Evolution of the mammary gland from the innate immune system?

Claudia Vorbach,[1]* Mario R. Capecchi,[2] and Josef M. Penninger[1]

Summary

The mammary gland is a skin gland unique to the class Mammalia. Despite a growing molecular and histological understanding of the development and physiology of the mammary gland, its functional and morphological origins have remained speculative. Numerous theories on the origin of the mammary gland and lactation exist. The purpose of the mammary gland is to provide the newborn with copious amounts of milk, a unique body fluid that has a dual role of nutrition and immunological protection. Interestingly, antimicrobial enzymes, such as xanthine oxidoreductase or lysozyme, are directly involved in the evolution of the nutritional aspect of milk. We outline that xanthine oxidoreductase evolved a dual role in the mammary gland and hence provide new evidence supporting the hypothesis that the nutritional function of the milk evolved subsequent to its protective function. Therefore, we postulate that the mammary gland evolved from the innate immune system. In addition, we suggest that lactation partly evolved as an inflammatory response to tissue damage and infection, and discuss the observation that the two signaling pathways, NF-kB and Jak/Stat, play central roles in inflammation as well as in lactation.

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Abbreviations: XOR, xanthine oxidoreductase; XD, xanthine dehydrogenase; XO, xanthine oxidase; ROS, reactive oxygen species; RNS, reactive nitrogen species; O_{2-}, superoxide anions; OH′, hydroxyl radicals; H_2O_2, hydrogen peroxide; NO, nitric oxide; hBD-1, human β-defensin-1; PRM, pattern recognition molecule; PRR, pattern recognition receptor; TLR, Toll-like receptor; NF-kB, Nuclear factor kappaB; IKK, IkB kinase; TNF-α, tumor necrosis factor α; UTR, untranslated region; RANKL, receptor activator of NF-kB ligand; C/EBPβ, CCAAT/enhancer binding protein; Jak, Janus tyrosine kinase; Stat, signal transducer and activator of transcription; IFN-γ, interferon-γ; Ig, immunoglobin.

Introduction

The innate immune system is an evolutionarily conserved, rapid defense system. A great majority of animal species rely exclusively on innate immune responses while dealing with microbial insults.[1] Unlike the acquired immune system, which evolved during vertebrate evolution and depends on specialized immune cells, innate immunity is primarily mediated by peptides, small proteins and pattern recognition receptors to fight off dangerous microbes. Intriguingly, various peptides, small proteins and enzymes with innate immune function are also present in milk. Milk has a dual role of nourishment and immunological protection of the mammalian newborn and is a unique body fluid within the animal kingdom.[2] Considering the complex biochemical mechanisms of milk formation and secretion, lactation appears to have evolved gradually in incremental steps. Although milk composition differs among various mammals, the strong caloric value of milk is primarily due to the occurrence of fat droplets and the milk sugar lactose.

Interestingly, both the immunological and the strong nutritional value of milk are largely due to unique evolutionary contributions of the two antimicrobial enzymes xanthine oxidoreductase (XOR) and lysozyme, which are both expressed in and secreted from the lactating mammary epithelium.[3] By outlining a dual role for XOR in lactation, we add new evidence for the hypothesis, originally proposed by Hayssen and Blackburn, that the nutritional value of milk evolved subsequently to its immunological function.[4–8] We suggest that milk and the mammary gland evolved from the innate immune system and that lactation and inflammatory responses share many common mechanisms.

Epithelial body barriers are major sites for continued exposure to microbes. Not surprisingly, protective skin glands are common in evolution. We discuss the theory that the mammary gland evolved as a mucus skin gland potentially with the goal of protecting the newly evolving mammalian skin from infectious disease or, as has been suggested, to protect the egg surface and the newborn.[4–8] We hypothesize that lactation, as we know it today, evolved as an inflammatory response to tissue damage and infection and that inflammatory molecules became key regulators of lactation.

Xanthine oxidoreductase (XOR) has protective and nutritional functions in milk

XOR is an evolutionarily conserved housekeeping enzyme with a primary role in purine catabolism and a central role in the innate immune system.[9] Interestingly, XOR is also essential for lactation and crucial for the unique process of milk fat droplet enveloping and secretion from the lactating mammary gland, which provides an example of gene sharing, the use of a gene and its encoded protein for more than one function.[10] XOR is not only present in the terminally differentiated mammary epithelium and associated with milk fat droplet membranes, but XOR is also found in large amounts in milk where it performs various antimicrobial functions. XOR from the secretory mammary tissue was reported to have anti-streptococcal activity and enhancing XOR activity in fresh milk results in a dose-dependent bacterial growth inhibition.[11,12]

XOR exists in two enzymatic forms, a xanthine dehydrogenase (EC 1.1.1.204; XD) and a post-translationally modified xanthine oxidase (EC 1.1.3.22; XO). Both forms, but particularly the XO form, are involved in the synthesis of numerous reactive oxygen species (ROS) and reactive nitrogen species (RNS). At low amounts, ROS and RNS perform numerous cellular functions such as second messengers but, at high levels, ROS and RNS can act as microbiocidals.[13] Moreover, ROS and RNS can generate an entire cascade of microbiocidal reactions and products by participating in the synthesis of additional antimicrobial and cytotoxic molecules.[14] XOR generates the antimicrobial ROS superoxide anions (O_2^-), hydroxyl radicals (OH′) and hydrogen peroxide (H_2O_2). These molecules can further increase their antibacterial effect in milk, e.g. by contributing to the lactoperoxidase system, a native milk enzyme particularly prominent in ruminants, which synthesizes bacteriocidal and bacteriostatic ROS.[15–17] XOR is also known to synthesize nitric oxide (NO). Human milk samples show a NO-generating capacity that can be terminated by applying an inhibitor for XOR.[12] Addition of nitrite to human milk increases its antibacterial properties, presumably due to XO-generated NO and the generation of additional potent oxidants derived from NO.[18] Milk XO has also been implicated in an anti-viral response associated with its ability to convert retinaldehyde to retinoic acid.[19] Hence, XOR has a dual role in the lactating mammary gland: a protective antimicrobial function and a 'nutritional' role by enabling milk fat droplets to be enveloped and secreted from the lactating mammary epithelium (Fig. 1).

Lysozyme and α-lactalbumin: dual function in immunity and lactation

The lactating mammary epithelium also secretes the antimicrobial enzyme lysozyme for various protective functions.[20] Interestingly, lysozyme also evolved various nutritional roles in the mammary gland. Over evolutionary time, lysozyme underwent gene duplications and the duplicated allele evolved into α-lactalbumin, an important nutritional whey protein unique to the lactating mammary gland. Besides being a dietary whey protein, α-lactalbumin together with β-1,4 galactosytransferase constitutes the unique lactose synthetase enzyme complex, which synthesizes the milk sugar lactose.[21] Lactose is a major calorie source for the offspring and has immunological and physiological advantages over glucose. For instance, lactose limits the occurrence of bacteria in the mammary gland and in the neonate, since it only promotes the growth of microorganisms that are capable of hydrolyzing this disaccharid. Attached to lactose are numerous oligosaccharides that can further inhibit pathogens and bacterial toxins rather than providing nutritional benefits. Furthermore, lactose and its derivatives are osmotically active and draw water into milk thereby increasing its volume.[22] Mice with a targeted disruption of α-lactalbumin produce highly viscous milk that cannot be removed from the mammary glands by the suckling pups.[23] Hence, the antimicrobial enzyme lysozyme also evolved a dual role in the lactating mammary gland: a protective antimicrobial role and, through gene duplications, numerous 'nutritional' roles leading to the presence of α-lactalbumin and lactose, and the accumulation of water in milk (Fig. 1).[4]

Evolution of the mammary gland from the innate immune system?

The innate immune system is a first-line defense system that provides organisms with various immediately available

Figure 1. Molecules that play a dual role in immunity and nutrition. Proposed origin of milk as an antimicrobial mucus secretion containing many evolutionarily conserved, protective molecules, which today comprise part of the whey proteins of milk (blue). Two of these molecules, xanthine oxidoreductase and lysozyme, evolved due to gene sharing and gene duplication additional functions in the ancient mammary epithelium such as milk fat droplet secretion and the unique synthesis and secretion of α-lactalbumin as well as the milk sugar lactose. The secretion of these calorie-rich components markedly increased the nutritional value to milk (green). The nutritional and protective importance of milk was further expanded by serum-derived proteins that comprise part of the whey proteins of milk (red). In particular, lactose and its derivatives are osmotically active and draw water into milk. Caseins are the major protein source of milk in higher mammals. While the calcium-sensitive α_1 α_2, β, γ, δ-caseins presumably originated *de novo*, the calcium-insensitive κ-casein shows structural and sequence similarities to fibrinogen (purple). Abbreviation: Ab, antibody; Ig, Immunoglobulin; PRM, Pattern Recognition Molecule; PRR, Pattern Recognition Receptor; XOR, Xanthine oxidoreductase.

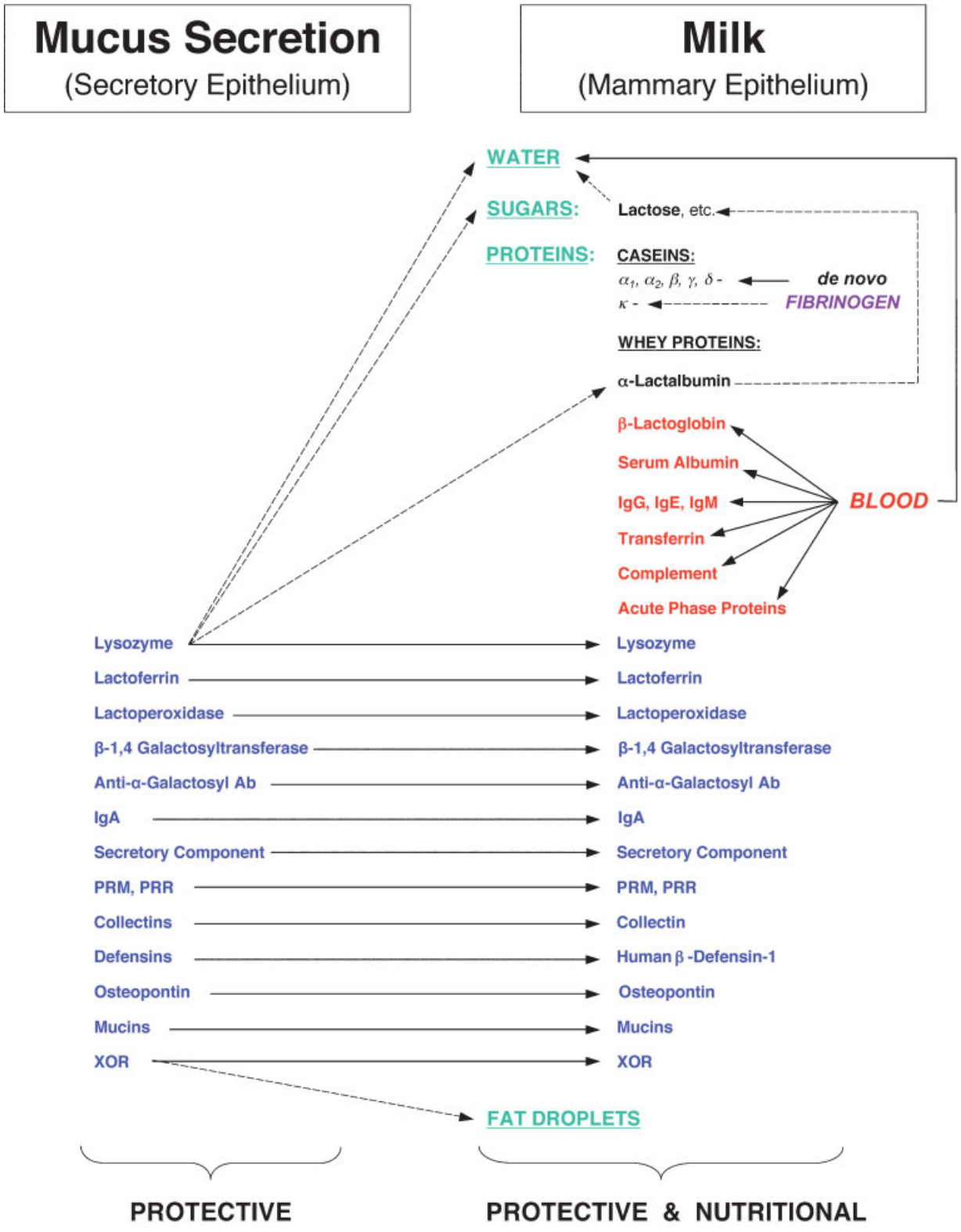
Mucus Secretion
(Secretory Epithelium)
Milk
(Mammary Epithelium)
WATER
SUGARS:
Lactose, etc.
PROTEINS:
CASEINS:
$\alpha_1, \alpha_2, \beta, \gamma, \delta$ -
de novo
κ -
FIBRINOGEN
WHEY PROTEINS:
α-Lactalbumin
β-Lactoglobin
Serum Albumin
IgG, IgE, IgM
Transferrin
Complement
Acute Phase Proteins
BLOOD
Lysozyme
Lactoferrin
Lactoperoxidase
β-1,4 Galactosyltransferase
Anti-α-Galactosyl Ab
IgA
Secretory Component
PRM, PRR
Collectins
Defensins
Osteopontin
Mucins
XOR
Lysozyme
Lactoferrin
Lactoperoxidase
β-1,4 Galactosyltransferase
Anti-α-Galactosyl Ab
IgA
Secretory Component
PRM, PRR
Collectin
Human β -Defensin-1
Osteopontin
Mucins
XOR
FAT DROPLETS
PROTECTIVE
PROTECTIVE & NUTRITIONAL

Figure 1.

defense mechanisms including mucus barriers and inflammatory responses. XOR and lysozyme are both abundant proteins in epithelial tissues, epithelial secretions and phagocytes, performing various important roles in innate immunity.[9] While the antimicrobial role of lysozyme is well established, the immunological role of XOR has so far received only little attention. As a housekeeping enzyme, XOR performs many cellular protective and signaling functions associated with its synthesis of uric acid and its synthesis of ROS and RNS. Not surprisingly, both enzymes are prominently expressed in the mammary epithelium and found in milk to help provide passive immunity for the nursing offspring and to counteract the development of mastitis during lactation.[9]

Numerous other evolutionary conserved antimicrobial molecules that are part of the innate immune system are also expressed by the mammary epithelium and/or are found in milk (Table 1 and Fig. 1). For instance, milk contains the metal-binding glycoproteins lactoferrin and transferrin, which act as bacteriostatic and bactericidal agents.[24] Lactoferrin is present in many body fluids and mucosal secretions, but its concentration is particularly high in milk.[25] The related protein transferrin occurs only in milk of some species, while it is a predominant serum protein in all mammals studied.[25] Lactoperoxidase is a member of a family of animal peroxidases/myeloperoxidases, which, similar to XOR, catalyze many important reactions for metabolic functions as well as for general host defense of mucosal surfaces. Lactoperoxidase is found in milk but also expressed by neutrophils for immune defense.[26,27]

Human milk contains human β-defensin-1 (hBD-1), which is also constitutively expressed in cultured mammary epithelial cells and in lactating and non-lactating mammary glands.[28] Defensins are antimicrobial peptides of the innate immune system present throughout the animal kingdom. They effect a broad spectrum of antibiotic activity, primarily by disrupting microbial cell membranes.[29] Moreover, mammary epithelial cells and even milk contain various pattern-recognition molecules and receptors (PRMs, PRRs) such as Toll-like receptors (TLRs) that recognize molecular patterns of the surface of microbes.[30,31] Whether TLRs play important roles in the local host defense of mammary epithelium or have yet unknown functions in mammary glands needs to be determined. Since all these innate immune factors have evolved early in evolution long before the advent of mammals, it appears that milk has evolved as an antimicrobial secretion and that the nutritional function of milk developed subsequently to its protective role (Fig. 1).[4]

Inflammatory molecules and signaling pathways as key regulators of lactation

Inflammation is a complex response that is coordinated by the concerted actions of soluble factors and cells to protect from microbial invasion or injury.[32] Throughout evolution, inflammatory responses are mediated by conserved cell signaling pathways. One such pathway that is essential for proper immune responses from *Drosophila* to mammals is the activation of the transcription factor NF-kB.[33] In the absence of signals, NF-kB resides in the cytoplasm associated with inhibitor proteins of the IkB family or p105 and p100. Following stimulation, the IkB kinase (IKK) complex phosphorylates NF-kB inhibitors leading to degradation of IkB or the partial degradation of the p100 and p105 proteins followed by nuclear translocation of NF-kB. The NF-kB pathway controls expression of various antimicrobial molecules, cytokines and costimulatory molecules.[33] Importantly, the NF-kB pathway also plays a central role in lactation. Transgenic mice overexpressing IkBα in the mammary epithelium as well as mice that harbour an IKKα knock-in mutation, which abolishes IKKα catalytic activity, exhibit lactation defects associated with decreased differentiation of mammary epithelial cells during pregnancy resulting in the death of the newborns.[34]

A large number of ligand–receptor pairs that activate NF-kB have critical immune functions and, at the same time, are also important for mammary gland development.[35] For instance, tumor necrosis factor α (TNF-α) is a proinflammatory cytokine with various immunomodulatory properties. TNF-α stimulates growth, as well as secondary and tertiary branching of mammary epithelial cells in primary cultures.[36] Moreover, TNFα stimulates the expression of XOR in renal as well as mammary epithelial cells.[37] Binding sites for NF-kB have been reported in the 5′ UTR sequence of the human XOR gene, suggesting XOR as a direct downstream target of the TNFα/NF-kB pathway in the mammary gland (Fig. 2).

RANKL, the receptor activator of NF-kB ligand (also known as TRANCE/OPGL/ODF/ TNFSF11), belongs to the family of tumor necrosis factors and binding of RANKL to its receptor RANK leads to the activation of NF-kB.[38] RANKL plays various important roles in bone formation, immunity, and as in lactation.[37–41] During pregnancy, mammary glands of mice with targeted deletions in RANKL or RANK show reduced epithelial proliferation and secretory differentiation leading to completely impaired lactation and the death of newborn pups.[42] Besides activation of the NF-kB pathway, RANKL directly regulates the expression of the milk protein β-casein by triggering the nuclear translocation of the CCAAT/enhancer binding protein C/EBPβ.[43] Mice deficient for C/EBPβ show aberrant ductal morphogenesis and decreased secretory differentiation of the mammary gland leading to impaired lactation.[44] Importantly, C/EBPβ not only plays an important role in lactation but is also involved in mediating inflammatory responses.[45] Moreover, C/EBPβ was shown to bind to the proximal rat XOR promoter that contains four C/EBP binding sites (Fig. 2).[46]

The Jak/Stat pathway (Janus tyrosine kinase (Jak)-signal transducers and activators of transcription (Stat) signaling pathway) was initially discovered in relation to interferon-γ

Table 1. Various protective molecules that participate in innate immunity are also found in the mammary gland and/or in milk

Molecule	Function in innate immunity	Occurrence in the mammary gland and/or in milk	References
Acute phase proteins (e.g. Mannose-binding lectin, MBL)	MBL is an acute phase protein that activates the complement system by the lectin pathway after the recognition of saccharides present on the surface of microorganisms. Lectins occur in many body fluids to agglutinate cells or precipitate glycoconjugates as part of innate defense mechanisms.	Found in milk.	(49,50,73–77)
Albumin	Serum protein and antioxidant due to oxidizable thiol groups.	Found in milk.	(51)
Anti-α-galactosyl Antibody	Natural human serum antibody synthesized in response to immune stimulation by enteric bacteria, also found in numerous body secretions presumably to prevent bacterial attachment to surface epithelia.	Found in milk.	(78)
Collectins (e.g. the lung surfactant protein-D, SP-D)	Collagen-like lectins (collectins) are expressed on numerous human epithelial tissues. Collectins inhibit microbial virulence and adherence, enhance phagocytosis and killing, are involved in chemotaxis and modulate cytokine production. SP-D is the major protein associated with mammalian pulmonary surfactant. SP-D has a role in surfactant homeostasis as well as innate immunity. SP-D is secreted onto luminal epithelium of many organs where it binds to oligosaccharides on the surface of microorganisms.	Expressed by the mammary epithelium.	(52,53)
Complement	Serum proteins that help to recognize and eliminate pathogens and toxic molecules.	Found in milk.	(54,79,80)
Defensins	One of the most important families of antimicrobial peptides of the innate immune system, conserved throughout the animal kingdom, permeabilize membranes of microbes, activate complement, stimulate cytokine and chemokine production.	Found in milk. Human ß-defensin-1 is constitutively expressed in cultured mammary epithelial cells, as well as in lactating and non-lactating mammary glands.	(55,81)
Immunoglobin A (IgA)	Major Ig in mucosal secretions. It functions as immunological barrier on mucous membranes, neutralizes toxins and viruses, inhibits microbial growth and adherence, opsonizes microbes, enhances phagocytosis and killing, and activates complement. Most of human IgA is secretory sIgA known to inhibit colonization of pathogenic bacteria on mucosal surfaces.	Found in milk where it is the dominant immunoglobin; IgA helps to protect the newborn against intestinal infections by blocking bacterial adhesion to the gut epithelium and by neutralizing microbial toxins.	(53,55–58,82)
β-Lactoglobin	Acetylation of β-lactoglobin, as well as α-lactalbumin generates molecules with strong antiviral activity.	Found in milk.	(46)
Lactoferrin	Bacteriostatic glycoprotein of many body fluids and mucosal secretions, releases LPS from the membrane of gram-negative bacteria, involved in immunomodulation, inhibition of adenovirus replication, activation of gene transcription, and functions as antioxidant.	Found at high concentrations in milk where it has bacteriostatic and bacteriocidal functions, Partial digestion of lactoferrin releases peptides with broad antimicrobial activities. The specific composition of amino acids gives lactoferrin also an important nutritional role.	(83–86)
Lactoperoxidase	Member of a family of animal peroxidases/myeloperoxidases that catalyze many important reactions for metabolic functions as well as innate immunity, it synthesizes antimicrobial molecules, protects mucosal surfaces, and is expressed by phagocytic cells.	Found in milk, where it has bacteriostatic and bacteriocidal functions.	(27,87,88)
Mucins	Membrane-bound or secreted glycoproteins of mucous surface epithelia that protect from infections and injuries. Mucins are the major component of mucus.	Expressed by the mammary epithelium and found in milk.	(89–91)
Osteopontin (OPN)	A secreted, adhesive, glycophosphoprotein that contains the arginine-glycine-aspartic acid sequence found in many extracellular matrix proteins. It is suggested to be involved in Th-1 type immune responses, it has anti-inflammatory activities and induces NF-kB.	Found in milk.	(92,93)

(*Continued*)

Table 1. (*Continued*)

Molecule	Function in innate immunity	Occurrence in the mammary gland and/or in milk	References
Pattern Recognition Molecules and Receptors (PRMs and PRRs)	Expressed in many body fluids, as well as in the intestine, lung, skin. PRRs recognize molecular arrays/patterns of the surface microbes and subsequently mediate the secretion of innate immune response molecules and couple innate to adaptive immunity.	Expressed by mammary epithelial cells and found in milk.	(30,31)
Secretory component (SC)	Exists as epithelial surface receptor and as a soluble form in various secretions, has a role in protecting sIgA against proteolytic degradation and ensures appropriate localization of sIgA. SC also neutralizes bacterial toxins and prevents bacterial adhesion by direct, nonspecific binding.	Found in milk.	(94,95)
Transferrin	Metal-binding glycoprotein, a predominant serum globin in mammals, chelates ferric iron and thereby acts as a defense for infections.	Found in milk.	(51,96,97)

(IFN-γ) signaling in immune cells.[47] Activation of Jak proteins leads to the phosphorylation of Stats and other proteins, which subsequently undergo nuclear translocation to regulate gene transcription.[47] Stats mediate responses to an array of diverse cytokine and non-cytokine stimuli, resulting in altered expression of inflammatory genes.[48] Subsequently, critical functions for Jak/Stats have been ascribed in multiple other tissues. In the mammary gland, Jak2 is activated by prolactin and responsible for nuclear translocation of Stat5.[49,50] Stat5 exists in the isoforms Stat5a and Stat5b.[51] Mice mutant for Stat5a show reduced proliferation of the mammary epithelium during pregnancy and a reduced secretory differentiation during lactation.[52] While mice mutant for Stat5b show no mammary gland defects, Stat5a/b double knockout mice show a reduced expression of milk proteins during lactation. Furthermore, Stat5a/b double knockout mice exhibit defects in the expansion of macrophages in inflammatory exudates.[53]

Prolactin is known as a key lactogenic hormone but, depending on the cellular context, prolactin can also act as an anti-inflammatory or proinflammatory cytokine.[54] Interestingly, it has been demonstrated that prolactin is involved in the protective as well as the nutritional role of milk. Prolactin participates in regulating the secretion of immunoglobin A (IgA), the prominent Ig in mucus and milk that inhibits the colonization of pathogenic bacteria on mucosal surfaces.[55] Changes in the secretion of IgA are associated with the anti-inflammatory potential of epithelial tissues.[56,57] In addition, prolactin stimulates the uptake of some amino acids and glucose, as well as the synthesis of casein, α-lactalbumin, lactose and milk fat droplets in the lactating mammary epithelium.[58] Finally, prolactin and IFN-γ also stimulate the expression of XOR in mammary epithelial cells via the Jak/Stat signaling pathway (Fig. 2). Thus, multiple small molecules and ligand–receptor systems that have critical roles in inflammatory responses exert dual and, in many cases, essential functions in immunity and mammary gland biology. Similar to the above-mentioned pathways that exhibit dual functions in mammary gland formation and inflammatory responses, the Toll–dorsal pathway provides another intriguing example of how signaling pathways involved in innate immunity exert additional functions in evolution. For example, Toll was first identified in the dorsal–ventral axis formation in a *Drosophila* screen and later recognized as a critical molecule that protect flies from fungal infections.[59,60]

Did lactation evolve as an inflammatory response?

Throughout evolution, inflammatory responses led to the production of conserved effector molecules, including lysozyme and XOR (Fig. 3). Following pathogen challenge, mice deficient for lysozyme show reduced inflammatory expression of lysozyme and increased inflammation compared to control mice.[61] XOR is not only upregulated in the terminally differentiating mammary epithelium and throughout lactation, XOR is also upregulated in other organs and epithelial tissues as part of an inflammatory response.[9] This upregulation of XOR is often associated with a conversion from the XD to the XO form, the potent enzymatic form that generates ROS and RNS. While XOR is an evolutionarily conserved enzyme, only in mammals is it possible to rapidly and reversibly convert XOR from the XD to the XO form making XOR an ideal component for a rapid innate immune response.[9]

Interestingly, the XO form of XOR predominates also in milk and is the isoform that is responsible for milk fat droplet enveloping and secretion.[62] Inflammation can also lead to the formation of fat droplets. For instance, endothelial cells and various immune cells that participate in an inflammatory and immunological response often contain numerous cytoplasmic fat droplets.[63] It has been suggested that those fat droplets play an important role in the formation of eicosanoid mediators

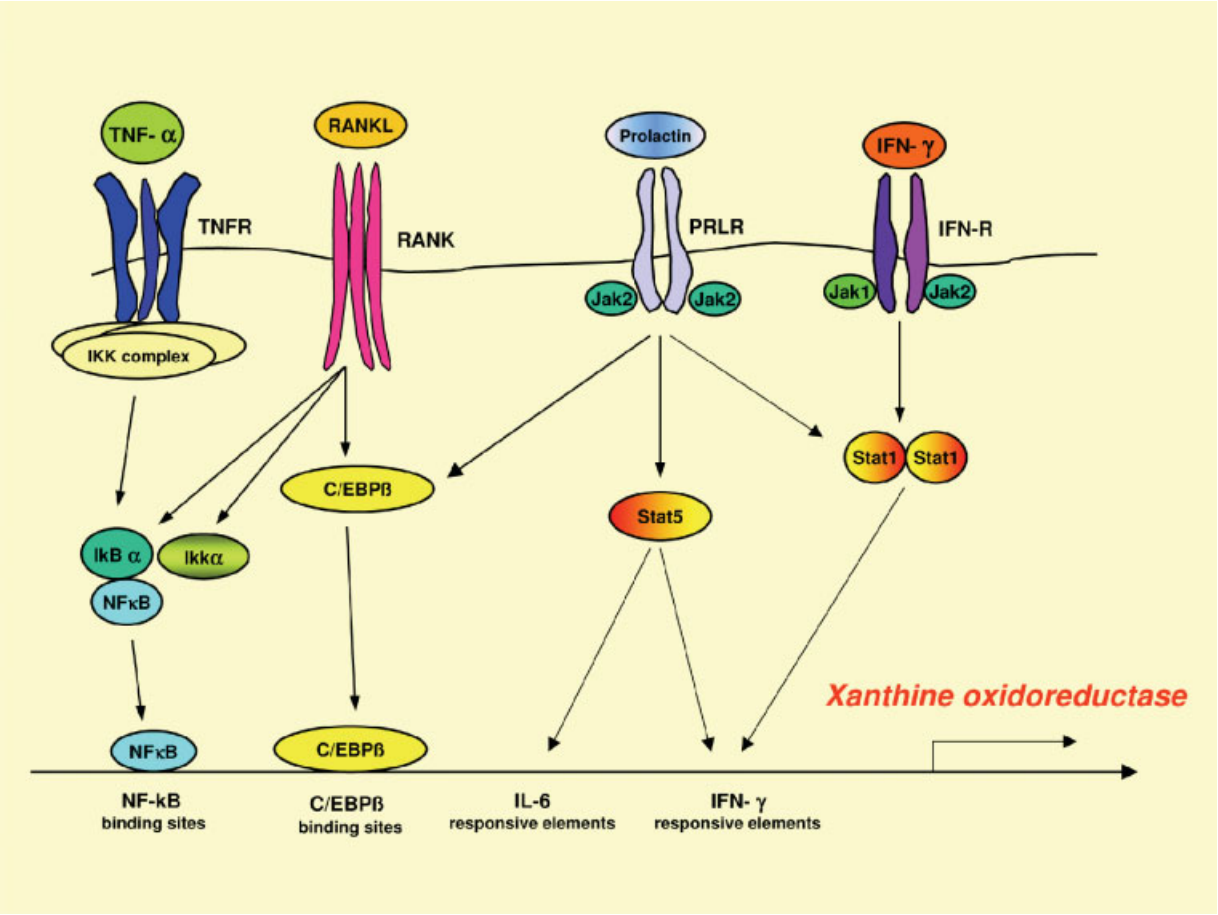

Figure 2. Schematic representation of signaling pathways involved in xanthine oxidoreductase (XOR) expression in the mammary gland and during inflammation. XOR is upregulated by TNFα in mammary epithelial cells and binding sites for NF-kB have been reported in the 5′ UTR of the human XOR gene, suggesting that XOR is a direct downstream target of the TNFα/NF-kB pathway in the mammary gland. NF-kB activation in the mammary gland is also stimulated by RANKL. In addition, RANKL as well as prolactin stimulate the translocation of the transcription factor C/EBPα into the nucleus of mammary gland epithelial cells, which leads to the expression of β-casein. C/EBPβ was shown to bind to the proximal rat XOR promoter. Prolactin and IFNγ are both known to activate the Jak/Stat pathway and are known to up regulate XOR in mammary epithelial cells. An IL-6 responsive element and five potential IFNγ responsive elements have been reported in the 5′ UTR of the human XOR gene suggesting that XOR may also be a direct downstream target of the Jak/Stat pathway in the mammary gland.[98] Abbreviations: C/EBPβ CCAAT/enhancer binding protein β; IFN, interferon; IKK, IκB kinase complex; IL, interleukin; Jak, Janus tyrosine kinase; PRLR, prolactin receptor; RANK, receptor activator of NF-kB; RANKL, receptor activator of NF-kB ligand; Stat, signal transducers and activators of transcription, TNFR, tumor necrosis factor receptor; UTR, untranslated region.

during the inflammatory response.[64] Very recently, uric acid, the end product of XOR synthesis, was shown to be an endogenous danger signal released by injured cells and essential for an inflammatory immune response.[65]

Protective skin glands are common within the animal kingdom. Tissue damage and infection may have triggered inflammatory reactions within these early mammary gland anlagen, associated with increased mucus secretion and increased expression of protective molecules. Thus, ancient mammary gland anlagen may have evolved as organs that provided large amounts of antimicrobial factors to protect the evolving mammalian skin and therefore the integrity of the whole organism.[4] Ancient mammary glands presumably had no nipples and milk flowed onto the ventral fur as in today's monotreme platypus.[66] Antibacterial skin and skin gland secretions are also well known from numerous other animal species such as salamanders, fish epidermis, or frogs.[67–70]

During reproduction, lactation and mucus secretion, in particular mucus hypersecretion as a result of an inflammatory response, may have helped to protect the egg surface of primitive mammals as well as the newborn, resulting in increased reproductive fitness.[4,6] Similarly, reptiles, birds, amphibians and fish also evolved specializations to immunologically

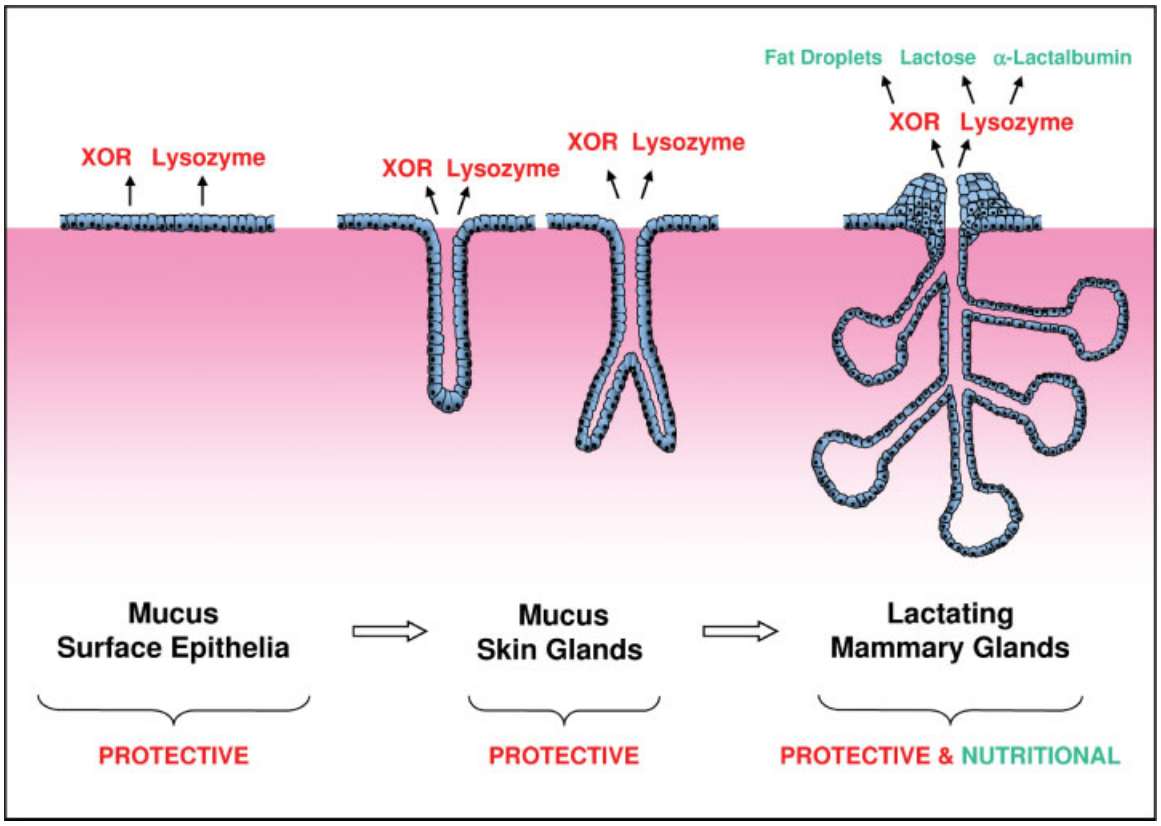

Figure 3. Proposed evolution of the mammary gland from a mucus-secreting epithelial gland. Mammary glands presumably evolved as mucus-secreting skin glands that similar to many mucus surface epithelia secreted antimicrobial enzymes such as XOR and lysozyme. The evolution of additional functions of XOR and lysozyme in the ancient mammary epithelium resulted in the secretion of fat droplets, α-lactalbumin and lactose. Consequently, the mammary gland evolved from a protective immune organ into a reproductive organ unique to the class mammalia.

protect their eggs.[4] Consequently, it appears that stimulation of mucus secretion/lactation as well as the development of the mammary gland came under the control of reproductive hormones and the mammary gland evolved from a primary immune "gland" into a major reproductive organ. Importantly, numerous gender-specific effects on the immune system have been reported, suggesting that sex-hormone-regulated immunity could have also contributed to the evolution of mammary glands in females.[71,72] Not surprisingly, reproductive and pituitary hormones were initially thought to be the key regulators of lactation. Yet, lactation is decisively regulated by molecules and signaling pathways that are central to an inflammatory response. Thus, we suggest that lactation initially reflected an inflammatory response to tissue damage and infection while its reproductive role may have evolved subsequently.

Conclusions

The mammary gland presumably evolved as a small integumental gland that, similar to other epithelial surface tissues and protective skin glands, was able to synthesize a mucous secretion containing a variety of antimicrobial molecules to protect the skin, the eggs and/or the newborns.[4–8] Due to gene sharing and gene duplication events, two of these antimicrobial enzymes, XOR and lysozyme, evolved unique additional roles in the mammary epithelium, resulting in the secretion of fat droplets, the presence of a whey protein and sugar, and the accumulation of water. The occurrence of these major nutritional components and calorie sources in a body fluid gave milk a unique nutritional value in addition to its protective immunological role.[4–8] New molecular evidence supports the notion that mammary glands and milk production evolved as part of the innate immune system and corroborates the initial hypothesis of Hayssen and Blackburn that the nutritional role of the mammary gland evolved subsequently to its protective function. Moreover, the critical involvement of conserved signaling pathways in immunity as well as the development and function of the mammary gland suggests that molecules controlling inflammatory responses were indeed key regulators and essential triggers for the evolution of modern lactation.

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