Exploration of Immunology

Open Exploration

Open Access Review

The mammary gland is intolerant to bacterial intrusion

Pascal Rainard*

Infectiology and Public Health (ISP), National Research Institute for Agriculture, Food and Environment (INRAE), Université de Tours, 37380 Nouzilly, France

***Correspondence:** Pascal Rainard, Infectiology and Public Health (ISP), National Research Institute for Agriculture, Food and Environment (INRAE), Université de Tours François Rabelais, 37380 Nouzilly, France. pascal.rainard@inrae.fr
Academic Editor: Jochen Mattner, FAU Erlangen-Nürnberg and UKER, Germany
Received: September 19, 2023 **Accepted:** January 4, 2024 **Published:** February 6, 2024

Cite this article: Rainard P. The mammary gland is intolerant to bacterial intrusion. Explor Immunol. 2024;4:59–72. https://doi.org/10.37349/ei.2024.00128

Abstract

Mammals depend on the secretion of milk to rear their offspring, which exposes the organ in charge of the function, the mammary gland (MG), to bacterial threat. The essential driving force that conditions the interactions of bacteria with the MG is the abundant secretion of milk, a nutritious fluid which endows the common mastitis-causing pathogens with a doubling time of less than 30 min. From this angle, mammals rely on a potential bacterial bioreactor for the survival of their offspring. The MG is lined with a two-layered epithelium devoid of protective mucus. This means that the mammary epithelium is exposed directly to bacteria once they have passed through the opening lactiferous canal. To cope with the threat, the MG resorts to neutrophilic inflammation to check bacterial proliferation in its lumen and at its epithelial lining. Promptness of neutrophil recruitment is a necessity, which requires a low threshold of activation on the part of the mammary epithelium. Constrained by natural selection, the MG has evolved an innate and adaptive immunity intolerant to bacteria regardless of their level of virulence. The evolutionary issue has been to find a compromise between the deleterious tissue-damaging side effects of inflammation and the maintenance of the secretory function indispensable for the offspring's survival. It appears that the MG relies mainly on neutrophilic inflammation for its protection and is regulated by type 3 immunity. Advances in knowledge of type 3 immunity in the MG will be necessary to induce immune protection adapted to the physiology of this peculiar organ.

Keywords

Mammary gland, mastitis, innate immunity, adaptive immunity, neutrophilic inflammation

Introduction

Mastitis, the inflammation of the mammary gland (MG), is usually triggered by the growth of bacteria in the lumen of the gland. Mastitis is the most common bacterial disease affecting the health of dairy cows, with a high impact on the productivity, product quality, and welfare of dairy animals [1]. Mastitis is also the first indication for antimicrobial treatments, with the potential risk of emergence of resistance [2]. Dairy animals do not have the exclusivity of mastitis, as this disease affects sows, rabbits or guinea pig does,

bitches, or mares [3–6]. Breastfeeding women are not spared, as about 20% of women experience a painful breast with fever during lactation [7, 8]. It is likely that most if not all mammals are confronted with mastitis. It may be asked whether the MG is particularly susceptible to infections. The MG likely developed from cutaneous glands, progressively specializing as an organ producing copious amounts of nutritious liquid [9]. It plays the dual role of providing nutrients and early passive immune protection to the offspring [10, 11]. This puts selective pressure on this organ whose function is essential for the reproduction of mammals. In particular, the MG must ensure the production of milk in all circumstances, even when it is infected, which is not without consequence on its mode of reaction to bacteria, whether these are considered pathogenic or harmless.

The MG is isolated from the external environment by a channel (opening lactiferous duct) equipped with a sphincter. The lumen of the gland is delimited by an epithelium which contributes to the creation of the blood-milk barrier. This barrier regulates the exchanges of soluble or cellular components between blood and milk and participates in the mammary immune response [12]. Whenever bacteria enter the MG lumen, the mammary epithelium reacts. Mammary responses to bacterial intrusions have been mainly studied in dairy animals or mouse models of experimentally induced mastitis, and much less, for ethical reasons, in the human breast. Due to large anatomical and physiological differences, extrapolations from mouse models to farm animals remain uncertain. However, some shared commonalities between species and responses to different pathogens do exist, which the remainder of this review will endeavor to highlight.

What makes the MG unique

Like most branched glands, the lactating MG comprises a duct system draining lobes that regroup lobules. A lobule consists of several alveolar acini composed of secretory mammary epithelial cells (MECs). The lactiferous ducts are lined by a simple columnar or cuboidal epithelium, except for the large collecting ducts and cisterns in dairy animals that comprise a stratified two-layered epithelium [13]. Ducts and alveoli are sheathed by a discontinuous outer layer of myoepithelial cells, whose contraction is responsible for milk ejection. The mammary epithelium is embedded in a loose connective tissue and surrounded by a capillary network.

The structure of the MG differs between species. In the cow, the lactiferous system ends in a gland cistern, then the teat cistern opens up through the opening lactiferous canal (*Ductus papillaris mammae*) which is about 10 mm long and is encircled by elastic connective tissue and smooth muscle cells forming a circular sphincter [13, 14]. Each of the four individual glands ("quarters") that make up the udder has a unique teat duct. In the mare, each of the two adjacent MGs separated by a septum has a teat, and the lactiferous ducts converge to a milk cistern at the base of the teat. Each of the two lobes that make up a gland has its own opening duct and orifice in the corresponding teat [15]. In the human breast, there are no cisterns or lactiferous sinuses next to the areolae, and from 4 to 18 ducts exit at the nipple [16]. These anatomical differences may have important consequences in relation to the infection process. Milk storage capacity (independently of milk ejection) varies tremendously, from 1 mL to 10 mL in breast to several hundred milliliters in goat and cow milk cistern cavities [17, 18]. Higher volumes of cisternal milk offer higher amounts of growth medium for bacteria (hence higher bacterial load) between milking or suckling. The number and structure of teat orifices are probably even more consequential. In effect, these orifices, indispensable for milk delivery to the young, are potential portals of entry for unfriendly intruders: Bacteria can pass the teat canal especially when the sphincter relaxes during milking or suckling. The teat canal is the interface between the MG and its environment. As it plays the role of primary barrier to infection, its integrity is of paramount importance for the health of the MG. Bacteria that can colonize the teat skin and teat canal have an increased probability of entering the gland. This is likely why coagulase-negative staphylococci are the most frequent agents of MG infections in dairy animals [19, 20].

Apart from its anatomy, the feature that makes the MG unique is the secretion of milk, which puts the MG at high risk of severe infection. The lumen of the lactating MG is filled with a very nutritious liquid that

allows literally dozens of bacterial species to proliferate [21]. Experimentally induced infections of the MG of dairy ruminants have shown that bacterial concentrations up to 10^9 colony-forming units (cfu)/mL can be reached in the milk of infected glands and that the dividing time of certain strains of *Escherichia coli* (*E. coli*) or *Staphylococcus aureus* (*S. aureus*) during the exponential growth phase *in vivo* is in the range of 20–30 min [22–24]. The MG must therefore be able to face this threat by quickly mobilizing effective immune defenses. Milk has a low antimicrobial activity, which requires the development of various techniques (fermentation, cheese making) for its preservation. A few cfu of bacteria such as staphylococci or coliforms (less than 100 cfu) are enough to cause mastitis [25, 26]. Bacteria that possess the fitness attributes enabling them to use milk nutrients (such as lactose fermentation and casein degradation) and to withstand iron-depriving defenses (such as the acquisition of iron from citrate) and the low level of complement do not need MG-specific virulence factors to thrive in the lumen of uninflamed MGs [19, 27–29]. The lactating MG can be likened to a bio-fermenter: MG cavities are supplied with a rich nutrient fluid maintained at constant temperature, pH, and oxygenation. In other words, mammals rely on a potential bacterial bioreactor for the survival of their offspring. Besides the bacterial load and its accompanying toxic metabolites, the predicament is still worsened by the destabilization of the milk matrix (decreased pH, proteases) leading to curdling that obstructs the lactiferous ducts, causing milk stasis. This precludes the emptying of the sequestered lobules and leads to their involution. This situation must be constantly monitored by immune surveillance and controlled very quickly by an inflammatory response to avoid overwhelming bacterial proliferation.

The dormant immune defense system of the MG

Few leucocytes populate the mammary tissue and milk of healthy MGs

One would expect that faced with such a high level of bacterial threat, the udder would have developed an imposing immune defense system. It is not the case at all. Compared with the digestive tract or the upper airways, the immune defenses of the uninfected healthy udder seem weak. The MG epithelium is devoid of cells specialized in the production of mucus or antimicrobial peptides, such as the goblet and Paneth cells present in the upper airways or gut epithelia. Consequently, the MG epithelial lining is not protected by a mucus layer that concentrates antimicrobial molecules or secretory immunoglobulin A (IgA) and isolates the cell surface from bacteria [30].

Accordingly, the MG is not a mucosal organ, even though it can be considered a member of the mucosal-associated lymphoid system due to its links with the gut-associated lymphoid tissue in some species (mouse or human MG) but not in others (MG of ruminants) [10, 31, 32]. In healthy MGs, most of the leucocytes are found in association with the epithelium, mainly ductal macrophages and intraepithelial cluster of differentiation 8 ($CD8^{pos}$) T cells [33]. A few $CD4^{pos}$ T cells can be found, but there are no organized immune formations [34, 35]. In milk, leucocytes are few, mainly macrophages and $CD4^{pos}$ and $CD8^{pos}$ lymphocytes that have a memory phenotype [36–38]. In dairy cows, the physiological baseline of cell concentration is usually less than 20,000 cells/mL at peak lactation, and there is reason to believe that more than 50,000 cells/mL in cow milk indicates some degree of inflammation [39], as loss of milk production begins above this threshold [1, 40, 41].

However, the mammary epithelium senses bacteria and bacterial products

The MG is equipped to sense the intrusion of bacteria and react promptly. This has been shown experimentally with the instillation of various bacterial agonists of the innate immune system (microbe-associated molecular patterns; MAMPs) into the lumen of the MG, which elicits a dose-dependent inflammatory response [42–45]. It has long been known that the MG is very sensitive to *E. coli* lipopolysaccharide (LPS) [46]. Expression of the Toll-like receptors (TLRs) 2, 4, and 9 in the mammary tissue has been documented [47]. MECs express TLR2 and TLR4 at their apical membrane [48] and bovine MECs in culture express TLR1, TLR2, TLR4, TLR6 (but not TLR5), and the oligonucleotide domain receptors nucleotide oligomerization domain 1 (NOD1) and NOD2 [44]. MECs do not express membrane CD14 when grown *in vitro*, but they secrete in milk-soluble CD14 which contributes to the innate recognition of bacteria

by TLR and reduces the severity of MG infection by *E. coli* [49–51]. CD14 protein expression *in vivo* is low in MECs of uninflamed mouse MG, but high quickly after intramammary infusion of *E. coli* LPS [52]. However, apical membrane expression remains to be documented.

The expression of several pattern recognition receptors (PRRs) by MECs enables them to play the role of a sentinel of the MG. However, the level of expression is not at its peak in healthy MGs and is upregulated under inflammatory conditions, such as the expression of CD14 in mouse MG during LPS-induced mastitis [30, 52]. It is likely that MECs are assisted in their sentinel role by intraepithelial and alveolar macrophages. The numerous ductal macrophages that are in close contact with luminal MECs are good candidates for this function, but their role remains to be established [53, 54]. The fact that MECs do not react to *Streptococcus uberis* but macrophages does argue in this direction since *S. uberis* triggers inflammation in the MG [55]. Besides MAMPs, the MG could sense bacterial metabolites, as mucosal-associated invariant T (MAIT) cells have been identified in human and bovine milk [56, 57]. These cells recognize metabolites produced by bacteria and fungi and possess antimicrobial capacities.

In a way, the immune system of the healthy MG can be considered dormant since the mammary tissue does not harbor lymphoid formations and hosts few tissue and milk leukocytes unless infection induces tertiary lymphoid structures [34, 58, 59]. It even appears rather disarmed, with little epithelial protection against bacteria: no mucus layer, few antimicrobial peptides, dilution, and quenching of immune effectors by milk. On the other hand, it is equipped for sensing bacteria and bacterial products, which indicates that it can be awakened. Many observational and experimental studies have established how the MG reacts to and manages bacterial intrusions, mainly in dairy ruminants but also in mouse mastitis models, as discussed in the following.

The intolerant mammary epithelium

The MG reacts strongly to bacterial proliferation

Bacteria proliferating in the MG lumen release MAMPs and metabolites that the mammary epithelium senses. This triggers a self-defense response on the part of MECs and intraepithelial leucocytes. This response involves the production of antimicrobial peptides (β-defensins and cathelicidins), lactoferrin, complement components [C3, factor B, complement C4b-binding protein (C4BP)], acute phase proteins [serum amyloid protein 3 (SAA3), pentraxin 3 (PTX3)], and calgranulins (S100A8, S100A9, S100A12) [60–65]. The reaction participates in the protection of the epithelium lining from invasion by bacteria and in slowing down their proliferation in milk. An efficient response relies on two additional and complementary components: the generation of chemokines and the lowering of the blood-milk barrier. Among chemokines, those recruiting neutrophils [chemokine C-X-C motif ligand 1 (CXCL1), CXCL2, CXCL3, CXCL8] are prominent, but others [such as chemokine C-C motif ligand 2 (CCL2), CCL5, or CCL20] are also produced, responsible for the influx of mononuclear leukocytes [51, 52, 66–68]. The modulation of barrier leakiness allows blood complement components and Igs (including specific antibodies) to access the gland lumen. These responses concur to make possible an efficient phagocytosis of bacteria by neutrophils in the lumen and the epithelium [12, 69, 70].

There are different ways the MG reacts to different pathogens. However, there are also commonalities in these responses [71]. Neutrophilic inflammation is an indispensable element of an efficient response to MG infection. Obstruction or delay in neutrophil recruitment results in life-threatening mastitis [72–74]. Neutrophilic inflammation is also a harbinger and a fixture of mastitis, and as such is used as a diagnostic tool of MG infection in dairy ruminants [75]. A massive influx of neutrophils is necessary to oppose the proliferation of bacteria, which may cause collateral damage to the mammary epithelium [76, 77]. Milk contains opsonins that help macrophages and neutrophils kill bacteria such as *E. coli* or *S. aureus*, although some encapsulated bacteria are not efficiently opsonized [78, 79]. Neutrophils contribute to the partial collapse of the blood-milk barrier, which is beneficial as this allows blood defense components (such as complement, antibodies, transferrin, and lectins) to access the MG lumen [80], but has also deleterious effects on the epithelium [12, 77]. The modulation of mammary neutrophilic inflammation by the immune system is thus of consequence for the secretory function of the MG.

The defense response is modulated by resident immune cells

The most numerous cells in a lactating MG are the MECs by far, and these cells can sense bacteria, mount self-defense, and trigger inflammation. They have a moderate capacity to produce inflammatory cytokines, but they express receptors for these cytokines [interleukin-6 (IL-6), IL-1β, tumor necrosis factor alpha (TNF-α)] [81–83]. They also respond to the lymphokines interferon-gamma (IFN-γ) and IL-17 [83, 84]. Thus, their defense activity can be modulated by leucocytes. Resident MG leucocytes are not abundant, but because they are positioned at the frontline of infection, they can respond as soon as bacterial intrusion is detected before inflammation recruits circulating leucocytes.

Besides stromal, ductal, and alveolar macrophages and dendritic-like cells, the MG harbors lymphoid cells. The proportion of B cells is variable across species and the lactation cycle. In the lactating mouse MG, only 2% of leucocytes are B lymphocytes, and 10% T lymphocytes [54]. This issue will not be developed here because B cells and locally produced antibodies have not been shown to modulate appreciably the response of the MG to bacterial intrusion. This role is mainly devoted to T lymphocytes. Unfortunately, knowledge of the localization and nature of T lymphocytes in healthy mammary tissue is limited. It has been reported that in the bovine MG, T lymphocytes are present in close contact with the epithelium and in the connective tissue, with a predominance of $CD8^{pos}$ over $CD4^{pos}$ cells, in particular within the epithelium [85]. A proportion of the $CD8^{pos}$ intraepithelial cells could be γδ T cells [86]. T cells, mainly $CD8^{pos}$, and macrophages are also intimately associated with the human breast epithelium [33, 87]. Their presence in healthy glands suggests that they play a role in the epithelium homeostasis and integrity, but this remains to be documented. In the murine MG, immature dendritic cells and retinoic acid receptor (RAR)-related orphan receptor gamma t ($ROR\gamma t^{pos}$) $CD4^{pos}$ (T helper 17; Th17) lymphocytes are found in nulliparous and lactating glands, with a transient increase at the onset of involution, and a proportion of these lymphocytes also express forkhead box P3 [FoxP3; Th17/regulatory T (Treg) cells] [88]. Little is known about the activities of MG lymphocytes, and most knowledge is derived from milk cells. In human, mouse, or cow milk, T lymphocytes display the phenotype ($CD45RO^{pos}$) and functional characteristics of memory T cells [36, 37, 89–91]. In cattle, a subpopulation of CD8 T cells has been shown to play a major immunoregulatory role [92]. In healthy lactating glands and during involution, most $CD8^{pos}$ T cells may have an immunoregulatory function, maybe to avoid self-reaction to milk components.

Unconventional T cells, which are not restricted to the classical major histocompatibility complex (MHC) molecules, are also present in the MG [93]. MAIT cells are activated by riboflavin synthesis metabolites presented by the antigen-presenting MHC-related protein 1 molecule (MR1) [93]. These cells contribute to the immune response to bacteria and fungi competent in the synthesis of vitamin B2 riboflavin. MAIT cells have been detected in breast milk where they represent a minor proportion of T cells [56]. In cow milk, only 0.8% of $CD3^{pos}$ T cells are MAIT cells, but this proportion increases fivefold in mastitis milk [57]. Moreover, bovine MAIT cells respond to *E. coli*, riboflavin-proficient bacteria responsible for mastitis, by producing IFN-γ and TNF-α, suggesting that they could participate in the defense of the MG [57].

γδ T cells can be considered as unconventional as most are unrestrained by classical MHC restriction [93]. Most γδ T cells produce IL-17 or IFN-γ [94]. γδ T cells from mice, humans, or cows can respond to microbial antigens and can produce IL-17 very rapidly in the absence of clonal expansion, contrary to αβ T cells [95]. This ability would make these cells the major initial IL-17 producers in acute infections [94]. γδ T cells have been shown to interact with epithelial barriers and contribute to the homeostasis and antimicrobial response intestinal intraepithelial γδ lymphocytes do [96]. γδ T cells are present in the tissue of uninfected MGs, but their functions and precise localization have not been determined [74]. γδ T cells are also found in colostrum and milk [97, 98].

At the onset of infection, neutrophils are the first cells to appear in milk in high numbers. Neutrophilic inflammation is characteristic of the MG response to bacterial intrusion, and the massive recruitment of neutrophils may mask the influx of other immune cells. However, mononuclear immune cells are also recruited [35]. All types of lymphocytes can be found in MG secretion. In general, $CD4^{pos}$ T cells became predominant over $CD8^{pos}$ cells, both displaying the $CD45RO^{pos}$ phenotype of effector/memory cells [99]. γδ

T cell numbers also increase, suggesting that they could participate in the immune defense of the MG, notably by producing IL-17 [74, 100]. The relative contribution of resident intraepithelial and subepithelial immune cells remains to be clarified.

Although knowledge is limited in this area, it can be hypothesized that resident immune cells play an important role in early responses to infection, as they do in other epithelia exposed to bacteria [101, 102]. The cells recruited by the local inflammatory reaction complete the immune response. Adaptive immune responses will also modify the response of the MG to infections. The immune memory induced by infections, chronic or recurrent, will largely depend on the pathogen in question. This area, which is very broad and complex, will not be treated here. The other mode of induction of immunological memory, deliberate this time, is vaccination. The objective is to induce a population of antigen-specific tissue-resident memory T lymphocytes capable of adaptive immunosurveillance [103]. Antigen-specific neutrophilic inflammation can be induced in the MG by systemic or local (intramammary) immunization [35]. It has been shown that luminal injection of a model antigen (ovalbumin) elicits a neutrophil influx in milk in sensitized but not naive animals [104, 105]. A comparable reaction can be induced with killed bacteria or bacterial extracts [106, 107]. The neutrophilic inflammation can accelerate the cure of infection in relation to IL-17-associated pathways detected in the tissue of immunized MGs [108, 109]. The mammary antigen-specific neutrophilic inflammation depending on the generation of Th17 cells is amplified by innate immunity and correlates with the production of IL-17 and IFN-γ in the milk of immunized cows [105, 110]. It can be put forward that mammary macrophages or dendritic cells present bacterial antigens to tissue-resident Th17 cells which, in response, secrete cytokines (IL-17A, IL-17F, IFN-γ) that stimulate MECs to amplify neutrophilic inflammation [35].

From the above, it is tempting to assume that type 3 immunity is involved during MG infections and adaptive responses. Type 3 immunity is mediated by the signature cytokines IL-17A, IL-17F, and IL-22 that are produced by a diversity of lymphoid cells such as innate lymphoid cell type 3 (ILC3), γδ T cells, CD4 helper (Th17) and CD8 (T cytotoxic 17; Tc17) αβ T cells [111, 112]. Type 3 immunity can elicit neutrophilic inflammation at sites of infection, stimulate epithelial cell self-defense, and contribute to epithelial homeostasis [113]. For these reasons, type 3 immunity appears to be particularly suited to protecting the MG against bacterial species that cause neutrophilic inflammation, such as staphylococci, streptococci, or coliforms [114].

Conclusions

Mastitis is a common disease of the MG. This results from the threats to which this organ is exposed and its response to infection is dictated by the way in which it fulfills its function. It has been speculated that lactation is evolutionarily related to the innate immune system, originating from skin glands producing a secretion rich in antimicrobial and other protective compounds [9]. The apparent contradiction between the possible evolutionary link of the MG development with the inflammatory innate immune response and the anti-inflammatory activity of milk [115] can be resolved if we consider that innate immunity is for the protection of the MG itself, and avoidance of inflammation is intended for the neonate. Human milk comprises antimicrobial factors but also anti-inflammatory and immunomodulating agents [116]. The repertoire of immune agents in milk is widely different between mammalian species [10], but the balance of pro-inflammatory and anti-inflammatory effects tends to provide a type of protection to the neonate that does not promote inflammation. The notion that milk has a potent and targeted defense activity against pathogenic bacteria [115] must be interpreted with discernment. After milking, colostrum and milk are rapidly spoiled by pathogenic, commensal, or environmental bacteria if left at room temperature. *In vivo* observations in dairy animals reveal that at the onset of infection, bacterial growth is exponential before neutrophilic inflammation develops. This shows that milk is prone to bacterial growth. Innate immune elements are not active (lactoferrin) or in sufficient concentration (complement) in the MG lumen [28, 117, 118]. Secretory IgA is likely to protect the neonate by interfering with the binding of bacteria to the digestive epithelium of the neonate [115], but is poorly effective in the MG due to the absence of mucus to

anchor to and its dilution in the MG secretion [30]. Milk is for the neonate, not the MG, thus it is logical that it conveys immunity adapted to the offspring. In this review, this is the immunity for the MG that is considered. This viewpoint offers perspectives different from that of the mother/infant dyad. The MG incorporates elements of the innate immune system, but the priority given to the function of nutrition and the production of relatively dilute milk reduces the efficacy of these elements. The evolution of the MG towards the production of a copious rich medium entailed the necessity of protection against bacterial growth. The mammary epithelium has an essential secretory function, and there is little use for defensive mucus and efficient barrier effect as bacteria can proliferate in the lumen, taking advantage of the nutritional richness of milk. A protected epithelium but milk curdled by proliferating bacteria and clogged ducts would be ineffective. A logical consequence is that the healthy udder is normally sterile and cannot accommodate a metabolically active bacterial community (a microbiota), which would necessarily curd the milk, clog the ducts, and trigger an inflammatory response. Natural selection favored the two major defense systems that protect extant mammalian MG. First, the anatomical sphincter of the opening lactiferous canal. It must function as a "one-way valve" [14], allowing suckling or milking but preventing bacterial ingress. Second, the prompt and massive mobilization of phagocytes to control the growth of bacteria in milk. The MG must be intolerant to bacteria because of the threat posed by a potentially huge bacterial load during infection and the degradation of milk quality which would prevent milk ejection. The anatomical and physiological characteristics of the MG dictate its mode of defense in response to the opportunities they offer to bacteria. The lactating mammalian female is in essence a "milk factory" [9], and the MG offers a bioreactor to bacteria equipped to use lactose and caseins as nutrients. Colostrum and milk cannot prevent bacterial proliferation, but phagocytic killing is the most efficient way to counter bacterial overgrowth. Phagocytosis in suspension in a liquid is a peculiarity of the MG. To be efficient, it requires very high numbers of neutrophils, because they rely on haphazard encounters with bacteria, contrary to the chemotactic approach that operates in solid tissue. Moreover, neutrophils are poor swimmers, contrary to some bacteria. Additionally, neutrophils tend to ingest casein micelles and fat globules, which hampers their bactericidal capacities [76]. Therefore, MG has developed a remarkable ability to mobilize phagocytic cells via neutrophilic inflammation, and this can best be achieved with type 3 immunity. Type 3 immunity encompasses innate and adaptive immunity, and its manifestations are finely tuned to be tissue-dependent organ-specific [119, 120]. Much remains to be explored about type 3 immunity in the MG, and the prospects of an application for the control of mastitis constitute a strong incentive to continue research in this direction.

Abbreviations

CCL2: chemokine C-C motif ligand 2

$CD8^{pos}$: cluster of differentiation 8

cfu: colony-forming units

CXCL1: chemokine C-X-C motif ligand 1

E. coli: *Escherichia coli*

IFN-γ: interferon-gamma

IgA: immunoglobulin A

IL-6: interleukin-6

LPS: lipopolysaccharide

MAIT: mucosal-associated invariant T

MAMPs: microbe-associated molecular patterns

MECs: mammary epithelial cells

MG: mammary gland

MHC: major histocompatibility complex

Th17: T helper 17

TLRs: Toll-like receptors

Declarations

Author contributions

PR: Writing—original draft, Writing—review & editing.

Conflicts of interest

The author declares that he has no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

Not applicable.

Funding

Not applicable.

References

1. Ruegg PL. *A 100-year review*: mastitis detection, management, and prevention. J Dairy Sci. 2017;100:10381–97.
2. Ruegg PL. What is success? A narrative review of research evaluating outcomes of antibiotics used for treatment of clinical mastitis. Front Vet Sci. 2021;8:639641.
3. Björkman S, Kauffold J, Kaiser MØ. Reproductive health of the sow during puerperium. Mol Reprod Dev. 2023;90:561–79.
4. Hughes K, Watson CJ. The mammary microenvironment in mastitis in humans, dairy ruminants, rabbits and rodents: a one health focus. J Mammary Gland Biol Neoplasia. 2018;23:27–41.
5. Vasiu I, Dabrowski R, Tvarijonaviciute A. Lactation-related mammary gland pathologies—a neglected emergency in the bitch. Reprod Domest Anim. 2021;56:208–30.
6. Canisso IF, Podico G, Ellerbrock RE. Diagnosis and treatment of mastitis in mares. Equine Vet Educ. 2021;33:320–6.
7. Cullinane M, Amir LH, Donath SM, Garland SM, Tabrizi SN, Payne MS, et al. Determinants of mastitis in women in the CASTLE study: a cohort study. BMC Fam Pract. 2015;16:181.
8. Wilson E, Woodd SL, Benova L. Incidence of and risk factors for lactational mastitis: a systematic review. J Hum Lact. 2020;36:673–86.
9. Oftedal OT. The evolution of milk secretion and its ancient origins. Animal. 2012;6:355–68.

10. Butler JE, Rainard P, Lippolis JD, Salmon H, Kacskovics I. Chapter 116 - The mammary gland in mucosal and regional immunity. In: Mestecky J, Strober W, Russell MW, Kelsall BL, Cheroutre H, Lambrecht BN, editors. Mucosal immunology (fourth edition). Boston: Academic Press; 2015. pp. 2269–306.
11. Hassiotou F, Geddes DT, Hartmann PE. Cells in human milk: state of the science. J Hum Lact. 2013;29: 171–82.
12. Wellnitz O, Bruckmaier RM. *Invited review*: the role of the blood–milk barrier and its manipulation for the efficacy of the mammary immune response and milk production. J Dairy Sci. 2021;104: 6376–88.
13. Kierszenbaum AL, Tres L. Histology and cell biology. An introduction to pathology. Philadelphia, PA: Elsevier Saunders; 2012.
14. Paulrud CO. Basic concepts of the bovine teat canal. Vet Res Commun. 2005;29:215–45.
15. Chavatte-Palmer P. Lactation in the mare. Equine Vet Educ. 2002;14:88–93.
16. Geddes DT, Gridneva Z, Perrella SL, Mitoulas LR, Kent JC, Stinson LF, et al. 25 Years of research in human lactation: from discovery to translation. Nutrients. 2021;13:3071.
17. Gooding MJ, Finlay J, Shipley JA, Halliwell M, Duck FA. Three-dimensional ultrasound imaging of mammary ducts in lactating women. J Ultrasound Med. 2010;29:95–103.
18. Bruckmaier RM, Blum JW. B-mode ultrasonography of mammary glands of cows, goats and sheep during α- and β-adrenergic agonist and oxytocin administration. J Dairy Res. 1992;59:151–9.
19. Vanderhaeghen W, Piepers S, Leroy F, Van Coillie E, Haesebrouck F, De Vliegher S. *Invited review*: effect, persistence, and virulence of coagulase-negative *Staphylococcus* species associated with ruminant udder health. J Dairy Sci. 2014;97:5275–93.
20. Bergonier D, de Crémoux R, Rupp R, Lagriffoul G, Berthelot X. Mastitis of dairy small ruminants. Vet Res. 2003;34:689–716.
21. Watts JL. Etiological agents of bovine mastitis. Vet Microbiol. 1988;16:41–66.
22. Kornalijnslijper JE, Daemen AJ, van Werven T, Niewold TA, Rutten VP, Noordhuizen-Stassen EN. Bacterial growth during the early phase of infection determines the severity of experimental *Escherichia coli* mastitis in dairy cows. Vet Microbiol. 2004;101:177–86.
23. Rainard P, Gitton C, Chaumeil T, Fassier T, Huau C, Riou M, et al. Host factors determine the evolution of infection with *Staphylococcus aureus* to gangrenous mastitis in goats. Vet Res. 2018;49:72.
24. Vangroenweghe F, Rainard P, Paape M, Duchateau L, Burvenich C. Increase of *Escherichia coli* inoculum doses induces faster innate immune response in primiparous cows. J Dairy Sci. 2004;87: 4132–44.
25. Newbould FHS, Neave FK. The response of the bovine mammary gland to an infusion of staphylococci. J Dairy Res. 1965;32:163–70.
26. Erskine RJ, Eberhart RJ, Grasso PJ, Scholz RW. Induction of *Escherichia coli* mastitis in cows fed selenium-deficient or selenium-supplemented diets. Am J Vet Res. 1989;50:2093–100.
27. Blum SE, Goldstone RJ, Connolly JPR, Répérant-Ferter M, Germon P, Inglis NF, et al. Postgenomics characterization of an essential genetic determinant of mammary pathogenic *Escherichia coli*. mBio. 2018;9:e00423-18.
28. Rainard P. The complement in milk and defense of the bovine mammary gland against infections. Vet Res. 2003;34:647–70.
29. Leimbach A, Poehlein A, Vollmers J, Görlich D, Daniel R, Dobrindt U. No evidence for a bovine mastitis *Escherichia coli* pathotype. BMC Genomics. 2017;18:359.
30. Rainard P, Gilbert FB, Germon P. Immune defenses of the mammary gland epithelium of dairy ruminants. Front Immunol. 2022;13:1031785.
31. Kang W, Kudsk KA. Is there evidence that the gut contributes to mucosal immunity in humans? JPEN J Parenter Enteral Nutr. 2007;31:246–58.

32. Kehrli ME Jr, Harp JA. Immunity in the mammary gland. Vet Clin North Am Food Anim Pract. 2001; 17:495–516.
33. Degnim AC, Brahmbhatt RD, Radisky DC, Hoskin TL, Stallings-Mann M, Laudenschlager M, et al. Immune cell quantitation in normal breast tissue lobules with and without lobulitis. Breast Cancer Res Treat. 2014;144:539–49.
34. Ferguson DJ. Intraepithelial lymphocytes and macrophages in the normal breast. Virchows Arch A Pathol Anat Histopathol. 1985;407:369–78.
35. Rainard P, Foucras G, Martins RP. Adaptive cell-mediated immunity in the mammary gland of dairy ruminants. Front Vet Sci. 2022;9:854890.
36. Bertotto A, Gerli R, Fabietti G, Crupi S, Arcangeli C, Scalise F, et al. Human breast milk T lymphocytes display the phenotype and functional characteristics of memory T cells. Eur J Immunol. 1990;20: 1877–80.
37. Taylor BC, Dellinger JD, Cullor JS, Stott JL. Bovine milk lymphocytes display the phenotype of memory T cells and are predominantly $CD8^+$. Cell Immunol. 1994;156:245–53.
38. Van de Perre P, Rubbo PA, Viljoen J, Nagot N, Tylleskär T, Lepage P, et al. HIV-1 reservoirs in breast milk and challenges to elimination of breast-feeding transmission of HIV-1. Sci Transl Med. 2012;4: 143sr3.
39. Schalm OW, Carroll EJ, Jain NC. Number and types of somatic cells in normal and mastitic milk. In: Schalm OW, Carroll EJ, Jain NC, editors. Bovine mastitis. Philadelphia: Lea & Febiger; 1971. pp. 94–127.
40. Hortet P, Seegers H. Calculated milk production losses associated with elevated somatic cell counts in dairy cows: review and critical discussion. Vet Res. 1998;29:497–510.
41. Jones GM, Pearson RE, Clabaugh GA, Heald CW. Relationships between somatic cell counts and milk production. J Dairy Sci. 1984;67:1823–31.
42. Larsen LB, Hinz K, Jørgensen AL, Møller HS, Wellnitz O, Bruckmaier RM, et al. Proteomic and peptidomic study of proteolysis in quarter milk after infusion with lipoteichoic acid from *Staphylococcus aureus*. J Dairy Sci. 2010;93:5613–26.
43. Wellnitz O, Baumert A, Saudenowa M, Bruckmaier RM. Immune response of bovine milk somatic cells to endotoxin in healthy quarters with normal and very low cell counts. J Dairy Res. 2010;77: 452–9.
44. Porcherie A, Cunha P, Trotereau A, Roussel P, Gilbert FB, Rainard P, et al. Repertoire of *Escherichia coli* agonists sensed by innate immunity receptors of the bovine udder and mammary epithelial cells. Vet Res. 2012;43:14.
45. Bougarn S, Cunha P, Harmache A, Fromageau A, Gilbert BF, Rainard P. Muramyl dipeptide synergizes with *Staphylococcus aureus* lipoteichoic acid to recruit neutrophils in the mammary gland and to stimulate mammary epithelial cells. Clin Vaccine Immunol. 2010;17:1797–809.
46. Carroll EJ, Schalm OW, Lasmanis J. Experimental coliform (*Aerobacter aerogenes*) mastitis: characteristics of the endotoxin and its role in pathogenesis. Am J Vet Res. 1964;25:720–6.
47. Goldammer T, Zerbe H, Molenaar A, Schuberth HJ, Brunner RM, Kata SR, et al. Mastitis increases mammary mRNA abundance of β-defensin 5, Toll-like-receptor 2 (TLR2), and TLR4 but not TLR9 in cattle. Clin Diagn Lab Immunol. 2004;11:174–85.
48. Tsugami Y, Wakasa H, Kawahara M, Watanabe A, Suzuki T, Nishimura T, et al. Adverse effects of LPS on membrane proteins in lactating bovine mammary epithelial cells. Cell Tissue Res. 2021;384: 435–48.
49. Labéta MO, Vidal K, Nores JE, Arias M, Vita N, Morgan BP, et al. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. J Exp Med. 2000;191:1807–12.

50. Lee JW, Paape MJ, Elsasser TH, Zhao X. Recombinant soluble CD14 reduces severity of intramammary infection by *Escherichia coli*. Infect Immun. 2003;71:4034–9.
51. Védrine M, Berthault C, Leroux C, Répérant-Ferter M, Gitton C, Barbey S, et al. Sensing of *Escherichia coli* and LPS by mammary epithelial cells is modulated by O-antigen chain and CD14. PLoS One. 2018;13:e0202664.
52. Zheng J, Watson AD, Kerr DE. Genome-wide expression analysis of lipopolysaccharide-induced mastitis in a mouse model. Infect Immun. 2006;74:1907–15.
53. Maxymiv NG, Bharathan M, Mullarky IK. Bovine mammary dendritic cells: a heterogeneous population, distinct from macrophages and similar in phenotype to afferent lymph veiled cells. Comp Immunol Microbiol Infect Dis. 2012;35:31–8.
54. Hassel C, Gausserès B, Guzylack-Piriou L, Foucras G. Ductal macrophages predominate in the immune landscape of the lactating mammary gland. Front Immunol. 2021;12:764661.
55. Günther J, Czabanska A, Bauer I, Leigh JA, Holst O, Seyfert HM. *Streptococcus uberis* strains isolated from the bovine mammary gland evade immune recognition by mammary epithelial cells, but not of macrophages. Vet Res. 2016;47:13.
56. Bedin AS, Molès JP, Rutagwera D, Nagot N, Kankasa C, Tylleskär T, et al. MAIT cells, TCR γδ+ cells and ILCs cells in human breast milk and blood from HIV infected and uninfected women. Pediatr Allergy Immunol. 2019;30:479–87.
57. Edmans MD, Connelley TK, Jayaraman S, Vrettou C, Vordermeier M, Mak JYW, et al. Identification and phenotype of MAIT cells in cattle and their response to bacterial infections. Front Immunol. 2021;12: 627173.
58. Collins RA, Parsons KR, Bland AP. Antibody-containing cells and specialised epithelial cells in the bovine teat. Res Vet Sci. 1986;41:50–5.
59. Fragkou IA, Dagleish MP, Papaioannou N, Cripps PJ, Boscos CM, Ververidis HN, et al. The induction of lymphoid follicle-like structures in the ovine teat duct following experimental infection with *Mannheimia haemolytica*. Vet J. 2010;184:194–200.
60. Kawai K, Korematsu K, Akiyama K, Okita M, Yoshimura Y, Isobe N. Dynamics of lingual antimicrobial peptide, lactoferrin concentrations and lactoperoxidase activity in the milk of cows treated for clinical mastitis. Anim Sci J. 2015;86:153–8.
61. Swanson K, Gorodetsky S, Good L, Davis S, Musgrave D, Stelwagen K, et al. Expression of a β-defensin mRNA, lingual antimicrobial peptide, in bovine mammary epithelial tissue is induced by mastitis. Infect Immun. 2004;72:7311–4.
62. Yang W, Molenaar A, Kurts-Ebert B, Seyfert HM. NF-κB factors are essential, but not the switch, for pathogen-related induction of the bovine β-defensin 5-encoding gene in mammary epithelial cells. Mol Immunol. 2006;43:210–25.
63. Molenaar AJ, Harris DP, Rajan GH, Pearson ML, Callaghan MR, Sommer L, et al. The acute-phase protein serum amyloid A3 is expressed in the bovine mammary gland and plays a role in host defence. Biomarkers. 2009;14:26–37.
64. Kelleher SL, Lönnerdal B. Immunological activities associated with milk. Adv Nutr Res. 2001;10: 39–65.
65. Ogundele M. Role and significance of the complement system in mucosal immunity: particular reference to the human breast milk complement. Immunol Cell Biol. 2001;79:1–10.
66. Gilbert FB, Cunha P, Jensen K, Glass EJ, Foucras G, Robert-Granie C, et al. Differential response of bovine mammary epithelial cells to *Staphylococcus aureus* or *Escherichia coli* agonists of the innate immune system. Vet Res. 2013;44:40.

67. Günther J, Esch K, Poschadel N, Petzl W, Zerbe H, Mitterhuemer S, et al. Comparative kinetics of *Escherichia coli*- and *Staphylococcus aureus*-specific activation of key immune pathways in mammary epithelial cells demonstrates that *S. aureus* elicits a delayed response dominated by interleukin-6 (IL-6) but not by IL-1A or tumor necrosis factor alpha. Infect Immun. 2011;79:695–707.
68. Farquhar C, Mbori-Ngacha DA, Redman MW, Bosire RK, Lohman BL, Piantadosi AL, et al. CC and CXC chemokines in breastmilk are associated with mother-to-child HIV-1 transmission. Current HIV Res. 2005;3:361–9.
69. Paape MJ, Shafer-Weaver K, Capuco AV, Van Oostveldt K, Burvenich C. Immune surveillance of mammary tissue by phagocytic cells. Adv Exp Med Biol. 2000;480:259–77.
70. Rainard P, Riollet C. Mobilization of neutrophils and defense of the bovine mammary gland. Reprod Nutr Dev. 2003;43:439–57.
71. Schneider P, Salamon H, Weizmann N, Nissim-Eliraz E, Lysnyansky I, Shpigel NY. Immune profiling of experimental murine mastitis reveals conserved response to mammary pathogenic *Escherichia coli*, *Mycoplasma bovis*, and *Streptococcus uberis*. Front Microbiol. 2023;14:1126896.
72. Jain NC, Schalm OW, Carroll EJ, Lasmanis J. Experimental mastitis in leukopenic cows: immunologically induced neutropenia and response to intramammary inoculation of *Aerobacter aerogenes*. Am J Vet Res. 1968;29:2089–97.
73. Hill AW, Shears AL, Hibbitt KG. The pathogenesis of experimental *Escherichia coli* mastitis in newly calved dairy cows. Res Vet Sci. 1979;26:97–101.
74. Porcherie A, Gilbert FB, Germon P, Cunha P, Trotereau A, Rossignol C, et al. IL-17A is an important effector of the immune response of the mammary gland to *Escherichia coli* infection. J Immunol. 2016;196:803–12.
75. Schukken YH, Wilson DJ, Welcome F, Garrison-Tikofsky L, Gonzalez RN. Monitoring udder health and milk quality using somatic cell counts. Vet Res. 2003;34:579–96.
76. Paape MJ, Mehrzad J, Zhao X, Detilleux J, Burvenich C. Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. J Mammary Gland Biol Neoplasia. 2002;7:109–21.
77. Zhao X, Lacasse P. Mammary tissue damage during bovine mastitis: causes and control. J Anim Sci. 2008;86:57–65.
78. Robinson JE, Harvey BA, Soothill JF. Phagocytosis and killing of bacteria and yeast by human milk cells after opsonisation in aqueous phase of milk. Br Med J. 1978;1:1443–5.
79. Hill AW, Heneghan DJ, Williams MR. The opsonic activity of bovine milk whey for the phagocytosis and killing by neutrophils of encapsulated and non-encapsulated *Escherichia coli*. Vet Microbiol. 1983;8:293–300.
80. Hill AW, Heneghan DJ, Field TR, Williams MR. Increase in specific opsonic activity in bovine milk following experimental *Escherichia coli* mastitis. Res Vet Sci. 1983;35:222–6.
81. Xu T, Dong Z, Wang X, Qi S, Li X, Cheng R, et al. IL-1β induces increased tight junction permeability in bovine mammary epithelial cells via the IL-1β-ERK1/2-MLCK axis upon blood-milk barrier damage. J Cell Biochem. 2018;119:9028–41.
82. Fitzgerald DC, Meade KG, McEvoy AN, Lillis L, Murphy EP, Machugh DE, et al. Tumour necrosis factor-α (TNF-α) increases nuclear factor kappaB (NFκB) activity in and interleukin-8 (IL-8) release from bovine mammary epithelial cells. Vet Immunol Immunopathol. 2007;116:59–68.
83. Bougarn S, Cunha P, Gilbert FB, Harmache A, Foucras G, Rainard P. Staphylococcal-associated molecular patterns enhance expression of immune defense genes induced by IL-17 in mammary epithelial cells. Cytokine. 2011;56:749–59.
84. Khalkhali-Ellis Z, Abbott DE, Bailey CM, Goossens W, Margaryan NV, Gluck SL, et al. IFN-γ regulation of vacuolar pH, cathepsin D processing and autophagy in mammary epithelial cells. J Cell Biochem. 2008;105:208–18.

85. Yamaguchi T, Hiratsuka M, Asai K, Kai K, Kumagai K. Differential distribution of T lymphocyte subpopulations in the bovine mammary gland during lactation. J Dairy Sci. 1999;82:1459–64.
86. Yamaguchi T, Hiratsuka M, Asai K, Kumagai K. A phenotype of mammary intra-epithelial lymphocytes(mIEL)in cows. Acta Histochem Cytoc. 2000;33:11–5.
87. Zirbes A, Joseph J, Lopez JC, Sayaman RW, Basam M, Seewaldt VL, et al. Changes in immune cell types with age in breast are consistent with a decline in immune surveillance and increased immunosuppression. J Mammary Gland Biol Neoplasia. 2021;26:247–61.
88. Betts CB, Pennock ND, Caruso BP, Ruffell B, Borges VF, Schedin P. Mucosal immunity in the female murine mammary gland. J Immunol. 2018;201:734–46.
89. Wirt DP, Adkins LT, Palkowetz KH, Schmalstieg FC, Goldman AS. Activated and memory T lymphocytes in human milk. Cytometry. 1992;13:282–90.
90. Sabbaj S, Ghosh MK, Edwards BH, Leeth R, Decker WD, Goepfert PA, et al. Breast milk-derived antigen-specific $CD8^+$ T cells: an extralymphoid effector memory cell population in humans[1]. J Immunol. 2005;174:2951–6.
91. Ikebuchi R, Fujimoto M, Moriya T, Kusumoto Y, Kobayashi K, Tomura M. T cells are the main population in mouse breast milk and express similar profiles of tight junction proteins as those in mammary alveolar epithelial cells. J Reprod Immunol. 2020;140:103137.
92. Hoek A, Rutten VP, Kool J, Arkesteijn GJ, Bouwstra RJ, Van Rhijn I, et al. Subpopulations of bovine $WC1^+$ γ T cells rather than $CD4^+CD25^{high}$ $Foxp3^+$ T cells act as immune regulatory cells *ex vivo*. Vet Res. 2009;40:6.
93. Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. The burgeoning family of unconventional T cells. Nat Immunol. 2015;16:1114–23. Erratum in: Nat Immunol. 2016;17:214. Erratum in: Nat Immunol. 2016;17:469.
94. Chien YH, Meyer C, Bonneville M. γδ T cells: first line of defense and beyond. Annu Rev Immunol. 2014;32:121–55.
95. Zeng X, Wei YL, Huang J, Newell EW, Yu H, Kidd BA, et al. γδ T cells recognize a microbial encoded B cell antigen to initiate a rapid antigen-specific interleukin-17 response. Immunity. 2012;37:524–34.
96. Ismail AS, Severson KM, Vaishnava S, Behrendt CL, Yu X, Benjamin JL, et al. γδ Intraepithelial lymphocytes are essential mediators of host–microbial homeostasis at the intestinal mucosal surface. Proc Natl Acad Sci U S A. 2011;108:8743–8.
97. Bertotto A, Castellucci G, Fabietti G, Scalise F, Vaccaro R. Lymphocytes bearing the T cell receptor gamma delta in human breast milk. Arch Dis Child. 1990;65:1274–5.
98. Asai K, Komine Y, Kozutsumi T, Yamaguchi T, Komine K, Kumagai K. Predominant subpopulations of T lymphocytes in the mammary gland secretions during lactation and intraepithelial T lymphocytes in the intestine of dairy cows. Vet Immunol Immunopathol. 2000;73:233–40.
99. Taylor BC, Keefe RG, Dellinger JD, Nakamura Y, Cullor JS, Stott JL. T cell populations and cytokine expression in milk derived from normal and bacteria-infected bovine mammary glands. Cell Immunol. 1997;182:68–76.
100. Jing XQ, Cao DY, Liu H, Wang XY, Zhao XD, Chen DK. Pivotal role of IL-17-producing γδ T cells in mouse chronic mastitis experimentally induced with *Staphylococcus aureus*. Asian J Anim Vet Adv. 2012;7:1266–78.
101. Schenkel JM, Fraser KA, Beura LK, Pauken KE, Vezys V, Masopust D. Resident memory CD8 T cells trigger protective innate and adaptive immune responses. Science. 2014;346:98–101.
102. Turner DL, Farber DL. Mucosal resident memory CD4 T cells in protection and immunopathology. Front Immunol. 2014;5:331.
103. Mueller SN, Mackay LK. Tissue-resident memory T cells: local specialists in immune defence. Nat Rev Immunol. 2016;16:79–89.

104. De Cueninck BJ. Expression of cell-mediated hypersensitivity in the lumen of the mammary gland in guinea pigs. Am J Vet Res. 1982;43:1696–700.
105. Rainard P, Cunha P, Ledresseur M, Staub C, Touzé JL, Kempf F, et al. Antigen-specific mammary inflammation depends on the production of IL-17A and IFN-γ by bovine CD4+ T lymphocytes. PLoS One. 2015;10:e0137755.
106. Targowski SP, Nonnecke BJ. Cell-mediated immune response of the mammary gland in guinea pigs. I. Effect of antigen injection into the vaccinated and unvaccinated glands. Am J Reprod Immunol. 1982; 2:29–38.
107. Herbelin C, Poutrel B, Gilbert FB, Rainard P. Immune recruitment and bactericidal activity of neutrophils in milk of cows vaccinated with staphylococcal α-toxin. J Dairy Sci. 1997;80:2025–34.
108. Herry V, Gitton C, Tabouret G, Répérant M, Forge L, Tasca C, et al. Local immunization impacts the response of dairy cows to *Escherichia coli* mastitis. Sci Rep. 2017;7:3441.
109. Cebron N, Maman S, Walachowski S, Gausserès B, Cunha P, Rainard P, et al. Mammary Th17-related immunity, but not high systemic Th1 response is associated with protection against *E. coli* mastitis. NPJ Vaccines. 2020;5:108.
110. Rainard P, Cunha P, Gilbert FB. Innate and adaptive immunity synergize to trigger inflammation in the mammary gland. PLoS One. 2016;11:e0154172.
111. Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. J Allergy Clin Immunol. 2015;135:626–35.
112. Gaffen SL. An overview of IL-17 function and signaling. Cytokine. 2008;43:402–7.
113. Eberl G. Development and evolution of RORγt$^+$ cells in a microbe's world. Immunol Rev. 2012;245: 177–88.
114. Rainard P, Cunha P, Martins RP, Gilbert FB, Germon P, Foucras G. Type 3 immunity: a perspective for the defense of the mammary gland against infections. Vet Res. 2020;51:129.
115. McClellan HL, Miller SJ, Hartmann PE. Evolution of lactation: nutrition *v*. protection with special reference to five mammalian species. Nutr Res Rev. 2008;21:97–116.
116. Goldman AS. The immunological system in human milk: the past—a pathway to the future. Adv Nutr Res. 2001:10:15–37.
117. Reiter B, Brock JH. Inhibition of *Escherichia coli* by bovine colostrum and post-colostral milk. I. Complement-mediated bactericidal activity of antibodies to a serum susceptible strain of *E. coli* of the serotype O 111. Immunology. 1975;28:71–82.
118. Reiter B, Brock JH, Steel ED. Inhibition of *Escherichia coli* by bovine colostrum and post-colostrum milk. II. The bacteriostatic effect of lactoferrin on a serum susceptible and serum resistant strain of *E. coli*. Immunology. 1975;28:83–95.
119. Amatya N, Garg AV, Gaffen SL. IL-17 signaling: the yin and the yang. Trends Immunol. 2017;38: 310–22.
120. McGeachy MJ, Cua DJ, Gaffen SL. The IL-17 family of cytokines in health and disease. Immunity. 2019; 50:892–906.