Importance of human milk for health and as antiviral immunotherapy - a narrative review

ABSTRACT

Background Immunological protection against novel mucosal pathogens is crucial to us as our immunity is unable to effectively defend against specific pathogens without previous immune encounter, as experienced in the SARS-CoV-2 pandemic. However, a nursing neonate is protected from many novel infections by exclusive human milk feeding despite having a naïve immune system without much previous pathogen exposure. It is observed that SARS-CoV-2 is not transmitted to the nursing infant through human milk, and that natural maternal infections produce specific antibody responses in human milk. Furthermore, maternal vaccination against SARS-CoV-2 may modify some of these responses compared to natural infections. In this setting, it was felt necessary to also explore if early, innate immunity in human milk can protect against SARS-CoV-2.To explore this hypothesis, I reviewed the pathogenic mechanisms of Covid-19 focusing on the methods of viral entry through the human mucosae, infection establishment, immune dysregulation and disease causation and integrated these with the early actions by human milk feeding on mucosal infections. I then extrapolated the relevant pathways of human milk immune protection as potentials to protect against SARS-CoV-2. **Methods** This was divided into three steps which firstly included a literature search, secondly a step wise analyses and synthesis of data, and thirdly, an integration of data to form a hypothesis. The first step searched articles in two areas which in the first area, included articles on the infection and pathogenesis of SARS-CoV-2 and in the second area, included articles on innate immunity in human milk. In the second step, I analysed the immunological actions in human milk against mucosal infections, on the whole, and synthesised some of these relevant actions against the pathogenesis of SARS-CoV-2 infections. In the third step, I integrated human milk immune pathways that could interfere with the establishment of SARS-CoV-2 infection, viral invasion, immune dysregulation and the progression of the disease **Results** Infection by SARS-CoV-2 can theoretically be reduced or mitigated by the effect of early immune constituents in human milk. Human milk feeding may confer protection against all stages of the disease including establishment of SARS-CoV-2 infection, invasion and immune dysregulation and these actions may benefit both the individual and the community **Limitations**The multifunctional and dynamic nature in which human milk constituents function in a nursing infant cannot be fully reproduced by studying isolated components under experimental conditions. Even when such factors can theoretically offer protection against the virus, this concept has to be further researched in large cohorts of nursing infants. **Conclusion** The role of human milk in preventing the infection must be explored further and if true , exclusive human milk feeding must be considered as a reason for the smaller number of infections observed in children compared to adults in the pandemic. The additional counselling of human milk feeding for protection against novel pathogens, besides its established role in reducing neonatal mortality, would enhance rates of exclusive human milk feeding. General health can be developed and promoted through the potential immunotherapy provided by exclusive human milk feeding.

Keywords:

human milk feeding, immunity, SARS-CoV-2, mucosal, immunotherapy

INTRODUCTION

The pathogenesis of Covid-19 explains its clinical manifestations and highlights the importance of timely preventive therapy to prevent the infection or to reduce its impact.

Covid-19 is primarily a mild disease of childhood but its pathogenesis can involve multiple organ systems, especially the respiratory system. Its peak infectivity coincides with maximum SARS-CoV-2 load, just before or within the initial five days of symptom onset [1]. The acute disease spectrum includes acute asymptomatic, mild and severe disease. Amongst children who develop severe disease, there are often predisposing factors such as obesity and bronchial asthma and other immunosuppressive conditions. There is a growing body of evidence that the virus is also temporally related to a post infective phenomena causing a novel disease now recognized to be a separate disease entity referred to as the Pediatric Multisystem Inflammatory disease which is temporally associated with Covid-19.Its manifestations are often acute, severe abdominal pain or high grade, often unremitting fevers refractory to antibiotics[1] Systemic impact can affect almost any system with the gastrointestinal, cardiac ,dermatological, renal, respiratory, haematological, or neurological systems involved by the disease [1].

Covid-19 may also impact long term health but while some children with Covid-19 continue to experience prolonged illness, most recover by day 56 of illness [2] .The spectrum of psychological impact of Covid-19 in children is well supported [3]

Children have a different susceptibility to the illness compared to adults [1] and this is attributed due to a number of factors[4].Lower exposures to SARS-CoV-2 and differences in innate and adaptive immune responses in children may explain this. The presence of cross reactivity from other coronavirus infections due to previous exposures in children who have a greater frequency of upper respiratory infections may explain this. The differences noted in intestinal microbial colonization in children with a less inflammatory immune profile in the gut or the presence of higher blood levels of some factors such as melatonin have been suggested. Moreover, nonspecific, off target protection from live vaccination[4]may work independently or along with other factors to explain this difference.

Additionally, this article considers the possibility that the immunological benefits of human milk may also be a mechanistic explanation for the differences observed in children in this mucosal infection.

Human milk feeding allows every mother to provide immune defenses innately present in her milk, even without infection exposure. This can systematically hinder invasion of mucosal portals, prime targets of the SARS-CoV-2, and provide a number of antiviral defences, protecting from early infections. At the same time, the adaptive immunity in human milk which develops after exposure to infection mainly by antibody formation, is ‘stimulated’ or bridged by powerful innate immune factors. Maternal vaccination against the infection may further sustain and modify useful mucosal protective immune potentials.

It is noted that human milk does not transmit the SARS-CoV-2 to the nursing infant, instead, infected mothers produce specific antibodies in the milk [5], which are likely to protect. While SARS-CoV-2 ribonucleic acid (RNA)was detected on several breast swabs it was not found in any breastmilk sample in women diagnosed with Covid-19. Moreover, SARS-CoV-2-specific antibodies of the immunoglobulin A (IgA) and immunoglobulin G (IgG) type were found correlating with SARS-CoV-2 neutralization activity [5]. Of added importance is that the vaccination of breastfeeding women against SARS-CoV-2 produced specific IgA and IgG antibodies in breast milk for 6 weeks after vaccination. IgA was detected 2 weeks after vaccination followed by a spike in IgG after 4 weeks coinciding with a week after the second given vaccine [6].

To prevent subsequent pathogenesis of Covid-19 which is multi-fold but fuelled by a proinflammatory cytokine response, human milk can potentially modulate this by its predominant antiinflammatory

content and this has the potential to have impact on acute disease, tissue preservation and some of its sequalae.

2. MATERIAL AND METHODS

The review was divided into three steps which included:

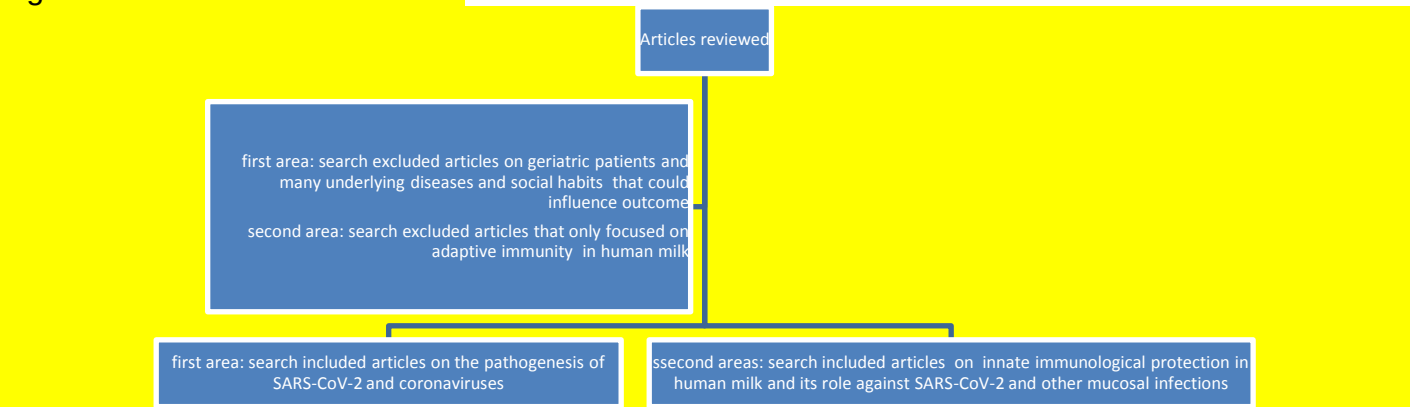
1) literature search comprising two areas of interest
2) step wise analyses and synthesis of data
3) exploring the domain of overlap by integration and extrapolation

In the first step, articles searched were in two areas of interest. The first area included articles which focused mainly but not exclusively on the pathogenesis of SARS-CoV-2, coronaviruses, and other comparable infective agents. The second area of interest were articles which reviewed the innate immunological protection in human milk and its role against relevant mucosal diseases

In the search of the first area of interest, articles included were clinical research articles, experimental work, expert and interim guides. Publications selected included case reports, meta-analyses, systematic reviews on human infections by coronaviruses and other mucosal pathogens with comparable pathogenicity. Articles describing the pathogenesis of SARS-CoV-2 and its impact in the pandemic were reviewed. How mucosal viruses gain entry into the human body, establish and infect and go on to produce invasive disease and complications were explored. Articles that were excluded were Webpages which provide the public with questions and answers and media releases. Except for diabetes mellitus which was considered to significantly influence the unborn infant, specific underlying chronic conditions which could influence the pathogenesis of Covid-19 were excluded. The pathogenetic mechanisms of SARS-CoV-2 on geriatric patients were excluded. Specific social habits or dietary factors other than the influence by human milk feeding were excluded.

In the search of the second area of interest, articles included searches on breastfeeding and innate immunity and breastmilk immunology related to mucosal infections and SARS-CoV- 2. Publications in this section included case reports, meta-analyses, systematic reviews, clinical research articles, reviews, and experimental work on humans and animals. The articles included reviewed the impact of innate immunity in human milk on infections and focused on those that involve the mucosal systems of the respiratory and gastrointestinal tract. Articles which elucidated how human milk factors prevent establishment of mucosal infections, can prevent mucosal invasion and modulate immune processes were reviewed. Except for a few articles that dealt with immunoglobulins in colostrum and human milk article which were included, articles that only dealt with adaptive immunity in breastmilk or on other aspects of breastmilk protection that was thought not to be directly relevant to the pathogenesis of mucosal infections were excluded.

Figure 1: Literature search areas



In the second step on data analyses and synthesis, the following methods were used:

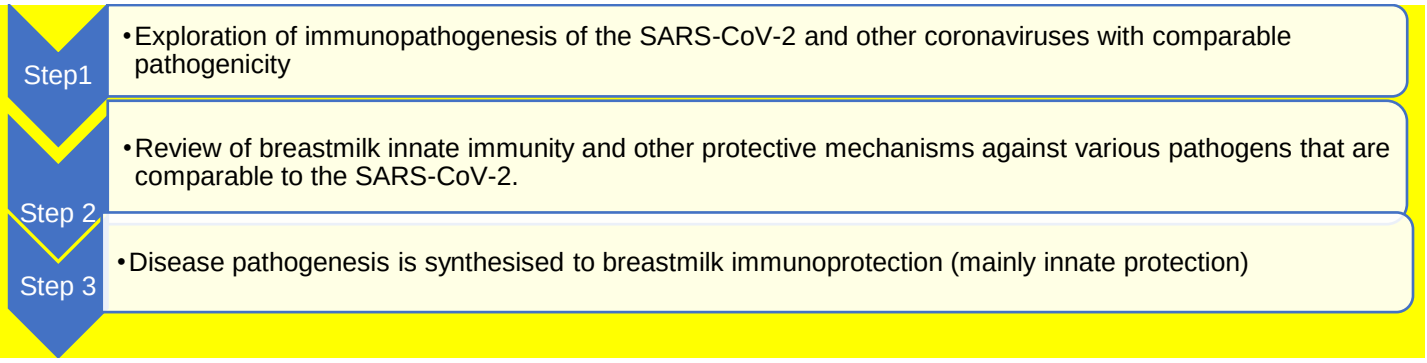
Information from the pathogenesis of Covid-19 and innate breastmilk immune protection was analysed in three steps.

The first step was to explore how mucosal infections in general, and the SARS-CoV-2, specifically, infect mucosae to establish disease.

The second step analysed actions of breastmilk on immunity against various mucosal pathogens that were considered comparable to the SARS-CoV-2. The similarities of such pathogens included, the mucosal portals of entry for disease causation, the systemic impact such as the organs affected, inflammatory responses stimulated by them,and the age of infection.

The third step synthesised some of these relevant actions against the pathogenesis of SARS-CoV-2 infections

Fig 2: Step wise analysis and synthesis of data

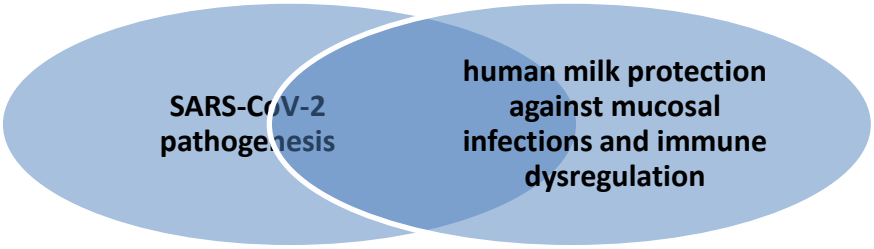


In the last step, the domain that overlapped between the pathogenesis of SARS-CoV-2 infection and the pathways in innate human milk actions against mucosal infection and immune dysregulation were integrated as a whole to form a hypothesis with practical importance.

Integration and extrapolation indicated that in theory, the pathogenicity of the disease could be reduced or halted in a systematic manner, by human milk feeding. Mucosal entry, receptor blockade,

immune modulation, innate immune recognition, immune cells and antiviral activity were protective pathways by human milk feeding against SARS-CoV-2

Fig 3: Hypothesis: Protective domains in human milk feeding against the pathogenesis of SARS-CoV-2



3 Results and discussion

3.1 SARS-COV-2 pathogenicity

SARS-CoV-2 is a large, spherical single-stranded RNA virus. It is a mucosal pathogen and genetically similar to SARS-CoV, but with a higher reproductive rate (R 0) [5] .There are four main structural proteins , the nucleocapsid protein that contains the viral genome and three envelope proteins of which the spike protein, attaches to host cells through a receptor binding domain[7]

If timely clearance of the virus at the respiratory mucosae does not occur, the virus attaches itself to target receptors which are also found in many organs with hazards of widespread disease. SARS-CoV-2 binds to angiotensin-converting enzyme-2 (ACE-2) receptors and fusion with a protein on host cell surfaces internalises the virus[8]. The virus is viable for days on smooth surfaces such as stainless steel, plastic or glass and at lower temperatures and humidity [8,9]. Of note is that cellular receptors of coronaviruses belong to the same protein family, and cellular entry occurs through the co-expression of other host peptidases which activate the coronavirus spike proteins [8]. The transmembrane serine proteases cleave and activate the coronavirus spike proteins during cell entry[8].Spike protein dismantling releases infective viral genome, and with proofreading mechanisms in this RNA virus, a lower mutation rate[9] in the early stages of the pandemic was quickly followed by the occurrence of many dangerous mutants[10].Droplets and contact with nasal ,oral or conjunctival mucosae propagate the virus[10,11].

Fever, myalgia, headache, respiratory symptoms and temporary anosmia with taste loss due to transient damage to olfactory cells may occur [11]. Intracellular viral replication releases infective virions which stimulate host's innate immune responses [7,11] Viral recognition stimulates T lymphocytes and dendritic cells to the site of infection for early viral clearance. Host immunity is induced to produce inflammatory factors, macrophages, maturation of dendritic cells, and the synthesis of interferons (IFNs), all vital for eliminating the virus. Neutralising antibodies are produced in SARS-CoV-2, possibly useful, but their exact duration and impact are yet unclear. The viral load may also be a factor in recovery [7].Immune suppression, viral evasion, high viral load and host variables influenced by underlying conditions may contribute to more severe disease with spread to the lower airway[7,12]. Pneumonic lung involvement is associated with inflammation, confirmed by pathological findings[1,4]. Some develop septic shock, multi-organ dysfunction [6] which are important complications and Guo et al found that specific cytokines and serum markers were significantly higher in diabetic patients predisposing them to the inflammatory storm [12], and extensive tissue destruction is linked to hyperferritinemia[13]

In acute severe SARS-CoV2 many children had underlying predisposing factors such as obesity, bronchial asthma, sickle cell anemia and immunosuppression[1].The other complication associated with the SARS -COV2 is multi-system inflammatory syndrome in children (MIS-C)[1].Support of an

immune phenomena in children who recovered from mild disease and who were relatively healthy. Clinical and laboratory observations suggest many hypotheses of the inflammatory state that occurs in the disease some 2-6 weeks following an acute illness or exposure to SARS-CoV-2. Immune dysregulation in the genesis of MIS-C resulting from an exaggerated hyperimmune response is supported by positive serology and negative PCR testing, while the antibody or T-cell recognition of self-antigens (molecular mimicry) leading to synthesis of autoantibodies and formation of circulating immune complexes have been proposed[14]. The role of genes in clinical expression is suggested by differences in disease incidence in the world [14].

3.1.1 Immunobiology in children against SARS-CoV-2

Natural immunobiological mechanisms of children against the SARS-CoV-2 are more favourable than in adults and at least partially explain the relatively fewer mortalities amongst children in this disease compared to adults.The protective mechanisms include reduced expression of receptors that the virus utilizes to enter target cells, the differences in innate and adaptive immunity in this age group compared to adults which contribute to different immune dynamics, protective against the pathogenesis of this virus. The differences in childhood immunity are suggested to also preserve the pulmonary endothelial barrier and prevent acute respiratory distress syndrome (ARDS) [14].

4. Importance of innate immunity in human milk

Early immune responses of innate immunity are critical to deter viral survival in the human \severe respiratory infections can occur with specific gene polymorphisms of innate immune responses [15] and such genetic polymorphisms can delay, diminish or exaggerate antiviral responses [16] resulting in severe , invasive disease. Adding to this challenge is that respiratory pathogens have evolved processes to suppress or evade these important innate responses.

Evolutionarily, human nutrition provides innate protection from novel infections and prevents tissue damage as a result of it [17]. The mammary glands extract substances from maternal blood or may actively synthesise constituents with direct or indirect immune capacity. These substances are present in its cellular content, in the milk fat globule membrane found in milk, in its growth factors and in encrypted peptides within bioactive components. Many milk factors seem to have evolved for multiple functions with capacity to adapt to the mother infant dyad [17,18,19]. This makes human milk, on the whole, not reproducible in its immune potential, cost effective, and uniquely responsive in biological potential.

Human milk is empowered, through its innate immunity to fortify primary mucosal sites where infective agents gain entry[18,19],an early defence that is mandatory in the young with relative immunodeficiency. The infected mother can protect, by early innate immune responses[18,19], and further this by priming adaptive immune protection.

At chief sites of major antigenic challenge in the respiratory and gastrointestinal tract, innate defences strengthen the infant's developing immunity and in some situations give added individual protection as its immune content varies from mother to mother or with feeding time, in colostrum, transitional milk and mature milk[18,19], for such dynamic immune-protective potentials. Colostrum is produced in the first five days after birth, and is rich in white blood cells, human milk oligosaccharides (HMOs), bioactive factors such as immunoglobulin A(IgA), lactoferrin, growth and colony stimulating factors and antioxidants. Following this, transitional milk is produced up to 2 weeks postpartum which contains decreasing amounts of protein and higher lactose, fat and water-soluble vitamins. Mature milk, with does not show variation with regards to its nutrient content after 6 weeks postpartum. There are also differences in the milk of mothers who deliver at term compared to preterm with preterm milk containing increased proteins and specific immune factors [18]

4.1 Enhancing physical and chemical barriers by mucosal protection

The ciliated pseudostratified columnar epithelium which line most of the respiratory tract has special functions as a barrier to pathogens and foreign antigens, preventing infections and tissue injury

[20].Goblet cells secrete mucous, and form a vital layer of vigorous innate defence through mucociliary clearance[21].

Mucociliary clearance at the airway epithelium is augmented by the formation of mucous gel , the glycosylated mucin glycoproteins, with multiple defensive roles and transmembrane mucins such as MUC1 and MUC 4 as innate defences[21,22,23].Underlying the mucosal epithelia are leukocytes which secrete antibodies, defensins and lysozyme , for early immune defences[22].These substances provide a dual protection , acting as a physical barrier and providing direct antimicrobial activity, with capacity to opsonize microbes and clear them[22] .

Inefficient mucociliary clearance or absence of sufficient mucin glycoproteins in the airway disrupts this early and vital defense.Viruses can develop methods that interfere with early defences as in SARS- CoV -2 infections resulting in cell fusion, epithelial destruction , cilium shrinkage and other pathological changes to the epithelium[24],weakening its defense.

In the infant energy dependent processes must be conserved for growth and development and human milk is an investment towards this as nutrition is enriched by early defence ingredients.

For instance, mucosal tissues utilize energy to produce mucins, and increase their energy requirements to produce the important early defensive shield by mucin glycoproteins to fight off infections [22].However human milk nutrition has defensive mucous enriching factors that augment the amount of mucous in the respiratory tract or that step up early immunity by coating epithelial surfaces with mucous that prevents viral entry [25], and an effective step for primary infection prevention. Mucins in human milk add on a layer to the developing immunity in the gastrointestinal and respiratory tracts by supporting the prevention of adhesion of pathogens to the cell surface [25]. MUC1 and MUC4 competitively inhibit receptor to viral interaction [25].Sialyated human milk mucin inhibits viral binding to the infant's cell surface glycan receptor and inhibits rotavirus in the gastrointestinal tract, blocking experimental adhesion of recombinant norovirus-like particles, possibly emulated in action for immunotherapy[25,26]

Human milk proteins impact at the core of immune defences by stimulating gene expression. β-casein, for example, stimulates MUC2 gene expression for increased goblet cell numbers [27,28] Additionally mucous secretion is also enhanced by substances such as epidermal growth factor (EGF) increasing mucin production by goblet cells [28], enhancing synthesis and secretion of mucous.

Trefoil factors, are cellular products that produce mucin in breastmilk and step up immunity by activating intestinal epithelial cells and healing of mucosae [29], enhancing tissue repair and limiting tissue damage. Mucosal barriers remove the virus by agglutination and expulsion through muco-ciliary action in the respiratory tract or peristaltic movement in the gut [30].

Epithelial adherence and colonization prevent infection and complications but could also control infection transmission. Human milk oligosaccharides (HMOs), which are found abundantly in human milk, coat epithelial surfaces and prevent pathogen contact and adherence to epithelia, an important mechanism which would otherwise permit viral replication. Soluble fucosylated and sialyated HMOs are bound by lectin receptors of fucose or sialyl-dependent pathogens, entrapping viruses so the host innate immune system cannot recognize them. HMOs are also prebiotics for commensal microbes, along with other factors in human milk, augment holistic health and fortify epithelia to prevent early steps of infection such as viral adherence [31].

4.2 Receptor blockage

Viral entry sabotages cellular machinery for viral replication. Blocking cellular entry by interrupting viral receptors, can prevent cellular internalisation of virus. The SARS-COV-2 requires specific ACE-2 receptors [4] to enter cells while heparan sulfate proteoglycan (HSPG) receptors assist in SARS-COV-2 cell entry [32], establishing infection.

Human milk has ingredients that are “receptor decoys” in early infection prevention [31]. Viruses utilize cell surface glycoconjugates as receptors to enter cells, while some human milk oligosaccharides (HMOs),abundant in early nutrition, express glycans that bind onto host cell surface lectins and prevent viral binding and invasion[33].Not all breastfeeding mothers can effectively provide protection through this route , as specific HMO glycosylation providing these receptors depends on factors such as the mother’s blood Lewis status[34].

The antiviral spectrum in human milk such as cytokines, monolaurin, Vitamin A, Tenascin C, lactadherrin, lactoferrin and others and this may be captured even where direct nursing is not feasible, through milk banks that collect milk with specific properties [35].

Lactoferrin is a multifunctional glycoprotein and is found abundantly in colostrum [35]. It fortifies human nutrition with antiviral action by direct and indirect methods. Its dynamic levels increase as lactation progresses to the second year [36], re-emphasising holistic importance of sustained breastfeeding. Lactoferrin acts against a gamut of non-enveloped and enveloped DNA and RNA viruses through numerous mechanisms; inhibiting cellular entry, by direct attachment to the virus or by blocking cellular receptors. Its action against human pathogens such as Herpes simplex virus, human papillomavirus, human immunodeficiency virus (HIV), and rotavirus from entering host cells, is notable at the step where these viruses enter cells utilising common cell surface receptors such as heparan sulfate glycosaminoglycan cell receptors (HSPG)[37].

SARS-CoV and SARS-CoV-2 are similar in their sequences and receptor-binding domain structure [38], hence they are compared when reviewing viral pathogencity.Lactoferrin protects against SARS-CoV infection by binding to HSPGs, interrupting the initial interaction between SARS-CoV and host cells[39]. Additionally, lactoferrin may also interrupt the step of cellular entry of SARS-CoV-2 which also utilises HSPG as cofactor, for cell anchor and entry [32].

The antiviral spectrum of lactoferrin includes fighting off infections that depend on iron. Lactoferrin interferes with iron utilization of pathogens. Viruses may also infect iron-acquiring cells by binding to another human milk protein, transferrin receptors during cell entry. Other viruses alter proteins involved in iron homeostasis. In coronavirus infection and inflammation, lactoferrin plays a preventive role in the respiratory and gastrointestinal tract [40].

While the levels of the various immunoglobulin fractions differ in colostrum and milk, IgA is generally the main immunoglobulin, immunoglobulin M(IgM)the second most dominant immunoglobulin and immunoglobulin G(IgG)is the least dominant compared to the IgA and IgG[41] Secretory immunoglobulin A (SIgA), is the main antibody in mucosal secretions and may engage multiple innate mechanisms for early antiviral protection .It consists of two or more IgA monomers joined by a J chain in association with SC which helps transport and release IgA into the intestinal lumen. It also protects IgA from proteolysis and anchors IgA in the mucus layer overlying the mucosal surfaces [42]

While SARS-CoV-2 SIgA responses in human milk follow maternal recovery from COVID-19[43], the protective mechanism by this tetrameric complex with secretory component (SC) primarily of mucosal adaptive immune functions in this scenario is not known. However, timely innate antimicrobial protection by SIgA at intestinal mucosal sites by blocking receptor binding, immune exclusion and interference with pathogen virulence determinants [42] has been demonstrated against other pathogens

A cohort study of lactating parents compared human milk mRNA vaccination responses to responses to Covid-19 infections .This study found differences in IgA and IgG antibodies in human milk between COVID-19 infection and mRNA vaccination and these differences were noted for up to 90 days. Infection was associated with a variable IgA-dominant response that was stable up to 90 days after diagnosis and vaccination was linked to an IgG-dominant response. The concentration of IgG response was noted to increase after each vaccine dose and to start to decline by 90 days after the second dose.There was increased human milk IgA only after the first dose following vaccination. Both infection and vaccination produced neutralization activity against live SARS-CoV-2 virus in human milk [44].

4.3 Innate immune recognition

Timely, selective and regulated pathogen recognition must differentiate from the plentiful commensals in the human body. Antiviral immune attack must also minimise destruction of normal tissue and commensal microbes. Cells infected with viruses must be destroyed early by focused immune recognition with effective and regulated antiviral responses. At the same time, where dysregulated immunity can cause invasive disease and tissue destruction, as in the immunopathology of invasive SARS- CoV-2 infection, the antiviral action of human milk along with its capacity for anti-inflammation and immunomodulation could be useful to counter or reduce this immunopathology.

The innate immune system recognizes pathogens by pathogen-recognition receptors (PRRs) that sense distinct pathogen-associated molecular patterns (PAMP).Toll-like receptors (TLR) are activated and signalled by RNA viruses through RNA sensors [45]. Members of the TLR family detect viruses and induce the production of interferons through several signalling proteins. Reproducing this, TLR agonists are suggested as therapy [45] The SARS-CoV-2 activates TLR2 signalling, which results in the robust expression of proinflammatory cytokines [46].

Human milk components can selectively recognize pathogens and differentiate them from commensals. TLR signalling is important for such differential and specific recognition [47,48]. In human milk there are specific TLR responses on different cellular components based on TLRs that are activated; such responses are not found in infant formulas [48].TLR2, TLR3, TLR5 and soluble cluster of differentiation (sCD)14, and human β-defensin-1 (hBD-1), function as pattern recognition receptors for innate immune recognition[18] . PRRs in human milk and other bioactive substances in the intestine of the breastfed infant create an anti-inflammatory environment , while TLR responses can be modified by soluble toll-like receptors (sTLRs) and sCD14[18,48,49].

The immunomodulation in human milk potentially fine tunes specific TLR mediated inflammatory responses, while focusing on defences against viruses, interacting sTLRs and sCD14 with a spectrum of bioactive factors.

When the impact of human milk is integrated with the pathogenesis of the disease in the gastrointestinal tract which is an important organ in Covid-19 pathogenesis in children, the benefits of human milk feeding on the intestinal microbiome adds on to the spectrum of innate defences. Lactoferrin, is one of a spectrum of substances in human milk , which can promote the gut milieu. It enhances the growth of enterocytes and along with anti-inflammatory and immunomodulatory actions, steps up mucosal immunity at the gut epithelial barrier [38].

4.4 Immune cells

Immune cells and their antiviral products prevent or limit the infection.

Maternal blood leukocytes that home to the mammary gland travel through epithelial cell spaces to be secreted into milk, whereas blood monocytes reaching the mammary gland become activated and

function as motile macrophages, secreted into breastmilk for various dynamic immune functions [18,19, 50,51]. Immune constituents are also guided into mother's milk by mucosal immunity as the lactating mammary gland and its products are integral to this immune compartment [18,19]. Holistic maternal health including the health of the mother's lactating mammary glands may be important for this.

Human milk immune cells include epithelial cells, the motile macrophage, neutrophils, lymphocytes, innate lymphoid cells (ILCs), hematopoietic progenitor cells and stem cells.[51,52] There are epithelial cells, macrophages (32.6%) , neutrophils (45.1%) and lymphocytes (21.3%), characterized mainly by CD3+ T cells (83%), distributed between CD4+ and CD8+[52, 53] , as well as Tγδ+ cells (11%), CD16+ NK cells (3-4%), and B cells (2%).[52, 54] While some immune cells transferred in milk last in the infant for about 6 days [51,55], human milk can continue to stimulate the proliferation and survival of milk neutrophils and macrophages through colony stimulating factors (CSF)[52].In the mother who expresses milk, milk cells remain viable for a few hours after expression , and do not typically survive after freezing or pasteurization[52], stressing advantages of direct human milk feeding.

Individual protection is evident in the milk of mothers whose infants have severe bronchiolitis where there are an increased number of live cells .Milk from mothers of infants who were hospitalized with bronchiolitis had increased viable cells compared to milk from mothers of healthy infants (1.3±0.4 vs. 0.3±0.03×10(6) cells/ml, mean±s.e.m.p≤0.001).Maternal milk cells from infants hospitalized with bronchiolitis produced a skewed cytokine profile *ex vivo* in response to stimulation by live respiratory syncytial virus but not when cultured with a non-specific mitogen [56]

Animal experiments indicate that pluripotent stem cells in human milk can replicate and differentiate outside the mammary cell lineage and can integrate into distant organs [57]. For instance, it is fascinating that human milk stem cells can reach the brain and differentiate into neurons and glial cells [54], and it is hypothesised that such cells can repair damaged or injured tissue in the aftermath of invasive viral infections.

4.5 Immune modulation

Human milk augments immune maturity and modulates immunity in the developing immune system. While there is still a lot more about immune modulation in human milk to be known, it is a dynamic process that seems to respond to the infant's needs.

Lactoferrin,as mentioned above, is a human milk constituent with multiple antiviral actions. It also binds viruses, blocks some viruses from attaching to target cells and can suppress intracellular viral replication. Lactoferrin receptors are found in many immune cells such as monocytes and lymphocytes. Lactoferrin activates antigen presenting cells (APC) and can link innate and adaptive immune functions for T and B cells. The anti-inflammatory action of lactoferrin on leukocytes that are stimulated with lipopolysaccharides (LPS) is associated signalling proteins[58]

Experimentally, infected bronchial cells are protected from inflammation and cell necrosis by lactoferrin [59]. Lactoferrin is an iron scavenger and modulates signalling pathways. It helps reduce the production of pro-inflammatory cytokines and reactive oxygen species (ROS)[59].

When these are extrapolated to the nursing infant, lactoferrin, in human milk, may similarly reduce immune dysregulation in the 'cytokine storm' recognised as a complication of SARS-CoV-2, through anti-inflammatory impact, and by this, could reduce lung damage in the infection.

In the gastrointestinal tract, lactoferrin transports iron and deprives iron from iron-dependent microbes, reducing their infectivity. Through its action on iron, lactoferrin may have a role in the

hyperferrinemia associated with Covid -19. Preventing thrombocytopenia, through platelet surface receptors may help in the hypercoagulable state, recognised in COVID-19[59] Lactoferrin and other substances stimulate effective immune responses by recruiting APCs to enhance adaptive immunity[58,59].

Responsive immune-protection is evident during active infections. In nursing infants, mother's milk showed increased total number of white blood cells, especially macrophages, and TNFα levels [50], a regulation that could accelerate recovery in the nursing infant. Individual protection through human milk occurs within the dynamics of a unique compartment distinct from maternal plasma, predominantly anti- inflammatory, differing among mammalian species, influenced by diet and stage of feeding, with notable differences in colostrum and mature milk [19,60].

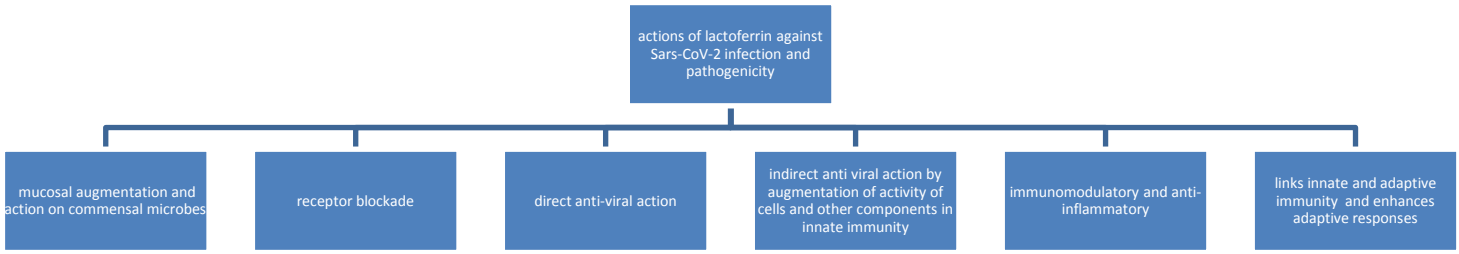
At the, the microscale, human milk is also dynamically regulated. Human milk microribonucleic acids (miRNAs) are mainly produced in the mammary gland, while small amounts are drawn from maternal blood with lactation-specific regulatory functions. Cells, exosomes and fat globules protect milk miRNA and transfer them to the infant's bloodstream [60]. A study found that while the total miRNA provided to the nursing infant is constant in the first six months of lactation, the miRNA concentrations are altered in the fourth month compared to the second and sixth month. The authors suggest that this may be due to remodelling of the mammary gland in response to the infant's changing feeding patterns, as an adaptation to the infant's needs [60]

Additionally, maternal diet can transfer into human milk subtypes of miRNAs codified by non-human genomes but still present in the circulation [61], and some have been noted to have antiviral immune activity such as the freely circulating xeno-miRNA (XenomiRs), against the Influenza A viruses (IAVs)[62].

Immunomodulatory activity in human milk is supported by some reduction to allergies and immune - related diseases in later life in the breastfed. A fortified mucosal layer induced by human milk microbes, sIgA, cytokines and immunomodulatory substances prevents microbial translocation across mucosal surfaces [63]. This may be important to prevent overstimulation of systemic immunity. Through exclusive human milk feeds, the establishment of a "mother-microbe-infant-microbe" link renders human milk as helpful against acute diseases and protective for long term health [64,65].

The framework of disease prevention by human milk feeding can be compared to the basic principles of vaccinology [66]. Human milk is an excellent vaccine as it has an incomparable safety profile and can prevent clinical infection. By preventing mucosal pathogens from gaining entry into the body by protecting against epithelial colonization, innate immunity can also decrease person-to- person and community transmission of the virus.
Through timely prevention, human milk antiviral factors potentially reduce the infective load which naturally prevents severe infection and its consequences.
Hence, human milk can act as primary and secondary prevention in such infections.

Fig 4: Possible defenses by lactoferrin in human milk against SARS-CoV-2



5. Conclusion

It is of interest that the integration of the pathogenesis of Covid-19 with the immunological actions of human milk feeding, supports a theoretical possibility for stepwise protection in human milk against the virus. While innate immune protection in human milk potentially protects the mucosae, against viral entry, viral invasion and disease complications, studies indicate that human milk responses may be modified by maternal vaccination in SARS-CoV-2 infections. This may be a strategy that could enhance human milk anti SARS-CoV-2 immunoprotection..

While much research in this area is needed, it is important to continue to emphasize exclusive human milk feeding as an important modifiable variable that promotes infection protection and holistic health.

COMPETING INTERESTS

There are no competing interests

AUTHOR'S CONTRIBUTION

The single author as named above formed the idea of the review, designed the review, did the literature search, reviewed the relevant papers, analysed , synthesised and integrated the information , wrote the first and final draft of the manuscript , then read and approved the final manuscript

CONSENT

Not applicable

CONFLICT OF INTEREST

All work is referenced correctly to the best of my knowledge.

The author declares no ethical issues or conflict of interest after the work is published

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