin G_1-producing cells were the predominant plasma cell type, followed by IgG_2- and IgA-producing cells. Immunoglobulin G_2-producing plasma cells were prevalent in Furstenberg's rosette, but were observed only occasionally in the parenchyma and streak canal.

Tissue from the streak canal contained the lowest number of Ig-producing plasma cells (Table I). No significant differences in numbers of Ig-producing plasma cells were observed between infected and uninfected quarters. Macrophage-like Ig-bearing cells located in the subepithelial stroma were more numerous in the streak canal.

Immunoglobulin concentrations in milk are shown in Table II. The concentration of Ig isotypes in milk from Staph. aureus infected quarters did not differ significantly from milk of uninfected quarters. However, the concentration of all Ig isotypes tended to be higher in milk from infected quarters. Immunoglobulin G_1 was the major Ig isotype in milk from both infected and uninfected quarters, followed by IgA.

The effect of Staph. aureus infection on the index of relative occurrence of milk Ig isotypes was not significant (Table III). The index of IgG_1 was slightly lower in infected quarters. Immunoglobulin A, IgM, and IgG_2 indices tended to be higher in milk from infected quarters.

TABLE II. Concentrations of Immunoglobulin Isotypes and Bovine Serum Albumin in Milk from *Staphylococcus aureus* Infected and Uninfected Quarters.

		Uninfected quarters (n=5)		Infected quarters (n=10)	
		$\bar{x}$	se	$\bar{x}$	se
		mg/ml			
Ig isotype	IgG_1	0.53	0.15	0.56	0.11
	IgG_2	0.04	0.13	0.18	0.10
	IgM	0.05	0.02	0.08	0.01
	IgA	0.11	0.12	0.37	0.08
BSA		0.18	0.04	0.28	0.03

TABLE III. Relative Indices of Occurrence of Immunoglobulin Isotypes in Milk from Staphylococcus aureus Infected and Uninfected Quarters.

Ig isotype	Uninfected quarters (n=5) $\bar{x}$	se	Infected quarters (n=10) $\bar{x}$	se
IgG_1	8.87	1.58	6.45	1.12
IgG_2	0.88	0.39	1.18	0.31
IgM	5.25	2.13	9.28	1.37
IgA	46.65	19.95	95.67	14.11

Immunoglobulin A had the highest index of relative occurrence in milk from both Staph. aureus infected and uninfected quarters while IgG_2 index was the lowest.

Results of plasma cell numbers and milk Ig concentration data evaluated with respect to duration of staphylococcal IMI are shown at Tables IV and V. These data suggest that in persistently infected quarters, IgA- and IgM-producing plasma cells tended to be higher, whereas in other quarters infected with Staph. aureus, IgG_1- and IgG_2-producing plasma cell numbers showed slight increases with the exception of IgG_1-producing plasma cells in Furstenberg's rosette. Quarters that became infected during lactation had significantly more IgG_1-producing plasma cell at Furstenberg's rosette.

Ultrastructural examination of plasma cells from secretory parenchyma (Figure 2b) showed typical characteristics of Ig-producing plasma cells (Bessis, 1961). Eccentrically located round nucleus with peripherally located heterochromatin were observed. Parallel lamellae of rough endoplasmic reticulum were noted in the cytoplasm.

TABLE IV. Plasma Cell Populations in Bovine Mammary Tissue from Uninfected and Staphylococcus aureus Infected Quarters.[1]

Tissue area	Ig isotype	Uninfected quarters (n=5) $\bar{x}$	se	Infected quarters: Persistent IMI (n=5) $\bar{x}$	se	Infected quarters: New IMI (n=5) $\bar{x}$	se
Secretory parenchyma	IgG_1	1.9	0.8	1.5	0.9	3.2	0.8
	IgG_2	0.6	0.5	0.4	0.6	1.9	0.5
	IgM	1.5[a,b]	0.5	2.4[b]	0.5	2.5[a]	0.5
	IgA	2.6[a,b]	0.5	3.8[b]	0.6	1.5[a]	0.5
Furstenberg's rosette	IgG_1	9.7[a]	6.0	18.2[a,b]	6.0	33.9[b]	6.7
	IgG_2	6.9	5.3	13.6	5.3	24.2	6.0
	IgM	1.5	0.6	2.1	0.6	2.8	0.7
	IgA	3.6	0.9	3.7	0.9	4.4	1.0
Streak canal	IgG_1	1.0	0.5	0.7	0.5	0.8	0.5
	IgG_2	0.2	0.2	0.2	0.2	0.2	0.2
	IgM	0.3	0.3	0.7	0.3	0.1	0.3
	IgA	0.4	0.4	1.2	0.4	0.5	0.4

[1] Mean number of plasma cells/5.3×10^4 um^2 tissue area.
[a,b] Means within tissue area and Ig isotype with different superscript differ ($P<0.05$).

TABLE V. Immunoglobulin and Bovine Serum Albumin Concentrations of Milk from Uninfected and Staphylococcus aureus Infected Quarters.

		Uninfected quarters (n=5)		Infected quarters: Persistent IMI (n=5)		Infected quarters: New IMI (n=5)	
		$\bar{x}$	se	$\bar{x}$	se	$\bar{x}$	se
		mg/ml					
Ig isotype	IgG_1	0.54	0.14	0.41	0.14	0.67	0.14
	IgG_2	0.04	0.13	0.24	0.13	0.15	0.14
	IgM	0.05	0.01	0.06	0.01	0.09	0.01
	IgA	0.11	0.11	0.35	0.11	0.39	0.11
BSA		0.18	0.04	0.29	0.04	0.28	0.04

5. DISCUSSION

Results of this study suggest that the antigenic effect of Staph. aureus IMI on the bovine mammary gland humoral immune system is minimal. Unresponsiveness of the bovine mammary gland to immunogenic stimulation even after multiple antigen administration has been reported (Brock et al.,

1975; Adlam et al., 1981). Whether suboptimal stimulation is associated with immunosuppression or with the ability of bacteria to escape immune surveillance is unknown. Chronic Staph. aureus mastitis has been reported to cause impaired blastogenic response of bovine mammary lymphocytes to various mitogens (Nonnecke and Harp, 1985). Since blast transformation of lymphocytes upon exposure to mitogens is a widely accepted indicator of a competent immune system, nearly complete loss of blastogenic response of lymphocytes to mitogens suggested a possible impaired immune system in Staph. aureus infected bovine mammary glands (Nonnecke and Harp, 1985). Supporting these findings, in our study, no significant plasma cell migration or proliferation in secretory parenchyma of Staph. aureus infected quarters were observed compared to control quarters. Also, Ig concentrations of milk from infected quarters were not significantly different from control quarters, indicating a possible unstimulated humoral immunity in the mammary gland against Staph. aureus IMI. Suppressive effects of several pathogens such as mycoplasmas, viral diarrhea virus, enzootic bovine leukosis virus, on the bovine immune system have been documented (Muscoplat et al., 1973; Roberts et al., 1973; Weiland and Straub, 1976).

Slight increases in plasma cell numbers of parenchymal tissue paralelled slight increases in milk Ig concentrations. However, this does not necessarily imply

that all Ig in milk were synthesized by plasma cells located in the gland during infection, since increases in both Ig transfer from serum and Ig production by plasma cells located in tissue may occur in the same quarter simultaneously (Butler, 1981). Thus, elevation in Ig concentrations of milk is most likely associated with both mechanisms. Relative indices of milk Ig isotypes were used to speculate on transfer mechanisms of Ig into milk (Guidry et al., 1980). Butler (1981) suggested that a high relative index of occurrence (>2) of milk Ig might indicate selective transfer of Ig from serum and/or sythesis of Ig by mammary gland plasma cells (Butler, 1981). The relative index of occurrence of milk IgA (Table III) suggests that in Staph. aureus infected quarters, selective accumulation mechanism may transfer a greater proportion of milk IgA from serum since the number of IgA-producing cells in tissue did not increase.

The source of IgA in ruminant lacteal secretions is thought to be mammary gland plasma cells (Butler et al., 1971; Mach and Pahud, 1971; Butler, 1973; Lascelles and McDowel, 1974; Lascelles, 1979), although there are reports indicating the contributory effects of serum derived IgA on total IgA concentrations in milk (Newby and Bourne, 1977; Sheldrake et al., 1984). Serum derived or locally synthesized IgA is thought to be transferred selectively by a secretory component mediated mechanism from intercellular

spaces into milk (Lascelles et al., 1981). However, evidence of sIgA transfer as well as transfer of the dimeric IgA molecule without secretory piece indicates the presence of other transfer mechanism(s) (Butler, 1986).

Immunoglobulin A-producing plasma cell numbers in the parenchyma from infected quarters did not differ from parenchymal tissues of uninfected quarters. Contrary to our results, Nickerson and Heald (1982) found significantly more IgA-producing plasma cells in secretory tissue during the early phase of experimental Staph. aureus infection. However, recently, Sordillo and Nickerson (1987) noted slight decreases in IgA-producing plasma cells in tissue from quarters infected chronically with major pathogens including Staph. aureus during the nonlactating period. In the present study, one half of the infected quarters had persistent staphylococcal IMI from the previous lactation, whereas the other quarters acquired the infection during lactation. Thus, the duration of infection may be an important factor in the immune response mounted against Staph. aureus.

In ruminant milk, the IgG_1:IgG_2 ratio is much greater than that of serum and a highly selective transfer mechanism for IgG_1 has been documented in the mammary gland (Sasaki et al., 1977). During acute clinical mastitis, nonselective transfer of IgG_2 increased while selective accumulation of IgG_1 was reduced markedly (Lascelles, 1979). As the

infection became chronic, the process was reversed (Lascelles, 1969). Results of the present study found only slight changes in relative indices of milk IgG subclasses indicating that staphylococcal IMI did not alter transfer mechanisms of IgG subclasses significantly which agreed with another study (Lascelles, 1969).

Previous research has shown that teat end tissues were important in resistance to mastitis by acting as physical barriers against invading pathogens as well as containing keratin which has antibacterial activity (Craven and Williams, 1985). More recent studies (Nickerson and Pankey, 1983; Nickerson at al., 1984; Collins et al., 1986) demonstrated the presence of plasma cells and other infiltrating leucocytes in teat end tissues and a possible immunological role of teat end tissues was suggested (Nickerson and Pankey, 1983). Morphometric analysis of teat end tissues revealed that occurrence of leucocytes in Furstenberg's rosette was striking (Nickerson and Pankey, 1983; Collins et al., 1986). Although IgG_2-producing plasma cells were not determined, most Ig-producing plasma cells in Furstenberg's rosette were IgG_1-producing plasma cells, followed by IgM, and IgA-producing plasma cells (Collins et al., 1986). In the present study, similar results were observed. Most plasma cells were found in Furstenberg's rosette and occasionally in the streak canal of infected and uninfected quarters. Immunoglobulin G_1-

producing plasma cells were more numerous compared to other plasma cell types in all quarters. In Staph. aureus infected quarters, IgG_1-producing plasma cell numbers were significantly elevated, indicating a possible immunostimulatory effect of infection on plasma cell migration to Furstenberg's rosette and/or on proliferation of plasma cells in Furstenberg's rosette. Assesment of the biological importance of plasma cell function in Furstenberg's rosette is difficult. However, the location of teat end tissues in terms of establishment of IMI might imply additional significance to these structures with respect to prevention of new IMI (Nickerson, 1985). Antibodies synthesized by plasma cells located in close proximity to the epithelial lining might have easier access to the lumen and subsequently to invading pathogens (Nickerson and Pankey, 1983) as well as function in tissues as barriers to bacterial invasion (Collins et al., 1986). Antibodies in the lumen could play an important role by inhibiting bacterial attachment to epithelial cells and/or neutralizing toxins (Nickerson and Pankey, 1983).

Cytophilicity of ruminant IgG subclasses was first recognized by Watson (1975, 1976) who demonstrated the cytophilicity of IgG_2 for neutrophils in sheep. Later, cytophilic activities of ovine IgG_1 but not IgG_2 were reported for macrophages (Yasmeen, 1980). Desiderio and Campbell (1980) found that bovine IgG_2, and to a lesser

extent IgG_1, were cytophilic for mammary gland macrophages. Thus, Ig-bearing cells in bovine mammary tissue should not be classified as Ig-producing plasma cells since other cell types with cytophilic and/or endocytosed Ig could stain positively for Ig isotypes (Butler, 1981; Leary et al., 1982). In this study, some IgG_1- and IgG_2-bearing cells with a macrophage-like appearence were noted, and these cells might be cytophilic Ig-bearing macrophages. Cytophilic Ig could provide a superior phagocytic capability for leucocytes in the elimination of specific antigens (Watson, 1976). In addition, depending upon the binding affinity of uncomplexed antibodies to Fc receptors on leucocytic cells, large amounts of Ig might be tranferred passively into milk by these cells (Colditz and Watson, 1985).

Our data suggests that hyporesponsiveness of the humoral immune system of the bovine mammary gland against Staph. aureus infection and possibly against other antigens infused for immunization purposes might result from insufficient plasma cell migration to mammary gland tissues and/or suboptimal proliferation of mammary gland plasma cells. Only slight increases in Ig concentrations of milk from infected quarters indicates that involvement of systemic humoral immune mechanisms in the process of elimination of infection is also minimal. Overall, the unstimulatory character of staphylococcal infection on

plasma cell function may help the bacteria to establish persistent infections.

REFERENCES

1. Adlam, C., Keery, J. B., Edkins, S. E., and Ward, P. D., 1981. Local and Systemic antibody responses in cows following immunization with staphylococcal antigens in the dry period. J. Comp. Path., 91: 105-113.
2. Bessis, M. C., 1961. Ultrastructure of ltmphoid and plasma cells in relation to globulin and antibody formation. Lab. Inv., 10: 1040-1067.
3. Brock, J. H., Steel, E. D., and Reiter, B., 1975. The effect of intramuscular and intramammary vaccination of cows on antibody level and resistance to intramammray infection by Staphylococcus aureus. Res. Vet. Sci., 19: 152-158.
4. Brown, R. W., Barnom, D. A., Jasper, D. E., McDonald, J. S., and Shultze, W. D., 1981. Microbiological procedures for use in the diagnosis of bovine mastitis. National Mastitis Council, Inc., Second Edition. Washington, D. C.
5. Butler, J. E., 1973. Synthesis and distribution of immunoglobulins. J. Am. Vet. Med. Assoc., 163: 795-798.

6. Butler, J. E., 1981. A concept of humoral immunity among ruminants and an approach to its investigation. In: J. E. Butler (Editor). The Ruminant Immune System. Plenum, New York, London. pp. 3-55.
7. Butler, J. E., Frenyo, V. L., Whipp, S. C., Wilson, R. A., and Koertge T. E., 1986. Metabolism and transport of bovine serum sIgA. Comp. Immunol. Micro. Inf. Dis., 9: 303-315.
8. Butler, J. E., Kiddy, C. A., Maxwell, C., Hylton, M. B., and Asofsky, R., 1971. Sythesis of immunoglobulins by various tissues of the cow. J. Dairy Sci., 54: 1323-1324.
9. Colditz, I. G., and Watson, D. L., 1985. Immunophysiological basis for vaccinating ruminants against mastitis. Aust. Vet. J., 62: 145-153.
10. Collins, R. A., Parsons, K. A., and Bland, A. P., 1986. Antibody containing cells and specialised epithelial cells in the bovine teat. Res. Vet. Sci., 41: 50-55.
11. Craven, N., and Williams, M. R., 1985. Defenses of the mammary gland against infection and prospects for their enhancement. Vet. Immunol. Immunopathol., 10: 71-127.

12. Desiderio, J. W., and Campbell, S. G., 1980. Bovine mammary gland macrophages; isolation, morphologic features, and cytophilic immunoglobulins. Am. J. Vet. Res., 41: 1595-1599.

13. Guidry, A. J., and Miller, R. H., 1985. Immunoglobulin isotypes concentrations in milk as affected by the stage of lactation and parity. J. Dairy Sci., 69: 1799-1805.

14. Guidry, A. J., Butler, J. E., Pearson, R. E., Weinland, B. T., 1980. IgA, IgG_1, IgG_2, IgM, and BSA in serum and mammary secretions throughout lactation. Vet. Immunol. Immunopathol., 1: 329-341.

15. Lascelles, A. K., 1969. Immunoglobulin secretion into ruminant colostrum. In: M. Reynolds, and S. F. Folley, (Editors), Lactogenesis; The Initiation of Milk Secretion at Parturition. Univ. Pennsylvania Press, Philedelphia, pp. 131-136.

16. Lascelles, A. K., 1979. The immune system of the ruminant mammary gland and its role in the control of mastitis. J. Dairy Sci., 62: 154-160.

17. Lascelles, A. K., and McDowell, G. H., 1974. Localized humoral immunity, with particular referenceto ruminants. Transplant. Rev., 19: 170-208.

18. Lascelles, A. K., Beh, K. J., and Husband, A. J., 1981. Origin of antibody in mammary secretion with particular reference to the IgA system. In: J. E. Butler (Editor). The Ruminant Immune System, Plenum, New York, London, pp. 493-511.

19. Leary, Jr, H. L., Larson, B. L., and Nelson, D. R., 1982. Immunohistochemical localization of IgG_1 and IgG_2 in peripartum and lactating bovine mammary tissue. Vet. Immunol. Immunopathol., 3: 509-514.

20. Mach, J. P., and Pahud, J. J., 1971. Secretory IgA; Major immunoglobulin in most bovine external secretions. J. Immunol., 106: 552-563.

21. Muscoplat, C. C., Johnson, D. W., and Stevens, J. B., 1973. Abnormalities of in vitro lymphocyte response during bovine viral diarrhea virus infection. Am. J. Vet. Res., 34: 753-755.

22. Newby, T. J., and Bourne, J., 1977. The nature of local immune system of the bovine mammary gland. J. Immunol., 118: 41-45.

23. Nickerson, S. C., 1985. Immune mechanisms of the bovine udder; An overview. J. Am. Vet. Med. Assoc., 187: 41-45.

24. Nickerson, S. C., and Heald, C. W., 1982. Cells in local reaction to experimental Staphylococcus aureus infection on bovine mammary gland. J. Dairy Sci., 65: 105-116.

25. Nickerson, S. C., and Pankey, J. W., Cytologic observations of the bovine teat end. Am. J. Vet. Res., 44: 1433-1441.

26. Nickerson, S. C., Pankey, J. W., and Boddie, N. T., 1984. Distribution, location, and ultrastructure of plasma cells in the uninfected, lactating bovine mammary gland. J. Dairy Res., 51: 209-217.

27. Nonnecke, B. J., and Harp, J. A., 1985. The effect of chronic staphylococcus mastitis on mitogenic responses of bovine lymphocytes. J. Dairy Sci., 68: 3323-3328.

28. Norcross, N. L., 1977. Immune response of mammary gland and the role of immunization in mastitis control. J. Am. Vet. Med. Assoc., 170: 1228-1231.

29. Oehme, F. W., and Coles, E. H., 1967. Field use and evaluation of a vaccine for bovine staphylococcic mastitis. J. Dairy Sci., 50: 1792-1797.

30. Oliver S. P., and Smith, K. L., 1982. Bovine mammary involution following intrammary infusion of colchicine and endotoxin at drying off. J. Dairy Sci., 65: 801-813.

31. Pankey, J. W., Boddie, N. T., Watts, J. L., and Nickerson, S. C., 1985. Evaluation of protein A and a commercial bacterin as vaccines aginst Staphylococcus aureus mastitis bt experimental challenge. J. Dairy Sci., 68: 726-731.

32. Roberts, D. H., Windsor, R. S., Masiga, W. N., and Kariavu, C. G., 1973. Cell-mediated immune response in cattle to Mycoplasma mycoides var. mycoides. Inf. Immun., 8: 349-354.

33. Sasaki, M., Larson, B. L., and Nelson, D. R., 1977. Kinetic analysis of the binding of immunoglobulins IgG_1 and IgG_2 to bovine mammary cells. Biochim. Biophys. Acta, 497: 160-170.

34. SAS User's Guide; Statistics, 1982 Edition. SAS Ins. Inc., Cary, NC.

35. Sheldrake, R. F., Husband, A. J., Watson, D. L., and Cripps, A. W., 1984. Selective transport of serum derived IgA into mucosal secretions. J. Immunol., 132: 363-368.

36. Sordillo, L. M., and Nickerson, S. C., 1987. Quantification and immunoglobulin classification of plasma cells in nonlactating bovine mammary tissue. J. Dairy Sci., (Accepted for publication).

37. Watson, D. L., 1975. Cytophilic attachment of ovine IgG_2 to autologous polymorphonuclear leucocytes. Aust. J. Exp. Biol. Med. Sci., 53: 527-529.

38. Watson, D. L., 1976. The effect of cytophilic IgG_2 on phagocytosis by ovine polymorphonuclear leucocytes. Immunol., 31: 159-165.

39. Weiland, F., and Straub, O. C., 1976. Biological properties of lymphocytes from leucotic cattle. Vet. Microbiol., 1: 387-388.

40. Willoughby, R. A., 1966. Bovine staphylococcic mastitis; An immunohistochemical study of the cellular sites of antibody formation. Am. J. Vet. Res., 27: 522-532.

41. Yasmeen, D., 1981. Antigen specificcytophilic activity of sheep IgG_1 and IgG_2 antibodies. Aus. J. Exp. Biol. Med. Sci., 59: 297-302.

42. Yurchak, A. M., Butler, J. E., and Thomasi, Jr, T. B., 1971. Fluorescent localization of immunoglobulins in the tissues of the cow. J. Dairy Sci., 54: 1324-1325.

APPENDIX

TABLE VI. Concentrations of Immunoglobulin Isotypes and Bovine Serum Albumin in Serum from Staphylococcus aureus Infected and Uninfected Animals.

		Uninfected (n=4)		Infected (n=4)	
		$\bar{x}$	se	$\bar{x}$	se
		----------mg/ml----------			
Ig isotype	IgG_1	19.67	2.57	10.85*	1.81
	IgG_2	14.45	1.78	15.12	1.26
	IgM	1.86	0.18	1.29*	0.13
	IgA	0.58	0.08	0.51	0.06
BSA		43.61	3.13	38.63	2.21

* $P < 0.05$ from uninfected.

TABLE VII. Distribution of Macrophage-like Immunoglobulin-bearing Cells in Bovine Mammmary Tissue from Staphylococcus aureus Infected and Uninfected Quarters.[1,2]

Tissue area	Ig isotype	Uninfected quarters (n=5) $\bar{x}$	se	Infected quarters (=10) $\bar{x}$	se
Secretory parenchyma	IgG_1	0.5	1.2	3.0	0.9
	IgG_2	1.4	2.0	5.6	1.5
Furstenberg's rosette	IgG_1	2.8	3.2	15.7*	2.4
	IgG_2	4.7	4.1	18.8**	3.1
Streak canal	IgG_1	6.4	4.1	10.3	2.9
	IgG_2	3.7	4.7	15.4	3.3

[1] Mean number of plasma cells/5.3×10^4 um^2 tissue area.

* $P < 0.01$ from uninfected.

** $P < 0.05$ from uninfected.

VITA

Mehmet Z. Doymaz was born in Harmancik, Bursa, Turkey, on March 8, 1962. Following graduation from Bursa Imam Hatip Lisesi, he was admitted to Ankara Universitesi Veteriner Fakultesi in September, 1978, and he received Veteriner Hekim Degree in June, 1983.

He has been studying at The University of Tennessee, Knoxville, since September, 1985 on a scholarship from The Turkish Ministry of Education.