SYMPOSIUM: BOVINE IMMUNOLOGY

Immunobiology of the Mammary Gland

L. M. SORDILLO,[1] K. SHAFER-WEAVER, and D. DeROSA
Department of Veterinary Science, Center for Mastitis Research,
The Pennsylvania State University, University Park 16802-3500

ABSTRACT

The mammary gland is a complex organ that provides neonatal offspring with milk for nourishment and disease resistance. Specific and innate immune factors associated with mammary gland tissues and secretion also play a vital role in protecting the gland from infectious disease. Through genetic selection and technological advances in milk removal, the bovine mammary gland yields far more milk than is needed to nourish the newborn calf. This excess is the basis of the dairy industry. Factors associated with the intense management of dairy cattle can profoundly affect mammary gland immunity and the ability of the host to resist mastitis. Technological advances in immunology have led to the availability of new research tools that can facilitate the study of mammary gland immunity and disease pathogenesis. In recent years, considerable research effort has focused on enhancing the natural defense mechanisms of the mammary gland during periods of heightened susceptibility to disease. This paper provides a comprehensive overview of mammary gland immunity with special emphasis on the bovine system. The underlying mechanisms of disease susceptibility and development of potential immunoregulatory strategies to control mastitis are discussed.

(**Key words**: mammary immunity, mastitis, vaccines, cytokines)

Abbreviation key: **ADCC** = antibody-dependent, cell-mediated cytotoxicity, **CSF** = colony-stimulating factor, **G** = granulocyte (used in combined form only), **GM** = granulocyte-macrophage, **IFN** = interferon, **IL** = interleukin, **MHC** = major histocompatibility complex, **NK** = natural killer, **rb** = recombinant bovine, **rh** = recombinant human, **TNF** = tumor necrosis factor.

INTRODUCTION

The prevention and treatment of mastitis are primary concerns of the dairy industry. Mastitis is the most devastating disease affecting adult dairy cows, and the associated economic losses continue to present a serious burden to producers. Current practices of mastitis control are based on proper milking hygiene, reduced exposure to environmental pathogens, and dry cow antibiotic therapy; these practices have reduced occurrence of the disease. However, the most recent estimates of the National Mastitis Council (15) suggest that mastitis affects one-third of every dairy cow ($\bar{X}$ = 1.5 quarters). Clearly, new and innovative approaches for mastitis control are needed.

One means of decreasing the impact of mastitis on the dairy industry is to increase the natural ability of the cow to resist infections. Defense of the mammary gland against mastitis-causing pathogens is mediated by several anatomical, cellular, and soluble protective factors. Once bacteria successfully penetrate the teat end opening, it is the efficiency of these defense mechanisms that determines the resistance of the mammary gland to new IMI. There are certain times in the lactation cycle when mammary gland defenses fail to operate properly and cows become more susceptible to mastitis. Strategies aimed at enhancing immune systems of the mammary gland during these periods of immunosuppression would greatly impact the ability of the cow to resist infection. This paper reviews mammary gland immunobiology and the potential role of immunomodulators in the control of mastitis.

DISCUSSION

Mammary Gland Immunity

The mammary gland is protected by a variety of defense mechanisms, which can be separated into two distinct categories: innate immunity and specific immunity. Innate immunity, also known as nonspecific responsiveness, is the predominant defense during the early stages of infection. Nonspecific responses are present or are activated quickly at the site of infection by numerous stimuli; however, they are not

Received July 16, 1996.
Accepted January 28, 1997.
[1]Corresponding author. Present address: 115 Henning Building, Department of Veterinary Science, The Pennsylvania State University, University Park 16802-3500.

augmented by repeated exposure to the same insult. Nonspecific or innate responses of the mammary gland are mediated by the physical barrier of the teat end, macrophages, neutrophil, natural killer (**NK**)-like cells, and by certain soluble factors. Conversely, the specific or acquired immune system recognizes specific determinants of a pathogen that facilitate selective elimination. Recognition of pathogenic factors is mediated by antibody molecules, macrophages, and several lymphoid populations. Because of the "memory" of certain lymphocytes, specific immune responses can be augmented by repeated exposure to a pathogen. In the mammary gland, both innate and acquired protective factors are coordinated to provide optimal protection from disease.

Anatomical defenses. Mastitis occurs when bacteria gain entrance to the mammary gland via the teat canal. For this reason, the teat end is considered to be the first line of defense against invading pathogens. The teat end contains sphincter muscles that maintain tight closure between milkings and hinder bacterial penetration. Increased patency of these muscles is directly related to increased incidence of mastitis (54).

The teat canal is lined with keratin, which is crucial to the maintenance of the barrier function of the teat end, and removal of the keratin has been correlated to increased susceptibility to bacterial invasion and colonization (6, 9). Teat keratin is a waxy material that is derived from stratified squamous epithelium. The keratin structure enables trapping of invading bacteria, thus hindering their migration into the gland cistern (33). Within the keratin lining, antimicrobial agents have been identified. The esterified and nonesterified fatty acids present in teat keratin, such as myristic acid, palmitoleic acid, and linoleic acid, are bacteriostatic (110). Additionally, cationic protein in the canal can bind electrostatically to mastitis pathogens, which alters the bacterial cell wall, thus rendering them more susceptible to osmotic pressure. The inability to maintain osmolarity causes lysis and death of the invading pathogens (54, 110).

Cellular defenses. Bacterial pathogens that are able to traverse the teat end opening must then escape the antibacterial activities of the mammary gland microenvironment in order to establish disease. The activities of resident and newly recruited leukocytes during the early stages of pathogenesis play a pivotal role in the establishment of IMI. Milk SCC consist of several cell types, including neutrophils, macrophages, lymphocytes, and a smaller percentage of epithelial cells. In the healthy lactating mammary gland, total SCC are often $<10^5$/ml of milk. During a bacterial IMI, however, total SCC can increase to $>10^6$/ml of milk within just a few hours (62, 67). Studies (29, 35) have shown that the severity and duration of mastitis is critically related to the promptness of the leukocyte migratory response and the bactericidal activity of SCC at the site of infection. Some bacteria release metabolic by-products, enterotoxins, or cell-wall components as they colonize and grow in the mammary gland. These bacterial factors either directly or indirectly serve as chemoattractants for leukocytes. If SCC move rapidly from the blood stream and are able to eliminate the inflammatory stimuli (bacteria), then recruitment of leukocytes ceases, and SCC returns to healthful levels. If bacteria are able to survive this immediate host response, then the inflammation continues, resulting in SCC migration between adjacent mammary secretory cells toward the alveolar lumen (10). Prolonged diapedesis of leukocytes causes damage to mammary parenchyma tissue, resulting in decreased production of milk (31, 96). The duration and severity of the inflammatory response have a major impact on the quantity and quality of the milk produced (97, 98).

Neutrophils are the predominant cell type found in mammary tissues and in mammary secretions during early inflammation and can constitute >90% of total mammary gland leukocytes (62, 97, 98). These nonspecific cells travel from the blood to the mammary gland in response to a variety of inflammatory mediators, such as cytokines, complement, and prostaglandins (4, 66). Once at the site of infection, neutrophils phagocytose and kill bacterial pathogens. Neutrophils exert their bactericidal effect through a respiratory burst that produces hydroxyl and oxygen radicals, which are key components of the oxygen-dependent killing mechanism. Bacteria are killed by the action of superoxide ions, hypochlorite, and hydrogen peroxide. During phagocytosis, bacteria also may be exposed to several oxygen-independent reactants such as peroxidase, lysozyme, various hydrolytic enzymes, and lactoferrin (see Soluble Defenses section). In addition to their phagocytic capabilities, neutrophils are a source of small antibacterial peptides, the defensins, which are able to kill a number of the pathogens that cause mastitis (83).

Macrophages are the predominant cell type found in the milk and tissues of healthy involuted and lactating mammary glands (42, 46, 96, 98). Like neutrophils, macrophages are active mammary gland phagocytic cells that are capable of ingesting bacteria, cellular debris, and accumulated milk components (96). The phagocytic rate of macrophages can be increased substantially in the presence of opsonic antibody for specific pathogens. Because of the

indiscriminate ingestion of fat, casein, and other milk components, mammary gland neutrophils and macrophages are less effective at phagocytosis than are blood leukocytes (62, 93, 114). The phagocytic and bactericidal activities of these cells are especially diminished during the periparturient period (62, 114).

In addition to their role in early nonspecific defenses, macrophages also play a key role in antigen processing and presentation (22, 70). Antigens from ingested bacteria are processed within macrophages and appear on the membrane in association with major histocompatibility complex (**MHC**) class II antigens. These MHC class II antigens are polymorphic membrane molecules that are required by other host cells (lymphocytes) for the recognition of foreign antigens.

Generation of effective specific immunity involves both antigen-presenting cells and lymphocytes. Lymphocytes are the only cells of the immune system that recognize antigens through membrane receptors that are specific for invading pathogens. There are two distinct subsets of lymphocytes, which differ in function and protein products, T and B lymphocytes. The T lymphocyte can be further subdivided into $\alpha\beta$ T lymphocytes, which include CD4+ (T-helper) lymphocytes and CD8+ (T-cytotoxic or T-suppressor) lymphocytes, and $\gamma\delta$ T lymphocytes. Depending on stage of lactation and tissue location, the percentages of these cells can vary significantly.

The T-helper lymphocytes produce cytokines in response to recognition of an antigen-MHC complex on B lymphocytes and macrophages. Through the ability to secrete certain cytokines, CD4+ cells play an important role in activating B-lymphocytes, T-lymphocytes, macrophages, and various other cells that participate in the immune response. The relative proportion of CD4+ cells from the total T-lymphocyte population of the mammary gland contrasts with that in peripheral blood (58, 64, 85, 115). Previous studies have shown CD8+ lymphocytes are the predominant phenotype in both tissues and secretions of healthy mammary glands; the ratio of CD4 to CD8 is <1. Cells obtained from blood have a higher ratio of CD4 to CD8, which is consistently >1.

The functional significance of the elevated frequency of CD8+ over CD4+ lymphocytes in milk and mammary tissue has not been defined thoroughly. However, it is well established that CD8+ lymphocytes can exert either cytotoxic or suppressor function (41). Cytotoxic T lymphocytes recognize and eliminate altered self cells via antigen presentation in conjunction with MHC class I molecules. Therefore, cytotoxic cells may act as scavengers, removing old or damaged secretory cells, the presence of which could increase the susceptibility of the mammary gland to infections (107). Suppressor T-lymphocytes are thought to control or modulate the immune response. Researchers (36, 39) have demonstrated that CD8+ lymphocytes, activated during bacterial infections, can suppress important host immune responses. Evaluation of lacteal secretions from mammary glands of dairy cows infected with *Staphylococcus aureus* also reveal a subpopulation of activated CD8+ lymphocytes that are capable of altering or suppressing the proliferative responses of CD4+ lymphocytes (65). Recent research [K. A. Shafer-Weaver and L. M. Sordillo, 1997, Bovine CD8+ suppressor lymphocytes alter immune responsiveness during the postpartum period. Vet. Immunol. Immunopathol. (in press)] with bovine mammary CD8+ lymphocytes suggests that the immunoregulatory roles of these cells depends on stage of lactation. Cells obtained from mid-lactation cows exhibited cytotoxic activity, but no cytotoxic activity was observed for CD8+ cells that were isolated from postpartum cows. Cells that were obtained during the postpartum period mainly expressed interleukin-4 mRNA, which is characteristic of the suppressor phenotype, as opposed to interferon (**IFN**)-γ mRNA, which is primarily expressed by cytotoxic cells (K. A. Shafer-Weaver and L. M. Sordillo, 1997, in press). Flow cytometric analysis also revealed that CD8+ lymphocytes have higher activation and higher expression of the β-chain during the postpartum period than during later lactation. Collectively, these data indicate that CD8+ lymphocytes immediately following parturition are of the suppressor type but from mid to late lactation are more of the type that have a cytotoxic nature. The preferential trafficking of CD8+ suppressor lymphocytes into mammary gland tissues and secretion may be responsible for the lower responsiveness of local leukocytes compared with those from peripheral blood.

The biological functions of $\gamma\delta$ T lymphocytes have been the subject of much speculation. Their functions are primarily associated with the protection of epithelial surfaces. The $\gamma\delta$ T lymphocytes preferentially migrate to epithelial surfaces and do not circulate extensively (2, 48). There are indications that $\gamma\delta$ T lymphocytes can mediate cytotoxicity with variable involvement of MHC (49) and can mediate some NK activity. The cytotoxic ability of $\gamma\delta$ T cells suggests that they may be able to destroy altered epithelial cells (48). In fact, IL-2 cultured $\gamma\delta$T lymphocytes are capable of recognizing and lysing malignant breast carcinoma cells lines (52). These cells may also play a role in infectious diseases and therefore provide an important line of defense against bacterial infections (48). Relative to the blood, both humans and ruminants express greater levels of $\gamma\delta$ T lymphocytes in mammary secretions and mammary parenchyma (78, 84). The finding that the percentages of $\gamma\delta$ T

lymphocytes decrease significantly in the mammary parenchyma during times of increased susceptibility to disease (84) suggests that these lymphocytes may constitute an essential line of defense against the bacteria causing mastitis.

The NK cells are large, granular, nonimmune lymphocytes that possess cytotoxic ability in the absence of MHC restriction. However, NK cells contain Fc receptors that enable these cells to participate in antibody-dependent, cell-mediated cytotoxicity (**ADCC**). Once the NK cell binds an antibody-bound target cell via Fc receptors, destruction of target cells is mediated by degranulation of granules containing perforin. The NK cells also can secrete various toxic molecules, such as tumor necrosis factor (**TNF**)-α, which may initiate apoptosis in altered cells.

The ability of NK cells to mediate ADCC suggests that they can play a role in bacterial infections. In fact, research has shown that human NK cells are capable of killing both Gram-positive and Gram-negative bacteria by an extracellular mechanism (25). Lymphoid cells that are isolated from bovine mammary gland with NK-like activity also exhibit a novel antibacterial property (94). Upon stimulation with IL-2, these lymphoid cells demonstrated an increased ability to kill *S. aureus* in a nonspecific manner (94). The mechanism by which these cells mediated bacterial killing has not been fully elucidated. However, this research suggests that NK-like cells may play an important role in eliminating bacteria from the mammary gland.

The primary role of B lymphocytes is to produce antibodies against invading pathogens. Unlike macrophages and neutrophils, B lymphocytes utilize their cell surface receptors to recognize specific pathogens. The B lymphocytes can internalize, process, and present antigen in the context of MHC class II molecules to T-helper lymphocytes. Upon presentation of the processed antigen to T-helper lymphocytes, IL-2 is secreted by the T lymphocytes, which in turn induce proliferation and differentiation of the B lymphocyte into either plasma cells that produce antibody or memory cells. Under certain conditions, differentiation of B lymphocytes can be directly stimulated by an antigen such as lipopolysaccharide. Unlike T lymphocytes, the percentages of B lymphocytes remain fairly constant between stages of lactation (84).

Soluble defenses. The soluble factors are associated with defense functions of the mammary gland in concert with cellular defenses in milk and tissue; each system modifies the effector functions of the other. These soluble factors can be divided into innate and specific components. Immunoglobulins function as the soluble effector of specific or humoral immune responses. These proteins are produced by antigen-activated B lymphocytes that subsequently proliferate and differentiate into antibody-secreting plasma cells. Antibodies in lacteal secretions are synthesized locally or are selectively transported or transudated from serum (3, 95). Four classes of Ig are known to influence mammary gland defense against bacteria causing mastitis: IgG_1, IgG_2, IgA, and IgM. Each of these classes differs in physiochemical and biological properties (26, 55).

The concentration of each Ig class in mammary secretion varies depending on stage of lactation and infection status of the mammary gland. In healthy glands, the concentration of Ig is low during lactation but slowly increases during the nonlactating periods and reaches peak concentrations during colostrogenesis (98). High concentrations of Ig also occur in the mammary gland during inflammation. The concentration of Ig in the gland is dependent upon the degree of permeability of secretory tissue and the number of Ig-producing cells that are present in the mammary gland (98). Although IgG_1 is the predominant isotype in healthy bovine lacteal secretion, neutrophils can transport IgG_2 to the mammary gland as they emigrate to the site of inflammation (26, 55).

Research has shown that IgG_1, IgG_2, and IgM can act as bacterial opsonins that enhance phagocytosis of neutrophils and macrophages. These antibodies can bind bacterial pathogens directly or with the C3b component of complement (40). Neutrophils and macrophages can bind antibody-bacteria complexes and antibody-C3b-bacteria complexes via their Fc receptors and subsequently more effectively phagocytize the invading bacteria. In contrast, IgA does not bind complement or opsonize bacteria. Instead, IgA appears to contribute to agglutination, preventing bacterial colonization and toxin neutralization (55). Because Ig are important for local defense of the mammary gland against pathogens, researchers are focusing on enhancing their concentrations in milk and sera (see Enhancing Specific Immunity section).

The mammary gland also contains nonspecific bacteriostatic components that work independently and in concert with Ig and cellular factors to provide protection to the mammary gland. These factors include lactoferrin, complement, lysozyme, and the lactoperoxidase-thiocyanate-hydrogen peroxide system. Lactoferrin is an iron-binding protein produced by epithelial cells and leukocytes and, in the presence of bicarbonate, sequesters free ferric ions present in milk. Lactoferrin is bacteriostatic by its ability to prevent growth of bacteria, such as staphylococci and coliforms, which have iron requirements (5, 8). With-

holding iron from bacteria may enhance killing by phagocytes by preventing the production of dismutase, a bacterial enzyme that inactivates superoxide radicals. Lactoferrin also may be active in modulation and regulation of macrophages, lymphocytes, and neutrophil function (88, 89). In ruminants, lactoferrin and specific IgG_1 antibodies act synergistically to inhibit the growth of *Escherichia coli* and *Klebsiella pneumoniae* (60). However, the bacteriostatic activity of lactoferrin can be abolished in the presence of citrate, a buffer produced by epithelial cells that chelates iron into a form that is readily usable by bacteria. Some bacteria, such as *Streptococcus agalactiae*, may be able to utilize lactoferrin as an iron source by binding lactoferrin via surface receptors (72). In the healthy mammary gland, the concentration of lactoferrin is low but increases during involution and inflammation (98). Because of the high concentrations of citrate and the low concentration of lactoferrin produced during lactation, the foremost role of lactoferrin apparently is in defense of the involuted mammary gland, particularly against coliforms (88).

Complement is a collection of proteins that is present in serum and milk, which functions in concert with a specific antibody to cause lysis of invading bacteria. Concentrations of complement are highest in colostrum, inflamed mammary glands, and during involution. In contrast, concentrations of complement are lowest during lactation. Therefore, because of its intermittent presence in milk, complement is thought to play only a minor bactericidal role in the mammary gland (76, 77); however, complement-sensitive organisms, including some strains of *E. coli*, are killed by the alternative complement pathway.

Lysozyme is a bactericidal protein that is present in milk and that functions by cleaving peptidoglycans from the cell wall of Gram-positive bacteria as well as the outer membrane of Gram-negative bacteria (76). Lysozyme may enhance the binding of lactoferrin to bacterial cell walls (80). In porcine and human milks, lysozyme, in combination with complement and secretory IgA, exhibited significant bactericidal activity to *E. coli* in vitro. However, whether this mechanism is active in the bovine mammary gland is unknown, especially considering that a study demonstrated that lysozyme limits chemotaxis and toxic oxygen production by neutrophils (28). Because ruminant milk contains only a small concentration of IgA and 300 times less lysozyme than human milk (11), this system may offer little protection to the bovine mammary gland.

The enzyme lactoperoxidase, in the presence of thiocyanate and hydrogen peroxide, is bacteriostatic for Gram-positive bacteria such as *S. aureus* and streptococci and bactericidal for Gram-negative bacteria such as coliforms (61). However, several factors can vary the effectiveness of this system in the mammary gland epithelial cells. Lactoperoxidase is produced in small concentration by mammary gland. The levels of thiocyanate in the mammary gland are dependent on the plane of nutrition. The source of hydrogen peroxide in the mammary gland is generated by enzymatic constituents of milk and, if present, by streptococci (34). The lactoperoxidase-thiocyanate-hydrogen peroxide system exerts its antibacterial properties through the production of hypothiocyanate, a reactive metabolite from the oxidation of thiocyanate (76). Myeloperoxidase produced by neutrophils also catalyzes the same reaction and additionally catalyzes the oxidation of chloride, the product of which provides the bacteriocidal activity of this system. In humans, myeloperoxidase is entirely responsible for the antimicrobial activity of this system (53). However, the low oxygen tension of the mammary gland can inhibit the production of hydrogen peroxide, thus limiting the effectiveness of this antimicrobial system against the pathogens that cause mastitis.

In recent years, the role of cytokines in the pathophysiology of bovine mastitis has been the subject of many studies. Cytokines are naturally produced proteins that play an important role in essentially all aspects of host defense by regulating the activity of cells that participate in specific and nonspecific immunity. The term "cytokine" describes a heterogeneous group of proteins produced by a spectrum of both immune and nonimmune cells under diverse circumstances. The immunomodulatory capacity of the cytokine network is complex. Individual cytokines can interact with other cytokines synergistically, additively, or antagonistically on multiple cell targets (45). Cytokines are often referred to as hormones because they are usually produced transiently and locally with potent biological activity at extremely low doses. Because of their extreme potency, elevated levels of certain cytokines can be detrimental to the host as well. To date, >30 cytokines have been identified, purified, and characterized by their regulatory activities. As large quantities of recombinant cytokines become available for research, more information is being generated concerning the potential immunotherapeutic application of cytokines for the control of bovine mastitis.

Numerous reports have shown the immunomodulatory capabilities of recombinant cytokines on important mammary immune cell functions (Table 1). The major groups of cytokines studied to date include interleukin (**IL**), colony-stimulating factor (**CSF**),

TABLE 1. Summary of cytokine effects on mammary immune cells.

Cytokine[1]	Observation	Reference
G-CSF	Increased milk SCC	(44)
	Increased numbers of milk neutrophils	(57)
GM-CSF	Enhanced neutrophil chemotactic and bactericidal activity	(91)
	Enhanced cytotoxic activity that was dependent on neutrophil antibody	(90)
	Enhanced neutrophil activity	(74)
IFN-γ	Enhanced neutrophil phagocytosis and bactericidal activity	(93)
	Enhanced neutrophil bactericidal activity	(23)
	Effective mastitis vaccine adjuvant	(69)
IL-1	Increased numbers of neutrophils	(17)
	No change in mononuclear cell proliferation	(109)
IL-2	Enhanced mammary mononuclear cell proliferation	(109)
	Enhanced cytotoxic and bactericidal activities of lymphocytes	(94)
	Increased plasma cell numbers	(56)
	Effective mastitis vaccine adjuvant	(69)

[1]G-CSF = Granulocyte colony-stimulating factor, GM-CSF = granulocyte-monocyte colony-stimulating factor, IFN-γ = interferon-γ, and IL = interleukin.

IFN, and TNF. The term "interleukin" was originally introduced to describe cell-free soluble factors that function as communicator molecules between leukocytes. Although all cytokines share this basic property, many of the well-characterized cytokines are designated as IL; IL-2 is the most extensively characterized of all the bovine cytokines. Originally described as T-cell growth factor, IL-2 is primarily produced by T lymphocytes of the helper phenotype and is responsible for clonal expansion of the initial T lymphocyte immune response and establishment of immune memory following mitogenic or antigenic stimulation. This cytokine also plays a role in B lymphocyte growth and differentiation, enhancing thymocyte proliferation, activating NK cells, and inducing cytotoxic T-cell activation (45, 50). There is evidence that altered endogenous IL-2 production contributes to diminished immune capabilities, which can lead to the development of disease (50). In fact, recent studies showed that colostrum samples that were obtained during the final week of gestation had low IL-2 activity, which correlates with diminished immune cell function and increased susceptibility to mastitis during this period (101). The possibility of enhancing bovine mammary gland defenses with IL-2 to increase resistance to mastitis has received considerable research attention. In vitro and in vivo studies indicate that recombinant bovine (**rb**) IL-2 may enhance functional capabilities of populations of mononuclear cells within the mammary gland (17, 85, 94, 109). Exposure to rbIL-2 markedly enhanced the proliferation of mononuclear cells isolated from milk to suboptimal concentrations of mitogens that normally do not elicit a proliferative response (109). Lymphocyte populations isolated from mammary tissues had increased cytotoxic and bactericidal activities following in vitro culture with IL-2 (85, 94). Enhanced expression of MHC class II molecules on the surface of IL-2-treated mononuclear cells was associated with the higher level of cell activation. When administered as an intramammary infusion, IL-2 can induce an infiltration of neutrophils into the milk in a dose-related manner (17, 103). Other changes in milk composition included a reduction in lactose concentration and a concomitant increase in BSA, pH, and total SCC. The range is narrow between therapeutic and toxic doses of IL-2 in the bovine mammary gland (103).

The CSF are a group of cytokines required for the proliferation and differentiation of a variety of hematopoietic stem cells. These growth factors are distinct glycoproteins that bind to cells by a common receptor and are produced by a variety of cells, including fibroblasts, endothelial cells, macrophages, and T cells. Each CSF tends to target a specific lineage to expand or activate its function. Originally characterized by its ability to stimulate myeloid colonies in soft agar, granulocyte (**G**)-CSF is required for the growth, survival, and differentiation of granulocytes phagocytic cells. The pronounced influence of G-CSF on phagocytic cell populations suggest possible clinical applications in the prevention of infectious bacterial diseases, such as mastitis. Both recombinant human G-CSF (14, 57) and rbG-CSF (44) have been administered subcutaneously to cows in doses ranging from 1 to 5 μg/kg per day. All studies demonstrated a two- to fivefold increase in peripheral blood neutrophils after 3 to 5 d of injections. Functionally,

Kehrli et al. (44) reported a decrease in random and directed neutrophil migration and an increase in phagocytosis and bactericidal activity after rbG-CSF treatment. The average SCC in milk of rbG-CSF treated cows was 50% higher than that of controls. No adverse clinical signs were reported in any of the studies of bovine animals, nor were the effects on milk production examined.

Granulocyte-macrophage (**GM**)-CSF was first identified by its capacity to induce hematopoietic progenitor cells to develop into granulocytes and macrophages (51). Several recent studies of dairy cows have shown that GM-CSF is not only an important molecule for inducing growth, but also affects a variety of functions of mature granulocytes. Treatment of bovine peripheral blood and mammary gland neutrophils with rbGM-CSF significantly increased the chemotactic and bactericidal capabilities of these cells (91). Others (74) have shown that GM-CSF treatment of neutrophils from mastitic cows increased the luminal-dependent chemiluminescence compared with that of untreated cells from the same cows. Intramammary infusion of rbGM-CSF at doses of up to 5 mg did not significantly affect total milk SCC but increased the ability of resident neutrophils to produce superoxide and also increased the percentage of phagocytic cells (17). Still others (90) have shown that systemic administration of rbGM-CSF to goats resulted in an increase in both total numbers and functional capabilities of mammary gland neutrophils during the early dry period. Most recently, researchers (106) have demonstrated that rbGM-CSF can stimulate bovine peripheral blood neutrophils for induced superoxide production. Because an early and rapid regression of functionally competent neutrophils from the blood stream is critical for the control of new IMI, enhancement of chemotaxis and phagocytosis of neutrophils with GM-CSF, as well as the kinetics of their induction, could increase the resistance of the mammary gland to invading pathogens.

Interferons are a group of closely related proteins of two major classes. Class I IFN consist of three closely related types: IFN-γ, IFN-β, and IFN-ω. The IFN-α and IFN-β are produced by a variety of cell types in response to several inducers, including viral infections, bacterial products, and tumor cells. In the bovine, the IFN-ω genes code for proteins produced by the early embryonic trophoblast, and these are referred to as IFN-τ (30, 79). The second class of IFN consists of a single protein, IFN-α, which is unrelated to the class I IFN. Interferon-γ is a cytokine derived from T lymphocytes that is often produced in response to stimulation by antigens or mitogens. Over 30 yr ago, the IFN were discovered and named based on their ability to induce antiviral states in vitro (45). Since then, IFN has been found to exhibit a variety of immunomodulatory properties to many aspects of the immune system. For example, IFN-γ enhances NK cell activity, ADCC, and cytotoxic T cell activity. This cytokine also enhances cytotoxicity mediated by macrophages against tumor cells, induces membrane-bound Fc receptors for IgG on macrophages, and stimulates the synthesis and release of reactive oxygen species from both macrophages and neutrophils. Recent evidence suggests that IFN-γ could elicit functional changes in phagocytic cells in the mammary gland that could make it effective in the control of bovine mastitis. The in vitro treatment of bovine mammary gland neutrophils with IFN-γ was shown to reverse the suppressive effects of mammary gland secretions and significantly increase the functional capabilities of these cells against *S. aureus* (93). Based on these preliminary findings, trials were conducted to determine the physiological and immunological effects of various doses of IFN-γ in the bovine mammary gland. The minimum biologically active dose of rbIFN-γ for intramammary use was assessed by evaluating biochemical and morphological changes in mammary glands in response to increasing doses of this cytokine. Results indicated that rbIFN-γ can be intramammarily infused at doses as high as 10^5 U per quarter without adversely affecting milk quality or normal mammary gland function of lactating dairy cows (102). Comparative results have been reported (71) when similar doses of IFN were infused into quarters of first lactation cows as well. Other researchers (23) have shown that intramammary administration of 10^5 U of rbIFN-γ also was an effective dose for enhancing the phagocytic and bactericidal capabilities of mammary gland neutrophils in vivo.

The role of TNF in the pathogenesis of coliform mastitis was studied recently. The acute symptoms most often associated with coliform mastitis are due to the rapid and unrestricted growth of the organism, the release of LPS, and the subsequent development of an unlimited inflammatory reaction. Release of LPS from Gram-negative bacteria initiates a nonspecific, acute phase response by evoking the synthesis and release of cytokines and eicosanoids at the foci of infection (47, 112, 117). Of the acute phase cytokine produced during the early stages of infection, TNF-α is a major mediator of endotoxic shock during peracute coliform mastitis. Elevated sera and milk concentrations of TNF-α were found in cows that had died from acute *E. coli* mastitis during the periparturient period (99). Recent studies (100) also showed that monocytes isolated from periparturient

dairy cows produced more TNF-α following LPS stimulation that cells isolated from cows in mid to late lactation. The enhanced ability of localized cell populations to produce this potent mediator around the time of calving may explain the greater frequency of clinical coliform mastitis during the periparturient period. The ability to modify the production of this potent mediator during coliform mastitis may lessen the morbidity and mortality associated with the acute form of this disease.

Enhancing Specific Immunity

Vaccination programs are designed to potentiate the immune system of the host toward a unique, specific antigen. For mastitis vaccines, eliciting a prompt recruitment of neutrophils to the site of infection can stimulate the production of specific antibody. Immunization can enhance neutrophil recruitment through the release of inflammatory mediators by localized antigen-specific lymphoid populations. Specific antibodies are required for the opsonization of bacteria and the promotion of phagocytosis by mammary gland populations of neutrophils. In addition to serving as opsonins, antibodies may neutralize bacterial toxins, interfere with adherence mechanisms of bacteria, and induce cell lysis of the invading pathogen (26). Both neutrophil numbers and antibody concentration are low in the healthy, uninfected mammary gland. Immunization protocols that are capable of potentiating these essential bactericidal components should contribute to the effective control of mastitis.

Mastitis vaccines are expected to eliminate chronic IMI, prevent the establishment of new IMI, and reduce the frequency and severity of clinical disease. Vaccines that are currently available apparently do not consistently reduce the incidence of new IMI or eliminate chronic mastitis. However, several recently available vaccines have effectively reduced the incidence of clinical mastitis.

Numerous attempts have been made to ameliorate or to prevent *S. aureus* mastitis through vaccination programs. Many of the earlier studies used systemically injected bacterins derived from in vitro grown cultures (1, 7). Although serum antibody titers increased following immunization, adequate antibody concentrations in milk were only achieved after inflammation to the challenge organisms had occurred. Increased milk antibody titers were effective in lessening the severity of disease but had no effect in preventing new IMI (1, 7). As more information became available concerning important *S. aureus* pathogenic mechanisms (104), different antigenic formulations were developed. The cell walls of *S. aureus* contain and produce a number of factors that are known to interfere with the ability of neutrophils to phagocytize and kill bacteria. Protein A is a cell-wall component of *S. aureus* that binds Ig by the nonspecific Fc receptor instead of by the antigen-specific Fab terminal. A vaccine against protein A may improve the opsonic activity of specific antibody by allowing the binding of Ig to the bacterial surface. Attempts to vaccinate dairy cows with a *S. aureus* protein A vaccine improved the spontaneous cure rate, but had no effect on the rate of new IMI (63).

Many *S. aureus* are able to produce an extracellular polysaccharide pseudocapsule that is known to have anti-phagocytic properties (116). Investigators have recently developed a *S. aureus* vaccine composed of bacteria that were cultured in vivo to promote the expression of important capsular antigens (113). The immunization of cows with this vaccine resulted in improved protection from experimental challenge with *S. aureus*, suggesting that altered in vitro growth conditions may interfere with the expression of important virulence factors that are normally expressed in vivo.

Evidence also suggests that staphylococcal toxins are important factors that can damage host tissues and promote bacterial growth. Studies (82) have shown that heifers vaccinated with an *S. aureus* vaccine formulated to stimulate anti-staphylococcal pseudocapsule antibodies; both α-toxin and β-toxin had reduced new IMI, and IMI were of shorter duration than IMI of an unvaccinated group. To date, the efficacy of these newer vaccine formulations used under field conditions has not be evaluated.

Considerable progress has been made over the last several years in the development of an effective mastitis vaccine against coliform mastitis. Initial studies (111) showed that cattle with low preexisting serum titers against common Gram-negative core antigens were more susceptible to clinical coliform mastitis than were cows with higher titers. From this observation, cows were then immunized with an R mutant *E. coli*, which resulted in a dramatic reduction in the incidence of clinical coliform mastitis (27). This bacterial strain is unique in that it lacks the enzymes that are required for normal bacterial cell-wall synthesis. As a consequence, the R mutant *E. coli* have nearly complete lipopolysaccharide assembly, but no O or somatic side chains. The exposed inner cell-wall structures are highly uniform, and vaccines containing killed R-mutant bacteria should provide broad-spectrum immunity against a wide variety of Gram-negative bacteria. To test this theory, a heat-killed *E. coli* J-5 mutant vaccine was

TABLE 2. Summary of micronutrient effects on mammary gland immunity.

Micronutrient	Observation	Reference
Se	Decreased efficiency in neutrophil function	(20, 38, 73)
	Improved bactericidal capabilities of neutrophils	(21, 38)
	Decreased severity and duration of mastitis	(21)
Vitamin E	Increased neutrophil bactericidal activity	(38, 87)
	Decreased incidence of clinical mastitis	(20, 87)
	In combination with Se, decreased prevalence of IMI at calving	(87)
Vitamin A	Decreased SCC	(73, 81)
	Moderated glucocorticoid levels	(81)
β-Carotene	Increased bactericidal function of phagocytes	(19)
	Increased mitogen-induced proliferation of lymphocytes	(73, 81)
Cu	Deficiency decreased neutrophil killing capability	(43)
	Deficiency increased susceptibility to bactericidal infection	(32)
Zn	Deficiency decreased leukocyte function	(32)
	Deficiency increased susceptibility to bacterial infection	(32, 73)

developed and tested in several field trials. Although the vaccine had little impact on the prevalence of coliform IMI, immunization with Gram-negative core antigens decreased the incidence and severity of clinical disease (13). The practical application of coliform mastitis vaccines is as a supplement to traditional methods of mastitis control based on good management and nutritional practices.

Micronutrient immunoregulation. The nutritional status of the cow is directly related to overall health, and proper nutrition has long been associated with the ability of an animal to fight disease. Although the study of the effects of specific nutrients is complicated by their diversified functions and complex interaction with other nutrients, researchers have been able to define more completely the role of several micronutrients in the process of infection and immunity. Because a variety of these substances and their deficiencies have been shown to have profound effects on the immune systems of many animals, adequate nutrition has received increasing attention as an essential element in the prevention and control of mastitis. Most of the available information on micronutrients and their immunomodulatory properties with regard to bovine mastitis focuses on Se, vitamin E, vitamin A, β-carotene, copper, and zinc. A summary of the primary roles of these micronutrients in mammary defense is outlined in Table 2.

Selenium is probably the best characterized micronutrient with regard to immunoregulatory effect. Selenium is a vital component of the antioxidant enzyme glutathione peroxidase, which is essential for the protection of cells and bodily tissues from autooxidative damage from the production of oxygen radicals by certain leukocytes during phagocytosis and killing (73). Deficiencies in Se result in compromised neutrophil function, which is a primary effector cell in the initial elimination of infections (20, 37). Because much of the soil in North America is deficient in Se, Se deficiency often results in animals for which the primary source of nutrition is derived from plants grown in these areas.

Based on the role of Se in important immune cell function and protection, deficiencies of this micronutrient can have serious consequences on mammary gland health. Many studies have documented the benefits of dietary Se supplementation for the control of bovine mastitis. Neutrophil killing of *S. aureus*, *Candida albicans*, and *E. coli* is greatly enhanced for dairy cows receiving an Se supplement compared with cows that were deficient in Se (20, 21, 38). Erskine et al. (21) also showed that the supplemented cows experienced clinical IMI of lesser severity and shorter duration than those of unsupplemented cows. The same study revealed lower peak bacterial numbers for cows provided with dietary Se when IMI did occur, probably because of the more rapid influx of neutrophils into the mammary gland upon bacterial infusion in cows receiving the Se supplement. These beneficial effects of Se can be attributed to the decreased damage to cells by oxygen radicals and peroxidases with an increased efficiency of the enzymes that are involved in intracellular killing mechanisms. Regardless of the means, overwhelming evidence for the protective role of Se against bovine mastitis clearly warrants inclusion of dietary Se supplementation in mastitis control protocols.

Vitamin E, which is similar to Se in its biological properties, is an important component of all cell membranes. Vitamin E provides stability and prevents the

debilitating peroxidation of membrane lipids. Vitamin E also plays a regulatory role in the biosynthesis of various inflammatory mediators (73), is necessary for the integrity of integument and wound healing, and has shown immunostimulatory effects, both cellular and humoral (75, 108). This essential antioxidant is found in high quantities in fresh, green foodstuffs; however, the concentration of vitamin E decreases as age of plants and length of storage increase, and vitamin E is often destroyed in silages. Therefore, deficiencies are common in unpastured cows and in cows during seasons when pasture is not available.

Because of its positive role in immunity and the widespread potential for deficiency in farm animals, vitamin E supplementation could provide great benefit to the control of bovine mastitis. Indeed, Hogan et al. (38) reported an increased intracellular kill of both *S. aureus* and *E. coli* when cow diets were supplemented with this micronutrient. Smith et al. (87) were able to demonstrate a reduction in the incidence of clinical mastitis by 37% when supplements of 1 g/d per cow were provided. Additionally, Se and vitamin E have a synergistic effect, reducing the prevalence of clinical mastitis, new IMI at calving, and SCC, as well as reducing the severity and duration of clinical mastitis to a greater degree than the supplementation of either micronutrient alone (20, 38). The necessity of nutritional supplementation, especially during the dry period before most deficiencies are likely to develop, should not be overlooked in the attempt to bolster the resistance of cows to mastitis.

Vitamin A and its precursor, β-carotene, have long been known for their effect on vision, normal cell growth, epithelial cells, and therefore mucosal surface integrity and stability. Vitamin A deficiency has been linked to increased glucocorticoid response to stress, which has an immunosuppressive effect (81). β-Carotene can act independently as an oxygen radical scavenger and is incorporated into cell membranes as such. Both vitamin A and β-carotene have been shown to have stimulatory effects on immune cell populations and have been correlated with a generally increased resistance to disease.

Deficiencies in both these nutrients have been related with severity of mastitis, and both decrease at a time when cows become increasingly susceptible to new IMI (12, 81). The role of vitamin A in epithelial health may be due to an affect on mammary gland defense mechanisms. Researchers (12) reported a negative correlation between concentrations of vitamin A and β-carotene and SCC in milk from lactating dairy cows. Supplementation with both of these nutrients improved the status of clinical mastitis over the provision of vitamin A alone, indicating a protective role of β-carotene that is independent of its function as precursor to vitamin A (59). The work of Daniel et al. (19) seems to support this idea, as the in vitro ability of phagocytic blood and milk leukocytes to kill *S. aureus* was enhanced in the presence of β-carotene but not various forms of vitamin A. Nevertheless, the importance of adequate dietary intake of both these micronutrients is still apparent, and mastitis control programs should ensure that proper levels are maintained in all cows.

Little information is available on the role of Cu in disease; however, its importance in normal biological function is recognized. Copper is required for the synthesis of hemoglobin and is an essential element in the antioxidant Cu-dependent enzyme superoxide dismutase. Copper is also present in the serum protein ceruloplasmin, which is recognized as an acute phase protein in cattle. The latter two proteins are important to immune function, partially because of their protection of cells from oxidative products released by phagocytosis and killing by leukocytes. Ceruloplasmin has also been indicated as a possible modulator of extracellular lysosomal enzyme activity as a result of inflammation (24).

Copper deficiencies have been shown (32, 73) to result in lowered bactericidal activity. As with vitamin A and β-carotene, Cu falls to its lowest concentrations at a time when cows are most vulnerable to mastitis. Studies at the University of Kentucky (32) showed that dietary Cu supplementation resulted in approximately a twofold reduction in the percentage of infected quarters at calving compared with that of untreated controls. The number of quarters infected by major mastitis pathogens was reduced more than fourfold. Somatic cell counts also tended to be lower in the supplemented group. Additionally, Suttle and Jones (105) report a decrease in proliferation of lymphocytes to mitogens in hypocupremic cows that was restored by Cu supplementation. These results indicate that Cu also may have a regulatory role in the immunity of the mammary gland.

Another micronutrient that is essential to various biochemical pathways, Zn has also been linked to proper immune function. Zinc is essential for the integrity of skin, the first physiologic barrier to infection. Zinc is also a component of the antioxidant Zn-dependent superoxide dismutase. Zinc is likely to have a stabilizing, antioxidative role in cellular membranes and therefore protects those membranes from damage (73). Zinc deficiency can result in the atrophy of thymus and other lymphoid tissue and in irregular profiles of serum antibodies and immune

TABLE 3. Summary of the efficacy of cytokines against bovine mastitis caused by *Staphylococcus aureus* or *Escherichia coli*.

Cytokine[1]	Mastitis model	Observation	Reference
G-CSF	*S. aureus*	No effect	(44)
	S. aureus	Reduction (47%) in new IMI	(57)
GM-CSF	*S. aureus*	Prevents new IMI	(18)
IFN-γ	*E. coli*	Reduced rate, duration, and severity of disease	(92)
IL-2	*S. aureus*	Improved antibiotic efficacy	(16)
IL-1 and IL-2	*S. aureus*	Prevents new IMI	(18)
IL-2	*S. aureus*	Dry cow treatment	(17)

[1]G-CSF = Granulocyte colony-stimulating factor, GM-CSF = granulocyte-monocyte colony-stimulating factor, IFN-γ = interferon-γ, and IL = interleukin.

cells (32, 73). Deficiencies in Zn predispose the cow to secondary infections, which can be reversed by supplementation (32, 73).

The problems associated with Zn insufficiencies can be exacerbated by high Ca diets, a common condition of cows during early stages of lactation. Therefore, an even greater concern is the proper dietary intake of Zn by dairy cows as a means to maintain mammary immunity. However, very few studies have been carried out that elucidate the specific relationship of Zn to bovine mastitis. Nevertheless, the interaction of Zn with immune cells and overall health indicates that Zn could certainly contribute to prevention or control of the disease.

Diet plays an important role in the ability of dairy cows to resist disease. Not only gross malnutrition, but also merely suboptimal levels of any one micronutrient is sufficient to affect mammary gland immunity adversely. Key to ensuring adequate levels of these important micronutrients is direct testing of animals at least at the herd level to delineate patterns in overall nutrient deficits. Provision of dietary supplements of the deficient vitamins or minerals in accordance with accepted doses is one practical means to enhance the inherent defense of the cow against invading mastitis pathogens.

Cytokine immunotherapy. Information also is available that describes the potential clinical application of recombinant cytokines for the control of experimental *S. aureus* and coliform mastitis of dairy cows (Table 3). Based on availability and species biological crossreactivity between human cytokines and bovine cells, much of the earlier work with recombinant cytokines in cattle involved the human recombinant forms.

One of the first cytokines to be tested in an experimental mastitis model was rhG-CSF. Administration of rhG-CSF to lactating dairy cows by subcutaneous injection reduced new IMI by 47% following experimental *S. aureus* challenge compared with placebo-treated controls (57). The reduction in new *S. aureus* IMI was related to the rhG-CSF recruitment of neutrophils induced into the mammary gland by rhG-CSF prior to challenge. There have been no reports on the prevention or treatment of *S. aureus* mastitis using an intramammary infusion of G-CSF.

Recent studies (56) have shown that intramammary administration of bovine IL-2 can enhance cellular and humoral immune responses in quarters infected with *S. aureus*. Prophylactic administration of IL-2 has been shown to protect the mammary gland from subsequent intramammary challenge with *S. aureus*. Therapeutic administration of these cytokines into quarters that were infected with *S. aureus* was less efficacious at eliminating preexisting IMI (17). However, evidence suggests that the combination of homologous cytokines with current antibiotic formulations may improve the overall efficacy of these therapeutic agents (16).

Interferon-γ can also regulate host responses to bacterial toxins. Depending on the dose and the timing of administration relative to challenge, IFN-γ was shown to decrease the morbidity and mortality associated with endotoxemia (45). Based on the biological actions of this cytokine, it was suggested that intramammary IFN-γ treatment may enhance bacterial clearance by mammary gland phagocytes and regulate acute inflammatory responses to bacterial toxins during acute coliform mastitis. To test this hypothesis, researchers (92) examined the influence of recombinant bovine IFN-γ treatment on the establishment and severity of experimentally induced *E. coli* mastitis in postpartum dairy cows. Dairy cows that were treated intramammarily with IFN-γ 24 h

before *E. coli* challenge had fewer infected quarters, infections of shorter duration, and lower clinical scores than cows treated with a placebo. All cows that had been treated with IFN-γ survived the experimental *E. coli* challenge, but the group receiving the placebo had a 42% mortality rate, which was attributed to coliform mastitis within 3 d of the experimental challenge (92).

These experiments clearly indicate the ability of recombinant cytokines to modify the outcome of mastitis when the immune system has been compromised. Recombinant cytokines are capable of modifying the outcome of mastitis through a combined effect of recruitment of effector cells to the mammary gland, enhanced bacterial clearance by phagocytic cell populations, and regulation of acute inflammatory reactions. However, research into the role of cytokines in bovine mastitis is in the beginning stages. The challenge that confronts researchers now is to gain a better understanding of the complex interaction between the pathogenesis of bacteria, host responses needed to eliminate pathogens from the mammary gland, and mechanisms by which cytokines can modulate these responses. Further developments in these areas are necessary before experimental findings can be transferred to field conditions. However, the information that has been generated thus far concerning the ability for effective manipulation and regulation of mammary immune functions suggests great potential for the future therapeutic application of cytokines for the control of bovine mastitis.

CONCLUSIONS

The development of immunomodulatory strategies for the control of bovine mastitis is an active area of research. The advent of recombinant DNA technology has allowed the production of large quantities of animal cytokines. An increased understanding of the role of cytokines in host defense leads to the most appropriate use proteins under field conditions. Mastitis vaccine technologies have improved considerably over the years, but, to date, mastitis vaccines are not widely used in programs for mastitis control. However, the recent development of the R mutant vaccines for Gram-negative mastitis should prove to be beneficial for the control of coliform mastitis on well-managed dairy operations. Although the role of nutrition in mammary resistance to infection has been best defined for antioxidants, supplementation with other micronutrients appears to hold promise as well. If immunomodulators can be used to augment immune function at critical periods during the production of food animals, then the economic loss caused by mastitis should be reduced. Enhancing the natural ability of the host to resist mastitis without introducing undesirable residues into the food chain is fully compatible with current public concerns and demands.

REFERENCES

1 Adlam, C. J., J. B. Kerry, S. Edkins, and P. D. Ward. 1981. Local and systemic antibody responses in cows following immunization with staphylococcal antigens in the dry period. J. Comp. Pathol. 91:105.

2 Allison, J. P., and W. L. Harvan. 1991. The immunobiology of T cells with invariant gamma delta antigen receptors. Annu. Rev. Immunol. 9:679.

3 Bastida-Corcvera, K. F. 1992. The enhancement of mammary gland immunity through vaccination. Page 335 *in* Bovine Medicine: Diseases and Husbandry of Cattle. A. H. Andrews, R. W. Blowey, H. Boyd, and R. C. Eddy, ed. Blackwell Sci. Publ., Cambridge, MA.

4 Baumann, H., and J. Graudie. 1994. The acute phase response. Immunol. Today 15:74.

5 Bishop, J. G., F. L. Schanbacher, L. C. Ferguson, and K. L. Smith. 1976. *In vitro* growth inhibition of mastitis-causing coliform bacteria by bovine apo-lactoferrin and reversal of inhibition by citrate and high concentrations of apo-lactoferrin. Infect. Immun. 14:911.

6 Bramley, A. J., and F. H. Dodd. 1984. Mastitis control: progress and prospects. J. Dairy Sci. 51:481.

7 Brock, J. H., E. D. Steel, and B. Reiter. 1975. The effect of intramuscular and intramammary vaccination of cows on antibody levels and resistance to intramammary infections by *Staphylococcus aureus* [mastitis by experimental challenge]. Res. Vet. Sci. 19:152.

8 Bullen, J. J., H. J. Rogers, and E. Griffiths. 1978. The role of iron in bacterial infection. Curr. Top. Microb. Immunol. 80:1.

9 Capuco, A. V., S. A. Bright, J. W. Pankey, D. L. Wood, R. H. Miller, and J. Bitman. 1992. Increased susceptibility to intramammary infection following removal of teat canal keratin. J. Dairy Sci. 75:2126.

10 Capuco, A. V., M. J. Paape, and S. C. Nickerson. 1986. *In vitro* study of polymorphonuclear leukocyte damage to mammary tissue of lactating cows. Am. J. Vet. Res. 47:663.

11 Chandran, R. C., K. M. Shahani, and K. G. Holly. 1964. Lysozyme content of human milk. Nature (Lond.) 204:688.

12 Chew, B. P., L. L. Hollen, and J. K. Hillers. 1982. Relationship between vitamin A and β-carotene in blood plasma and milk and mastitis in Holsteins. J. Dairy Sci. 65:2111.

13 Cullor, J. S. 1991. The role of vaccines in the prevention and moderation of clinical mastitis. Page 68 *in* Proc. 30th Annu. Mtg. Natl. Mastitis Counc., Reno, NV. Natl. Mastitis Counc. Inc., Arlington, VA.

14 Cullor, J. S., N. Fairley, W. L. Smith, S. L. Wood, J. D. Dellinger, M. S. Inokuma, and I. M. Souza. 1990. Hemogram changes in lactating dairy cow given human recombinant granulocyte colony-stimulating factor. Vet. Pathobiol. 27:311.

15 Current Concepts of Bovine Mastitis. 1996. Natl. Mastitis Counc., Inc., Madison, WI.

16 Daley, M. J., G. Furda, R. Dougherty, P. Coyle, T. Williams, and P. Johnston. 1992. Potentiation of antibiotic therapy of *Staphylococcus aureus* mastitis by recombinant bovine interleukin-2. J. Dairy Sci. 75:3330.

17 Daley, M. J., T. Williams, R. Dougherty, P. Coyle, G. Furda, and P. Hayes. 1991. *Staphylococcus aureus* mastitis: pathogenesis and treatment with bovine interleukin-1 and interleukin-2. J. Dairy Sci. 74:4413.

18 Daley, M. J., T. Williams, R. Dougherty, G. Furda, P. Hayes, and P. Coyle. 1993. Prevention and therapy of *Staphylococcus aureus* infections with recombinant cytokines. Cytokine 5:276.

19 Daniel, L. R., B. P. Chew, T. S. Tanaka, and L. W. Tjoelker. 1991. β-Carotene and vitamin A effects on bovine phagocyte function in vitro during the peripartum period. J. Dairy Sci. 74:124.
20 Erskine, R. J. 1993. Nutrition and mastitis. Vet. Clin. North Am. Food Anim. Pract. 9:551.
21 Erskine, R. J., R. J. Eberhart, P. J. Grasso, and R. W. Scholz. 1989. Induction of *Escherichia coli* mastitis in cows fed selenium-deficient or selenium-supplemented diets. Am. J. Vet. Res. 50:2093.
22 Fitzpatrick, J. L., P. J. Cripps, A. W. Hill, P. W. Bland, and C. R. Stokes. 1992. MHC class II expression in bovine mammary gland. Vet. Immunol. Immunopathol. 32:13.
23 Fox, L. K., H. D. Liggit, T. Yilma, and L. B. Corbeil. 1990. The effects of interferon intramammary administration on mammary phagocyte function. J. Vet. Med. 37:28.
24 Galdston, M., V. Levytska, and M. S. Schwartz. 1984. Ceruloplasmin: increased serum concentration and impaired antioxidant activity in cigarette smokers, and ability to prevent suppression of elastase inhibitory capacity of α-proteinase inhibitor. Am. Rev. Respir. Dis. 129.
25 Garcia-Penarrubia, P., F. T. Koster, R. O. Kelley, T. D. McDowell, and A. D. Bankhurst. 1989. Antibacterial activity of human natural killer cells. J. Exp. Med. 169:99.
26 Gershwin, L. J., S. Krakowka, and R. G. Olsen. 1995. Immunoglobulins. Page 34 *in* Immunology and Immunopathology of Domestic Animals. A. Miller, ed. Mosby Press, St. Louis, MO.
27 Gonzalez, R. N., J. S. Cullor, D. E. Jasper, and R. B. Bushnell. 1989. Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. Can. J. Vet. Res. 53:301.
28 Gordon, L. I., S. D. Douglas, N. E. Kay, Y. Osamu, H. S. Jacob, and E. F. Osserman. 1979. Modulation of neutrophil function by lysozyme. Potential negative feedback of inflammation. J. Clin. Invest. 64:226.
29 Grommers, F. J., D. van de Geer, H. van de Vliet, P. A. Henricks, and F. P. Nijkamp. 1989. Polymorphonuclear leukocyte functions: relationship between induced migration into the bovine mammary gland and in vitro cell activity. Vet. Immunol. Immunopathol. 23:75.
30 Hansen, T. R., D. W. Leaman, J. C. Cross, N. Mathialagan, J. A. Bixby, and R. Roberts. 1991. The genes for trophoblast interferons and the related interferon-α II possess distinct 5′-promoter and 3′-flanking sequences. J. Biol. Chem. 226: 3060.
31 Harmon, R. J., and C. W. Heald. 1982. Migration of polymorphonuclear leukocytes into bovine mammary gland during experimentally induced *Staphylococcus aureus* mastitis. Am. J. Vet. Res. 43:992.
32 Harmon, R. J., and R. M. Torre. 1994. Copper and zinc: do they influence mastitis? Page 54 *in* Proc. 33rd Annu. Mtg. Natl. Mastitis Counc., Inc., Orlando, FL. Natl. Mastitis Counc., Inc., Arlington, VA.
33 Hibbitt, K. G., C. B. Cole, and B. Reiter. 1969. Antimicrobial proteins isolated from the teat canal of the cow. J. Gen. Microbiol. 56:365.
34 Hibbitt, K. G., N. Craven, and E. H. Batten. 1992. Anatomy, physiology, and immunology of the udder. Page 273 *in* Bovine Medicine: Diseases and Husbandry of cattle. A. H. Andrews, R. W. Blowey, H. Boyd, and R. G. Eddy, ed. Blackwell Sci. Publ., St. Louis, MO.
35 Hill, A. W. 1981. Factors influencing the outcome of *Escherichia coli* mastitis in cows. Res. Vet. Sci. 31:107.
36 Hisatsune, T., A. Enomoto, K. Nishijima, Y. Minai, Y. Asano, T. Tada, and S. Kaminogawa. 1990. CD8+ suppressor T cell clone capable of inhibiting the antigen- and anti-T cell receptor-induced proliferation of Th clones without cytolytic activity. J. Immunol. 145:2421.
37 Hogan, J. S., K. L. Smith, W. I. Weiss, D. A. Todhunter, and W. L. Schockey. 1990. Relationships among vitamin E, selenium, and bovine blood neutrophils. J. Dairy Sci. 73:2372.
38 Hogan, J. S., W. P. Weiss, and K. L. Smith. 1993. Role of vitamin E and selenium in host defense against mastitis. J. Dairy Sci. 76:2795.
39 Holly, M., Y. S. Lin, and T. J. Rogers. 1988. Induction of suppressor cells by staphylococcal enterotoxin B: identification of a suppressor cell circuit in the generation of suppressor effector cells. Immunology 64:643.
40 Howard, C. J., G. Taylor, and J. Brownlie. 1980. Surface receptors for immunoglobulin on bovine polymorphonuclear neutrophils and macrophages. Res. Vet. Sci. 29:128.
41 Inoue, T., Y. Asano, S. Matsuoka, M. Furutani-Seiki, S. Aizawa, H. Nishimura, T. Shirai, and T. Tada. 1993. Distinction of mouse CD8+ suppressor effector T cell clones from cytotoxic T cell clones by cytokine production and CD45 isoforms. J. Immunol. 150:2121.
42 Jensen, D. L., and R. J. Eberhart. 1981. Total and differential cell counts in secretions of the nonlactating bovine mammary gland. Am. J. Vet. Res. 42:743.
43 Jones, D. G., and N. F. Suttle. 1981. Some effects of copper deficiency on leukocyte function in sheep and cattle. Res. Vet. Sci. 31:151.
44 Kehrli, M. E., J. P. Goff, M. G. Stevens, and T. C. Boone. 1991. Effects of granulocyte colony stimulating factor administration to periparturient cows on neutrophils and bacterial shedding. J. Dairy Sci. 74:2448.
45 Lawman, M.J.P., M. Campos, H. Bielefeldt Ohmann, P. Greibel, and L. A. Babiuk. 1989. Recombinant cytokines and their potential therapeutic value in veterinary medicine. Page 663 *in* Comprehensive Biotechnology. Pergamon Press, London, England.
46 Lee, C. S., F.B.P. Wooding, and P. Kemp. 1980. Identification, properties, and differential counts of cell populations using electron microscopy of dry cow secretions, colostrum, and milk from normal cows. J. Dairy Res. 47:39.
47 Lohuis, J.A.C.M., J.H.M. Verheijdon, C. Burvenich, and A.S.J.P.A.M. Van Miert. 1988. Pathophysiological effects of endotoxins in ruminants. II. Metabolic aspects. Vet. Q. 10:117.
48 Mackay, C. R., and W. R. Hein. 1991. Marked variations in γδ T cell numbers and distribution throughout the life of sheep. Curr. Top. Microbiol. Immunol. 173:107.
49 Mackay, C. R., W. R. Hein, M. H. Brown, and P. Matzinger. 1988. Unusual expression of CD2 in sheep: implications for T cell interactions. Eur. J. Immunol. 18:1681.
50 Magnuson, N. S., A. G. Spies, M. S. Nissen, C. D. Buck, A. D. Weinberg, P. J. Barr, J. A. Magnuson, and R. Reeves. 1987. Bovine interleukin-2; regulatory mechanisms. Vet. Immunol. Immunopathol. 17:183.
51 Metcalf, D. 1985. The granulocyte-macrophage colony stimulating factors. Science (Washington, DC) 229:16.
52 Miescher, S., M. Schreyer, C. Barras, C. Capasso, and V. Von Fliedner. 1990. Sparse distributions of gamma delta T lymphocytes around human epithelial tumors predominantly infiltrated by primed memory T cells. Cancer Immunol. Immunother. 32:81.
53 Moldoveanu, Z., J. Tenovun, J. Mestecky, and K. M. Pruitt. 1982. Human milk peroxidase is derived from milk leukocytes. Biochem. Biophys. Acta 718:103.
54 Murphy, J. M., and O. M. Stuart. 1953. The effect of introducing small numbers of *Streptococcus agalactiae* (Cornell Strain 48) directly into the bovine teat cavity. Cornell Vet. 43:290.
55 Musoke, A. J., F. R. Rurangirwa, and V. M. Nantulya. 1987. Biological properties of bovine immunoglobulins and systemic antibody responses. Page 393 *in* The Ruminant Immune System in Health and Disease. W. I. Morrison, ed. Cambridge Univ. Press, Cambridge, England.
56 Nickerson, S. C., P. A. Baker, and P. Trinidad. 1989. Local immunostimulation of the bovine mammary gland with interleukin-2. J. Dairy Sci. 72:1764.
57 Nickerson, S. C., W. E. Owens, and J. L. Watts. 1989. Effects of recombinant granulocyte colony-stimulating factor on *Staphylococcus aureus* mastitis in lactating dairy cows. J. Dairy Sci. 72:3286.

58 Oksenbergy, J. R., E. Persitz, and C. Brautbar. 1985. Cellular immunity in human milk. Am. J. Reprod. Immunol. Microbiol. 8:125.
59 Oldham, E. R., R. J. Eberhart, and L. D. Muller. 1991. Effects of supplemental vitamin A or β-carotene during the dry period and early lactation on udder health. J. Dairy Sci. 74:3775.
60 Oliver S. P., and T. Bushe. 1987. Growth inhibition of *Escherichia coli* and *Klebsiella pneumoniae* during involution of the bovine mammary gland: relation to secretory composition. Am. J. Vet. Res. 48:1669.
61 Outteridge, P. M., and C. S. Lee. 1988. The defense mechanisms of the mammary gland of domestic ruminants. Prog. Vet. Microbiol. Immun. 4:165.
62 Paape, M. J., W. P. Wergin, and A. J. Guidry. 1981. Phagocytic defense of the ruminant mammary gland. Adv. Exp. Med. Biol. 137:555.
63 Pankey, J. W., N. T. Boddie, J. L. Watts, and S. C. Nickerson. 1985. Evaluation of protein A and a commercial bacterin as vaccines against *Staphylococcus aureus* mastitis by experimental challenge. J. Dairy Sci. 68:726.
64 Park, Y. H., L. K. Fox, M. J. Hamilton, and W. C. Davis. 1992. Bovine mononuclear leukocyte subpopulations in peripheral blood and mammary gland secretions during lactation. J. Dairy Sci. 75:998.
65 Park, Y. H., L. K. Fox, M. J. Hamilton, and W. C. Davis. 1993. Suppression of proliferative response of BoCD4+ T lymphocytes by activated BoCD8+ T lymphocytes in the mammary gland of cows with *Staphylococcus aureus* mastitis. Vet. Immunol. Immunopathol. 36:137.
66 Persson, K., I. Larsson, and C. Hallen Sandgren. 1993. Effects of certain inflammatory mediators on bovine neutrophil migration *in vivo* and *in vitro*. Vet. Immunol. Immunopathol. 37:99.
67 Persson, K., C. H. Sandgren, and H. Rodriquez-Martinez. 1992. Studies of endotoxin-induced neutrophil migration in bovine teat tissues using indium-III–labeled neutrophils and biopsies. Am. J. Vet. Res. 53:2235.
68 Pighetti, G. M., and L. M. Sordillo. 1994. Enhanced antigen-specific responses in bovine mammary glands following administration of interleukin-2. J. Dairy Sci. 78:528.
69 Pighetti, G. M., and L. M. Sordillo. 1995. Enhanced mammary gland immunity following primary immunization with interferon-γ. J. Dairy Sci. 78:528.
70 Politis, I., X. Zhao, B. W. McBride, and J. H. Burton. 1992. Function of bovine mammary macrophages as antigen-presenting cells. Vet. Immunol. Immunopathol. 30:399.
71 Quiroga, G. H., L. M. Sordillo, R. W. Adkinson, and S. C. Nickerson. 1993. Cytologic responses of *Staphylococcus aureus*-infected mammary glands of heifers to interferon-gamma and interleukin-2 treatment. Am. J. Vet. Res. 54:1894.
72 Rainard, P. 1992. Binding of bovine lactoferrin to *Streptococcus agalactiae*. FEMS Microbiol. Lett. 98:235.
73 Reddy, P. G., and R. A. Frey. 1990. Nutritional modulation of immunity in domestic food animals. Adv. Vet. Sci. Comp. Med. 35:255.
74 Reddy, P. G., D. S. McVey, M. M. Chengappa, F. Blecha, H. C. Minocha, and P. E. Baker. 1990. Bovine recombinant granulocyte-colony stimulating factor enhancement of bovine neutrophil functions *in vitro*. Am. J. Vet. Res. 51:1395.
75 Reddy, P. G., J. L. Morrill, and R. A. Frey. 1987. Vitamin E. is immunostimulatory in calves. J. Dairy Sci. 70:993.
76 Reiter, B. 1978. Review of nonspecific antimicrobial factors in colostrum. Ann. Rech. Vet. 9:205.
77 Reiter, B., and J. D. Oram. Bacterial inhibitors in milk and other biological fluids. Nature (Lond.) 216:328.
78 Richie, E. R., R. Bass, M. L. Meistrich, and D. K. Demmison. 1982. Distribution of T-lymphocyte subsets in human colostrum. J. Immunol. 129:1116.
79 Roberts, R. M., D. W. Leaman, and J. C. Cross. 1992. Role of interferons in material recognition of pregnancy in ruminants. Proc. Soc. Exp. Biol. Med. 200:7.
80 Schanbacher, F. L., and K. L. Smith. 1975. Formation and role of unusual whey proteins and enzymes: relation to mammary function. J. Dairy Sci. 58:1048.
81 Scherf, H., T. M. Frye, and S. N. Williams. 1994. Vitamin A and β-carotene: a nutritional approach to the control of mastitis in dairy cattle. Page 77 *in* Proc. 33rd Annu. Mtg., Natl. Mastitis Counc., Orlando, FL. Natl. Mastitis Counc., Inc., Arlington, VA.
82 Sears, P. M., N. L. Norcross, K. Kenny, B. Smith, R. N. Gonzalez, and M. N. Romano. 1990. Resistance to *Staphylococcus aureus* infections in staphylococcal vaccinated heifers. Page 69 *in* Proc. Intl. Symp. Bovine Mastitis, Indianapolis, IN. Natl. Mastitis Counc., Inc., Arlington, VA.
83 Selsted, M. E., Y. Q. Tang, W. L. Morris, P. A. McGuire, M. J. Nonotny, W. Smith, A. H. Henschen, and H. S. Cullor. 1993. Purification, primary structures, and antibacterial activities of the beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. J. Biol. Chem. 268:6641.
84 Shafer-Weaver, K. A., G. M. Pighetti, and L. M. Sordillo. 1996. Diminished mammary gland lymphocyte functions parallel shifts in trafficking patterns during the postpartum period. Proc. Soc. Exp. Biol. Med. 212:271.
85 Shafer-Weaver, K. A., and L. M. Sordillo. 1996. Enhancing bactericidal activity of bovine lymphoid cells during the periparturient period. J. Dairy Sci. 79:1347.
86 Reference deleted in proof.
87 Smith, K. L., H. R. Conrad, B. A. Amiet, and D. A. Todhunter. 1985. Incidence of environmental mastitis as influenced by dietary vitamin E and selenium. Kiel. Milchwirtsch. Forschungsber. 37:482.
88 Smith, K. L., and S. P. Oliver. 1981. Lactoferrin: a component of nonspecific defense of the involuting bovine mammary gland. Page 535 *in* The Ruminant Immune System. J. E. Butler, ed. Plenum Press, New York, NY.
89 Smith, K. L., and D. A. Todhunter. 1982. The physiology of mammary gland during the dry period and the relationship to infection. Page 87 *in* Proc. Annu. Mtg. Natl. Mastitis Counc., Louisville, KY. Natl. Mastitis Counc., Inc., Arlington, VA.
90 Sordillo, L. M. 1992. Cytokines at drying off: potential role in mastitis control. Page 160 *in* Proc. 31st Annu. Mtg. Natl. Mastitis Counc., Arlington, VA. Natl. Mastitis Counc., Inc., Arlington, VA.
91 Sordillo, L. M., G. Afseth, G. Davies, and L. A. Babiuk. 1992. Effects of recombinant granulocyte-macrophage colony-stimulating factor on bovine peripheral blood and mammary gland neutrophil function *in vitro*. Can. J. Vet. Res. 56:16.
92 Sordillo, L. M., and L. A. Babiuk. 1991. Controlling acute *Escherichia coli* mastitis during the periparturient period with recombinant bovine interferon-gamma. Vet. Microbiol. 28:189.
93 Sordillo, L. M., and L. A. Babiuk. 1991. Modulation of mammary neutrophil function during the periparturient period following *in vitro* exposure to recombinant bovine interferon-gamma. Vet. Immunol. Immunopathol. 27:393.
94 Sordillo, L. M., M. Campos, and L. A. Babiuk. 1991. Antibacterial activity of bovine mammary gland lymphocytes following treatment with interleukin-2. J. Dairy Sci. 74:3370.
95 Sordillo, L. M., and S. C. Nickerson. 1988. Quantification and immunoglobulin classification of plasma cells in nonlactating bovine mammary tissue. J. Dairy Sci. 71:84.
96 Sordillo, L. M., and S. C. Nickerson. 1988. Morphometric changes in the bovine mammary gland during involution and lactogenesis. Am. J. Vet. Res. 49:1112.
97 Sordillo, L. M., S. C. Nickerson, and R. M. Akers. 1989. Pathology of *Staphylococcus aureus* mastitis during lactogenesis: relationship with bovine mammary structure and function. J. Dairy Sci. 72:228.
98 Sordillo, L. M., S. C. Nickerson, R. M. Akers, and S. P. Oliver. 1987. Secretion composition during bovine mammary involution and the relationship with mastitis. Int. J. Biochem. 19: 1165.
99 Sordillo, L. M., and J. E. Peel. 1992. Effect of interferon-γ on the production of tumor necrosis factor during acute *Escherichia coli* mastitis. J. Dairy Sci. 75:2528.
100 Sordillo, L. M., G. M. Pighetti, and M. R. Davis. 1995. Enhanced production of bovine tumor necrosis factor-α during

the periparturient period. Vet. Immunol. Immunopathol. 49: 263.
101 Sordillo, L. M., M. J. Redmond, M. Campos, L. Warren, and L. A. Babiuk. 1991. Cytokine activity in the bovine mammary gland secretions during the periparturient period. Can. J. Vet. Res. 55:298.
102 Sordillo, L. M., M. Snider, and L. A. Babiuk. 1992. Physiological and morphological changes in bovine mammary glands following intramammary infusion of recombinant interferon-gamma. Can. J. Vet. Res. 56:22.
103 Sordillo, L. M., M. Snider, H. Hughes, G. Afseth, M. Campos, and L. A. Babiuk. 1991. Pathological changes in bovine mammary glands following intramammary infusion of recombinant interleukin-2. J. Dairy Sci. 74:4164.
104 Sutra, L., and B. Poutrel. 1993. Virulence factors involved in the pathogenesis of bovine intramammary infections due to *Staphylococcus aureus*. J. Med. Microbiol. 40:79.
105 Suttle, N. F., and D. G. Jones. 1986. Copper and disease resistance in sheep: a rare natural confirmation of interaction between a specific nutrient and infection. Proc. Nutr. Soc. 45: 317.
106 Tao, W., R. Dougherty, P. Johnston, and W. Pickett. 1993. Recombinant bovine GM-CSF primes superoxide production, but not degranulation induced by recombinant bovine interleukin-1 in bovine neutrophils. J. Leuk. Biol. 53:679.
107 Taylor, B. C., J. D. Dellinger, J. S. Cullor, and J. L. Scott. 1994. Bovine milk lymphocytes display the phenotype of memory T cells and are predominantly CD8+. Cell. Immunol. 156:245.
108 Tengerdy, R. P., D. L. Meyer, L. H. Lauerman, D. C. Lueker, and C. F. Nockels. 1983. Vitamin E enhances humoral antibody response to *Clostridium perfringens* type D in sheep. Br. Vet. J. 139:147.
109 Torre, P. M., P. K. Konur, and S. P. Oliver. 1992. Proliferative response of mammary gland mononuclear cells to recombinant bovine interleukin-2. Vet. Immunol. Immunopathol. 32:351.
110 Treece, J. M., G. E. Morse, and C. Levy. 1966. Lipid analyses of bovine teat canal keratin. J. Dairy Sci. 49:1240.
111 Tyler, J. W., J. S. Cullor, and B. I. Osburn. 1988. Relationship between serologic recognition of *Escherichia coli* 0111:B4 (J5) and clinical coliform mastitis in cattle. Am J. Vet. Res. 49: 1950.
112 VanMiert, A.S.J.P.A.M. 1991. Acute phase response and noncellular defense mechanisms. Flemish Vet. J. 62 (Suppl. 1):69.
113 Watson, D. L., and C. L. Schwartzkoff. 1990. A field trail to test the efficacy of a staphylococcal mastitis vaccine in commercial dairies in Australia. Page 73 *in* Proc. Intl. Symp. Bovine Mastitis. Indianapolis, IN. Natl. Mastitis Counc., Inc., Arlington, VA.
114 Weber, L., E. Peterhans, and R. Wyler. 1983. The chemiluminescent response of bovine polymorphonuclear leukocytes isolated from milk and blood. Vet. Immunol. Immunopathol. 4:397.
115 Yang, T. J., J. F. Mathers, and E. D. Rabinovsky. 1988. Changes in subpopulations of lymphocytes in peripheral blood, and supramammary and prescapular lymph nodes of cows with mastitis and normal cows. Vet. Immunol. Immunopathol. 18:279.
116 Yoshida, K., Y. Ichiman, and S. Narikawa. 1984. Staphylococcal capsular vaccine for the preventing mastitis in two herds in Georgia. J. Dairy Sci. 67:620.
117 Zia, S., S. N. Giri, J. Cullor, P. Emau, B. I. Osborn, and R. B. Bushnell. 1987. Role of eicosanoids, histamines, and serotonin in the pathogenesis of *Klebsiella pneumoniae*-induced bovine mastitis. Am. J. Vet. Res. 48:1617.