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Immune Components of Colostrum and Milk—A Historical Perspective

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Abstract Key developments in the understanding of the immune functions of milk and colostrum are reviewed, focusing on their proteinaceous components. The topics covered include the immunoglobulins, immune cells, immunomodulatory substances, and antimicrobial proteins. The contributions of new technologies and the introduction of fresh approaches from other fields are highlighted, as are the contributions that mammary biology research has made to the development of other fields. Finally, a summary of some current outstanding questions and likely future directions of the field are given.

Keywords Lactation · Mammary · Antimicrobial

Abbreviations

BPI	bactericidal/permeability increasing protein
DHA	docosahexaenoic acids
EPA	eicosapentaenoic acid
IgA	immunoglobulin A
IgG	immunoglobulin G
LBP	lipopolysaccharide binding protein
LC-PUFA	long chain-polyunsaturated fatty acids

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Introduction

The concept that milk, mammary secretions and the mammary gland have major roles in immune defense is an old one. The bactericidal property of milk was recorded in the scientific literature in the late nineteenth century [1, 2]. Moreover, observations at this time on the ability of milk to provide immunity to the newborn [3] played a key role in the development of modern immunology. The aim of this review is to provide a modern audience with a timeline for the key discoveries in milk immunology, illustrating how the current understanding of the immune function of milk evolved, and to offer some pointers for the future direction of the field.

This review is divided into three sections: the immunoglobulins and immune cells, immunomodulatory components, and antimicrobial components, covering elements of both innate and adaptive immunity, immune defenses in the mammary gland and the participation of the mammary gland in the mucosal defense system. In this we focus largely on the proteinaceous components of milk, some of which are depicted in Fig. 1. Reviews describing physical barriers, the role of probiotics, and the carbohydrate and lipid components of milk that have host defense functions have recently been presented elsewhere [4, 5].

Immunoglobulins in Milk

Today it is largely forgotten that the immune properties of milk helped lay the foundation of modern immunology. In 1892, Paul Ehrlich demonstrated that mice immunized against plant toxins passed immunity to the fetus in utero as well as via the milk. These observations were subsequently shown to be due to an ammonium sulfate precipitable

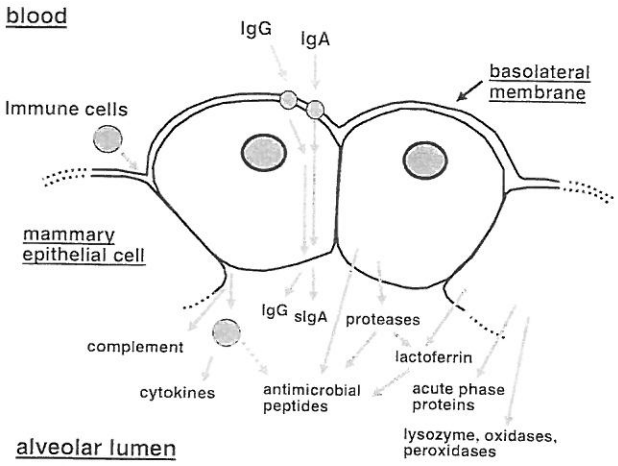

Fig. 1 Schematic showing some of the principal known proteinaceous components of the host defense system in milk and colostrum.

substance, termed an antibody [6]. Ehrlich extended this work to develop the concept of passive and active immunisation via antibodies, for which he was awarded the Nobel Prize in 1908. Many other researchers made important contributions through this early period (see Campbell and Petersen [7] for an excellent review of the history of immune milk). For example, Famulener [8] showed that goats immunized prior to parturition could transmit this immunity to the sera of their offspring via colostrum and that this transfer could also take place if goat serum was fed. Further experiments around this time established the concept of passive immunisation through the transfer of milk-derived antibodies from the intestinal lumen into the bloodstream. Colostrum was shown to contain a greater concentration of antibodies than mature milk, and the origin of these substances from serum was confirmed [9]. The importance of colostrum in providing protection from bacterial infection was shown by Smith and Little [10] who reported that a calf deprived of colostrum lacks "something" which allowed intestinal bacteria to invade the body and multiply in various organs. Allied work showed that this "something" was specific agglutinins (substances causing agglutination of cells) in the blood, derived from the mother's milk [11].

It was appreciated early on that there were significant differences between mammals in the immunological function of the mammary gland. For example, it was noted that colostrum was important for immune transfer to the newborn only in mammals with multi-layered placentae (for example the ungulates) [12–14]. In these species, the antibodies were shown to be absorbed into the bloodstream via the neonatal intestinal mucosa in the first 24–48 h of life [15]. Thus, by 1930, the role of the mammary gland in conferring protection to the neonate through the accumulation and secretion of agglutinating antibodies directed against pathogens was firmly established.

The bivalent structure of antibodies and the nature of their interaction with antigens were gradually elucidated through the 1940s. The highly specific nature of the antibodies in mammary secretions was demonstrated through analysis of the antibodies in colostrum fed to calves that had died from infections with different strains of *E. coli* [16]. While the presence of antibodies in milk and colostrum was indisputable, at this time there was still debate over their source and the nature of the therapeutic benefit that could be derived from them [7, 17].

The development of polyacrylamide gel electrophoresis and size-exclusion chromatography by the 1960s greatly accelerated the characterisation of the immune components of milk. These techniques led to the definition of individual classes of immunoglobulins (i.e. the agglutinating globulins) as well as comparisons between species. Thus, in sheep the predominant antibody in colostrum was shown to be IgG_1 [18], while in humans it was IgA [19]. Moreover, mammary secretions and serum were shown to have distinct immunoglobulin compositions (see Table 1). Despite much additional data being accumulated, there were still questions about the fundamental reason for the difference in milk immunoglobulins between species.

In the 1960s, Campbell and Petersen [7] were enthusiastic advocates for the therapeutic benefits of colostrum and milk

Table 1 Comparison of levels of immunoglobulin in human and cow colostrum, milk and serum.

Species	Ig	Concentration (g l)			% of total Ig		
		Colostrum	Milk	Serum	Colostrum	Milk	Serum
Human[a]	IgG	0.43	0.04	12.10	2.0	3.0	78.0
	IgA	17.35	1.00	2.50	90.0	87.0	16.0
	IgM	1.59	0.10	0.93	8.0	10.0	6.0
Cow[b]	IgG_1	46.40	0.58	11.20	75.5	71.6	47.0
	IgG_2	2.87	0.06	9.20	4.7	7.4	38.6
	IgA	5.36	0.08	0.37	8.8	9.9	1.6
	IgM	6.77	0.09	3.05	11.0	11.1	12.8

Ig immunoglobulin. [a] [20]. [b] [21].

from immunized cows. In particular, and most controversially, systemic responses to oral consumption of immune milk were claimed, with beneficial effects on symptoms of arthritis and hay fever [7]. This work was commercialized by Ralph Stolle, whose company (http://www.smbimilk.com) still markets 'hyperimmune milk' to the present day.

The origin of the immunoglobulins in milk was the source of some controversy. Campbell and Petersen believed that the majority of antibodies in bovine mammary secretions were synthesized within the udder. They based this premise, in part, on their observation that 'adequate' numbers of plasma cells are present in the udder immediately before parturition [20]. However, later workers found only low levels of plasma cells [21]. These differences may have been due to differences in the health status of the gland between the experiments. Others favoured a humoral source of the immunoglobulins in colostrum and milk [17], based on the observed decrease in serum immunoglobulin levels before parturition and a corresponding increase in the udder [22]. While IgG was shown to be transported to the mammary gland from the serum, IgA was found to be synthesized within the mammary gland by plasma cells which had migrated into the gland from the gastrointestinal tract (see below). It was found that in species where IgG is transferred to the foetus prior to birth (e.g. humans), IgA is the predominant immunoglobulin in colostrum, whereas in those born with no circulating IgG (e.g. cattle and sheep), IgG is the predominant immunoglobulin in colostrum. A 10-fold increase in the IgG_1:IgG_2 ration in bovine colostrum over that in serum suggested a specific IgG_1 transport mechanism [23].

By the 1970s, the mechanism for the transport of IgG into the mammary gland had been elucidated. The IgG receptor, FcRn, was identified and shown to be present on the apical surface of the gut of the suckling rat [24] as well as on the basolateral surface of the secretory epithelial cell during colostrogenesis [25]. Immunohistochemical analysis showed that FcRn expression coincided with Stage 1 lactogenesis (the onset of colostrogenesis) and was decreased during stage 2 lactogenesis (the onset of copious secretion; [26]).

The hormonal regulation of immunoglobulin transport into colostrum and milk has been investigated but remains incompletely described. Smith and co-workers [27] suggested that changing serum concentrations of estrogen and progesterone in late pregnancy exerted a controlling influence on the selective transport of IgG_1 to bovine lacteal fluid and thus colostrum formation. However, others suggested that the rate of IgG_1 transfer was a consequence of mammary gland development [28], which is itself under the control of estrogen and progesterone. Investigation into the role of prolactin in IgG transport showed that in the presence of the prolactin release inhibitor, bromoergocryptine, colostrum-like secretion continued post-partum for several days and mammary IgG_1 receptor activity was maintained [26]. Addition of prolactin to mammary cells in culture resulted in down-regulation of the receptor [29]. Thus, it seems that prolactin plays a role in regulating the IgG_1 receptor during Stage 2 lactogenesis. However, regulation of the induction of IgG receptor during Stage 1 lactogenesis remains poorly understood.

Advances in the knowledge of cellular immunity in mucosal tissues led to greater understanding of the origins of plasma cells in the mammary gland. These cells were shown to migrate into the mammary gland from the gut-associated lymphoid tissues (GALT) [30]. Adoptive transfer studies revealed that lymphocytes from the GALT populate many mucosal effector sites including the mammary gland. The precursors of plasma cells destined to produce IgA were shown to originate from GALT and traffic into the mammary gland near the time of parturition as well as in middle and late lactation [31]. In the late 1970s the concept of the 'common mucosal immune system' was proposed [32] in which the antigenic experience at one mucosal surface was deemed to lead to effector responses at a distant mucosal tissue, a concept that was supported by the discovery of cell surface receptors and cytokines. Thus, the idea emerged that cellular immune defense in the mammary gland is a local feature of an organism-wide integrated system.

In the mouse, T-cell migration to the mammary gland was shown to be mediated by mucosal addressin cell-adhesion molecule-1 (MAdCAM-1) expressed on the mammary vascular endothelium [33], while IgA plasma cell recruitment was found to be facilitated by vascular cell-adhesion molecule (VCAM-1) [34]. These adhesion molecules were found to be present in different proportions in the ruminant mammary gland [35], possibly accounting for some of the differences between species of the immunoglobulin isotypes in colostrum or milk. Recent studies have established a role for chemokines in directing immune cells to the mammary gland. Mouse mammary tissue has been shown to express the CCL28 (MEC) chemokine receptor, which binds to the CCR10 ligand on IgA lymphocytes [36]. Despite these advances, the nature of the stimulus for the trafficking of IgA plasma cells from the intestine to the mammary gland, particularly in late-pregnancy, is as yet unknown.

Transport of IgA from immune cells in the mammary gland into colostrum and milk has been elucidated. Key to this was the identification of the polymeric immunoglobulin receptor (pIgR), a transmembrane glycoprotein selectively expressed on mucosal and secretory epithelial basolateral cell surfaces. Earlier studies had identified a 'secretory component' that was linked to dimeric IgA following its

secretion into mucosal fluids [37]. This secretory component was later identified as the extracellular domain of pIgR. The transport of IgA involved binding to pIgR at the basolateral membrane, passage to the apical membrane then release into the alveolar lumen by cleavage of the complex, with IgA remaining linked to secretory component [38]. The pIgR protein was found to function as a sacrificial receptor, with one molecule of pIgR synthesised for every molecule of immunoglobulin secreted. The production of pIgR appears to limit the amount of IgA that is transported. Transgenic mice overexpressing pIgR in their mammary glands had double the IgA concentration in their milk compared with non-transgenic mice [39]. The expression of pIgR was shown to be under endocrine control in some tissues [40]. Prolactin and glucocorticoids were shown to enhance mammary pIgR protein and mRNA levels during lactation in the sheep [41].

Secretory component was found to be important for the function of IgA. When bound to IgA, it conferred protection from proteolytic degradation in the intestine [42, 43], and its presence facilitated localization of IgA to mucus [44]. Free secretory component has antimicrobial properties in its own right. It was first isolated from bovine milk [45] and subsequent studies have demonstrated that it binds to fimbrial colonization factors on bacterial surfaces, thereby reducing their pathogenicity [46]. Free secretory component also binds the cytokine IL-8 thereby modifying the pro-inflammatory effects of this cytokine [47].

Immunomodulatory Properties of Milk

The immune cells in milk are an important aspect of the immunomodulatory activity of milk. The presence of cells in milk and their association with mammary infection was described in the early twentieth century [48, 49]. However, the types of cells, their origin and their role in prevention of infection within the gland were debated for many years, particularly in non-bovine species. Significant advances in immunological analytical techniques eventually led to characterisation of the cell types in milk of humans as well as several other species. It is now clear that the cells in mammary secretions of all species studied consist of neutrophils, macrophages, lymphocytes and a smaller percentage of epithelial cells. In general, macrophages are the major cell population in milk from the healthy lactating mammary gland, whereas neutrophils predominate during early inflammation. These cells play an important role in signalling the presence of pathogens to the systemic immune system and thereby mounting a local immune response against pathogens.

Neutrophils and macrophages also phagocytose and kill bacteria directly, and this activity is enhanced by opsonic immunoglobulins and complement present in milk. By the 1970s, most components of the complement system were described in human [50] and bovine milk and colostrum [51].

The immune cells in milk may also modulate the neonatal immune system. This idea was first raised as a result of observing an increased level of neutrophils in the colostrum of non-nursing mothers in the absence of infection [52]. Evidence of transfer of tuberculin sensitivity to suckling infants [53] was used to support this hypothesis, as was transfer of partial tolerance and graft-versus-host reactions in rodents [54]. Subsequent studies have confirmed that milk cells can traverse the neonatal intestinal epithelium in a range of species [55, 56]. However, other studies found no evidence for transfer of cells across the gut [57] or tuberculin-sensitivity via milk [58].

A more recent explanation for modulation of the neonatal immune system via the cells themselves, is the production of soluble immune mediators. These "lymphokines" were first described in the 1980s and shown to stimulate the immune cells in the suckling animal [58, 59]. Many studies have since revealed the large range of these proteins (now termed "cytokines") present in human colostrum or milk. These include IL-1β, IL-6, IL-8, IL-10, IL-12, IL-18, IFN-γ, TNF-α, TGF-β, G-CSF, M-CSF, GM-CSF. These cytokines are thought to regulate the neonatal immune system in a variety of ways. For example, TGF-β has been proposed to reduce inflammatory reactions in the gut [60], to reduce allergy [61] and to stimulate intestinal IgA production [62]. Cyokines are also present in cow's milk, with increased levels during infection (reviewed in [63]).

The advent of recombinant technology enabled the production of cytokines such as Il-1, IL-2, IFN-γ and GM-CSF which induce higher numbers of neutrophils and macrophages when infused into the bovine udder. This finding has led to the idea that cytokines may be used as immunotherapy for prevention of mastitis in cattle, but to date none have been commercialized for routine use.

In vitro studies carried out over the last 20 years suggest that the repertoire of immune factors in milk also includes immunomodulatory peptides derived from caseins or whey proteins. These peptides are receiving much attention as possible sources of 'natural' bioactivity with health benefits for the consumer (see [64]). Casein peptides have also been used to stimulate the innate immune system within the mammary gland and prevent infections within the udder of cows at drying off [65]. However, peptides from the A1 variant of β-casein have been proposed to be involved in the development of type 1 diabetes, an auto-immune related disease, largely on the basis of epidemiological evidence [66].

In the 1980s, nucleotides were found in milk and proposed as potential immune regulators. By the mid

1980s, dietary nucleotide/nucleosides were shown to modulate cell-mediated immunity and prevent infection in animals [67]. Since then several infant feeding studies using formula supplemented with nucleotides suggest they can influence immune cell development and reduce the incidence of diarrhea.

Long chain poly-unsaturated fatty acids (LC-PUFA) such as DHA (docosahexaenoic acids) and EPA (eicosapentaenoic acid) were also identified in the 1980s as possible candidates for immune modulation. This notion was based on the observation that the diets of Eskimo children have a high proportion of fish containing these fatty acids, as well as a low incidence of asthma [68]. Subsequent studies have shown that infants fed formula supplemented with DHA also have decreased incidence of bronchitis than infants fed un-supplemented formula [69]. Supplementation of a mother's diet with LC-PUFA to enhance the immunomodulatory profile of breast milk is also receiving interest as a means to modify the infant's immune system [70].

A common feature of the studies over the years is that human milk is a rich source of many of the above-mentioned immunomodulatory components. The elucidation of many of these compounds was driven by the idea that breast-feeding prevents gastrointestinal and respiratory infectious disease in infants, as initially documented in 1930s [71]. This concept has now been broadened to include potential role of immunomodulatory elements in development of atopic disease (allergy) in children. Concentrations of LCPUFA, cytokines, nucleotides and polyamines in breast milk have all been associated with development of atopy in infants. In addition, the proportion of neutrophils is significantly higher and macrophages is lower in milk from mothers with an infant suffering from atopic dermatitis [72]. As human milk cells are a major source of some of the cytokines, it is possible that this finding could explain the link between cytokines and allergy. In some studies, an infant's risk of developing allergy has been linked to the mother, but not the father, having an allergy [73]. Thus, the association of a specific cytokine or fatty acid profile in breast milk with subsequent development of atopy in infants may simply relate to the mother's history of atopy.

Interestingly, one study has suggested a reduction in atopic dermatitis in at risk infants (when at least one parent has an allergy) after 6 months feeding of a partially hydrolysed infant formula as opposed to breast milk [74]. In addition, it has been suggested that prolonged (>7.5 months) exclusive breast-feeding of high risk infants has been associated with increased risk of IgE mediated food allergy, asthma and atopy, and atopic dermatitis [75], although shorter periods of breast-feeding are likely to be protective [76]. These observations support the notion that the reduction in exposure of the infant's immune system to foreign antigens at critical time points may be as important as the supply of immune factors from milk in the subsequent development of allergy in the infant. Much further work in these areas will be required to unravel the role of immune factors in colostrum and milk and development of mucosal immunity in infants.

Antimicrobial Proteins in Milk

Initial characterisation of the bactericidal components in milk was carried out in the 1920s. In 1922, Alexander Fleming [77] described a bacteriolytic activity which was present in a number of biological fluids, which he named lysozyme. Not long after, this activity was also described in milk [78]. About this time, what appeared to be a distinct antimicrobial action in milk was also described. This activity against streptococci was termed "lactenin" [79].

Improved techniques for protein analysis in the 1940s and 1950s led to the characterisation of these activities. The bactericidal activity of lysozyme was shown to be largely due to its ability to digest the complex polysaccharide component of the bacterial cell wall (reviewed in [80]). The isolation of lysozyme from human and cow's milk was reported in the 1960s [81, 82], with human milk having by far the greater abundance. Milk was shown to contain at least two distinct lactenins [83]. One of these was an oxidase, termed lactoperoxidase [84], which had earlier been purified from cow's milk [85]. Lactoperoxidase was subsequently shown to have antimicrobial activity against streptococci in the presence of peroxide and thiocyanate [86], thereby contributing to the antimicrobial activity of milk.

Other milk antimicrobial activities were also discovered. A fraction of cow's milk with a distinctive red colour was described in 1939 [87] and iron-chelating activity in milk was noted in 1951 [88]. Later workers characterised the protein responsible and termed it red milk protein, lactotransferrin, lactosiderophilin or lactoferrin. Lactoferrin was shown to have a bacteriostatic effect against *E. coli* [89] as well as the fungus, *Candida albicans* [90]. The antimicrobial properties of lactoferrin were attributed to its ability to sequester iron from the surrounding solution, thereby depriving bacteria of a mineral necessary for its growth. Xanthine oxidoreductase activity was first reported in milk, in 1902 [91]. Its antimicrobial activity, when supplied with exogenous substrate, was demonstrated in 1943, and was attributed to the formation of peroxide [92, 93].

As a result of these studies, by the mid 1960s, there was an appreciation that the defense property of milk was more complicated than simply the presence of immunoglobulins [94, 95]. Nevertheless, immunoglobulin transfer to the

neonate still dominated most studies on this topic at that time.

Further work in the 1970s provided more detail as to the function and biological role of antimicrobial milk proteins. Lactoferrin was found to be present in a number of additional secretions and fluids that are subject to pathogenic challenge [96], as well as in neutrophils [97], which play an important role in host defense. The level of lactoferrin in cow's milk was shown to rise significantly during mastitis [98]. The iron binding properties, predominance of the apo form of the protein in milk and the iron sequestering mechanism for its bacteriostatic effect were all corroborated [99, 100]. Lactoferrin was also shown to have bactericidal as well as bacteriostatic activity, and that it could act on a wider range of microbes than just *E. coli* [101]. Its antimicrobial activity was found to be due in part to alternative mechanisms such as membrane disruption, which was attributed to proteolytic cleavage products of lactoferrin, termed lactoferricins [102]. Lactoferrin has also been shown to have activity against a range of viruses, an activity that appears largely due to its binding to viral particles, thereby blocking virus entry to the cell (reviewed in [103].

The bactericidal actions of the enzymes in milk have been characterised in greater detail. The substrates for lactoperoxidase, hydrogen peroxide and thiocyanate, were shown to be produced by streptococci and liver detoxification pathways acting on dietary glucosinolates, respectively [104]. Lactoperoxidase was shown to produce a range of highly reactive groups which were thought to react with and disrupt the bacterial cell membranes (reviewed in [105, 106]). In addition to streptococci, this antimicrobial system was also found to act against a range of coliforms and Pseudomonas species, providing there was a source of peroxide [107, 108]. Xanthine oxidoreductase was shown to contribute to the lactoperoxidase antimicrobial system by supplying it with hydrogen peroxide [109]. Further evidence for the host defense role of xanthine oxidoreductase included: its production of a range of potentially bactericidal reactive oxygen species [110]; its increased abundance in neutrophils during infection [111]; and its inhibition being associated with an increase in microbial activity [112]. Lysozyme was shown to attack the cell walls of Gram positive bacteria by cleaving the glycosidic bond of *N*-acetylmuramic acid within the peptidoglycan molecule [113]. Lysozyme was identified in the secretory granules of neutrophils [114], associated with lactoferrin [115]. Proteolytic fragments of the caseins in milk were also shown to have antimicrobial activity (reviewed in [116]), suggesting that the proteases in milk as well as the major milk proteins themselves may also play a role in host defense.

By the late 1990s, a considerable body of knowledge had been accumulated as to the composition and mode of action of a range of antimicrobial proteins in milk. Yet, the understanding of host defense in milk was essentially the same as in the 1960s. However, around this time significant new insights were gained into the nature of innate immunity in vertebrates. These included the extensive cross-talk between the innate and acquired immune system, the first elucidation of the molecular mechanisms for pathogen recognition and identification of components of the signal transduction cascade leading to specific effector responses (reviewed in [117]). Identification of some of the components of host defense mechanisms in plants, insects and amphibians led to the discovery of equivalent systems in mammals, including the β-defensin and cathelicidin families of cationic antimicrobial peptides (reviewed in [118, 119]). This rapid series of discoveries energized the field of mammalian innate immunity, and provided a fresh approach to investigating the host defense properties of milk.

As a result, additional immune components of milk were identified. Members of the β-defensin family were found to be expressed by mammary epithelial cells with expression of their genes induced during mastitis [120, 121]. Members of the β-defensin and cathelicidin families were also found in milk and some at elevated levels in colostrum [122, 123]. A variant of an acute phase protein, serum amyloid A3, was found to be expressed in mammary cells in response to pathogens or pathogen-derived lipoteichoic acid and to be present in milk during mastitis [124], suggesting a role for this milk protein in host defense.

The repertoire of putative immune factors in milk continues to grow. A member of the RNAse family of proteins, angiogenin, has been known to be present in milk since the 1980s [125]. More recently, a host defense role was claimed for this protein, based on the discovery that mouse and human angiogenins have antimicrobial activity [126]. This role in milk is still to be verified, but is supported by the observation that other members of the RNase family are found in neutrophils and have antimicrobial as well as antiviral activities [127, 128].

Future Directions

It is almost axiomatic that much remains to be discovered about the innate host defense system in the mammary gland and its secretions. The potential of the recently developed technologies of genomics, transcriptomics and proteomics to shed light on the biology of milk and the mammary gland have yet to be fully realised. Gene expression studies using microarrays and proteomics published to date have revealed a hitherto hidden complexity of the host defense-associated proteins in milk and the immune response of the mammary gland [129–134]. These techniques are likely to

result in the identification of yet more components contributing to the host defense property of milk and colostrum.

A few fundamental questions remain to be answered. The regulation of the milk host defense system is not well understood. For example, the mechanisms responsible for differences in the composition of colostrum and milk between species and between stages of lactation have yet to be described. It is conceivable that these might involve epigenetic mechanisms. Also, the mechanisms by which pathogens are recognised by the host defense system is still a relatively unexplored area. Proteins such as Lipopolysaccharide Binding Protein (LBP) and Bactericidal/Permeability-Increasing Protein (BPI) have a key role in the systemic response to pathogens by binding to pathogen-derived compounds and presenting them to receptors on immune cells. It seems likely that an analogous system for pathogen recognition exists in milk, but to date this has not been described.

The biological roles of many of the known milk host defense components have still to be fully elucidated. The multifunctional nature of some of these has recently been suggested, for example the immunomodulatory properties of lactoferrin and some antimicrobial peptides known to be present in milk [135, 136]. To date most work on the proteins in mammary secretions has concentrated on the suppression of pathogens, while other possible functions have been under-represented in the literature. The immune system is known to play a role in mammary development and involution, as well as responding to neoplasia. It will be interesting to learn to what extent the proteins in milk contribute to these processes. Another current question is the extent of co-operativity and complementarity between the various components, as well as the nature of the mechanisms by which collectively they suppress the viability, growth, or virulence of pathogens of the mammary gland and neonatal GI tract. The possible synergies between the different proteins have not been thoroughly explored. Rather than consisting simply of immunoglobulins and a few minor milk proteins with inherent antimicrobial properties, it is now perhaps appropriate to view the defense system in mammary secretions as comprising a single integrated system. Thus in the future the application of a systems biology approach may be useful.

The host defense proteins in milk have many possible practical applications. The use of bovine milk extracts as natural food preservatives and functional food ingredients are two potential uses, and these are already beginning to be explored. One example of this is the supplementation of infant's formula and other consumer milk-based products with lactoferrin. Individual components of milk are already being used therapeutically. For example, colostral IgG purified from cows immunised with *E. coli* is being marketed as a product to prevent travellers' diarrhea. IgA has perhaps even more potential in this area, as it has better stability in the GI tract [137]. In the future, milk proteins with intrinsic bactericidal activity may be alternatives or supplements to traditional antibiotics, for which there is an increasing need [138]. It has also been suggested that the LPS-binding activity of lactoferrin could be used therapeutically [139]. Other potential uses are in dairying. For example, antimicrobial proteins have been expressed in cow's milk, either through production of transgenic cattle or by transfection of mammary cells in vivo, thereby enhancing the animal's resistance to mastitis [140, 141]. It is conceivable that in the future, the level of the endogenous host defense components in milk could be enhanced by inducing their over-expression through animal management approaches. Finally, detection of components of the host defense in milk could conceivably be used to detect mastitis in dairy cows. Similarly, their genes could be used as the basis for genetic selection for resistance to mastitis. Each of these applications has particular functional requirements that will need to be met. Future work will determine to what extent these potential uses are realised.

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