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The potential for Lactoferrin to reduce SARS-CoV-2 induced cytokine storm

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ABSTRACT

The COVID-19 pandemic is a serious global health threat caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Symptoms of COVID-19 are highly variable with common hyperactivity of immune responses known as a “cytokine storm”. In fact, this massive release of inflammatory cytokines into the pulmonary alveolar structure is a main cause of mortality during COVID-19 infection. Current management of COVID-19 is supportive and there is no common clinical protocol applied to suppress this pathological state. Lactoferrin (LF), an iron binding protein, is a first line defense protein that is present in neutrophils and excretory fluids of all mammals, and is well recognized for its role in maturation and regulation of immune system function. Also, due to its ability to sequester free iron, LF is known to protect against insult-induced oxidative damage induced during COVID-19 disease states.

Lactoferrin (LF) is a multifunctional, iron binding protein, contained in neutrophils and mammalian excretions. It is long since known that LF has direct anti-microbial functions [26,27], and pertinent to this review, AlKhzinder commented on the possible benefits of LF to boost natural responses to SARS-CoV-2 [23]. Wang, et al [24] theorized a utility for LF as a tool to control development of pathologies due to coronavirus infection, acting as an anti-inflammatory, anti-infective and immune-regulator. And Chang, et al., suggested that LF may serve to augment intracellular anti-viral mechanisms directly [25]. We agree with this assessment, and further explore mechanisms underlying LF as a therapeutic to function prophylactically or therapeutically to control tissue damage induced during COVID-19 disease states.

Lactoferrin (LF) is a front-line, natural immune modulator [1-3] that has ability to counteract excessive immune responses [4,5] and also facilitate the development of adaptive immunity [6].

The cytokine storm is the main cause of mortality and morbidity of patients admitted to intensive care units following septic shock, injury or postsurgical trauma [7,8]. Excessive cytokine release by hyper-reactive individuals is also common in COVID-19 patients [9,10] with interleukin (IL) –6 and IL-10 serving as best markers of the disease prognosis [11]. Other complications resulting from the aberrant response of patients to the viral infection include, among others, increased thrombosis associated with microcoagulation [12] and intestinal microflora dysbiosis [13]. The therapeutic approaches and suggested treatments [14,15] aimed at prevention or amelioration of this violent immune response encompass steroids [16], nonsteroidal drugs [17], low dose radiation [18], convalescent plasma [19], IL-6 inhibition [20], vitamins [21], probiotics [22] and other nutraceuticals [13]. Recently, AlKhzinder commented on the possible benefits of LF to boost natural responses to SARS-CoV-2 [23]. Wang, et al [24] theorized a utility for LF as a tool to control development of pathologies due to coronavirus infection, acting as an anti-inflammatory, anti-infective and immune-regulator. And Chang, et al., suggested that LF may serve to augment intracellular anti-viral mechanisms directly [25]. We agree with this assessment, and further explore mechanisms underlying LF as a therapeutic to function prophylactically or therapeutically to control tissue damage induced during COVID-19 disease states.

Lactoferrin (LF) is a multifunctional, iron binding protein, contained in neutrophils and mammalian excretions. It is long since known that LF has direct anti-microbial functions [26,27], and pertinent to this review, has also demonstrated direct anti-viral capabilities [28-30]. An overall summary of LF immunoregulatory activities is listed in Table 1. Specifically, the importance of lactoferrin in immune cell responses has been detailed in a way to link cellular activation for natural killer and T lymphocytes, mononuclear cells migration, monocytes and neutrophils recruitment to improve overall immune responses to elicit varied aspects of antimicrobial effects [31,32]. LF is deeply involved in many critical physiological functions, namely immune regulatory and the redox homeostasis. By virtue of iron sequestration, LF attenuates the production...
Table 1
Immunoregulatory actions of LF in the context of infections and clinical insults.

| Model                                      | LF treatment  | Effects                                                                 | Reference |
|--------------------------------------------|---------------|-------------------------------------------------------------------------|-----------|
| **Mouse and human in vitro cell models**   |               |                                                                         |           |
| human whole blood culture                  | rhLF in culture | regulation of gene expression involved in oxidative stress            | Kruzel, et al. 2013 |
| PBMC activated by LPS                      | rhLF in culture | regulatory effect on LFA-1 expression mediated by TNF α               | Zimecki, et al. 1999 |
| human macrophages stimulated with EBV      | rhLF in culture | inhibition of chemokine production via suppression of NFκB            | Zheng, et al. 2014 |
| hydrogen peroxide induced oxidative damage in epithelial intestinal cells | rhLF in culture | differentiation of monocytes into macrophages with decreased TLR-4 expression, TLR signaling and proinflammatory cytokine production | Wisgrill, et al. 2018 |
| monocyte derived dendritic cells           | rhLF in culture | generation of dendritic cells with impaired ability to promote Th1 type response | Puddu, et al. 2011 |
| primary human megakaryocytes               | rhLF in culture | inhibition of platelet production in matured megakaryocytes          | Matsumura-Takeda, et al. 2008 |
| **Viral infections**                       |               |                                                                         |           |
| human cytomelagovirus (HCMV) and human herpes virus 1 (HSV-1) infection of human embryonic lung cells | rhLF in culture | inhibition of virus infection and replication by preventing virus adsorption and cell penetration | Hasegawa, et al. 1994 |
| rotavirus infection of enterocyte HT-12 cell line | apo and holo rhLF in culture | inhibition of replication stronger by apo LF, binding of LF to virus particles | Superetti, et al. 1997 |
| adenovirus infection of HEF-2 cells        | rhLF and lactoferrin in culture | inhibition of infection by binding of LF to glycosaminoglycan receptor | Di Base, et al. 2003 |
| HSV infection of Vero cells                | rhLF and lactoferrin in culture | BLF inhibits only virus entry to cells and lactoferrin both entry and replication | Andersen, et al. 2004 |
| rhadomyosarcoma and neuroblastoma SK-N-SH cells infected with EU71 enterovirus | rhLF in culture | inhibition of replication by binding to the target cells and VP1 viral protein | Weng, et al. 2005 |
| influenza A virus infection in vitro       | rhLF in culture | protection by apoptosis inhibition, block of nuclear transcript | Pietraoniti, et al. 2010 |
| Egyptian avian lethal influenza A (HSN1) infection of MDCK cell line | rhLF in culture | dose-dependent inhibition of replication | Taha, et al. 2010 |
| influenza virus infection in mice          | rhLF administered daily by gavage from 1 day before infection to day 6 postinfection | improved histology, lower number of leukocytes infiltrating BALF, lower IL-6 serum level | Shin, et al. 2005 |
| hepatitis B virus infection of cultured human hepatocytes (PH5CH8) | rhLF or hLF in culture | prevention of the infection by preincubation of cells but not the virus by LF | Hara, et al. 2002 |
| hepatitis C infection of Hep G2 cells      | human, camel, bovine or sheep LF in culture | prevention of HCV entry into cells by all LFs via direct interaction with the virus | El-Fakhary, et al. 2013 |
| echovirus infection of human cells in various assay systems | rhLF digested with gastrointestinal enzymes in culture | inhibition of replication with a better effect of digested BLF than intact BLF | Taha, et al. 2011 |
| norovirus (MNV) infection of RAW 264.7 cells | rhLF in culture | dose-dependent decrease or block of cell damage and virus titles in the medium and cells, increase of IFN α and IFN β production by the cells | Ishikawa, et al. 2013 |
| Encephalomyocarditis virus infection of splenocytes | rhLF and BLF in culture | both LFs protective to the same degree in young and old mice | Zarycinska, et al. 2017 |
| SARS pseudovirus infection of HEK293E/ACE2-Myc cells | rhLF in culture | block of the binding of spike protein A to host cells at 4 °C, lack of spike protein /ACE2 integration disruption, role of heparin sulfate block by BLF | Lang, et al. 2011 |
| SARS-CoV-2 infection in Caco-2 cells       | rhLF in culture | induction of gene expression for IFNA1, IFNB1, IL12, TRIF, TR7, IRT3, IRF7 and MAV, partial inhibition of virus infection and replication | Salari, et al. 2021 |
| **Endotoxemia and bacterial infections**   |               |                                                                         |           |
| LPS-induced endotoxia                      | rhLF given i.v. 24 h prior to LPS | significant reduction of TNF α serum level, BLF alone induces serum IL-6 reduction of TNF α, IL-6, IL-10 and nitric oxide after 1 h pretreatment with hLF, other treatments not effective in reduction of IL-6 and IL-10 | Machnicki, et al. 1993 |
| LPS-induced endotoxia                      | rhLF given before, together or after LPS challenge | protection of gut mucosal integrity, increased survival | Kruzel, et al. 2002 |
| LPS-induced inflammation                   | rhLF administered i.p. before LPS | protection of gut mucosal integrity, increased survival | Kruzel, et al. 2000 |
| acute LPS-induced lung injury              | i.p. injection of rhLF 1 h before or after LPS | both protocols effective in decreasing histological, biochemical and immunological inflammatory parameters | Li, et al. 2012 |
| Mycobacterium tuberculosis TDM-induced lung pathology | rhLF given iv. together with TDM | reduction in granuloma formation, increased levels of IL-10 and TGF β | Welsh, et al. 2010 |
| Mycobacterium tuberculosis infection       | rhLF in drinking water starting from infection, for 1–3 weeks | lung inflammation and bacteria number significantly reduced, increased total lymphocyte number and CD4+ IFN γ and IL-17 producing cells | Welsh, et al. 2011 |
| Mycobacterial TDM-induced lung pathology   | rhLF or rmLF delivered by gavage at day 4 and 6 after TDM | reduced production of IL-12p40, increased IL-6 and CXCL1, increased number of macrophages and diminished granuloma formation | Hwang, et al. 2017 |
| human monocytes and granulocytes infected with methicillin-resistant Staphylococcus aureus | rhLF in culture | increased production of INF γ and IL-2, decrease of TNF α, IL-6, IL-10, IL-12p40, and IL-10 | Hwang, et al. 2014 |

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of reactive oxygen species (ROS) and subsequent oxidative stress that may lead to wild “cytokine storm”. However, at this time, there is little evidence for direct interaction of LF with SARS-CoV-2 and/or the pathologically related Middle East respiratory syndrome coronavirus (MERS-CoV), although, LF may exhibit some inhibition of ACE2 binding via non-specific interaction with surface glycans [33]. It has been shown that LF is able to prevent internalization of some viruses by binding to heparan sulfate proteoglycans (HSPGs) which is present on many cell phenotypes, thus providing generalized protection against a variety of viral infection [34]. The role of LF in controlling fundamental biological processes has been revealed in recent years with emphases on its immunoregulatory functions. In this context, LF is theorized as an important component in the toolbox of therapeutics to combat virions due to its maintenance of physiological homeostasis and its link to regulate innate and adaptive immunity [35].

2. Biology of LF in context of multifunctional properties

Lactoferrin is a well conserved, monomeric 80-kDa single polypeptide chain contained in most mammalian exocrine secretions, including milk, tears, saliva, bronchial and intestinal secretions and also in the secondary granules of neutrophils. It is considered a first line defense protein involved in protection against microbial infections [36,37] and subsequent development of systemic inflammatory response syndrome (SIRS) and sepsis [38-40]. LF has been implicated in immunoregulatory functions [35,41-44], as a modulator of vaccine kinetics, and containing chemoprotective activity [45]. The primary structure of human LF (hLF) is characterized by a single polypeptide chain containing 692 amino acids organized in two highly homologous lobes, each capable of binding single ferric ion (Fe3+). In this regard, LF is considered as an antioxidant because its iron binding ability may prevent the iron-catalyzed formation of ROS namely H2O2 and -OH [47-50]. Lactoferrin is a glycoprotein and in humans the glycans are the N-acetyllactosaminic type, α1,3-fucosylated on the N-acetyl-glucosamine residue linked to the peptide chain. The oligosaccharide component of glycoprotein is often critical for determination of pharmacological properties including in vivo activity, pharmacokinetics, and immunogenicity. Of particular importance is that LF bridges innate and adaptive immune functions by regulating target cell responses, using mechanisms that are highly dependent on the type of carbohydrates attached to the protein.

Bovine lactoferrin (bLF), widely available on nutritional market, shares ~ 70% amino acid sequence homology with human LF (hLF), albeit with a different glycosylation pattern. Although good for nutritional supplementation, bLF cannot be used for parenteral applications in humans due to potential risk of anaphylactic shock. The major concern for LF clinical utility relates not only to amino acid incompatibility but, more importantly, to the glycan structure. Thus, there remains no clinical data on effectiveness of systemically (parenterally) delivered LF in humans. Recombinant human LF (rhLF) has been produced in various expression systems [51], including transgenic plants [52] and animals, but none of the resultant products have been tested and approved for human use for intravenous (systemic) administration. Although there are two rhLFs available for larger studies, one from transgenic rice [53] and the other from Aspergillus awamori [54], both present a health threat if administered intravenously due to the antigenic nature inherent within their non-homologous glycosylation patterns. In 2013, Kruzel, et al. reported production of fully compatible, non-antigenic rhLFs obtained in a mammalian expression system using the Chinese Hamster Ovary cell line (CHO), yet no clinical trials with this molecule have been reported [55].

### Table 1 (continued)

| Model | LF treatment | Effects | Reference |
|-------|--------------|---------|-----------|
| *Pseudomonas aeruginosa* acute and chronic infection in mice | intranasal administration of aerosolized bLF | significant decrease of the neutrophil recruitment and proinflammatory cytokine levels, faster recovery and body weight gain | Valenti, et al. 2017 [76] |
| peritonitis induced by Methicillin-resistant *Staphylococcus aureus* | rhLF given 2 h post-infection | modest increase in survival, reduced IL-6 and IL-17 serum levels | Hwang, et al. 2014 [82] |
| Normal and manipulated rodents | | | |
| surgery in mice | bLF given i.v. or per o.s. before thymectomy or splenectomy | suppression of IL-6 and TNFα after thymectomy, deeper after per o.s. treatment, weaker effects on splenectomy | Zimecki, et al. 1998 [75] |
| ovalbumin (OVA) induced pleurisy in mice | bLF or hLF given buccally 24 h and 3 h before OVA | decrease of clinical manifestation of pleurisy, decrease of IL5 level in pleural exudates | de la Rosa, et al. 2008 [32] |
| ovalbumin (OVA) responses in mice | bLF given i.p. before OVA | neutrophil and macrophage recruitment; Th1 polarization | Zimecki, et al. 2012 [81] |
| hyperoxia in CD-1 mice | bLF in aerosol | reduction in lung edema, cell number in BALF, IL-1β, IL-6, pulmonary fibrosis and DNA fragmentation, increase of survival from 20% to 66.7% | Chen, et al. 2014 [80] |
| transcriptome profiling in rat spleen | rhLF per os or i.v. | largely overlapping changes in gene expression by both treatments, associated with upregulation of genes involved in oxidative stress and inflammation | Kruzel, et al. 2020 [69] |
| Healthy persons and patients | | | |
| healthy volunteers | bLF chewable tablets for 14 days | normalization of spontaneous TNFα and IL-6 production by PBMC cultures depending on initial immunological reactivity | Kruzel, et al. 2007 [4] |
| healthy males | microencapsulated bLF, per os in two four week periods | significant effect for 200 mg daily doses, lowering CD69α activation marker on CD4+ cells, increase in the volume of most frequent intestinal bacteria species | Dix, et al. 2018 [100] |
| healthy volunteers, indomethacin-induced enteropathy | ingestion of 5 g rhLF, followed by 2 doses of indomethacin after 9 h and overnight | reduction of the increased by indomethacin small intestine permeability | Troost, et al. 2003 [97] |
| PMBC from patients with systemic inflammatory response syndrome | bLF in culture | inhibition of both spontaneous and LPS-induced PBMC proliferation, upregulation of IL-6 and TNFα production in septic survivors | Adanik, et al. 1998 [88] |
| blood from patients diagnosed with severe sepsis or septic shock | rhLF in culture | LPS-induced TNFα production upregulated in patients with sustained energy, inhibited or without effect in moderately reactive patients | Aytin, et al. 2018 [89] |
| COVID-19 patients | bLF as nutritional supplement, 4-6 daily doses for 10 days | complete and fast recovery within the first 4-5 days, prevention of the disease at lower doses in healthy persons having contacts with the patients | Serrano, et al. 2020 [136] |
3. Lactoferrin and immune homeostasis

There are two major metabolic pathways by which LF may support immune homeostasis during environmental insult: a) by controlling the iron-dependent production of ROS, and b) by activating or reducing receptor binding capacity on immune cells. There is convincing evidence of severe oxidative stress in patients with pre-septic conditions. Dysregulation of the immuno-inflammatory response, as seen during the development of sepsis, may culminate in host cell and organ damage. Testing the anti-oxidant properties of LF in human embryonic diploid fibroblasts (MRC5) using 2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-D), it was demonstrated that LF reduced intracellular ROS levels in a dose-dependent manner. LF also decreases ROS-induced apoptosis [4] and attenuates mitochondrial dysfunction [56].

By acting as a homeostatic agent, LF can work both ends of the immunological spectrum to increase low response or dampen aggressive ones. High affinity of LF to ferric ions (Fe++++) makes it important for the maintenance of iron dependent oxidative stress. Oxidative stress occurs when the production of destructive reactive oxygen species (ROS) surpasses the body’s own natural antioxidant defenses, resulting in cellular damage and death. Thus, by virtue of iron sequestration LF protects against tissue injury (Fig. 1), which may be critical during the development of infection such as COVID-19. It has direct capacity to regulate both pro-inflammatory and anti-inflammatory responses [38,57]. The utility of such pleiotropic immune mediator represents a novel therapeutic approach, which is dependent on elicited responses for outcome. Of particular importance is LF’s ability to bind to various cell receptors including CD14 [58], Toll-like receptor-2 and TLR-4 [59], as well as possible interaction with receptors involved in coronavirus binding; heparan sulfate proteoglycans (HSPG) [60,61] and angiotensin-converting enzyme 2 (ACE2) [62,63]. The role of LF in correction of the immune status of healthy individuals and patients seems indispensable since the protein can normalize its function in a sophisticated manner by sensing degrees of reactivity of the immune system and act accordingly [4,6]. As a component of the innate immunity, LF exerts diverse immunomodulatory functions. In particular, it can act in an adjuvant capacity. We and others demonstrated that LF (bovine-derived and recombinant human) augments vaccine efficacy by promoting both T and B lymphocytic responses that culminate in subsequent pathological protection against challenge with virulent pathogens [6,64]. Indeed, studies in neonatal mice show that LF had a significant effect to induce influenza neutralizing antibodies when combined with hemagglutinin antigen [65]. This bodes well for theoretical incorporation of LF into existing SARS-CoV-2 vaccines to boost neutralizing antibodies post immunization. In fact, LF secreted into all human mucosal sites is considered a natural adjuvant enhancing immunity to all infectious agents as we encountered during the period of infancy onward [66-68]; it assists in the acquisition and development of protective immunity against infectious-related pathologies during early stages of our ontogeny. Accordingly, exogenous LF given to population during this COVID-19 pandemic may be of great benefit to acquire immunity; this can be accomplished in safe and accelerated matter, especially when considering the safety profile of bovine LF which has been well documented in clinical trials for enterocolitis (discussed below).

Recently we demonstrated that LF given orally to experimental rats may account for the anti-inflammatory properties of LF [55]. Such a bidirectional action is rather unusual and may well explain the immunoregulatory nature of LF. Inhibition of immune cell reactivity by LF can also be accomplished by downregulation of toll like receptors, and even PAMPs [6,70]. For example, LF can inhibit Epstein-Barr virus infection not only by blocking CD21 receptor on B cells but also by interference with the activation of TLR-2 and TLR-9 [71]. Monocytes from umbilical cord blood cultured with LF differentiate into macrophages that have decreased TLR-4 expression, TLR signaling and cytokine production; they acquire an anergic state. A similar phenomenon can be observed with monocytes cultured with LF, where they differentiate into poor antigen presenting cells [72].

4. Effects of LF on cytokine production and lung injury in mice

Lactoferrin regulates insult-induced, cytokine-driven inflammation (Fig. 2). Early studies demonstrated that pretreatment of mice with LF...
More importantly, LF was effective in lowering serum TNF-α levels and endotoxemia was associated with induction of IL-10 production [74]. It also appeared that the protective effect of LF prevented lipopolysaccharide (LPS) molecule by augmentation of low level responses or modulation of overaggressive cytokine response. Both effects serve to assist with tissue repair after insult. Adapted from [115].

Fig. 2. Potential for LF to regulate insult-induced inflammation. Exogenous LF can modulate insult-induced inflammation in a similar way as endogenous molecule by augmentation of low level responses or modulation of overaggressive cytokine response. Both effects serve to assist with tissue repair after insult. Adapted from [115].

5. Immunoregulatory effects of LF in human volunteers

A specific study to determine clinical relevance for use of LF to treat sepsis used human blood derived monocytes and granulocytes infected with MRSA, and examined cytokine changes when treated with homologous recombinant human LF [82]. The outcome of treatment with LF led to increased production of IFN-γ and IL-2, with a concurrent decrease in IL-6, TNF-α, IL-1β, IL-12p40, and IL-10. The monitoring of the course of COVID-19 infection revealed that high levels of IL-6 and IL-10, but perhaps not always TNF-α, as markers of morbidity and mortality of patients [11]. These reported markers confirm what was identified in earlier reports from septic patients [8]. It also became clear that very low or exceptionally high reactivity of peripheral blood mononuclear cells (PBMC) significantly correlated with mortality rate in septic shock patients, and were accompanied by a low ability to produce IL-6 and TNF-α by septic non-survivors [8]. Such a phenomenon probably resulted from an immune exhaustion following excessive cytokine production and release into circulation. LF may have direct activity to interfere with SARS-CoV-2 infection, perhaps by preventing virion attachment to cell surface ACE2 receptor [63] or binding to a heparin sulfate co-receptor [61]. There is speculation that LF alone may function as a nutritional supplement to ward infection [83]. More likely, LF functions in a manner to regulate/modulate cytokines in the tissue environment.

Since serum levels of IL-6, together with IL-10, represent only the marker of host’s response to COVID-19 virus, it is not clear whether these cytokines are important for pathology of the infection or they rather fulfill a protective role. Therefore, it may not be advisable to completely block activity of IL-6. However, the effects of treatment of patients with tocilizumab or sarilumab, IL-6 inhibitors [20,84], seem disputable. Indeed, it may be critical to utilize LF in combination therapies with other agents [85]. For example, hypo-thiocyanite combined with LF can limit pulmonary damage in models of respiratory infections [86]. In an analogous line of thought, there were no significant effects registered with blocking TNF-α production in septic patients [87]. Our experience showed that LF could regulate LPS-induced IL-6 and TNF-α production by PBMC cultures in septic and trauma patients [88,89], and breaking anergic responsiveness of blood cells by LF was of particular interest. It should also be mentioned that the ability of LF to inhibit platelet aggregation [90] may be of advantage to prevent microcoagulation as a major cause of organ failure [12].

The most intriguing data demonstrating regulatory effects of LF on spontaneous production of IL-6 and TNF-α by PBMC, derives from studies in healthy volunteers [4]. In one trial, volunteers received chewable tablets containing bLF for several days and the production of cytokines by PBMC cultures was measured before, on day 1 and on day 14 after treatment. The subjects were divided into categories of low, moderate and high responders, according to levels of TNF-α production following LF treatment. The study revealed that production of IL-6 successively rose in low responders but diminished in high responders, attaining the same level of reactivity in both categories on day 14. The regulatory action of LF was also evident by elevating the cytokine production in the low responders, transient stimulation in the moderate and a very strong inhibition in the high responder category. In conclusion, it seems that a more reasonable approach to prevent consequences due to
an induced cytokine (septic) shock should be aimed at optimization of immune reactivity of patients with pathogens inducing clinical insults.

6. Gut integrity and microbiota during clinical insult: Preventive and therapeutic utility of LF

We are of the opinion that LF would be most beneficial when applied as a prophylactic nutraceutical supplement to correct the immune status in healthy subjects and improve composition of gut microflora, crucial in maintenance of homeostasis. General health conditions, such as age and nutritional status of individuals, are typically associated with gut microflora composition that can potentially affect the critical course of SARS-CoV-2 infection [91] or trauma patients [92]. Others turn attention to proper function of microbiota-gut-liver-lung axis and provide preliminary evidence that disturbances in this axis may be linked to COVID-19 infection [93]. In fact, human brush border intestinal cells are particularly susceptible to SARS-CoV-2 infection [94]. Preventive and therapeutic actions of LF are directed both to protection of gut mucous layer integrity and composition of microflora. The integrity of gut wall is protected in the model of endotoxemia in mice [95] and rats [96], and is attenuated in healthy volunteers treated with ibuprofen [97]. In vitro [98], in turn, LF was shown to protect Caco-2 cells against intracellular oxidative stress. Since gut microbiota dysbiosis may also contribute to pathogenicity of COVID-19 infection [13], the beneficial effects of LF on composition of gut microbiota is of utmost importance [99]. An interesting trial was conducted on volunteers receiving bLF preparations for two 2-week periods separated by 2-week off term [100]. The authors found beneficial effects of LF on the volume of physiological bacterial species and lower expression of activation CD69 marker on CD4+ cells. Thus, application of nutraceutical preparations containing LF, displaying beneficial effects on human microbiota and microbiota would be highly recommended as a preventive measure to optimize the immune system function [101]. Nevertheless, a therapeutic approach by oral or intracheal administration of LF may also be effective considering promising results from animal models and human in vitro studies. Indeed, it is well established that oral administration of LF increases antimicrobial related NK cell activity in the small intestines of mice [102],

7. LF in clinical trials for neonates and enterocolitis

Although it is beyond the scope of this review, it should be mentioned that bovine LF has been successfully used in clinical trials of neonates to investigate utility to reduce incidence of enterocolitis. Multiple studies have shown the potential for utility; beginning with landmark studies by Manzoni, et al. [103,104]. Specific studies to note include those reported by Pammi and Shiresh [105], and trials completed by Ochoa, et al. [106] where oral delivered LF prevented sepsis in neonates of very low birth weight (VLBW). Kaur, et al. also demonstrated efficacy of oral bLF to reduce incidence of sepsis in neonates of less than 2000 g [107]. And Akin, et al. reported success in studies on VLBW infants, with an interesting twist showing increased Treg populations in the treated group, albeit a smaller patient was investigated [108]. Finally, use of a recombinant human lactoferrin (talactoferrin) showed some promise in low weight infants [109], although this study had limited enrollment and therefore no statistical difference between groups. Talactoferrin differs in human glycosylation patterns, and may not be fully compatible with human lactoferrin. Overall conclusions comparing all clinical studies to date is limited due to study terms, demographics, LF manufacturer, number of enrolled neonates, and set outcomes for specific pathogens. The ELFIN study represents one of the largest clinical trials, and offers solid hope for general support to use enteral LF to limit preterm infections post birth [110,111]. While promising, questions still remain [112]. Recent meta-analyses indicate further need for continued data collection and coordination of trial parameters to determine overall success in this clinical arena [113,114].

8. LF lessens lung pathology during the acute respiratory distress Syndrome (ARDS)

Finally, there may be insights for utility of LF as a therapeutic for SARS-CoV-2 induced pathology garnered from our studies investigating pulmonary ARDS occurring in models of granulomatous inflammation (reviewed in [115]). Specifically, LF was able to diminish or abolish cytokine excess and lung pathology cause by Mycobacterium tuberculosis [116,117]. Of course, pathology developed post mycobacterial infections are unique from viral induced tissue damage. However, it should be noted that LF was also able to diminish hyperc reactive immunopathology developed after administration of isolated surface mycotic acid components [118,119]. Direct reduction of proinflammatory IL-1β, IL-6 and TNF-α were identified post LF treatment, while IL-10 and TGF-β were maintained or even enhanced.

9. Conclusion

Based on the previously collected data from the animal and human studies, we postulate that LF may be of clinical benefit in prevention and amelioration of the cytokine storm and its devastating consequences in lungs and other vital organs. LF can be applied in prophylaxis, as well as a therapeutic agent, by multiple routes of administration (including oral delivery) for individuals at risk of infection, and especially for those demonstrating impairment of innate immune function.

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