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Re-thinking benign inflammation of the lactating breast: A mechanobiological model

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Abstract

Despite the known benefits of breastfeeding for both infant and mother, clinical support for problems such as inflammation of the lactating breast remains a research frontier. Breast pain associated with inflammation is a common reason for premature weaning. Multiple diagnoses are used for inflammatory conditions of the lactating breast, such as engorgement, blocked ducts, phlegmon, mammary candidiasis, subacute mastitis, mastitis and white spots, which lack agreed or evidence-based aetiology, definitions and treatment. This is the first in a series of three articles which review the research literature concerning benign lactation–related breast inflammation. This article investigates aetiological models. A complex systems perspective is applied to analyse heterogeneous and interdisciplinary evidence elucidating the functional anatomy and physiology of the lactating breast; the mammary immune system, including the human milk microbiome and cellular composition; the effects of mechanical forces during lactation; and the interactions between these. This analysis gives rise to a mechanobiological model of breast inflammation, in which very high intra-alveolar and intra-ductal pressures are hypothesized to strain or rupture the tight junctions between lactocytes and ductal epithelial cells, triggering inflammatory cascades and capillary dilation. Resultant elevation of stromal tension exerts pressure on lactiferous ducts, worsening intraluminal backpressure. Rising leucocyte and epithelial cell counts in the milk and alterations in the milk microbiome are signs that the mammary immune system is recruiting mechanisms to downregulate inflammatory feedback loops. From a complex systems perspective, the key mechanism for the prevention or treatment of breast inflammation is avoidance of excessively high intra-alveolar and intra-ductal pressures, which prevents a critical mass of mechanical strain and rupture of the tight junctions between lactocytes and ductal epithelial cells.

Keywords

blocked ducts, breastfeeding, breast inflammation, human milk microbiome, human milk somatic cells, lactation, mastitis, mechanobiology

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Introduction

Breast pain is one of the most common reasons women give for premature weaning.[1,2] Despite the known benefits of breastfeeding for both infant and mother, clinical interventions for problems such as breast inflammation and pain remain a research frontier.[3]

Multiple diagnoses are used for benign inflammatory conditions of the lactating breast, including engorgement, blocked ducts, phlegmon, mammary candidiasis, subacute mastitis, mastitis and white spots. Yet these diagnoses lack agreed or evidence-based definitions and treatment. There is no consensus in the research literature on causes of benign lactation–related breast inflammation (BLBI, pronounced 'bill-bee'), including mastitis.[4,5]

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Overuse of medical, surgical and pharmaceutical interventions is an increasingly serious international problem in health care.[6,7] Both patients and clinicians typically overestimate the benefits of medical interventions and underestimate potential harms.[8–10] It is not surprising then, given the relative lack of research into clinical breastfeeding support, that overmedicalization and overtreatment are significant problems in the care of breastfeeding women and their babies, including when clinical breast inflammation emerges.[5,11–18]

This is the first of three articles which consider aetiology, classification and management of benign lactation–related inflammatory conditions. Ethics approval has not been required since this is a theoretical investigation. This article addresses aetiology. The second article addresses clinical classification, prevention and management.[19] The third article addresses aetiology, classification, prevention and management of lactation-related inflammation of the nipple–areolar complex.[20]

These articles assume that pathology such as malignancy, which is not BLBI or end-stage non-malignant lactation-related breast inflammation (abscess, fistula or galactocoele) has been excluded. Identification, differential diagnoses and management of these excluded conditions in the lactating breast are detailed in the Academy of Breastfeeding Medicine Clinical Protocol #30: *Breast Masses, Breast Complaints, and Diagnostic Breast Imaging in the Lactating Woman*.[21]

The complex systems approach to BLBI detailed in this three-part series forms part of the breastfeeding domain of the programmes known as Neuroprotective Developmental Care (NDC or 'the Possums programs'), developed and delivered in Australia since 2011. NDC synthesizes the evidence concerning early life care across the domains of breastfeeding, cry-fuss problems, infant sleep and parental mood by applying the theoretical frames of evolutionary biology and complexity science, translating this evidence into clinical practice.[5,11,22–35] Applying an evolutionary perspective, breastfeeding is foundational to, and interacts with, each other domain.

The pathogenic microbiota theory of BLBI

By the 1980s, a disease-centric view of human milk had taken hold. Because human milk was believed to be sterile, any bacteria cultured from milk was considered to be either infective or contaminant washed back from the infant oral cavity and maternal skin.[36,37] Applying this pathogenic model of BLBI, antibiotics are commenced if:

1. Signs and symptoms of mastitis, however defined, persist for more than 12 to 24 h;
2. The woman has concurrent nipple damage; or
3. The woman feels acutely unwell, for example, with fever.[38–40]

As knowledge of the human milk microbiome has grown, proponents of a pathogenic microbiota model of breast inflammation have hypothesized that the irregular, branching and densely interlaced human lactiferous ductal system (Appendix 1) favours the growth of biofilm-forming bacteria, perhaps in association with *Candida albicans* (Appendix 2). Biofilm is theorized to result in sticky milk and narrowed or blocked lactiferous ducts, causing cascades of epithelial inflammation and stromal oedema.[41–44] Clinical protocols based on the pathogenic microbiota model recommend long courses of antibiotics and antifungals when lactating women experience persistent breast inflammation or nipple pain. Protocols also advise patients to use mechanical pressure (e.g. localized lump massage or vibration), therapeutic ultrasound, therapeutic breast massage or manual lymphatic drainage to disperse theorized lactiferous duct plugs or 'lactoliths' and associated fluid.[38–40,45]

But attempts to unblock ducts with lump massage or vibration may worsen BLBI, due to microvascular trauma and stromal pressure effects. Emerging research contests the pathogenic microbiota model of breast inflammation, discussed below and in Appendix 2.[18,46–48] There is no physiological rationale or evidence to support the hypothesis that milk thickens or curdles or becomes sticky in the ducts, causing clinical inflammation. Although ultrasound studies show that milk may have rich fat droplet content as it passes through the ducts, there is no evidence to suggest that fat droplets coalesce to block milk flow, causing clinical inflammation.[49] Evidence demonstrating the lack of efficacy of clinical strategies which are based upon the pathogenic microbiota model of BLBI is examined in the second article of this series.[19]

An updated aetiological model is required in order to classify, prevent and effectively manage BLBI. This article synthesizes the latest evidence concerning, first, mechanical forces of lactation and, second, the microbiome and cellular composition of human milk, which play immunomodulatory roles within the mammary gland immune system. To make sense of interactions between mechanobiology and the immunoregulatory role of human milk within the breast, a thorough understanding, third, of the functional anatomy of the lactating breast is required, detailed in Appendix 1.

The immune system of the lactating breast: nested complex adaptive systems

A mother and her infant are best conceptualized as a complex adaptive system, in which multiple biobehavioural and physiological complex adaptive systems are nested, interacting together. Each complex adaptive system contains a myriad of interacting elements and feedback loops. In the study of complex systems, the function of the whole cannot be explained by the behaviour of any single component. A small perturbation may have amplified and unpredictable effects over time ('butterfly effect'). Health

problems emerge when myriad interacting feedback loops fail to stabilize the system.

Lactocytes take up plasma components and manufacture constituents of breast milk to secrete a nutritive and immune-factor-rich fluid into the alveoli and duct lumens. The mammary gland immune system provides defence against both endogenous tissue damage and exogenous infection, for both the breast and the infant. Applying a complexity lens, clinical inflammation emerges as a host immune response to physiological stress, which then acts to downregulate perturbation and restore homeostasis in the lactating breast.

The perturbations within the mammary gland immune system which lead to clinical inflammation result from a complex network of interactions, including between two key systems:

1. Mammary gland mechanobiology
2. Human milk, itself comprised of multiple complex adaptive systems, including the microbiome, somatic cells, oligosaccharides, exosomes and metabolome.

Simplistic, linear interventions into complex adaptive systems (e.g. instructions to massage or vibrate a breast lump) risk unintended outcomes (e.g. worsened inflammation and abscess). Strategies for both prevention and treatment of BLBI promote resilience and stabilize systems by multilateral downregulation of certain emergent feedback loops and upregulation of other protective feedback loops.

Mechanobiology of the lactating breast

Mechanosensing and the healthy lactating breast

Mechanical signals are a constant feature of the natural world, resulting in finely tuned coordination among signalling networks and genes. But the critical role of mechanical factors in the signalling networks of lactation is only beginning to be elucidated.[50]

In 1987, Wilde hypothesized that a protein in the whey fraction, named the Feedback Inhibitor of Lactation, acted as a master key in the synthesis and suppression of milk synthesis. However, it is now understood that milk synthesis and suppression are not controlled by a single entity but are complex systems (Appendix 1).[51]

It is possible that bioactive factors within milk (such as growth factors, parathyroid hormone–related protein and serotonin) act as inhibitors, regulating milk secretion. It is also accepted that progesterone, prolactin, oxytocin and leukaemia inhibitory factor modulate cell signalling and function in the mammary gland. But these modulatory factors appear to have indirect and time-delayed effects on milk synthesis, relative to the immediate and powerful local control exerted by pressure and stretching negative-feedback mechanisms. Three-dimensional time-lapse imaging of the mammary gland of lactating mice supports the existence of a multifaceted system of mechanical sensing through chemical signals in the mammary gland.[50,51]

Cell signalling and function during lactation are affected by mechanical stressors from:

1. Cell-intrinsic forces, for example, contractile forces exerted by the actin–myosin skeleton of myoepithelial cells;[50]
2. Cell-extrinsic forces, for example, lactocyte stretching and inter-lactocyte tight junction rupture arising from elevated intraluminal pressure;[50,51]
3. Stromal substrate mechanics, that is, stromal tissue density and tension; and
4. Environmental force on stroma and ducts, for example,
   a. Intra-oral mechanical forces during suckling,[11,28,29]
   b. Direct external pressure on an area of the breast resulting in microvascular trauma and elevated stromal tension[52] or
   c. Direct external pressure on an area of the breast resulting in prolonged ductal compression.

Lactation and the body's inflammatory response share many common mechanisms; the healthy lactating mammary gland is a proinflammatory environment.[53,54] This article integrates Weaver and Hernandez's proposal that mammalian species downregulate milk by apoptosis,[51] with Jindal et al.'s proposal that partial gland involution occurs prior to the complete cessation of breastfeeding in response to decreasing milk removal[54] and Stewart et al.'s work on mechanosensing in the murine mammary gland,[50] to propose a mechanobiological model for downregulation of milk synthesis in the lactating human breast.

Before an alveolus fills, lactocytes present rounded apices to the lumen. When a lactocyte takes this columnar or triangular shape, fat droplets bud off from the apical cell membrane. As intra-alveolar pressure builds due to milk accumulation, lactocyte calcium-permeable ion channels are activated; lactocytes absorb the increasing mechanical load by stretching and losing their apices. This protects inter-lactocyte tight junction integrity but prevents fat droplet extrusion.

The mechanical effects of severe stretching of the lactocyte cell membrane are not yet clearly elucidated. It is not known if mechanical forces exert an immediate downregulatory effect upon lactocyte cell membrane's capacity to exocytose protein and lactose in Golgi-derived secretory vesicles or upon cell membrane permeability to water and ions. It seems most likely that lactocytes steadily secrete lactose and proteins into alveolar lumens, with continued passage of ions and water across the cell membrane in response, even as tight junctions stretch. Tight junction

strain triggers chemical signals, such as cytokines, chemokines and adhesion molecules, which warn the host immune system of early cell and tissue damage, recruiting local hyperaemia and increased leucocytes. Sodium, chloride and albumin from the plasma may pass directly through the tight junctions as they open up under mechanical strain, increasing intra-alveolar volume.[46]

Increasing milk accumulation exerts shearing or compression forces on tight junctions, which finally break under severe mechanical stress, and the alveolus and its basement membrane rupture. This precipitates a dynamic wound-healing inflammatory response in the stroma and milk, proteolytic degradation of the alveolar basement membrane and lactocyte apoptosis. Immune cells and, perhaps more importantly, other mammary epithelial cells phagocytose debris from these small subclinical areas of involution. Lactocytes are irreversibly replaced with adipocytes as tissue is repaired and remodelled.[50,51,53,54] Applying the mechanobiological theory of BLBI, normal wound-healing processes occur microscopically throughout the course of a healthy and successful lactation in response to intermittent excessively high intra-alveolar and intra-ductal pressures, without the development of clinical signs and symptoms.

Approaching 6 months post birth, an infant begins to ingest solids. At this time, maternal milk secretion decreases through the same mechanism of elevated intraluminal pressures, tight junction rupture, alveolar collapse and lactocyte death. Complete cessation of breastfeeding, whenever this occurs, triggers one of the largest cascades of programmed cell death to occur in mammals: 80% to 90% of remaining lactocytes switch from milk secretion to apoptosis. During complete weaning, breast stroma is characterized by a heightened inflammatory or wound-healing environment, including activation of macrophages, lymphangiogenesis, and fibroblasts for tissue repair and remodelling. The post-weaning cascade of inflammatory activity and cell death peaks 2 weeks after the last breastfeed and is largely resolved by 4 weeks after the last breastfeed.[53–55]

Mechanosensing and the clinically inflamed lactating breast

Fetherstone[56] proposed that mastitis results when intra-alveolar pressures rise so high that lactocyte tight junctions leak large milk proteins back into the stroma, triggering an inflammatory response. But building on new research about the mechanobiology of the lactating breast and the role of mechanosensing in the mammary gland immune response,[50] a complex system perspective proposes that the mechanical effects of high intra-alveolar and intra-ductal pressure are a major regulator of the dynamic homeostasis of the lactating breast.

Once a critical mass of microscopic tight junction strain and alveolar rupture is reached within part of the breast, a clinically significant area of inflammation with hyperaemia, stromal tension, and perhaps tenderness or pain emerges. If milk is not able to be extracted from a duct, for example, due to the compressive force of stromal tension or restrictive feeding practices, upstream ductal lumens and alveoli continue to dilate as lactocytes secrete more milk. When inter-lactocyte tight junctions and alveolar basement membranes break, cell and molecular debris, leucocytes and interstitial fluid gather in the stroma. Cellular and molecular waste and fluid pass into activated and dilated lymphatic capillaries. A cascade of hyperaemia, increased interstitial fluid and lymphatic capillary dilation, increased stromal tension, increased ductal compression, increased intra-alveolar and intra-ductal pressure, and, finally, alveoli rupture ensues (Appendix 1).

The mechanobiological model is consistent with Ingman et al.'s hypothesis that partial involution occurs during BLBI, resulting in decreased milk synthesis, which is observed post mastitis. Ingman et al.[46] proposed that inflammatory processes rather than pathogenic bacteria trigger BLBI. They observed that macrophages in the stroma surrounding the alveoli express Toll-like receptors, as do lactocytes and mammary epithelial cells. But Toll-like receptors are activated not only by bacterial and other stressors but by mechanical stress signals, initiating an inflammatory response. Toll-like receptors are just one of multiple crosstalk mechanisms which detect and respond to endogenous cell and tissue damage, sensing and signalling within the complex adaptive system of the mammary gland immune system–milk interface.

Translating the mechanobiological model of BLBI into clinical practice, the following key mechanical factors elevate intra-alveolar and intra-ductal pressures and predispose to clinically relevant breast inflammation:

1. Any factor which causes external compression of lactiferous ducts (e.g. conflicting intra-oral vectors of force during suckling, which compress ducts);
2. Any factor which increases internal stromal tension and occludes lactiferous ducts (e.g. microvascular trauma in the stroma resulting from lump massage or vibration);
3. Any factor which decreases frequency of alveolar contraction and ductal dilations (e.g. spacing of feeds or of milk removal opportunities) (Appendix 1).

The implications for clinical management are discussed in detail in the second article of this series.[19]

The microbiome and cells of human milk downregulate inflammation caused by high intraluminal pressures

Human milk cells

Multicolor flow cytometry demonstrates that healthy human milk contains up to 4000 living cells (which are not

microorganisms) per millilitre. Milk cell populations are highly dynamic, with high levels of inter-individual variability, and altered by stage of lactation, infant milk removal and the health of both the mother and infant.[37,57–59]

Up to 98% of milk cells are mature lactocytes and myoepithelial cells exfoliated from the constantly renewing mammary epithelium. Exfoliated lactocytes may continue to secrete milk proteins. Although there are high numbers of leucocytes in colostrum, leucocyte counts fall by four-fifths to comprise just 2% of cells in mature milk. They migrate into the alveolar lumen through inter-lactocyte tight junctions and protect the mammary gland by phagocytosis and production of bioactive compounds. Up to 6% of the cells in human milk are stem and progenitor stem cells, which have the capacity to repair tissue by differentiating into lactocytes and myoepithelial cells (Appendix 1).[57,58,60–62]

Human milk microbiome

Over the past decade, research has shown that human milk contains a dynamic site-specific microbiome, low in microbial load relative to other sites in the healthy human body but richly diverse (Appendix 2). Although human milk has some commonality with other body site microbiota, it is a distinctly unique microbial ecosystem. The maternal skin, infant oral and human milk microbiomes share some features but remain very different ecosystems. Directions of influence are still being elucidated and are likely multidirectional (Appendix 2).

The milk microbiome interacts with other complex systems in human milk, for example, oligosaccharides, the metabolome, exosomes and leucocytes, to exert powerful immunomodulatory effects on the mammary gland, protecting mammary immune homeostasis. Fluctuating and dynamic microbiome diversity promotes host resilience when perturbations arise, as diverse inter-microbial interactions reduce the probability of specific organisms becoming dominant.[36,47,63–65]

Milk microbiomes vary enormously in taxonomic composition between healthy mothers and may also vary substantially within the one lactation. Because of the high inter-individual variability in human milk microbiomes, including in response to a range of environmental factors, variations in milk microbiome have not been found to be clinically meaningful, including in breast inflammation (Appendix 2).[36,47,63–65]

Gut microbiome studies show that microbial ecosystems are more conserved at functional than at taxonomic levels. Different taxonomic profiles in the microbiome of a specific human niche have been shown to result in microbial ecosystems which display similar behaviour. Researchers are increasingly investigating interactions between microbes in human milk rather than attempting to catalogue exactly which microbes are present, recognizing that microbial functions within the human milk microbiome may be better biomarkers for health-disease states than taxonomical composition.[47,64,66–68]

Biofilms

Biofilms are a normal part of healthy human microbiomes (Appendix 2). A biofilm may be a community of just a few dozen microorganisms, or hundreds of thousands or more. A biofilm gives the members of its organization adhesion and cohesion capabilities, nutritional niches, protection from environmental stresses and host immune attacks, and capacity for cellular communication. The skin of normal healthy volunteers, for example, is rich in biofilm, and the absence of skin biofilm has been associated with disease.[69]

Much of what we know about pathologic biofilm derives from the hospital setting, where biofilms typically form on chronic wounds, such as decubital and diabetic ulcers and burns, or from medical prostheses and implants inserted into the body. In these contexts, a biofilm may grow into a strong and dynamic ecological structure created by dense network associations within the microbiome, including the mycobiome. These pathogenic biofilms produce an extracellular matrix of glycoproteins, glycolipids, saccharides, minerals and extracellular DNA, and may also contain host-derived components, such as human saliva, vaginal secretions or serum. The pathogenic biofilm matrix protects bacteria which operate as pathogens, making it more difficult for antibiotics to reach bactericidal concentration in the wound bed or on the implanted medical device. In these settings, antimicrobials, particularly in sub-effective doses, can even induce biofilm formation and expression of additional virulence attributes.[69] Mature biofilms of *C. albicans*, for example, when challenged with sub-minimum inhibitory concentrations of fluconazole, secrete higher quantities of aspartic peptidase, a multitask virulence factor, compared to untreated biofilms.

But this article argues that the research concerning pathogenic biofilm in chronic wounds, burns and medical prostheses should not be extrapolated into the radically different, uniquely immune-factor-rich environment of the lactating mammary gland. It has been hypothesized that most bacteria in human milk are planktonic, that is, floating freely within the fluid, though it is possible that some bacteria are associated with milk immune cells in vivo. There is no evidence to support the hypothesis that pathogenic biofilm causes sticky milk, duct blockage and breast inflammation. Biofilm formation is a potential property of the various *Staphylococcus* strains which have been isolated from human milk (Appendix 2), but pathologic biofilm formation in a lactating breast is likely to be a late-stage manifestation of severe inflammation or tissue necrosis, not causative.[36,63,65]

The protective role of benign lactation–related inflammation

Milk leucocytes respond to stress

Human milk leucocytes form a complex system, operating within the many complex adaptive systems of mammary gland immunity. During the clinical presentation of BLBI typically diagnosed as mastitis, leucocytes increase to comprise 95% of human milk cells. Antimicrobial proteins, granulysin, perforin and other granzymes released by leucocytes in human milk are also elevated. Leucocyte concentrations return to normal with resolution of clinical symptoms. Milder lactation-related inflammatory conditions such as painful nipples or blocked ducts show less dramatic but measurable leucocyte count increases in milk.[59,70,71]

The milk microbiome responds to stress

Human microbiome researchers are increasingly critical of the term *dysbiosis*, since the concept of dysbiosis is based on an outdated assumption of a normative eubiotic state. Human microbiomes are not yet taxonomically categorized due to their astonishing complexity and are highly variable between individuals and over time (Appendix 2). Researchers also point out that microbial diversity is not always associated with improved health, as is currently assumed.[72,73]

The pathogenicity of most bacterial species depends, first, on the state of the host, and second, the strain of the bacteria. The terms *commensal* and *pathogenic* are unhelpful in discussions of milk microbiome and BLBI, because pathogenicity in the context of human milk is on a spectrum and context specific. A potentially pathogenic microbe which exists quite normally within the human milk microbiome is only pathogenic when regulatory feedback loops have been overwhelmed, resulting in prolonged or severe illness and the need for antibiotics.

Bacterial communities are highly dynamic. For example, antimicrobial-induced disturbance of milk microbiota is quickly reversed. During an episode of BLBI, total bacterial counts climb, with decreased diversity of species, and higher counts of those species identified, often including *Staphylococcus*.[19,36,74] Applying a complex systems lens, these perturbations characterize an ecosystem adapting under stress, acting to restore equilibrium through upregulation of some feedback loops and downregulation of others. The milk microbiome participates in the activation of myriad immune feedback loops within multiple complex systems (e.g. microorganisms interact together, with the milk metabolome, with milk oligosaccharides, with milk leucocytes and many other factors) to maintain physiological integrity and health.[47] Although it is not clear yet why counts of some bacteria increase, this does not necessarily signal a pathogenic process which requires antimicrobial intervention. For example, toxins and degrading enzymes secreted by specific bacteria promote the wound-healing environment required for rapid degradation of involuted alveoli and cell debris.

Reduced milk synthesis after clinically significant inflammation occurs irrespective of whether or not specific bacteria are cultured. This finding corroborates the hypothesis that perturbed milk supply is a consequence of a critical mass of alveolar rupture or involution due to mechanical pressure effects rather than bacterial infection.[46]

The complex adaptive systems of mammary immunity respond to stress

Leucocytes pass through lactocyte or mammary epithelial cell tight junctions into milk, in response to mechanical strain or rupture of tight junctions. They are recruited to downregulate inflammation in the ensuing wound-healing environment. This article proposes that high leucocyte counts are associated with decreased bacterial diversity because leucocytes phagocytose bacteria and secrete antimicrobial factors.[59] Certain bacteria, for example, *Staphylococcus aureus*, are well adapted in human environments and more resilient despite high leucocyte counts.[19] From a complex systems perspective, when the mammary gland immune system is stressed by areas of alveoli rupture, a wound-healing inflammatory response ensues: multiple feedback loops within the microbiome and cells of the milk are activated to reassert homeostasis or equilibrium. From an evolutionary perspective, activation of the milk microbiome, milk cells, milk metabolome and other aspects of the mammary gland immune system are expected to successfully suppress positive feedback loops and protect the host.

When BLBI emerges, the health of the breastfeeding woman and her infant are best served by strengthening the resilience of multiple immune system feedback loops rather than by unilateral elimination of an emergent organism.[75] Stabilization is much more likely if disruptive external factors which promote inflammation are removed, such as lump massage or vibration, in order to support mammary gland resilience. Management strategies are discussed in detail in the second article of this series.[19]

Fever enhances the mammary immune system response to stress

Kvist et al.[76] conducted a study of 154 lactating women presenting to a midwifery clinic with breast inflammation, who had been symptomatic for between 1 and 7 days prior to presentation. Although 52% had an elevated temperature at their initial visit, no association was found between fever at presentation and antibiotic use or outcomes. In a

2010 analysis, Kvist[48] points out that the high levels of leucocytes and C-reactive protein associated with mastitis indicate inflammation, not bacterial load. Kvist et al.'s findings are supported by recent work on the immune homeostatic role of fever.

Fever may be activated either by pathogenic microorganisms or by internal cell and tissue damage. Applying the mechanobiological model of BLBI, when alveolar breakdown is identified either by mammary epithelial cells, milk microbiota or stromal leucocytes, signalling networks are activated and proinflammatory cytokines are released. When a critical mass of alveolar collapse has occurred, clinical inflammation along a spectrum of signs emerges, developing into hyperaemia, pain and fever.

Higher body temperatures are known to drive the activity of proteins which switch on genes responsible for further recruitment of the body's cellular immune response, in particular neutrophils, which phagocytose cell debris.[77] Overly aggressive use of antipyrectics may interfere with the homeostatic role of fever in the human immune system, and this is likely to be the case in the mammary immune system too.[78]

Conclusion

BLBI has been previously explained by a pathogenic microbiota model, resulting in overuse of antibiotics and antifungals. But integration of recent research concerning the mechanobiology of the lactating breast and the mammary gland immune system, which includes the milk microbiome and cells, suggests that the mechanical effects of rising intra-alveolar and intra-ductal pressures trigger complex inflammatory cascades. Rising stromal tension exerts intramammary pressure on lactiferous ducts, worsening intraluminal backpressure. Rising milk leucocyte counts and alterations in the microbiome composition are signs that the mammary immune system is responding protectively to stress by recruiting mechanisms which downregulate inflammatory feedback loops. From a complex systems perspective, when the mammary gland immune system, which includes milk leucocytes and the milk microbiome, encounters perturbation or threat, the inflammatory response is a robust and complex set of feedback loops designed to reassert homeostasis or equilibrium.

Applying this mechanobiological model of lactation-related breast inflammation, the key mechanism for the prevention or treatment of breast inflammation is avoidance of excessively high intra-alveolar and intra-ductal pressures, in order to prevent a critical mass of inter-lactocyte tight junction strain and rupture. The implications of this revised aetiological model for classification, prevention and management are examined in the second article of this three-part series.

Rigorously debated theoretical models are necessary to determine which clinical approaches are worth the investment of the precious research dollar. The mechanobiological model of BLBI has been developed as part of the foundational breastfeeding domain of NDC (or 'the Possums programs'). Breastfeeding has health benefits for infants, both short term and long term, and for their mothers. Currently, much of the advice received by breastfeeding women is experience or opinion-based, in the context of a historical gendered failure of health systems to prioritize investment in clinical breastfeeding research. There is an urgent need for high-quality evaluation of interventions for BLBI, build upon solid theoretical frames, in order to optimize the long-term protective benefits an infant receives from his or her mother's milk.

Author contribution

Pamela Douglas: Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft; Writing – review & editing.

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Appendix 1

The stroma of the lactating breast is exposed to frequent and irregular alterations of pressure gradients

Selected functional anatomy. Mechanical milk removal and 24-h test weighing studies have elucidated the range of rates of milk synthesis typical for the breasts of successfully

breastfeeding women.[79,80] Milk is removed from the breasts by the intermittent negative mechanical pressure of suckling and the intermittent positive mechanical pressure of milk ejection, working in tandem. Sometimes the breasts leak milk in the absence of suckling, due to positive pressure of milk ejection.

The lactating mammary gland is a highly dynamic and adaptive environment. Milk ejection, for example, is not machine-like and precise: as in all biological systems, there is a great deal of asynchrony and variability, though it remains well coordinated and robust. Degrees of milk synthesis differ randomly between different parts of the glandular tissue. Between 20% and 100% of the glandular tissue of lactating women is comprised of highly productive lobules, which are collections of alveoli emptying into a common ductule. These are characterized as type 3 or type 4 lobules. But 20% of lactating women have less than 60% of type 3 and type 4 lobules; the remainder are undifferentiated or less mature lobules, labelled as type 1 and type 2, from which lower amounts of milk are secreted. Milk ejection is asynchronous across the breast, with heterogeneous emptying of alveoli and lobules. Adipose tissue is highly variable within breasts and is mostly not interspersed with glandular tissue.[54,81–83]

In a series of pioneering ultrasound imaging studies, Geddes et al. (also Ramsay et al.) have demonstrated that about two-thirds of alveoli and their lobules within a lactating breast are located within a 3-cm radius of the nipple, and that lactiferous ducts travel back from nipple orifices to the alveoli in densely interlaced branching patterns.[49,81,84] Ducts ranging from 0.1 to 10 mm in diameter at rest have been identified under the areola with ultrasound, but even narrower ductules run to the alveoli. Because there is little subcutaneous tissue under the dermis of the nipple–areolar complex, ducts are often just 1 to 2 mm beneath the surface, and are highly compressible with even very light external touch, much like veins on the back of the hand.

It is likely that many ductules rest for a time between feeds in an occluded or closed down state, much like the 50% of lymphatic vessels which are collapsed and quiescent in the lactating breast.[54] Ducts may also gradually fill with milk which is constantly secreted by the lactocytes and which flows out along pressure gradients between feeds. The ducts of each breast may fill with up to 30 mL of milk, transferred to the infant by vacuum application of suckling prior to oxytocin activation, but there are no 'lactiferous sinuses' which store milk.[81]

Milk ejection. The main mechanism of milk transfer occurs from alveoli contraction and ductal dilation, in tandem with the vacuum of suckling. Because both alveoli contraction and milk duct dilation occur asynchronously in response to oxytocin impulses, milk flows from different parts of the breast, heterogeneously.

The lactocytes which line the alveolar lumen are surrounded by star-shaped, oxytocin-sensitive, contractile myoepithelial cells. The myoepithelial cells are enveloped by a thin but dense collagen basal membrane. Similarly, the cuboidal epithelial cells which line the lactiferous ducts are surrounded by myoepithelial cells, again enveloped by a basal membrane. When oxytocin is released in response to nipple stimulation, myoepithelial cells contract, although not all myoepithelial cells contract in response to a pulse of oxytocin, and alveoli are not uniformly dilated or contracted at any one time.

Contraction of alveolar myoepithelial cells results in contraction of the alveolus and its lumen, ejecting milk into the ducts. Stewart et al.'s three-dimensional (3D) imaging of mice mammary glands found that lactocytes contracted by about a third, repetitively and unpredictably warping and stretching under the stochastic contractile mechanical pressure of the myoepithelial cells in response to a pulse of oxytocin.[50]

Geddes and colleagues[85] demonstrated that contraction of ductal myoepithelial cells results in shortening and dilation of ducts, minimizing resistance to milk flow. Dilation may be augmented by intraluminal pressure of flow from the alveoli. Main ducts dilate by 0.5 to 1.9 mm in diameter, though Geddes et al. comment that contraction of myoepithelial cells surrounding alveoli which are less full may result in lower intra-ductal pressure and smaller duct dilation. Ductal dilation may last 45 s to 3.5 min. Ductal dilation is also, like alveolar contraction, asynchronous: a 2- to 8-s difference has been observed between the initial oxytocin burst and the timing of flow in main ducts.

Most milk ejections are not felt by women, and some women do not feel milk ejections at all.[49] But human milk ejection patterns are innately programmed and physiologically robust. Duct diameter changes during milk removal from a healthy breast are stable and do not relate to the infant's milk intake, time to milk ejection, time since last breastfeed, stage of lactation or milk production. An individual mother's timing, pattern and number of milk ejections is consistent over time and between lactations, whether breastfeeding or pumping.[86–88]

The number of milk ejections detected during a breastfeed is highly variable between successfully breastfeeding mothers, and is the only factor related to the amount of milk the infant consumes for that breastfeed, regardless of the length of feed. Many milk ejections can occur in a short period of time: between 1 and 17 episodes of increased intra-ductal pressure have been measured in breastfeeds of up to 25 min.[86] The median time from the end of one milk ejection to the beginning of the next is 90 s, with a range of 40 to 203 s. The first two milk ejections in a feed produce 62% of total milk removal in a 15-min period. The first milk ejection contributes a greater volume and greater total percentage of milk expression than each subsequent milk ejection.

Milk synthesis. When exclusive breastfeeding is successful, the amount of milk secreted by a woman's breasts rapidly stabilizes from day 11 postpartum at an average of 788 g daily, though the range in successful breastfeeding is highly variable (440–1220 g) depending on the mother–infant pair.[80,89]

Although this article hypothesizes that downregulation of milk secretion throughout the course of lactation is predominantly mechanobiological, upregulation of milk secretion is believed to be facilitated by sensory stimulation of suckling and the hormonal effects of more frequent milk removal on stem cells. Stem cells are located in the myoepithelial and epithelial layers of alveoli and ducts, presumably not only in type 3 and type 4 but also in underdeveloped type 1 and type 2 lobules. Human stem cells are derived from the maternal haematopoietic system and remodel the breast when development of lactocytes and myoepithelial cells is required. Prolactin stimulates not only milk synthesis but also cell proliferation, theorized as the mechanism which allows regeneration and differentiation of the lactating epithelium and dynamic maintenance and turnover of the secretory tissue during the course of the lactation.[57,58,60–62]

The capacity to generate more alveoli and ducts throughout the course of lactation appears to be limited by baseline numbers of prolactin receptors, set in the first hours and days of life. In 140 healthy term Japanese newborns, 7 to 11 opportunities for milk removal in the first 24 h postpartum was associated with increased 24-h milk production, decreased weight loss and decreased serum bilirubin by days 5 to 7.[90] In 358 healthy term Nigerian newborns who breastfed about 13 times in the first 24 h showed improved weight gain and lower serum bilirubin levels by day 7 compared to those who fed less frequently.[91] Rate of milk production at 2 weeks post birth in 98 healthy term infants in the United States correlated with frequency of milk removal and predicted the rate of milk production at 6 weeks.[92]

Intra-lobular stroma is exposed to frequent and irregular alterations in pressure gradients due to alveolar contractions and ductal dilations. Inter-lobular stroma is dense fibrous connective tissue, in which adipose tissue is embedded. The intra-lobular stroma in the lactating mammary gland is a loose but highly vascular connective tissue, highly responsive to mammary epithelial signals.[93] Intra-lobular stroma also contains fibroblasts, abundant lymphocytes, macrophages and lymphatic vessels. The resting stromal density and tension exerted on lactiferous ducts and lymphatic vessels varies from woman to woman, influenced by genetic predispositions.[54] Arterial capillaries lace closely around the basement membrane of the alveoli. This proximity allows oxygen, proteins and nutrients to diffuse into the lactocytes, and carbon dioxide, unused proteins and some waste products to diffuse from the alveoli back into venous capillaries.

Ninety percent of the arterial blood carried into the mammary gland returns to the venous circulation, but 10% diffuses out of the capillaries into the stroma, as interstitial fluid. During lactation, half of the lymphatic vessels are collapsed at any one time, though Jindal et al.[54] observed that in the weeks immediately after weaning, all lymphatic vessels are dilated and contain cellular debris. New research in other parts of the human body show that lymphatic vasculature downregulates local inflammation through multiple pathways, including through removal of inflammatory products and lymphangiogenesis. This article proposes that lymphatic vasculature is another complex adaptive system within the lactating breast immune system.[94–97]

Blunt-ended lymphatic capillaries are composed of a single layer of specialized lymphatic endothelial cells with sparsely intermittent valves, anchored by filaments to the stroma and sensitive to pressure dynamics. Their sparse basement membrane and discontinuous intercellular junctions (known as buttons) allow passive intake of interstitial fluid, forming intra-vascular lymph. In addition to diffusion of fluid from the higher pressure of the stroma into the lymphatic capillary, entities too large to cross back through the tight junctions of venous capillaries pass through the large button junctions, including cell debris, protein complexes, lipids, macromolecules, immune cells and bacteria. Lymphatic capillaries are sensitive to contextual signals and have the capacity to tighten up intercellular junctions and limit transport of fluid and macromolecules. In response to increased interstitial fluid load and inflammatory mediators, lymphatic vessels adapt their pumping activity to increase or decrease transport, regulating the inflammatory state of the tissue they drain.[94–97]

Lymph moves under pressure gradients from the lymphatic capillaries into lymphatic collection vessels, which have a basement membrane, valves and lymphatic muscle cells. Lymphatic collection vessels are intrinsically contractile, and pump lymph towards the lymph nodes. Extrinsic pumping by pressure changes in surrounding tissues also contributes.[94–97] Although pectoral muscle movement and the movement of breathing are likely to play a minor role, this article hypothesizes that two dominant sources of breast tissue and stromal movement support extrinsic pumping of lymph: the vibratory effects of gravity acting on the breast, and the dynamic and variable pressure gradients formed across stromal tissue by repetitive, irregular and widespread alveolar contractions and lactiferous duct dilations.

One-way valves direct the flow of lymph towards the lymph nodes, where it is filtered in preparation for return into the blood stream. Seventy-five percent of mammary lymph drainage is superficial or cutaneous, draining into the axillary nodes; the other 25% is in the deep tissue, particularly of the medial breast, draining into the internal mammary nodes.

Appendix 2

The human milk microbiome

Half of the cells in the human body are microbial, with each niche's microbiome as unique to individuals as fingerprints. Microbiomes contribute many more genes than genomes to the human body, and shape phenotypic traits of the host, including nutritional and metabolic traits. Within the adaptive immune system, microbiomes use chemical and metabolic signals to regulate the abundance and activities of lymphocytes. As lymphocytes respond to encounters with antigens, they regulate the constantly changing genetic sequences produced in immunoglobulins. The complex crosstalk between a microbiome and the body's adaptive immune system determines whether the body recognizes a specific molecular pattern as non-self, or as a sign of endogenous cell and tissue damage, and how vigorously the immune system responds.[98]

Methods of human milk microbiome sampling are not yet standardized. Culture-based methods select out bacteria from expressed breast milk on specific growth media, identifying species and numbers of colony forming units. Molecular polymerase chain reaction (PCR) methods of analysing expressed breast milk identify DNA, which may be from viable or non-viable bacteria, non-cultivable bacteria and bacterial fragments. Non-viable bacteria are thought to be a significant component of the mammary gland immune system, acting as antigens which interact with host immune cells, much like inactivated vaccines. However, neither culture nor PCR is yet able to determine the true composition of a human milk microbiome *inside* a lactating woman's breast.

Despite these serious methodological limitations, researchers agree that the composition of the human milk microbiome is impacted by genetic predisposition, ethnicity, geographical location, circadian rhythm, age, body mass index and maternal nutrient intake, including fatty acids, carbohydrates or proteins. Some studies have found differences in the milk microbiome depending on the infant's mode of delivery, others haven't. Human milk microbiome composition also differs between colostrum, transitional and mature milk.[36]

Human milk has a median bacterial load of 10^6 cells/mL, and about 200 different species of bacteria have been identified in the healthy human milk, including a high load of bacteria which have been previously labelled as pathogenic (*Staphylococcus aureus*, *Escherichia coli*, *Group B streptococci*). It is generally agreed that the core bacterial genera of the milk microbiome, universal across lactating women, are *Staphylococcus*, *Streptococcus* and *Propionibacterium*. Much smaller and more variable populations of *Lactobacillus* and *Bifidobacterium* may occur, but not in all breastfeeding women.[36,47,63–65]

The human milk microbiome also comprises organized networks of viruses, fungi, archaea and protozoa. The viral fraction of human milk, the virome, is dominated by bacteriophages, which comprise 95% of human milk viruses. Bacteriophages modulate bacterial ecology by killing certain species. The fungal fraction of human milk, the mycobiome, interacts with and stabilizes the microbial domain in protective association networks, which together strengthen host health and immunity and resist overgrowth of any particular bacterial species (previously referred to as pathogen colonization). *Candida albicans* is the most common fungal commensal in the human body, and *Candida* spp. including *C. albicans* occur commonly in human milk, having a beneficial, probiotic effect, interacting with and containing bacteria.[5,36,66,67,99–101]

Theories about the origins of the human milk microbiome

Stroma. Viable bacteria are found in mammary tissue of women who have never breastfed, suggesting the mammary gland itself may be a source of bacteria for milk.[46,102] The human milk ecosystem is exposed to the internal environment of the breast stroma through lactocyte tight junctions which are permeable at birth and only close over the next few days as the colostrum changes to transitional milk. A lactocyte tight junction leak in response to the mechanical effects of rising intra-alveolar pressure may facilitate bacterial translocation; alveolar rupture ensures bacterial presence in the stroma.[56,65]

Retrograde spread from infant oral cavity and the nipple–areolar complex. In light of the research, the hypothesis that the human milk microbiome is predominantly seeded by retrograde movement of planktonic oral bacteria remains unconvincing.

First, the milk microbiome is exposed to the external environment through duct orifices in the nipple. Coagulase-negative *Staphylococcus*, *Candida* and *Streptococcus* of *mitrus* and *salivarious* groups inhabit healthy nipple–areolar complex skin, the infant's mouth and also human milk. However, these same organisms have also been isolated in antenatal colostrum, prior to contact with the newborn.[36]

Second, the neonatal oral microbiome is highly dynamic and altered by formula feeding. Although one study suggested that diversity increased in human milk microbiota after the first breastfeed, with an increased presence of oral microbes in the human milk microbiome attributed to retrograde seeding, other studies have not corroborated this finding.[103]

Third, during milk ejection, ultrasound analysis shows that milk in the breast not subject to mechanical or suckling milk removal flows first towards the nipple, but then backwards into other ducts which have a lower milk volume.[104] This finding of backward flow according to pressure gradients in a non-suckled breast cannot be interpreted as support for the hypothesis that bacteria spread by retrograde flow from the infant's mouth into human milk.

Fourth, infant saliva contains multiple soluble factors which protect the body from potential pathogens, including antibacterial salivary lysozyme and pattern-recognition molecules which regulate inflammation. A 2018 study demonstrated that a range of microorganisms growth was inhibited by saliva mixed with breast milk, regardless of whether the organisms were considered to be commensal or pathogenic.[105] Microbial growth including of *C. albicans* is inhibited for up to 24 h when breast milk and saliva are mixed. Breast milk contains an abundance of the enzyme xanthine oxidase, which acts upon the high levels of xanthine and hypoxanthine in neonatal saliva to release hydrogen peroxide. Hydrogen peroxide is a key oxidative radical and antibacterial, which damages bacterial cell wall integrity. This known antibacterial activity of infant saliva also makes the retrograde seeding hypothesis less than convincing.

Enteromammary transportation. The dominant source of bacteria for the infant gut is now believed to be the maternal gastrointestinal tract. Enteromammary traffic of immune cells occurs during late pregnancy and lactation, as the lactating breast becomes part of the body's mucosal immune system. Maternal mononuclear cells transport bacteria and also fragments of gut-derived bacteria to the breast during pregnancy and lactation. It is hypothesized that selected bacteria from the maternal gut colonize milk through this endogenous route. This is supported by the finding that faeces from infants share a common bacterial signature with their mother's milk.[36,47,63]

S. aureus *and lactation-related breast inflammation*

S. aureus is found in the healthy microbiomes of approximately one-third of the human population and may also result in invasive disease in many different sites of the human body. *S. aureus* displays metabolic plasticity and a range of virulence attributes, which make it particularly successful in counteracting immune mechanisms and dominating nutrient sources.[73] For example, *S. aureus* produces toxins and leukocidins, forms biofilm and rapidly acquires antibiotic resistance. *S. aureus* influences the metabolism of leucocytes, including neutrophils; it both elicits the formation of neutrophil extracellular traps, which enhance an immune response, and reduces the activity of neutrophil extracellular traps.[106] *S. aureus* survives within neutrophils but, from this intracellular niche, also extends the life of neutrophils.[107] These examples illustrate the complexity of interactions between *S. aureus* and the human immune system.

The significant methodological limitations complicating identification of and taxonomic classification of microbiota in human milk are discussed above. Nevertheless, it is agreed that *S. aureus* is more likely to be cultured in the milk of women with mastitis.[4]

Kvist et al.[75] cultured the milk of 192 women with mastitis and 466 without *S. aureus* and *Group B streptococci* were found more often in the women with breast inflammation, but 31% of healthy women had *S. aureus* and 10% had *Group B streptococcus*. No significant correlations were found between scores for erythema, breast tension, pain or for the total severity of symptoms at first contact, and the type of bacteria found in the breast milk. There was only an increased odds for a less favourable outcome when *Group B streptococci* were present in the milk.

Delgado et al.[108] investigated the microbial diversity of breast milk in 20 women with lactational mastitis and found that from the 149 bacterial species identified in their milk, 70% by culture and PCR analysis were *Staphylococci*, of which *Staphylococcus epidermidis* (previously known as a skin commensal) was the dominant species. In fact, *S. epidermidis* was isolated in 17 of the 20 women, *S. aureus* in only 5. No *Candida* spp. were identified. *Streptococcus* was isolated only in four samples and always outnumbered by *Staphylococcus*. The authors suggest that breast inflammation was accompanied by a process where some of the bacterial species usually present in human milk overgrow (*Staphylococcus*) while others disappear (in particular, *Lactobacilli* or *Lactococci*).

Cullinane et al.[109] found that 59% (16 of 27) of milk samples collected from women who reported mastitis at the time of collection or the day prior cultured positive for *S. aureus*. However, Cullinane et al. also found that 32% of milk samples collected at week 1 in asymptomatic lactating women (207 of 657 milk samples) cultured positive for *S. aureus*, and 15% at week 4.

Rimoldi et al.[110] found that in 26 lactating women with mastitis and another 34 with breast abscess, *S. aureus* was the most common microorganism identified, with methicillin resistance identified in 44.7% of *S. aureus* strains, including in 80.9% of the cases of abscess. Hospitalization was required more frequently in methicillin-resistant *S. aureus* cases.

Much remains to be elucidated about the role of *S. aureus* in benign lactation–related breast inflammation (BLBI). *S. aureus* is just one of myriad microorganisms which inhabit a woman's milk and which respond to and coordinate with the mammary gland immune system as it regulates the inflammatory cascades triggered by raised intraluminal pressures. *S. aureus* is, however, also a microorganism in human microbiomes particularly well placed to benefit from an inflammatory response and becomes increasingly dominant as the leucocyte count rises in the milk. This does not mean that *S. aureus* or other microorganisms are causative of BLBI and does not mean that *S.*

aureus needs to be eliminated with antibiotics. Usually, inflammation will resolve with application of the key principles discussed in the second article of this series.

It is possible that occasionally, over time, an extremely high cell count in milk composed of leucocytes and epithelial cells results in end-stage thickened or inspissated milk, which may be detected during milk expression and which also contains biofilm. But from a complex systems perspective, this is a very late sign of disruption, likely to be a consequence of high intraluminal pressures, ductal occlusion and the ensuing inflammatory cascade rather than a cause. It would also not usually require antimicrobial treatment, depending on the clinical picture.[19]