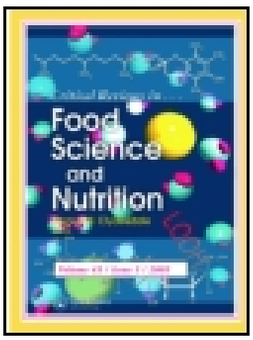

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REVIEW

Lactoferrin: an overview of its main functions, immunomodulatory and antimicrobial role, and clinical significance

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ABSTRACT

Lactoferrin (LF), a glycoprotein found in mucosal secretions, is characterized by a wide range of functions, including immunomodulatory and anti-inflammatory activities. Moreover, several investigations confirmed that LF displays high effectiveness against multiple bacteria and viruses and may be regarded as a potential inhibitor of enveloped viruses, such as presently prevailing SARS-CoV-2. In our review, we discuss available studies about LF functions and bioavailability of different LF forms in in vitro and in vivo models. Moreover, we characterize the potential benefits and side effects of LF use; we also briefly summarize the latest clinical trials examining LF application. Finally, we point potential role of LF in inflammatory bowel disease and indicate its use as a marker for disease severity.

KEYWORDS

Lactoferrin; lactoferrin receptor; anti-inflammatory; antiviral; antibacterial; immunomodulatory; inflammatory bowel disease; IBD

Lactoferrin: structure, distribution and main functions

Lactoferrin (LF) is an approximately 76 kDa iron-binding glycoprotein first isolated and purified from milk (Lönnerdal 2013; Legrand 2016). Due to its structural similarity with serum transferrin (Tf) and ability to bind Fe^{3+} ions, LF was classified as a member of transferrin family (Lambert, Perri, and Meehan 2005; Wang et al. 2019). It is made up of two homologous domains, termed the N- and C-lobes (Figure 1). Each lobe can be further divided into two similarly sized domains in the N-lobe (N1 and N2), and in the C-lobe (C1 and C2), respectively (Rastogi et al. 2016; Wally and Buchanan 2007). One LF molecule can bind two Fe^{3+} ions together with two CO_3^{2-} ions (Lauterbach et al. 2016).

One of the main LF activities is based on its influence on iron homeostasis. LF, by binding with iron, enhances intestinal iron absorption and thus may improve hemoglobin and total serum iron levels. Moreover, LF limits iron availability for several pathogens, e.g. LF is capable of inhibiting the growth of intracellular bacteria, through iron-dependent and iron-independent mechanisms (Voswinkel, Vogel, and Kulozik 2016). To date, several studies confirmed that LF possesses anti-inflammatory, immunomodulatory, antiviral, antibacterial, and antioxidant activities (Legrand et al. 2005; Zimecki and Artym 2005; Żelechowska, Agier, and Brzezińska-Błaszczyk 2016). Beyond that, recent reports indicate that the consumption of LF and its derivatives may modulate oral (Nakano et al. 2017) and intestinal (Vega-Bautista et al. 2019; Haiwen et al. 2019) microbiota composition in humans.

The presence of LF is species- and lactation-stage-dependent; however, the main sources of LF include human and bovine colostrum and milk (Cheng et al. 2008). Of note, the content of LF in human colostrum (>5 g/L) and milk (2–3 g/L) is significantly higher than in bovine colostrum (0.3–2.1 g/L) and milk (0.03–0.49 g/L) (Legrand 2016; Artym and Zimecki 2013). LF is also abundant in other exocrine fluids, such as saliva, tears, and semen. Moreover, it has been found at the mucosal surfaces and in granules of certain polymorphonuclear leukocytes, such as neutrophils, degranulation of which has been related to LF release into the serum (Ward, Uribe-Luna, and Conneely 2002). LF concentration in the blood of healthy humans is about 0.02 to 2.00 ug/mL and increases rapidly up to 200 ug/mL during inflammation, infection, tumor growth, or iron-overload (Cutone et al. 2020; Embleton et al. 2013; Latorre et al. 2010).

The safety of LF application

Numerous studies in both animals and humans evaluating the safety, allergenicity, and toxicity of LF application showed that its high doses were safe and well-tolerated (Cutone et al. 2020; Kaufman et al. 2021). Importantly, due to its high homology (69% amino acid sequence identity based on protein sequence alignment analysis) and similar activity to endogenous LF (Lönnerdal, Jiang, and Du 2011), bovine LF (bLF) has been suggested as an additional source of LF in humans and has commonly been used in infant

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

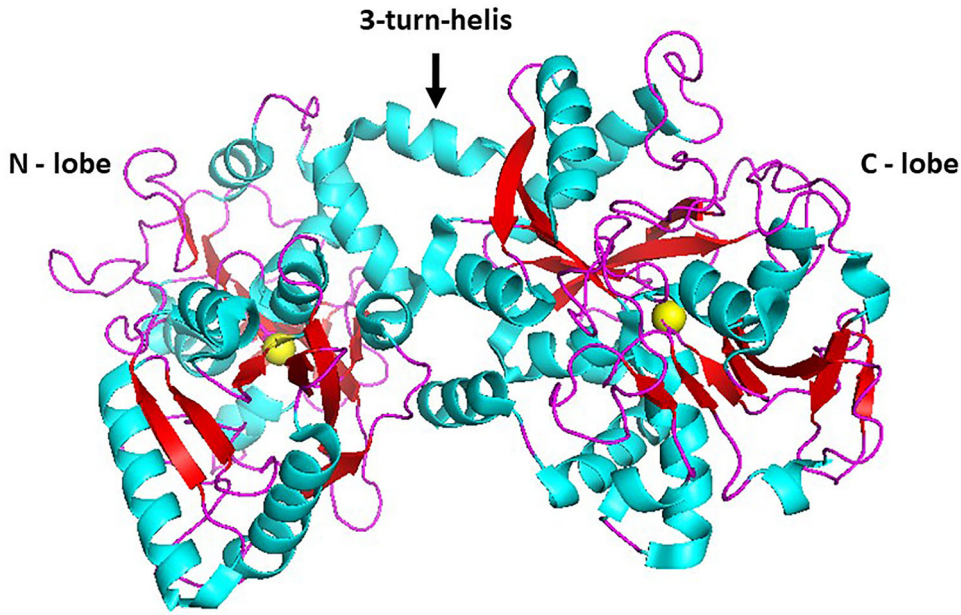

Figure 1. Three-dimensional structure representation of diferric human lactoferrin (1B0L Protein Data Bank code). The protein is presented in ribbon and ferric ions are presented as van der Waals spheres. Colors: Sheet – red, Loop – pink, Helix – blue.

formulas, and nutrition drinks (Legrand 2016; Artym and Zimecki 2013; Cornish et al. 2004; Lönnerdal 2016). Additionally, alluding to its antioxidant abilities, bLF is now commercially added not only to food products, but also to pharmaceuticals and cosmetics (Wang et al. 2019; Zimecki and Artym 2005; Latorre et al. 2010). Of note, bLF has been approved as a Generally Recognized as Safe (GRAS) compound by The United States Food and Drug Administration (FDA or USFDA) and as a dietary supplement by European Food Safety Authority (Cutone et al. 2020; Superti 2020).

Studies in preterm/term newborns and in older infants proved that oral application of bLF is not only safe, but due to its immunomodulatory activities may also protect against enteric infections. For example, Kaufman et al. (2021) conducted a prospective, dose escalation (100, 200, and 300 mg/kg/day) safety study of bLF dissolved in sterile water (100 mg/mL) for 30 days in preterm infants <1500 g, and noticed that supplementation of bLF up to 300 mg/kg/day was safe. In a randomized controlled trial on the safety and tolerance of a 28-day bLF supplementation with bovine whey and immunoglobulin (Ig) supplements carried out in free-living adults (n = 115) it has been evaluated that short-term bLF/Ig supplementation was generally well tolerated (Cox et al. 2017). In this investigation, measures of body composition remained stable and indices of glycemic control and blood lipids revealed fluctuations of <5% but were not significantly different in studied patients. On the contrary, Gaudian et al. (2008) stressed that patients with cow's milk allergy (CMA) were characterized by high immunoglobulin E (IgE)-mediated immune response elicited by bLF and that bLF may be classified as a strong milk allergen. However, the clinical significance of the presence of LF-specific IgE should be further demonstrated by positive oral challenges to bLF ingestion and positive skin prick tests to bLF.

Although the majority of the in vitro (Sessa et al. 2017; Lepanto et al. 2019) and in vivo studies (Hayes et al. 2006) on LF safety have been carried out using bLF, other studies indicated that the recombinant human LF (rhLF, talalactoferin) form is also safe to use (Hayes et al. 2006; Venkatesh and Rong 2008; Zhou et al. 2014). For example, the administration of rhLF (1.5–9 g/kg) per 15 days in humans with refractory solid tumors gave no adverse effects (Hayes et al. 2006). Moreover, the treatment with rhLF was also correlated with reduced average tumor growth rate in 7 of 10 patients compared to the time before LF administration. Similar results were shown when rhLF was administered to assess allergenicity in rats (2 g/kg) for 13 days (Zhou et al. 2014).

Contrarily, a number of clinical trials have not demonstrated the beneficial effects of rhLF administration. The first trial, a phase II/III randomized, double-blind, placebo-controlled study of the safety and efficacy of talactoferrin in patients with severe sepsis was suspended and Agennix stopped further recruiting and treating. This decision has been issued due to recommendation of The Data Safety Monitoring Board which was based on a review of available trial data and indicated that the 28-day mortality in the rhLF group was greater than in the placebo group (unpublished data). Another phase III study of oral rhLF administration in advanced non-small cell lung cancer patients (after two or more prior regimens) was conducted on a group of 742 patients (rhLF = 497, placebo = 245) (Paz-Ares et al. 2012). In this investigation rhLF did not extend overall survival or progression-free survival. The results of the following multicenter, randomized, placebo-controlled, phase II/III clinical study with the use of oral rhLF application in severe sepsis showed that oral administration of rhLF was not associated with a reduction in 28-day mortality in patients with severe sepsis and may even be harmful. This study was conducted on a group of 305 patients (rhLF = 153, placebo = 152) (Vincent et al. 2015).

Albeit several studies indicate that both bLF and rhLF are safe and may be regarded as nutritional supplements, the efficiency of their application needs further experiments and clinical trials. Moreover, subsequent studies should also take into consideration potential and possible adverse effects (e.g. skin rash, loss of appetite, fatigue, nausea, and constipation), as these have not been examined yet (Superti 2020; DI Mario et al. 2006).

Factors influencing LF bioavailability

LF Stability

The stability of LF may depend on a wide range of elements related to LF structure, e.g. iron saturation as well as environmental factors such as pH value, temperature, ionic strength, and the presence of other compounds such as proteins or polysaccharides (Legrand 2016; Wang et al. 2019; Lauterbach et al. 2016; González-Chávez, Arévalo-Gallegos, and Rascón-Cruz 2009). The method and time of processing, extraction and storage may also considerably affect the LF activity (Wang et al. 2019; Rastogi et al. 2016).

The binding of iron by LF changes its molecular conformation. LF may attach one iron atom to its both C- and N- lobes each and may exist in three basic forms: iron-saturated (holo-LF), iron-depleted (apo-LF) and intermediate, which binds iron only in either of the two lobes (mono-LF) (Voswinkel, Vogel, and Kulozik 2016; Steijns and Van Hooijdonk 2000; Bokkhim et al. 2013). Commercially available LF typically attains a saturation level of 10–20%. Importantly, the higher the saturation, the higher LF resistance to thermally induced denaturation and proteolysis.

LF is the only transferrin that possesses the ability to retain Fe^{3+} ions in a wide range of pH values although functional properties of LF have been documented within pH 2.0–8.0, and it was noted that the iron-binding ability of LF started to decline in pH 5.0–6.5 whereas at pH 2.0 only 10% of iron was attached (Wang et al. 2019; González-Chávez, Arévalo-Gallegos, and Rascón-Cruz 2009).

LF was found to be able to maintain its iron-binding capacity after its heating at temperatures ranging from 65 to 90 °C and ionic strength of about 0.01 or below (Sabra and Agwa 2020). Upon increasing the temperature, partial precipitation of LF was observed, with a notable decrease in its iron-binding capacity.

Current studies do not unequivocally define the exact degree of LF denaturation through thermal processes such as pasteurization, or ultra-high temperature processing (UHT) but indicate that high pressure processing, and producing powder form may reduce the loss of its functional properties (Wang et al. 2019). Conesa et al. (2009) and Paulsson et al. (1993) found that the bacteriostatic activity of bLF and hLF was retained after pasteurization (72 °C, 15 s); however, this functional property was lost after UHT treatment (135 °C, 4 s). In turn, Saito et al. (1994) found LF to be very thermally stable at a pH close to 4 and that it could be pasteurized or UHT-sterilized without a significant loss of physicochemical properties. The results obtained by Oria et al. (1993) demonstrated that heat treatment under conditions used in industrial processing does not significantly affect LF ability to interact with monocytic cells and to stimulate them, and that other milk proteins do not generally affect the interaction of LF with monocytes, which is the rationale for its use in infant formulas. In turn, Brisson et al. (2007) stressed that the binding of iron by LF improved its thermal stability and its isolation from whey. Noteworthy, they also observed that after the high-heat treatment, LF aggregates with cysteine-containing proteins (κ-casein, α-lactalbumin, and β-lactoglobulin) by thiol/disulfide-exchange reactions that lower its recovery from whey.

LF is capable of binding to various compounds such as DNA, proteins, proteoglycans, lipopolysaccharides, heparin, and metal ions e.g. Al^{3+}, Cu^{2+}, Mn^{3+}, and Zn^{2+} (Baker and Baker 2009) what may affect its stability and biological activity. Consequently, it has been evidenced that the number and type of sugars bound to LF through N-glycosylation impacts the LF ability to neutralize pathogens and influences its susceptibility to thermal denaturation and proteolysis (Van Veen et al. 2004; Barboza et al. 2012; Bengoechea et al. 2011). Furthermore, the formation of LF complexes and coacervates with different proteins or polysaccharides seems an interesting way to maintain its activity and structural integrity (González-Chávez, Arévalo-Gallegos, and Rascón-Cruz 2009; Baker and Baker 2009; Van Veen et al. 2004; De Kruif, Weinbreck, and De Vries 2004). Moreover, the LF stability may be increased by microencapsulation and PEGylation, which have been recognized as the most effective methods to protect LF properties so far (Wang et al. 2019). For example, in a recent study on encapsulated LF in rapeseed phospholipid–based liposomes it was showed that these liposomal particles have preserved 80% of LF in an intact form after 120 min of digestion, thus emphasizing that encapsulation can be an efficient tool for improving the bioavailability of LF through oral delivery (Vergara et al. 2020).

Digestion and absorption

Several in vitro and in vivo studies demonstrated that LF activities may be affected by gastric and intestinal digestion (Embleton et al. 2013; Baker and Baker 2009; Sreedhara et al. 2010). Interesting evaluations were conducted by Troost et al. (2001; 2002) who investigated digestion of either bLF or rhLF in adults. In the first study (Troost et al. 2001), 12 volunteers administered either apo-bLF with buffer, apo-bLF, or holo-bLF; it was observed that more than 60% of all bLF forms tested passed through the stomach in an intact form. In a further study (Troost, Saris, and Brummer 2002), it was noticed that rhLF was completely digested in the gastrointestinal (GI) tract of female ileostomy patients (n = 8). It is difficult to compare the differences between the digestion of bLF and rhLF due to varying factors such as risk of bias and incomparable study groups; however, these disparities may arise from different iron saturation levels of LF, which were described above (Bokkhim et al. 2013; Sabra and Agwa 2020).

Notwithstanding the outcomes of these studies, the gastric digestion may also demonstrate a positive impact on LF

activity because of the formation of antimicrobial LF derivatives such as lactoferrampin and lactoferricin in the stomach (Lizzi et al. 2016). In line, in in vitro and in vivo studies Kuwata et al. (1998; 1998; 2001) stressed that digestion of bLF can result in the formation of lactoferricin. In contrary, Furlund et al. (2013) did not detect bioactive lactoferricin in human gastric and duodenal juices in in vivo and in vitro digestion of bLF. Nevertheless, the authors emphasized that the gastric bLF digestion may generate more than 40 different peptides (6–22 amino acid restudies) with small molecular weight until about 2.5 kDa, whilst in vitro digestion, depending on the used conditions, may produce from 4 to 33 peptides.

In turn, other investigations on newborns, who do not have fully developed GI system, revealed that LF (bLF/hLF) was not completely digested in this group and retained its iron-binding capacity (Spik et al. 1982; Gisbert, McNicholl, and Gomollon 2009). Thus, these studies allow to assume that the benefits of LF oral application will be far more noticeable in infants compared to adults.

Bearing in mind that LF may have altered properties because of GI digestion, some studies defined the possibilities of intravenous LF administration. One of the analyses was conducted on mice (Prieels et al. 1978) and rats (Peen et al. 1998), and evaluated the distribution of hLF after intravenous injection. A higher hLF plasma concentration was observed in treatment groups compared to controls; however, it was also indicated that hLF was quickly cleared from the circulation, and then from the liver. Concurrently, in another investigation on bLF tissue accumulation, following intragastric intubation in mice, it was shown that the accumulation of bLF was not limited to liver but also observed in kidneys, spleen, gall bladder, or brain (Fischer et al. 2007). Considering that both bLF and hLF revealed a short half-life in plasma, frequent intravenous administration would be needed to attain a therapeutic effect. Therefore, oral application of LF is a more reasonable option as it is the most safe and noninvasive way for providing LF (Bokkhim et al. 2016; Yao et al. 2013; Kilic et al. 2017). However, further investigations shall conclusively clarify whether GI digestion may maintain LF basic or modified functions in humans, and if peptides that are generated during digestion may have subsequent impact on immune cells, or microbial pathogens.

Lactoferrin receptors

Numerous functions of LF depend on its target cells and on the presence of specific receptors (LFRs) at their surfaces. One of LFRs is 100% homologous to the intelectin 1 receptor (ITLN1, INTL1) (Liao et al. 2007). This receptor, alternatively named LF receptor (LFR) or Omentin-1, was initially found in the pig (Suzuki and Lönnerdal 2002) and human intestine brush-border membrane (Liao et al. 2007; Gíslason et al. 1993). Further analysis in the mouse model showed a significant expression of ITLN1 in the major organs and multiple systems, such as brain, heart, lungs, testes and ovaries, while in the GI tract it was found in esophagus, stomach, liver, pancreas, and small and large intestine (Suzuki and Lönnerdal 2004). In humans, the presence of ITLN1 was observed in Paneth and goblet cells (Suzuki, Lopez, and Lönnerdal 2005), biliary epithelium (Mancinelli et al. 2018), and adipose tissue (De Souza Batista et al. 2007). An interesting evaluation was conducted by Shin et al. (2008), who observed that bLF possesses higher affinity to recombinant human intelectin receptor (rhITLN) compared to hLF. Moreover, some evaluations on ITLN1 confirmed decreased expression of this receptor in obese and overweight people (De Souza Batista et al. 2007), and indicate its possible role in insulin-sensitivity of the adipose tissue. Additionally, a recent study indicated potential function of ITLN1 in the prevention of inflammation-induced osteoporosis in mice (Rao et al. 2018).

Low-density lipoprotein (LDL) receptor-related protein (LRP), termed LRP1 (Lin and Hu 2014; Herz and Marschang 2003) also belongs to the group of LFRs. This receptor is extensively distributed in different tissues including hepatocytes, smooth muscle cells, fibroblasts and neurons. LRP1 is a multi-functional signaling receptor involved in the transport and metabolism of cholesterol and apolipoprotein E (apoE)-containing lipoproteins (Herz and Marschang 2003). Of note, LRP1 is also capable of transporting LF from plasma to the liver in a rat model (Meilinger et al. 1995). LRP1-mediated transport of LF was also suggested in an in vitro model of the blood-brain barrier (BBB) (Fillebeen et al. 1999). Moreover, it has been evaluated that the mitogenic effect of LF in osteoblastic cells is mediated mainly through LRP1, but the anti-apoptotic actions of LF may be LRP1-independent (Grey et al. 2004; Cornish et al. 2006).

Noteworthy, there are several reports on the overexpression of LRP1 in numerous human diseases, e.g. Alzheimer's disease (Meilinger et al. 1995; Pires et al. 2012; Donahue et al. 2006). Furthermore, in a recent study on APP/PS1 transgenic mice it has been observed that silencing of brain LRP1 led to exacerbated neuroinflammation and enhanced production of pro-inflammatory cytokines such as interleukin 1 (IL-1β), tumor necrosis factor (TNF), and IL-6 (He et al. 2020). Therefore, further analyses assessing the anti-inflammatory potential of LF shall also investigate the linkage between LF and LRP1.

Besides the above, there are other LFRs, such as heparan sulfate proteoglycans (HSPGs) (Milewska et al. 2014), asialoglycoprotein receptor (ASGPR) (Cutone et al. 2020; Prieels et al. 1978), cluster of differentiation 14 (CD14) (Rawat et al. 2012; Sanui et al. 2017), CD184 (Chea et al. 2018; Seo et al. 2019), Toll-like receptors (TLR2 and TLR4) (He et al. 2020; Zhang et al. 2009; He, Lawlor, and Newburg 2016). Thus, LF may trigger diverse cellular effects depending on the receptor it binds to. Furthermore, endocytosed LF may be aimed at the nucleus where it could bind with DNA and serve as a transcriptional activator (Bennett et al. 1983; He and Furmanski 1995). It is noteworthy that due to the low levels of LF in the blood and internal tissues of healthy individuals in which LF likely reflects neutrophil activity, its binding with receptors expressed in organs such as brain,

heart, liver, pancreas, or small and large intestines is very limited.

Role of LF

Immunomodulatory and anti-inflammatory activity of LF

The majority of scientific evidence related to immunomodulatory and anti-inflammatory activity of LF comes from studies on LF deficiency in human and mouse models as well as from LF application in in vivo models. The results of these studies proved that LF may affect both innate and acquired immune response and modulate acute and chronic inflammation (Kruzel et al. 2006; García-Montoya et al. 2012). Moreover, the expression and secretion of LF on mucosal surfaces and its release at inflammatory sites have established its role as an agent that may be helpful, e.g. in viral and bacterial infections. LF can modify specific and nonspecific expression of antimicrobial proteins, and through TLRs may bind with pathogen associated microbial patterns (PAMPs) as well as mediators from innate immune cells that subsequently impact adaptive immune responses. Several in vivo and in vitro studies revealed that the positive charge of LF allows for binding with negatively charged regions of different immune cells and various pathogens, thereby triggering signaling pathways that result in cellular responses such as activation, differentiation and proliferation (García-Montoya et al. 2012; Arnold et al. 1982; Elass-Rochard et al. 1998; Van Hooijdonk, Kussendrager, and Steijns 2000; Eriksen et al. 2010). A detailed description of these mechanisms will be presented in the following paragraphs (Sections "Antiviral effects of LF" and "Antibacterial properties of LF").

Data available from in vivo and in vitro models indicates that LF is capable of cooperating with granulocytes, lymphocytes, macrophages, and Natural Killers (NK), thereby enhancing their functions, such as migration, proliferation, maturation, cytokine production and cytotoxicity (Shau, Kim, and Golub 1992; Zaczyńska et al. 2014; Wakabayashi et al. 2014). Noteworthy, the action of both hLF and bLF may not only increases the number of cytotoxic cells essential to innate immune system, for instance NK, but also affects cells of the adaptive immune system (Marek et al. 2009; Groot et al. 2005; Saidi et al. 2006; Actor, Hwang, and Kruzel 2009). Consequently, LF may promote the maturation of T-cell precursors into competent helper cells and may influence the differentiation of immature B-cells into efficient antigen-presenting cells (APCs). Also, the application of bLF to mitogen activated T-cells lowers total cytokine secretion (Kobayashi et al. 2005). In addition, as demonstrated in other studies, LF may work as a chemoattractant for immune cells, such as polymorphonuclear neutrophils (PMNs) and APCs, e.g. the application of rhLF chemoattract human monocytes in vitro and activate the recruitment of mouse phagocytes in vivo (Puddu, Valenti, and Gessani 2009; de la Rosa et al. 2008).

On the basis of the assessment of the expression of cytokines in in vivo and in vitro studies, it has been evaluated that LF can either increase (Legrand 2016; Actor, Hwang, and Kruzel 2009; Sorimachi et al. 1997; Zimecki et al. 2001) or inhibit (Chea et al. 2018; Kruzel et al. 2002; Håversen et al. 2002) the secretion of pro-inflammatory cytokines such as TNF, IL-6, and IL-1β, as well as augment the secretion of IL-12, which is secreted by APCs upon facing pathogenic agents, and stimulate the secretion of anti-inflammatory IL-10. Moreover, LF is able to change the balance of Th1 and Th2 cellular subsets, thereby limiting excessive inflammatory responses (Fischer et al. 2006), and due to iron scavenging properties may also be helpful in lowering reactive oxygen species (ROS) production (Siqueiros-Cendón et al. 2014). Noteworthy, such variety of LF activities may highly depend on the host immune status.

Besides that, LF may also play a significant role in the inhibition of neutrophil extracellular traps (NETs), which protect against inflow of pathogens but may be also correlated with the development of inflammatory and autoimmune diseases (Okubo et al. 2016). The authors of one study observed that normally expressed LF can bind to NETs via charge-charge interactions, which usually occurs prior to NETs release, but endogenous LF may be insufficient to inhibit excess NETs in pathological conditions. Simultaneously, it should be stated that uncontrolled inhibition of NETs may have implications on increased susceptibility to infections (Okubo et al. 2016; Bennike et al. 2015). On the other hand, LF itself represents high antimicrobial activity, so further studies shall examine the correlation between LF and NETs during inflammatory processes.

The mechanisms of LF cooperation with immune cells are still largely unknown and may be correlated with the interactions of LF with gut associated-lymphoid tissue as well as with intestinal microbiota (Lönnerdal 2016; Ganal-Vonarburg and Duerr 2020). Moreover, some studies demonstrated potential benefits of LF application while administered with probiotics (Vega-Bautista et al. 2019; Superti 2020), or even proved its prebiotic effect given alone (Chen, Ku, and Chu 2014; Chen, Liu, et al. 2017). Thus, the anti-inflammatory and immunomodulatory effects of LF supplementation shall be further examined in different chronic diseases such as inflammatory bowel disease (IBD).

Inflammation and iron homeostasis

During inflammation LF exerts anti-inflammatory activity against IL-6, thus up-regulating ferroportin (Fpn) and transferrin receptor 1 (TfR1) and down-regulating ferritin (Ftn), key players in iron and inflammatory homeostasis (IIH). In turn, some evaluations on LPS-inflamed macrophages compared to uninflamed have proven that the absence of bLF resulted in an increase of IL-6 levels related with a decrease of TfR, Fpn, and ceruloplasmin, whereas an intracellular Ftn and iron concentration were up-regulated (Miller 2013; Paesano et al. 2012). Simultaneously, the addition of bLF in inflamed macrophages reversed changes caused by IL-6, indicating its pivotal role in iron homeostasis and inflammation (Cutone et al. 2017).

Moreover, some evaluations on pregnant women suffering from iron deficiency anemia (IDA) and affected by

hereditary thrombophilia showed encouraging effects of LF oral application on selected hematological values including increased levels of hemoglobin, red blood cells, or hematocrit with simultaneous reduction of IL-6 (Lepanto et al. 2018; Rosa et al. 2017; Paesano et al. 2009; Paesano, Berlutti, Pietropaoli, Pantanella, et al. 2010).

In turn, in an interesting in vivo study comparing the effects of apo-bLF, holo-bLF and iron sulfate ($FeSO_4$) supplementation in Kenyan infants it has been evaluated that both LF forms may facilitate iron absorption (Mikulic et al. 2020). Additionally, the authors noticed that oral application of holo-bLF enhanced iron uptake comparatively to $FeSO_4$, whilst the addition of apo-LF to a test meal containing iron from $FeSO_4$ considerably increased (+56%) iron absorption.

Besides promising effects of LF application, the complexity of interactions between LF and iron remains largely unknown, thus it should be further precisely assessed with reference to iron-dependent pathologies in humans. Moreover, it is worth noting that despite encouraging results of LF role in inflammatory processes yielded in in vitro studies, there are also several investigations with inconsistent results, due to number of factors e.g. variable responsiveness of different cell lines to PAMPs (Cutone et al. 2017; Rosa et al. 2017). For example, phagocytes are very responsive to LPS, whereas epithelial cells represent considerably lower immune response to the same stimulus. Moreover, pro-inflammatory cytokines are secreted by epithelial cell monolayers when challenged with invasive pathogens, while no considerable levels are found following adherent bacteria challenge (Rosa et al. 2017). Thus, it is also indisputably very important to distinguish and comprehend the exact effects of LF on different cell lines in order to prevent the inappropriate interpretation.

Antiviral effects of LF

Currently, the majority of literature evaluating the antiviral activity of LF implies that the protein protects viral entry to cells rather than acting on further viral replication.

Surface of both, bLF and hLF possesses a comparatively high net positive charge and may influence anionic compounds such as glycosaminoglycans (GAGs) expressed on host cells (Baker and Baker 2009; Lang et al. 2011). GAGs are polysaccharides including five main classes: heparan-, chondroitin-, dermatan-, keratan sulfate, and hyaluronic acid, that are associated with binding proteins to form proteoglycans (Wakabayashi et al. 2014; Lang et al. 2011; Pietrantoni et al. 2015). Cell-surface heparan sulfate proteoglycans (HSPGs) are co-receptors used by pathogens such as bacteria, and certain viruses e.g. Herpesvirus, Human immunodeficiency virus (HIV), Echovirus, Adenovirus 2 and 5 (Lang et al. 2011; Pietrantoni et al. 2015). Another group of viruses using HSPGs are enveloped viruses such as Toscana Virus (TosV) or SARS-CoV. Despite the fact that viruses often require several binding mechanisms to enter the cell, LF ability to bind with HSPGs may result in limiting, or preventing virus cell entry (Pietrantoni et al. 2015). Furthermore, it was stated that LF may also inhibit viral infections by binding to dendritic cell-specific intercellular adhesion molecule 3-grabbin non-integrin (DC-SIGN) (Groot et al. 2005) as well as LDL receptors (Chen, Fan, et al. 2017).

Potential role of LF in COVID-19

Due to the global severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-triggered COVID-19 pandemic, with more than 100 million people infected worldwide and over 2 million fatalities as of February 10th 2020, auxiliary therapies aimed at reducing the spread of disease are urgently required.

SARS-CoV-2 is made up of four major structural proteins: spike (S), envelope (E), nucleocapsid (N), and membrane (M), approximately sixteen nonstructural proteins (nsp1–16), and five to eight accessory proteins. The S protein plays a vital role in viral attachment to the target cell and is crucial for further fusion and transport into the cell (Figure 2a). It comprises two subunits, which respectively recognize ACE2 as its receptor (S1) and mediate the fusion between SARS-CoV-2 and target cell (S2) (Lang et al. 2011; Jia et al. 2005). ACE2 is highly expressed in multiple epithelial cell types including human lung alveolar epithelial cells. It is also present in enterocytes of the small intestine and the brush border of the proximal tubular cells of the kidney (Lang et al. 2011; Jia et al. 2005). Other important factors influencing the viral entry are HSPGs and proteases such as transmembrane protease serine 2 (TMPRSS2) (Hoffmann et al. 2020; Tavassoly, Safavi, and Tavassoly 2020). Aforementioned, HSPGs provide the first anchoring sites and ensure adhesion of virus to cell membrane, whilst TMPRSS2 induces further cleavage of the S glycoprotein to enhance efficient viral transmission (Tiwari et al. 2020). Since the presence of TMPRSS2 has been noticed only in ACE2+ cells and its role in virus attachment and fusion is significant, there are also some suggestions that SARS-CoV-2 could pass into the cell using alternative pathways e.g. cathepsin B/L7 (Sungnak et al. 2020).

To date, only Lang et al. (2011) demonstrated that LF can inhibit SARS-CoV pseudovirus (spike protein-bearing pseudotyped virus) infection in a dose-dependent manner (Moore et al. 2004). The authors indicated that moieties of LF, by attaching to HSPGs, are capable of limiting the binding of viruses to ACE2 receptor proteins, thereby inhibiting further viral infection. In turn, in a recent preprint, Zhang et al. (2020) demonstrated that inhibition of HSPGs by heparin, a heparan sulfate-related glycan, or genetic ablation of their biosynthetic enzymes is associated with diminished ACE2-mediated coronavirus infection in murine leukemia viruses expressing spike proteins from SARS-CoV and SARS-CoV-2.

Very recently, Serrano et al. (2020) investigated the potential role of liposomal lactoferrin (LLF) in 75 patients with typical symptoms of COVID-19 who were tested positive to IgG/IgM. In this study LLF (256–384 mg/day) was administered with liposomal zinc (due to reduced levels of this microelement in COVID-19 patients) and its potential role in lowering viral entry as well as fusion with the cell.

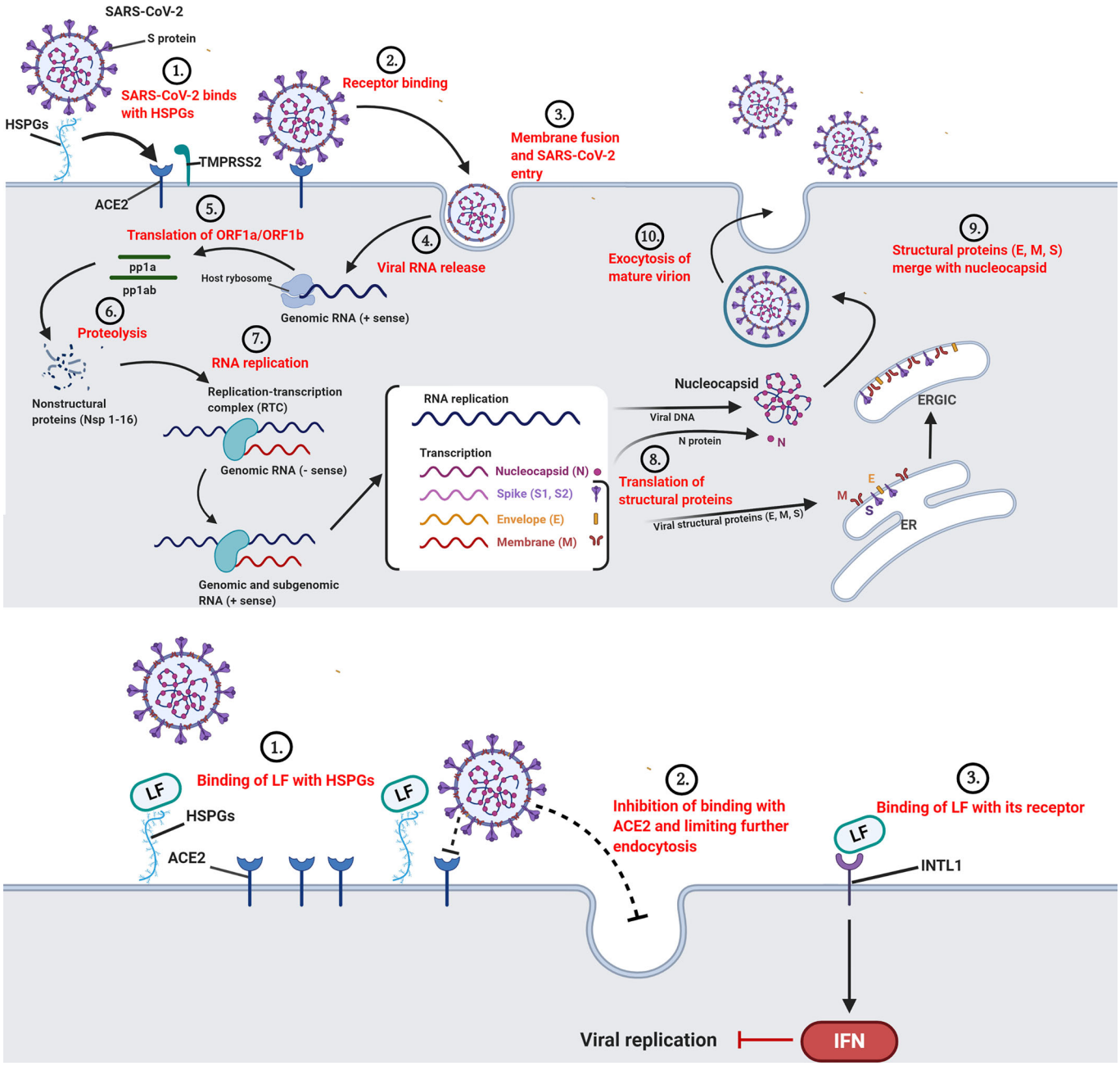

Figure 2. LF and SARS-CoV-2. (a) SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) via its spike protein (S) (Step 1). Proteases such as Transmembrane protease serine 2 (TMPRSS2) and cathepsin activate viral spikes to dissociate into subunits (S1 and S2), which respectively, S1: recognizes ACE2 as its receptor, S2: mediates the fusion between SARS-CoV-2 and target cell (Step 2). After receptor binding, the viral RNA is unveiled in the cytoplasm (Step 3). Ribosomal frameshift during translation elongation allows to produce ORF1a and ORF1ab-encoded replicase protein (pp) (Step 4). PP1a and PP1ab encoded by ORFs (1a and 1b) undergo proteolytic cleavage to constitute 16 nonstructural proteins (Nsp 1-16) (Step 5) that form the RNA replicase-transcriptase complex (RTC) (Step 6). During replication, full-length (−) genomic RNA is used as a template for full-length (+) genomic and subgenomic RNA. Viral structural proteins (E, M, S) are translated from the RNA and inserted into the endoplasmic reticulum, while genomic RNA with N protein form nucleocapsid in the cytoplasm (Step 7). Budding of nucleocapsids with structural proteins in the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) allows to release new virions (Step 8) through exocytosis (Step 9). Created with biorender.com. (b) Potential anti-viral activities of lactoferrin (LF). LF binds to heparan sulfate proteoglycans (HSPGs) and thus prevents the binding of SARS-CoV-2 with angiotensin-converting enzyme 2 (ACE2); thereby limits further endocytosis and viral replication (Steps 1 and 2). LF may also bind with its receptor e.g. Intelectin-1 receptor (INTL1), thus inducing intracellular signals such as the activation of Interferon (IFN), which inhibits viral replication as well. Created with biorender.com

The authors evaluated that LF has shown to be effective in reducing several symptoms, e.g. such as dry cough, muscular pain, or headache after 4–5 days of treatment, whereas the influence of LF on loss of taste and smell was not significant. The authors also suggested that the use of LF nasal drops, mouth spray, or nebulization may also be beneficial in the treatment of COVID-19.

Based on the studies discussed above, we propose a simplified diagram showing potential LF activity (Figure 2b). Noteworthy, although in several studies on other viruses it has been evidenced that LF may bind to their structure (Sano et al. 2003; Pietrantoni et al. 2003; Weng et al. 2005), and some researchers indicate that LF interacts with the ACE2 receptor, currently it is yet unknown if LF indeed

binds to SARS-CoV-2, or ACE2. Indisputably, a growing interest on HSPGs inhibitors in SARS-CoV-2 treatment (Tavassoly, Safavi, and Tavassoly 2020) shows that further research on LF role in viral infections is urgently needed. A wide spectrum of LF activity also allows to assume that LF plays a significant role inside the cells, and is capable of preventing the cytokine storm, thus avoiding systemic, lung or intestinal iron homeostasis disorders (Campione et al. 2020). Therefore, it is of a paramount importance to evaluate the implications of LF application at early stages of viral infection in humans.

Antibacterial properties of LF

The antibacterial activity of LF has been described in in vitro and in vivo studies on both G(+) and G(-) bacteria (González-Chávez, Arévalo-Gallegos, and Rascón-Cruz 2009; Lizzi et al. 2016). Of note, LF may act through iron-dependent, or iron-independent mechanisms. LF is capable of binding or chelating iron, which inhibits growth of bacteria (Sabra and Agwa 2020) and initially it was thought that LF antibacterial activity is linked with its affinity to Fe^{3+} ions, but more recent evidence shows that not only apo-LF, which can acquire iron from pathogens, but also holo-LF possesses the ability to inhibit the growth of certain bacterial strains. For instance, apo-LF, by chelating iron, was shown to be able to inhibit *Pseudomonas aeruginosa* adhesion and biofilm formation (Singh et al. 2002; Berlutti et al. 2005).

The antibacterial activity of LF may also result from its interaction with both lipoteichoic acid (LTA) of G(+) bacteria and LPS which is present in G(-) bacteria. Moreover, it was shown that the positively charged N-lobe of LF may also avert the interaction between LPS and the cations (Mg^{2+} and Ca^{2+}) essential for bacterial growth, which leads to LPS release from the cell wall and increased permeability of the membrane, thereby incurring the damage of pathogens (Roussel-Jazédé et al. 2010; Morgenthau, Beddek, and Schryvers 2014; Ostan, Morgenthau, et al. 2017). Aside from this, certain Gram-negative bacteria such as *Neisseria* spp. and *Moraxella* sp. have developed defensive mechanisms against peptides and are capable of acquiring iron from the host during infection (Brooks, Arutyunova, and Lemieux 2014; Hall and Mah 2017). Hence, LF application may not represent its antibacterial properties in these cases. For example, Ostan, Yu, et al. (2017) have recently demonstrated that hLF interacts with lactoferrin binding protein B (LbpB), which is a bi-lobed outer membrane-bound lipoprotein present in some of Gram-negative bacteria such as *Neisseria meningitides* and which exhibits a significant role in protecting bacteria against cationic peptides due to large regions rich in anionic residues in the N-terminal lobe through binding with C-lobe of hLF.

Finally, it was evidenced that LF can inhibit the signal transduction between LPS and the CD14–TLR complex (Siqueiros-Cendón et al. 2014; Peterson and Artis 2014). Consequently, this interaction protects against overexpression of LPS-induced inflammation and may be helpful in septic shock treatment. Additionally, LF is capable of activating dendritic cell (DC) differentiation into tolerogenic DC (Perdijk et al. 2018). This in turn leads to reduced reactivity to TLR ligands and diminished production of pro-inflammatory cytokines (Puddu et al. 2007).

Furthermore, the linkage between LF and LPS strengthens the effects of other antibacterials, such as lysozyme, which displays high enzymatic activity on both G(+) and G(-) bacteria, especially when administered with LF (Żelechowska, Agier, and Brzezińska-Błaszczyk 2016; Gajda and Bugla-Płoskońska 2014). Noteworthy, LF is not only capable of counteracting harmful bacteria strains but also can stimulate the growth of host beneficial bacteria such as *Lactobacilli* spp., and therefore may be potentially regarded as prebiotic (Superti 2020; Superti and De Seta 2020).

Antifungal properties of LF

LF and LF-derived peptides have demonstrated robust antifungal and anti-carcinogenic activity (Legrand 2016). Due to iron-binding activity, LF was capable of inhibiting the growth of some *Candida* species (*C. albicans, C. krusei and C. tropical*) (Al-Sheikh 2009), and *Aspergillus fumigatus* (Mohanraj and Sivasankar 2014). It was demonstrated that LF may increase fungal membrane permeability and thereby is capable of neutralizing fungal cells. These activities have been demonstrated also in vivo in mice, in which hLF protected against experimental oral infection with *C. albicans* (Velliyagounder et al. 2015). Moreover, co-administration of LF with antifungals has reinforced their inhibitory effect on *Candida* species (Venkatesh and Rong 2008; Fernandes and Carter 2017).

Anti-tumor properties of LF

In several in vitro and in vivo studies it was noted that LF and its derivatives exhibit anti-tumor effects through multiple mechanisms inducing apoptosis on various cancer cell lines, e.g. intestinal (HT-29) (Jiang and Lönnerdal 2017), gastric (AGS) (Pan et al. 2013), lung (A549), or breast (MDA-MB-231) (Tung et al. 2013). For example, in the latter LF has been demonstrated to inhibit the activity of cyclin-dependent kinases (CDK2 and CDK4) and it was suggested that LF may induce growth arrest at the G1 to S transition of the cell cycle (Damiens et al. 1999). Also, the ability of LF and its derivatives to upregulate caspase 3 expression were found in in vivo and in vitro models (Sabra and Agwa 2020; Damiens et al. 1999; Oh et al. 2004). In turn, in VEGF-A165-induced lung tumor transgenic mice, the reduction of vascular endothelial growth factor (VEGF) after oral administration of bLF (300 mg/kg of body weight; 3 times a week for 1.5 month) was observed (Tung et al. 2013). The reduced expression of VEGF may limit the transport of nutrients and oxygen to the tumor, and therefore slow down the speed of tumor growth. The reduction of VEGF was also noted in the rat model by oral application of bLF (Norrby et al. 2001).

It is worth noting that bLF suppresses the expression of a key protein in cancer progression, named survivin

(baculoviral inhibitor of apoptosis repeat-containing 5, BIRC5), in different cancer lines (Gibbons, Kanwar, and Kanwar 2015).

Clinical studies on LF application in humans

Investigations concerning LF supplementation in humans are quite vague. For example, although some evaluations have confirmed that oral application of bLF may be effective in iron deficiency anemia in pregnant women (Paesano, Berlutti, Pietropaoli, Goolsbee, et al. 2010; Rezk et al. 2016), and it is expected that LF may be a good source of iron, current studies do not unambiguously define whether LF promotes the absorption of iron (Wang et al. 2019). Indisputably, LF may cooperate with immune cells and modulate their functions (Legrand 2016; Lönnerdal 2016). Some studies suggest that higher concentration of LF in human colostrum is linked with ensuring protection to breast-fed infants against infections and inflammation processes (Zimecki and Artym 2005). However a recent randomized clinical trial (RCT) by The Enteral Lactoferrin in Neonates (ELFIN) group, evidenced that supplementation of bLF (150 mg/kg per day; maximum 300 mg/day) was not associated with a reduced infection rate in preterm infants (Griffiths et al. 2019). The ELFIN trial findings contradict available evidence, stressing that earlier reviews were relatively small and include methodological weaknesses that might have impacted on results. In another study comparing LF levels in preterm infants with and without late-onset sepsis (LOS) it has been evaluated that plasma LF levels were comparable in term and preterm infants with ($n = 32$) and without ($n = 53$) LOS (Strunk et al. 2020). Consequently, the authors pointed out that endogenous LF expression in preterm infants does not seem to influence the risk of developing LOS. In turn, in the newest meta-analysis of over 5000 infants, LF application did not improve death or major morbidity, although the authors have stressed out that they cannot exclude that some LF products might have diverse efficiency because of statistical heterogeneity observed in included trials (Tarnow-Mordi et al. 2020). Moreover, it was concluded that in further studies LF doses should be differentiated (up to 600 mg/kg per day), and more attention should be paid to products of demonstrated biological activity.

Lactoferrin in inflammatory bowel disease

With a view of multiplicity of LF effects on digestive and immune system, its potential role also seems to be relevant in IBD. IBD is classified as chronic relapsing condition of the GI tract, of which the two main forms are ulcerative colitis (UC) and Crohn's disease (CD) (Holtmann, Ford, and Talley 2016). The pathogenesis of IBD involves environmental and genetic factors, altered microbiota, and excessive immune response (Fichna 2016). The intestinal inflammation associated with disrupted homeostasis leads to loss of intestinal barrier integrity and increased production of pro-inflammatory cytokines (e.g. IL-1, IL-6, TNF, chemokine (C-C motif) ligand 2) and other factors such as NF-κB (Peterson and Artis 2014; Zielińska et al. 2019). Until now, the majority of studies on the role of LF in IBD were conducted in vitro and in animal models.

Regarding in vitro studies, in a model of inflamed human macrophage-like THP-1 cells (mimicking the infiltrating macrophages in IBD) which was stimulated with a mixture of LPS and IFN-γ, or LPS alone, it has been demonstrated that application of bLF not only led to reduced inflammation and rebalanced iron homeostasis in these cells but was also involved in a phenotype shift from pro-inflammatory to tolerogenic macrophages (Cutone et al. 2017). In another study, the effects of bLF application on adherent invasive *Escherichia coli* strain LF82 adhesion and entry into Caco-2 cells as well as cultured mucosal explants from CD patients infected with LF82 have been evaluated (Bertuccini et al. 2014). The authors demonstrated that bLF had no effect on bacterial adhesion, but strongly prevented invasion of Caco-2 cells in a dose-dependent manner. Moreover, it has been stressed that bLF inhibited the expression of IL-6, IL-8, and TNF in both cultured and CD-derived intestinal cells.

The anti-inflammatory and immunomodulatory activities of LF application have also been studied in animal models. For example, in the dextran sulfate sodium (DSS)-induced rat model, oral administration of bLF (200 mg/kg per 7 days) attenuated colitis in a dose-dependent manner (Togawa, Nagase, Tanaka, Inamori, Nakajima, et al. 2002). The results of this study also demonstrated that bLF may weaken the activation of pro-inflammatory cytokines, diminish inflammation, and reduce activity of myeloperoxidase (MPO), which is a lysosomal protein used as a biochemical marker of neutrophil infiltration. The ability of bLF to diminish pro-inflammatory cytokines such as IL-1β and TNF with concurrent upregulation of anti-inflammatory IL-4 and IL-10 was also found by the same authors in the 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis rat-model (Togawa, Nagase, Tanaka, Inamori, Umezawa, et al. 2002). Oral administration of hLF also resulted in reduced inflammation in DSS-induced model of colitis in mice compared to control group treated by bovine serum albumin or water (Håversen et al. 2003). In this investigation, the shortening of the colon was significantly less noticeable in the hLF-treated mice compared to control. In another study in DSS-induced colitis in mice, the anti-inflammatory role of oral LF treatment was linked with decreased mRNA levels of IL-1β and TNF, particularly when apo-LF form was used (Li et al. 2013).

With regards to the effect of bLF on the damaged epithelial barrier, the results of some in vitro (Zhao et al. 2019) and animal (Wu et al. 2014) studies revealed improvement of the intestinal epithelial barrier function. In a study on human intestinal epithelial crypt cells (HIECs) and Caco-2 cells it has been demonstrated that bLF significantly increased the expression of three tight junction proteins; claudin-1, occludin, and zonula occludens-1 (ZO-1) (Zhao et al. 2019). Additionally, by arresting cell cycle at the G2/M-phase, bLF promoted cell growth.

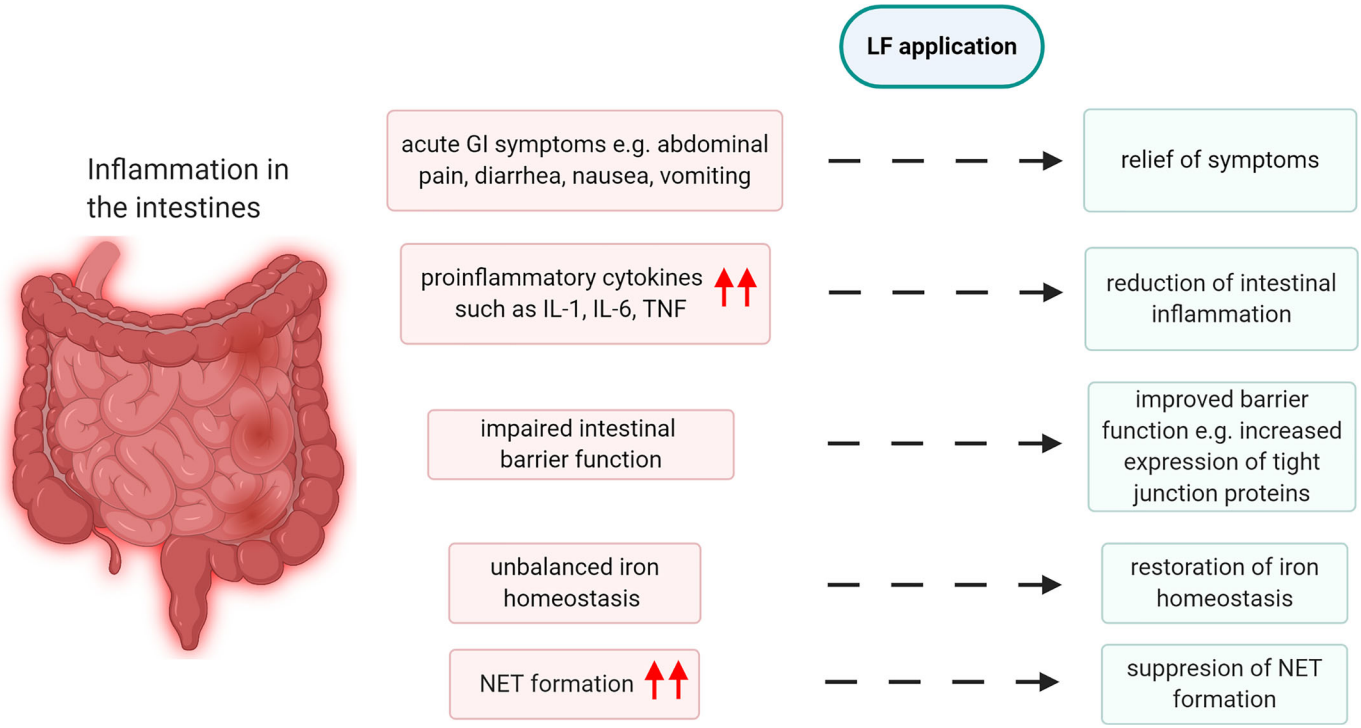

Figure 3. The most common inflammatory bowel disease (IBD) symptoms and theoretical benefits of lactoferrin (LF) application. Gastrointestinal (GI); interleukin (IL); tumor necrosis factor (TNF); neutrophil extracellular traps (NETs). Created with biorender.com.

The complex interactions of both endogenous and administered LF with immune cell as well as the impact of LF on intestinal mucosa in IBD patients is very poorly investigated. Moreover, considering that endogenous LF is exocytosed by neutrophils at the site of inflammation (Legrand 2016), the activity of administered LF may be significantly lowered in IBD patients. Thus, limited data with inconclusive results do not permit to consider LF as a supplement, even though it could potentially be recommended in IBD, particularly during flare-ups, or as a form of self-medication. However, the lack of studies cannot overshadow its promising multifunctional character that needs to be further elucidated (Figure 3). For example, a significantly higher abundance of NET formation was observed in intestinal biopsies of UC patients compared with controls (Bennike et al. 2015).

LF may also play an important role in the context of anemia, which is one of the most common extraintestinal manifestations of IBD, most often linked with iron deficiency and developed as a result of an ongoing inflammation in these patients (Paesano et al. 2014; Jimenez and Gasche 2019). Recent studies demonstrated that oral iron therapy may induce inflammation and alter the microbiota composition (Lee et al. 2017; Zimmermann et al. 2010), and thus lead to flare-ups. Moreover, oral iron exacerbated colitis and promoted carcinogenesis in animal models of colitis (Seril et al. 2002; Mahalhal et al. 2018). Also, it was shown that oral ferrous sulfate may significantly increase oxidative stress in human colitis (Goldsmith and Sartor 2014). Therefore, current approach to treat IDA in IBD patients is mainly focused on intravenous administration of iron (Jimenez and Gasche 2019). However, as discussed above, oral bLF application in ID/IDA women may increase total serum iron levels comparable to ferrous sulfate ($FeSO_4$) therapy, but with no significant adverse GI effects (Paesano et al. 2014). Moreover, it was demonstrated that bLF – unlike ferrous sulfate – can decrease the levels of IL-6 in serum (Zimecki et al. 2003; Paesano et al. 2014; Hunninghake et al. 2010). Altogether, there is a clear linkage of bLF efficacy with anemia in IBD. However, it should be noted that LF application does not directly increase iron serum levels, but may participate in complex indirect immune-modulating effects, which should be precisely examined in further studies (Lee et al. 2017; Abu Hashim, Foda, and Ghayaty 2017).

Finally, although there are no studies on LF supplementation in IBD patients, investigations into alleviation of symptoms typical for IBD have been undertaken. The latest investigation conducted by Mikulic et al. (2020) indicated that LF may be a safe way to improve iron intake and decrease the prevalence of anemia. Moreover, in a RCT conducted in 260 infants it has been proven that LF may even diminish the occurrence of diarrhea-related illnesses ($p < 0.05$) (Chen et al. 2016). Also, the most recent RCT demonstrated that LF application may be useful for the prevention of acute GI symptoms e.g. abdominal pain, diarrhea, nausea, and vomiting among childcare workers (Mizuki et al. 2020).

Hence, further research should also clarify whether LF oral supplementation could become an adjuvant to the diet of IBD patients, e.g. for simultaneous fulfillment of iron deficiencies and alleviation of intestinal inflammation.

LF as a potential marker in IBD

Another reason supporting the need for extensive clinical studies on LF applies to its role as a potential marker for

IBD (Borkowska et al. 2015; Walker et al. 2007; Langhorst and Boone 2012). Several studies confirmed that fecal LF (measured in μg per gram of stool) is significantly higher in IBD patients, which may imply its role in chronic inflammation (Borkowska et al. 2015; Buderus, Boone, and Lentze 2015; Dai et al. 2007). In one investigation, fecal LF levels were substantially higher in active UC (1126 ± 431 μg/g, n = 42) and CD (1035 ± 457 μg/g, n = 13) than inactive UC (97 ± 82 μg/g, n = 17) and CD (133 ± 89 μg/g, n = 5), respectively (Dai et al. 2007). Even greater differences were noted comparing these values to IBS patients (2.54 ± 1.49 μg/g, n = 25), and healthy volunteers (3.15 ± 1.60 μg/g, n = 34). The authors concluded that when the cutoff for fecal LF levels was above 324 μg/g for UC patients, the sensitivity and specificity rates of fecal LF as an IBD biomarker increased to 90% and 88%, respectively. For CD patients, the cutoff for fecal LF more than 240 μg/g also affected the sensitivity and specificity, which would be 92% and 80%, respectively.

In a study evaluating LF levels in children with CD significantly higher fecal LF in active CD (median 280 μg/g, n = 23) compared to inactive CD (22 μg/g, n = 23), and non-IBD (0.56 μg/g, n = 37) were found (Pfefferkorn et al. 2010). All of the patients with active CD had fecal LF levels above the reference cutoff level of 7.25 μg/g, consistent with the high sensitivity and negative predictive value of fecal LF. The authors also suggest that to increase the specificity for differentiating active from inactive CD, a higher fecal LF level of 60 μg/g should be used as a cutoff.

In another investigation in pediatric IBD patients the mean levels of fecal LF were markedly higher for CD (314 μg/g, SD 212.8, n = 21) and UC (371 μg/g, SD 181.5, n = 15) compared with control group (n = 20), which was only 1.3 (SD 2.4) (Buderus, Boone, and Lentze 2015).

Borkowska et al. (2015) demonstrated that children with IBD are characterized by considerably higher fecal LF compared to control (1.43 μg/g, n = 41); moreover, the authors indicated that CD patients had higher fecal LF (173.96 μg/g, n = 56) than UC (104.31 μg/g, n = 58).

The latest systematic review and meta-analysis of the 10 eligible studies confirmed that fecal LF has good potential as a future diagnostic tool due to its noninvasiveness as well as patient satisfaction, risk minimization, cost reduction, and hospitalization avoidance. Also, it is worth mentioning that LF is a reproducible, and inexpensive tool, which is highly relevant for IBD management.

Conclusions

Lactoferrin plays a substantial role in counteracting inflammatory processes and maintaining immune homeostasis. Moreover, it possesses extensive anti-microbial activity. Therefore, it seems crucial to further assess the effects of LF supplementation in humans with reference to viral, bacterial, and fungal infections as well as to compare the outcomes of these studies with earlier divergent results. Considering the safety of LF oral application and its low supply with diet, it is also necessary to examine LF efficiency as auxiliary therapy in cancer patients. Furthermore, it needs to be pointed out that due to the differences in digestive capability between infants and adults, the most benefits of oral LF supplementation will be noticeable in the former group. Several properties of LF may be beneficial in IBD as well, however due to limited number of data, we suggest conducting future studies focused particularly on the linkage between endogenous and administered LF with the immune system and iron homeostasis. Finally, another very pertinent path in the research on LF is to develop novel forms and formulations of LF with increased stability and bioavailability.

Authors' contributions

MS, JF and AJ wrote the paper and gave the study concept and design. AT critically revised the paper. All authors have read and approved the final version of the manuscript.

Disclosure statement

The authors report no conflicts of interest.

Abbreviations

APCs	antigen-presenting cells
ASGPR	asialoglycoprotein receptor
BBB	blood-brain barrier
bLF	bovine lactoferrin
CD	Crohn's disease
CD14	cluster of differentiation 14
CDK	cyclin-dependent kinase
DC	dendritic cells
DSS	dextran sulfate sodium
Ftn	ferritin
Fpn	ferroportin
GAGs	glycosaminoglycans
GI	gastrointestinal
hLF	human lactoferrin
HSPGs	cell-surface heparan sulfate proteoglycans
IBD	inflammatory bowel disease
IIH	iron and inflammatory homeostasis
IDA	iron deficiency anemia
ITLN1	intelectin 1 receptor
IL	interleukin
LbpB	lactoferrin binding protein B
LF	lactoferrin
LDL	low-density lipoprotein
LOS	late-onset sepsis
LPS	lipopolysaccharide
LRP	low-density lipoprotein receptor-related protein
LTA	lipoteichoic acid
NET	neutrophil extracellular traps
NF-κB	nuclear factor kappa B
NK	Natural Killers
PAMPs	pathogen associated microbial patterns
PMNs	polymorphonuclear neutrophils
RCT	randomized clinical trial
RES	reticuloendothelial system
ROS	reactive oxygen species
rhlntL	recombinant human intelectin receptor
rhLF	recombinant human lactoferrin
Tf	transferrin
TfR1	Tf receptor 1
TLRs	Toll-like receptors
TMPRSS2	transmembrane protease serine 2
UC	ulcerative colitis

UHT	ultra-high temperature
VEGF	vascular endothelial growth factor
ZO-1	zonula occludens-1

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References

Abu Hashim, H., O. Foda, and E. Ghayaty. 2017. Lactoferrin or ferrous salts for iron deficiency anemia in pregnancy: A meta-analysis of randomized trials. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 219:45–52. doi: 10.1016/j.ejogrb.2017.10.003.

Actor, J., S.-A. Hwang, and M. Kruzel. 2009. Lactoferrin as a natural immune modulator. *Current Pharmaceutical Design* 15 (17):1956–73. doi: 10.2174/138161209788453202.

Al-Sheikh, H. 2009. Effect of lactoferrin and iron on the growth of human pathogenic Candida species. *Pakistan Journal of Biological Sciences: PJBS* 12 (1):91–4. doi: 10.3923/pjbs.2009.91.94.

Arnold, R. R., J. E. Russell, W. J. Champion, M. Brewer, and J. J. Gauthier. 1982. Bactericidal activity of human lactoferrin: Differentiation from the stasis of iron deprivation. *Infection and Immunity* 35 (3):792–9. doi: 10.1128/IAI.35.3.792-799.1982.

Artym, J., and M. Zimecki. 2013. Milk-derived proteins and peptides in clinical trials. *Postepy Higieny i Medycyny Doswiadczalnej* 67:800–16. doi: 10.5604/17322693.1061635.

Baker, E. N., and H. M. Baker. 2009. A structural framework for understanding the multifunctional character of lactoferrin. *Biochimie* 91 (1):3–10. doi: 10.1016/j.biochi.2008.05.006.

Barboza, M., J. Pinzon, S. Wickramasinghe, J. W. Froehlich, I. Moeller, J. T. Smilowitz, L. R. Ruhaak, J. Huang, B. Lönnerdal, and J. B. German. 2012. Glycosylation of human milk lactoferrin exhibits dynamic changes during early lactation enhancing its role in pathogenic bacteria-host interactions. *Molecular & Cellular Proteomics* 11 (6): M111-015248.

Bengoechea, C., O. G. Jones, A. Guerrero, and D. J. McClements. 2011. Formation and characterization of lactoferrin/pectin electrostatic complexes: Impact of composition, pH and thermal treatment. *Food Hydrocolloids* 25 (5):1227–32. Juldoi: 10.1016/j.foodhyd.2010.11.010.

Bennett, R. M., J. Davis, S. Campbell, and S. Portnoff. 1983. Lactoferrin binds to cell membrane DNA. Association of surface DNA with an enriched population of B cells and monocytes. *The Journal of Clinical Investigation* 71 (3):611–8. doi: 10.1172/jci110807.

Bennike, T. B., T. G. Carlsen, T. Ellingsen, O. K. Bonderup, H. Glerup, M. Bøgsted, G. Christiansen, S. Birkelund, A. Stensballe, and V. Andersen. 2015. Neutrophil extracellular traps in ulcerative colitis. *Inflammatory Bowel Diseases* 21 (9):2052–67.

Berlutti, F., C. Morea, A. Battistoni, S. Sarli, P. Cipriani, F. Superti, M. G. Ammendolia, and P. Valenti. 2005. Iron availability influences aggregation, biofilm, adhesion and invasion of Pseudomonas aeruginosa and Burkholderia cenocepacia. *International Journal of Immunopathology and Pharmacology* 18 (4):661–70. doi: 10.1177/039463200501800407.

Bertuccini, L., M. Costanzo, F. Iosi, A. Tinari, F. Terruzzi, L. Stronati, M. Aloi, S. Cucchiara, and F. Superti. 2014. Lactoferrin prevents invasion and inflammatory response following E. coli strain LF82 infection in experimental model of Crohn's disease. *Digestive and Liver Disease* 46 (6):496–504. doi: 10.1016/j.dld.2014.02.009.

Bokkhim, H., N. Bansal, L. GrØndahl, and B. Bhandari. 2013. Physico-chemical properties of different forms of bovine lactoferrin. *Food Chemistry* 141 (3):3007–13.

Bokkhim, H., N. Bansal, L. Grøndahl, and B. Bhandari. 2016. In-vitro digestion of different forms of bovine lactoferrin encapsulated in alginate micro-gel particles. *Food Hydrocolloids* 52:231–42. doi: 10.1016/j.foodhyd.2015.07.007.

Borkowska, A., A. Liberek, G. Łuczak, A. Jankowska, K. Plata-Nazar, M. Korzon, and B. Kamińska. 2015. Fecal lactoferrin, a marker of intestinal inflammation in children with inflammatory bowel disease. *Acta Biochimica Polonica* 62 (3):541–5. doi: 10.18388/abp.2015_982.

Brisson, G., M. Britten, and Y. Pouliot. 2007. Effect of iron saturation on the recovery of lactoferrin in rennet whey coming from heat-treated skim milk. *Journal of Dairy Science* 90 (6):2655–64. doi: 10.3168/jds.2006-725.

Brooks, C. L., E. Arutyunova, and M. J. Lemieux. 2014. The structure of lactoferrin-binding protein B from Neisseria meningitidis suggests roles in iron acquisition and neutralization of host defences. *Acta Crystallographica. Section F, Structural Biology Communications* 70 (Pt 10):1312–7. doi: 10.1107/S2053230X14019372.

Buderus, S., J. H. Boone, and M. J. Lentze. 2015. Fecal lactoferrin: Reliable biomarker for intestinal inflammation in pediatric IBD. *Gastroenterology Research and Practice* 2015:1–4. doi: 10.1155/2015/578527.

Campione, E., T. Cosio, L. Rosa, C. Lanna, S. Girolamo, R. Di, Gaziano, P. Valenti, and L. Bianchi. 2020. Lactoferrin as protective natural barrier of respiratory and intestinal mucosa against coronavirus infection and inflammation. *International Journal of Molecular Sciences* 21 (14):4903–14. doi: 10.3390/ijms21144903.

Chandra Mohan, K. V. P., H. Devaraj, D. Prathiba, Y. Hara, and S. Nagini. 2006. Antiproliferative and apoptosis inducing effect of lactoferrin and black tea polyphenol combination on hamster buccal pouch carcinogenesis. *Biochimica et Biophysica Acta* 1760 (10): 1536–44. https://pubmed.ncbi.nlm.nih.gov/16905260/. doi: 10.1016/j.bbagen.2006.06.009.

Chea, C., M. Miyauchi, T. Inubushi, N. F. Ayuningtyas, A. Subarnbhesaj, P. T. Nguyen, M. Shrestha, S. Haing, K. Ohta, and T. Takata. 2018. Molecular mechanism of inhibitory effects of bovine lactoferrin on the growth of oral squamous cell carcinoma. *PLoS One* 13 (1):e0191683.

Chen, J. M., Y. C. Fan, J. W. Lin, Y. Y. Chen, W. L. Hsu, and S. S. Chiou. 2017. Bovine lactoferrin inhibits dengue virus infectivity by interacting with heparan sulfate, low-density lipoprotein receptor, and DC-SIGN. *International Journal of Molecular Sciences* 18 (9): 1957.

Chen, K., L. Chai, H. Li, Y. Zhang, H. M. Xie, J. Shang, W. Tian, P. Yang, and A. C. Jiang. 2016. Effect of bovine lactoferrin from iron-fortified formulas on diarrhea and respiratory tract infections of weaned infants in a randomized controlled trial. *Nutrition* 32 (2): 222–7. doi: 10.1016/j.nut.2015.08.010.

Chen, P. W., Y. W. Ku, and F. Y. Chu. 2014. Influence of bovine lactoferrin on the growth of selected probiotic bacteria under aerobic conditions. *Biometals* 27 (5):905–14. doi: 10.1007/s10534-014-9758-z.

Chen, P. W., Z. S. Liu, T. C. Kuo, M. C. Hsieh, and Z. W. Li. 2017. Prebiotic effects of bovine lactoferrin on specific probiotic bacteria. *Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine* 30 (2):237–48.

Cheng, J. B., J. Q. Wang, D. P. Bu, G. L. Liu, C. G. Zhang, H. Y. Wei, L. Y. Zhou, and J. Z. Wang. 2008. Factors affecting the lactoferrin concentration in bovine milk. *Journal of Dairy Science* 91 (3):970–6. doi: 10.3168/jds.2007-0689.

Conesa, C., C. Rota, E. Castillo, M. D. Pérez, M. Calvo, and L. Sánchez. 2009. Antibacterial activity of recombinant human lactoferrin from rice: Effect of heat treatment. *Bioscience, Biotechnology, and Biochemistry* 73 (6):1301–7. doi: 10.1271/bbb.80814.

Cornish, J., K. E. Callon, D. Naot, K. P. Palmano, T. Banovic, U. Bava, M. Watson, J.-M. Lin, P. C. Tong, Q. Chen, et al. 2004. Lactoferrin is a potent regulator of bone cell activity and increases bone formation in vivo. *Endocrinology* 145 (9):4366–74. doi: 10.1210/en.2003-1307.

Cornish, J., K. Palmano, K. E. Callon, M. Watson, J. M. Lin, P. Valenti, D. Naot, A. B. Grey, and I. R. Reid. 2006. Lactoferrin and bone; structure-activity relationships. *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire* 84 (3):297–302. doi: 10.1139/o06-057.

Cox, A. J., A. M. Watts, P. Zhang, L. T. Williams, A. W. Cripps, and N. P. West. 2017. Effects of short-term supplementation with bovine

lactoferrin and/or immunoglobulins on body mass and metabolic measures: A randomised controlled trial. *International Journal of Food Sciences and Nutrition* 68 (2):219–26. doi: 10.1080/09637486.2016.1224230.

Cutone, A., L. Rosa, G. Ianiro, M. S. Lepanto, M. C. B. Di Patti, P. Valenti, and G. Musci. 2020. Lactoferrin's anti-cancer properties: Safety, selectivity, and wide range of action. *Biomolecules* 10 (3):456. doi: 10.3390/biom10030456.

Cutone, A., L. Rosa, M. S. Lepanto, M. J. Scotti, F. Berlutti, M. C. B. di Patti, G. Musci, and P. Valenti. 2017. Lactoferrin efficiently counteracts the inflammation-induced changes of the iron homeostasis system in macrophages. *Frontiers Immunology* 8:705.

Dai, J., W. Z. Liu, Y. P. Zhao, Y. B. Hu, and Z. Z. Ge. 2007. Relationship between fecal lactoferrin and inflammatory bowel disease. *Scandinavian Journal of Gastroenterology* 42 (12):1440–4. doi: 10.1080/00365520701427094.

Damiens, E., I. El Yazidi, J. Mazurier, I. Duthille, G. Spik, and Y. Boilly-Marer. 1999. Lactoferrin inhibits G1 cyclin-dependent kinases during growth arrest of human breast carcinoma cells. *Journal of Cellular Biochemistry* 74 (3):486–98. %3C486::AID-JCB16%3E3.0.CO;2-6 doi: (19990901)74:3.

De Kruif, C. G., F. Weinbreck, and R. De Vries. 2004. Complex coacervation of proteins and anionic polysaccharides. *Current Opinion in Colloid & Interface Science* 9 (5):340–9. doi: 10.1016/j.cocis.2004.09.006.

de la Rosa, G., D. Yang, P. Tewary, A. Varadhachary, and J. J. Oppenheim. 2008. Lactoferrin acts as an alarmin to promote the recruitment and activation of APCs and antigen-specific immune responses. *The Journal of Immunology* 180 (10):6868–76. doi: 10.4049/jimmunol.180.10.6868.

De Souza Batista, C. M., R. Z. Yang, M. J. Lee, N. M. Glynn, D. Z. Yu, J. Pray, K. Ndubuizu, S. Patil, A. Schwartz, M. Kligman, et al. 2007. Omentin plasma levels and gene expression are decreased in obesity. *Diabetes* 56 (6):1655–61. doi: 10.2337/db06-1506.

DI Mario, F., G. Aragona, N. Dal Bo, L. Cavallaro, V. Marcon, P. Olivieri, E. Benedetti, N. Orzes, R. Marin, G. Tafner, et al. 2006. Bovine lactoferrin for Helicobacter pylori eradication: An open, randomized, multicentre study. *Alimentary Pharmacology and Therapeutics* 23 (8):1235–40. doi: 10.1111/j.1365-2036.2006.02851.x.

Donahue, J. E., S. L. Flaherty, C. E. Johanson, J. A. Duncan, G. D. Silverberg, M. C. Miller, R. Tavares, W. Yang, Q. Wu, E. Sabo, et al. 2006. RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. *Acta Neuropathologica* 112 (4):405–15.

Elass-Rochard, E., D. Legrand, V. Salmon, A. Roseanu, M. Trif, P. S. Tobias, J. Mazurier, and G. Spik. 1998. Lactoferrin inhibits the endotoxin interaction with CD14 by competition with the lipopolysaccharide-binding protein. *Infection and Immunity* 66 (2):486–91. PMC][10.1128/IAI.66.2.486-491.1998] [9453600]

Embleton, N. D., J. E. Berrington, W. McGuire, C. J. Stewart, and S. P. Cummings. 2013. Lactoferrin: Antimicrobial activity and therapeutic potential. *Seminars in Fetal & Neonatal Medicine* 18 (3):143–9. doi: 10.1016/j.siny.2013.02.001.

Eriksen, E. K., H. Holm, E. Jensen, R. Aaboe, T. G. Devold, M. Jacobsen, and G. E. Vegarud. 2010. Different digestion of caprine whey proteins by human and porcine gastrointestinal enzymes. *The British Journal of Nutrition* 104 (3):374–81. doi: 10.1017/S0007114510000577.

Fernandes, K. E., and D. A. Carter. 2017. The antifungal activity of lactoferrin and its derived peptides: Mechanisms of action and synergy with drugs against fungal pathogens. *Frontiers in Microbiology* 8:2. doi: 10.3389/fmicb.2017.00002.

Fichna, J. 2016. Inflammatory bowel disease treatment. *Pharmacological Reports PR* 68 (4):787–8. doi: 10.1016/j.pharep.2016.05.008.

Fillebeen, C., L. Descamps, M. P. Dehouck, L. Fenart, M. Benaïssa, G. Spik, R. Cecchelli, and A. Pierce. 1999. Receptor-mediated transcytosis of lactoferrin through the blood-brain barrier. *The Journal of Biological Chemistry* 274 (11):7011–7. doi: 10.1074/jbc.274.11.7011.

Fischer, R., H. Debbabi, A. Blais, M. Dubarry, M. Rautureau, P. N. Boyaka, and D. Tome. 2007. Uptake of ingested bovine lactoferrin and its accumulation in adult mouse tissues. *International Immunopharmacology* 7 (10):1387–93. doi: 10.1016/j.intimp.2007.05.019.

Fischer, R., H. Debbabi, M. Dubarry, P. Boyaka, and D. Tomé. 2006. Regulation of physiological and pathological Th1 and Th2 responses by lactoferrin. *Biochemistry and Cell Biology* 84:303–11.

Furlund, C. B., E. K. Ulleberg, T. G. Devold, R. Flengsrud, M. Jacobsen, C. Sekse, H. Holm, and G. E. Vegarud. 2013. Identification of lactoferrin peptides generated by digestion with human gastrointestinal enzymes. *Journal of Dairy Science* 96 (1):75–88. doi: 10.3168/jds.2012-5946.

Gajda, E., and G. Bugla-Płoskońska. 2014. Lizozym-wystepowanie w przyrodzie, właściwości biologiczne i mozliwosci zastosowań. *Postępy Higieny i Medycyny Doświadczalnej* 68:1501–15. doi: 10.5604/17322693.1133100.

Ganal-Vonarburg, S. C., and C. U. Duerr. 2020. The interaction of intestinal microbiota and innate lymphoid cells in health and disease throughout life. *Immunology* 159:39–51.

García-Montoya, I. A., T. S. Cendón, S. Arévalo-Gallegos, and Q. Rascón-Cruz. 2012. Lactoferrin a multiple bioactive protein: An overview. *Biochimica et Biophysica Acta (Bba) - General Subjects* 1820 (3):226–36. doi: 10.1016/j.bbagen.2011.06.018.

Gaudin, J. C., H. Rabesona, Y. Choiset, G. Yeretssian, J. M. Chobert, V. Sakanyan, M. Drouet, and T. Haertlé. 2008. Assessment of the immunoglobulin E-mediated immune response to milk-specific proteins in allergic patients using microarrays. *Clinical and Experimental Allergy* 38 (4):686–93. doi: 10.1111/j.1365-2222.2008.02952.x.

Gibbons, J. A., J. R. Kanwar, and R. K. Kanwar. 2015. Iron-free and iron-saturated bovine lactoferrin inhibit survivin expression and differentially modulate apoptosis in breast cancer. *BMC Cancer* 15 (1):1–16.

Gisbert, J. P., A. G. McNicholl, and F. Gomollon. 2009. Questions and answers on the role of fecal lactoferrin as a biological marker in inflammatory bowel disease. *Inflammatory Bowel Diseases* 15:1746–54.

Gíslason, J., S. Iyer, T. W. Hutchens, and B. Lönnerdal. 1993. Lactoferrin receptors in piglet small intestine: Lactoferrin binding properties, ontogeny, and regional distribution in the gastrointestinal tract. *Journal of Nutritional Biochemistry* 4 (9):528–33. doi: 10.1016/0955-2863(93)90089-F.

Goldsmith, J. R., and R. B. Sartor. 2014. The role of diet on intestinal microbiota metabolism: Downstream impacts on host immune function and health, and therapeutic implications. *Journal of Gastroenterology* 49:785–98.

González-Chávez, S. A., S. Arévalo-Gallegos, and Q. Rascón-Cruz. 2009. Lactoferrin: Structure, function and applications. *International Journal of Antimicrobial Agents* 33 (4):301.e1–e8. doi: 10.1016/j.ijantimicag.2008.07.020.

Grey, A., T. Banovic, Q. Zhu, M. Watson, K. Callon, K. Palmano, J. Ross, D. Naot, I. R. Reid, and J. Cornish. 2004. The low-density lipoprotein receptor-related protein 1 is a mitogenic receptor for lactoferrin in osteoblastic cells. *Molecular Endocrinology (Baltimore, Md.)* 18 (9):2268–78. doi: 10.1210/me.2003-0456.

Griffiths, J., P. Jenkins, M. Vargova, U. Bowler, E. Juszczak, A. King, L. Linsell, D. Murray, C. Partlett, M. Patel, et al. 2019. Enteral lactoferrin supplementation for very preterm infants: A randomised placebo-controlled trial. *The Lancet* 393 (10170):423–33. doi: 10.1016/S0140-6736(18)32221-9.

Groot, F., T. B. H. Geijtenbeek, R. W. Sanders, C. E. Baldwin, M. Sanchez-Hernandez, R. Floris, Y. van Kooyk, E. C. de Jong, and B. Berkhout. 2005. Lactoferrin prevents dendritic cell-mediated human immunodeficiency virus type 1 transmission by blocking the DC-SIGN-gp120 interaction. *Journal of Virology* 79 (5):3009–15. doi: 10.1128/JVI.79.5.3009-3015.2005.

Haiwen, Z., H. Rui, Z. Bingxi, G. Qingfeng, Z. Jifeng, W. Xuemei, and W. Beibei. 2019. Oral administration of bovine lactoferrin-derived lactoferricin (Lfcin) B could attenuate enterohemorrhagic Escherichia coli O157:H7 induced intestinal disease through improving intestinal barrier function and microbiota. *Journal of*

Agricultural and Food Chemistry 67 (14):3932–45. doi: 10.1021/acs.jafc.9b00861.

Hall, C. W., and T. F. Mah. 2017. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews* 41 (3):276–301. doi: 10.1093/femsre/fux010.

Håversen, L. A., L. Baltzer, G. Dolphin, L. A. Hanson, and I. Mattsby-Baltzer. 2003. Anti-inflammatory activities of human lactoferrin in acute dextran sulphate-induced colitis in mice. *Scandinavian Journal of Immunology* 57 (1):2–10. doi: 10.1046/j.1365-3083.2003.01162.x.

Håversen, L., B. G. Ohlsson, M. Hahn-Zoric, L. Å. Hanson, and I. Mattsby-Baltzer. 2002. Lactoferrin down-regulates the LPS-induced cytokine production in monocytic cells via NF-κB. *Cellular Immunology* 220 (2):83–95. doi: 10.1016/S0008-8749(03)00006-6.

Hayes, T. G., G. F. Falchook, G. R. Varadhachary, D. P. Smith, L. D. Davis, H. M. Dhingra, B. P. Hayes, and A. Varadhachary. 2006. Phase I trial of oral talactoferrin alfa in refractory solid tumors. *Investigational New Drugs* 24:233–40. doi: 10.1007/s10637-005-3690-6.

He, J., and P. Furmanski. 1995. Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA. *Nature* 373 (6516): 721–4. https://www.nature.com/articles/373721a0. doi: 10.1038/373721a0.

He, Y. Y., N. T. Lawlor, and D. S. Newburg. 2016. Human milk components modulate toll-like receptor-mediated inflammation. *Advances in Nutrition* 7 (1):102–11. doi: 10.3945/an.115.010090.

He, Y., J. B. Ruganzu, Q. Zheng, X. Wu, H. Jin, X. Peng, B. Ding, C. Lin, S. Ji, Y. Ma, et al. 2020. Silencing of LRP1 exacerbates inflammatory response via TLR4/NF-κB/MAPKs signaling pathways in APP/PS1 transgenic mice. *Molecular Neurobiology* 57 (9):3727–43. doi: 10.1007/s12035-020-01982-7.

Herz, J., and P. Marschang. 2003. Coaxing the LDL receptor family into the fold. *Cell. Cell Press* 112:289–92.

Hoffmann, M., H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T. S. Schiergens, G. Herrler, N.-H. Wu, A. Nitsche, et al. 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor article SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181 (2): 271–10. doi: 10.1016/j.cell.2020.02.052.

Holtmann, G. J., A. C. Ford, and N. J. Talley. 2016. Pathophysiology of irritable bowel syndrome. *The Lancet. Gastroenterology & Hepatology* 1 (2):133–46. doi: 10.1016/S2468-1253(16)30023-1.

Hunninghake, G. W., K. C. Doerschug, A. B. Nymon, G. A. Schmidt, D. K. Meyerholz, and A. Ashare. 2010. Insulin-like growth factor-1 levels contribute to the development of bacterial translocation in sepsis. *American Journal of Respiratory and Critical Care Medicine* 182 (4):517–25. doi: 10.1164/rccm.200911-1757OC.

Jia, H. P., D. C. Look, L. Shi, M. Hickey, L. Pewe, J. Netland, M. Farzan, C. Wohlford-Lenane, S. Perlman, and P. B. McCray. 2005. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *Journal of Virology* 79 (23):14614–21. doi: 10.1128/JVI.79.23.14614-14621.2005.

Jiang, R., and B. Lönnerdal. 2017. Bovine lactoferrin and lactoferricin exert antitumor activities on human colorectal cancer cells (HT-29) by activating various signaling pathways1. *Biochemistry and Cell Biology* 95 (1):99–109. doi: 10.1139/bcb-2016-0094.

Jimenez, K. M., and C. Gasche. 2019. Management of iron deficiency anaemia in inflammatory bowel disease. *Acta Haematologica* 142 (1):30–6.

Kaufman, D. A., A. Berenz, H. L. Itell, M. Conaway, A. Blackman, J. P. Nataro, and S. R. Permar. 2021. Dose escalation study of bovine lactoferrin in preterm infants: Getting the dose right. *Biochemistry and Cell Biology* 99 (1):7–13. doi: 10.1139/bcb-2020-0217.

Kilic, E., M. V. Novoselova, S. H. Lim, N. A. Pyataev, S. I. Pinyaev, O. A. Kulikov, O. A. Sindeeva, O. A. Mayorova, R. Murney, M. N. Antipina, et al. 2017. Formulation for oral delivery of lactoferrin based on bovine serum albumin and tannic acid multilayer microcapsules. *Scientific Reports* 7:1–10.

Kobayashi, S., R. Sato, O. Inanami, T. Yamamori, O. Yamato, Y. Maede, J. Sato, M. Kuwabara, and Y. Naito. 2005. Reduction of concanavalin A-induced expression of interferon-gamma by bovine lactoferrin in feline peripheral blood mononuclear cells. *Veterinary Immunology and Immunopathology* 105 (1-2):75–84. doi: 10.1016/j.vetimm.2004.12.016.

Kruzel, M. L., A. Bacsi, B. Choudhury, S. Sur, and I. Boldogh. 2006. Lactoferrin decreases pollen antigen-induced allergic airway inflammation in a murine model of asthma. *Immunology* 119 (2):159–66. doi: 10.1111/j.1365-2567.2006.02417.x.

Kruzel, M. L., Y. Harari, D. Mailman, J. K. Actor, and M. Zimecki. 2002. Differential effects of prophylactic, concurrent and therapeutic lactoferrin treatment on LPS-induced inflammatory responses in mice. *Clinical and Experimental Immunology* 130 (1):25–31. doi: 10.1046/j.1365-2249.2002.01956.x.

Kuwata, H., K. Yamauchi, S. Teraguchi, Y. Ushida, Y. Shimokawa, T. Toida, and H. Hayasawa. 2001. Functional fragments of ingested lactoferrin are resistant to proteolytic degradation in the gastrointestinal tract of adult rats. *The Journal of Nutrition* 131 (8):2121–7. doi: 10.1093/jn/131.8.2121.

Kuwata, H., T.-T. Yip, M. Tomita, and T. W. Hutchens. 1998. Direct evidence of the generation in human stomach of an antimicrobial peptide domain (lactoferricin) from ingested lactoferrin. *Biochimica et Biophysica Acta (Bba) - Protein Structure and Molecular Enzymology* 1429 (1):129–41. doi: 10.1016/S0167-4838(98)00224-6.

Kuwata, H., T. T. Yip, C. L. Yip, M. Tomita, and T. W. Hutchens. 1998. Bactericidal domain of lactoferrin: Detection, quantitation, and characterization of lactoferricin in serum by SELDI affinity mass spectrometry. *Biochemical and Biophysical Research Communications* 245 (3):764–73. doi: 10.1006/bbrc.1998.8466.

Lambert, L. A., H. Perri, and T. J. Meehan. 2005. Evolution of duplications in the transferrin family of proteins. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology* 140 (1): 11–25. doi: 10.1016/j.cbpc.2004.09.012.

Lang, J., N. Yang, J. Deng, K. Liu, P. Yang, G. Zhang, and C. Jiang. 2011. Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. *PLoS One* 6 (8):e23710. doi: 10.1371/journal.pone.0023710.

Langhorst, J., and J. Boone. 2012. Fecal lactoferrin as a noninvasive biomarker in inflammatory bowel diseases. *Drugs of Today* 48 (2): 149–61. doi: 10.1358/dot.2012.48.2.1732555.

Latorre, D., P. Puddu, P. Valenti, and S. Gessani. 2010. Reciprocal interactions between lactoferrin and bacterial endotoxins and their role in the regulation of the immune response. *Toxins* 2 (1):54–68. doi: 10.3390/toxins2010054.

Lauterbach, R., E. Kamińska, P. Michalski, and J. P. Lauterbach. 2016. Lactoferrin - A glycoprotein of great therapeutic potentials. *Developmental Period Medicine* 20 (2):118–25.

Lee, T., T. Clavel, K. Smirnov, A. Schmidt, I. Lagkouvardos, A. Walker, M. Lucio, B. Michalke, P. Schmitt-Kopplin, R. Fedorak, et al. 2017. Oral versus intravenous iron replacement therapy distinctly alters the gut microbiota and metabolome in patients with IBD. *Gut* 66 (5):863–71. doi: 10.1136/gutjnl-2015-309940.

Legrand, D. 2016. Overview of lactoferrin as a natural immune modulator. *Journal of Pediatrics* 173:S10–S5. doi: 10.1016/j.jpeds.2016.02.071.

Legrand, D., E. Elass, M. Carpentier, and J. Mazurier. 2005. Lactoferrin: A modulator of immune and inflammatory responses. *Cellular and Molecular Life Sciences: CMLS* 62 (22):2549–59. doi: 10.1007/s00018-005-5370-2.

Lepanto, M. S., L. Rosa, A. Cutone, M. P. Conte, R. Paesano, and P. Valenti. 2018. Efficacy of lactoferrin oral administration in the treatment of anemia and anemia of inflammation in pregnant and nonpregnant women: An interventional study. *Frontiers Immunology* 9: 2123.

Lepanto, M. S., L. Rosa, A. Cutone, M. J. Scotti, A. L. Conte, M. Marazzato, C. Zagaglia, C. Longhi, F. Berlutti, G. Musci, et al. 2019. Bovine lactoferrin pre-treatment induces intracellular killing of AIEC LF82 and reduces bacteria-induced DNA damage in

differentiated human enterocytes. *International Journal of Molecular Sciences* 20 (22):5666. doi: 10.3390/ijms20225666.

Li, L., F. Ren, Z. Yun, Y. An, C. Wang, and X. Yan. 2013. Determination of the effects of lactoferrin in a preclinical mouse model of experimental colitis. *Molecular Medicine Reports* 8 (4): 1125–9. doi: 10.3892/mmr.2013.1632.

Liao, Y., V. Lopez, T. B. Shafizadeh, C. H. Halsted, and B. Lönnerdal. 2007. Cloning of a pig homologue of the human lactoferrin receptor: Expression and localization during intestinal maturation in piglets. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* 148 (3):584–90. doi: 10.1016/j.cbpa.2007.08.001.

Lin, L., and K. Hu. 2014. LRP-1: Functions, signaling and implications in kidney and other diseases. *International Journal of Molecular Sciences* 15 (12):22887–901.

Lizzi, A. R., V. Carnicelli, M. M. Clarkson, C. Nazzicone, B. Segatore, G. Celenza, M. Aschi, V. Dolo, R. Strom, and G. Amicosante. 2016. Bovine lactoferrin and its tryptic peptides: Antibacterial activity against different species. *Applied Biochemistry and Microbiology* 52 (4):435–40. doi: 10.1134/S0003683816040116.

Lönnerdal, B. 2013. Bioactive proteins in breast milk. *Journal of Paediatrics and Child Health* 49 (SUPPL. 1):1–7. http://doi.wiley.com/10.1111/jpc.12104. doi: 10.1111/jpc.12104.

Lönnerdal, B. 2016. Bioactive proteins in human milk: health, nutrition, and implications for infant formulas. *The Journal of Pediatrics* 173: S4–S9. doi: 10.1016/j.jpeds.2016.02.070.

Lönnerdal, B., R. Jiang, and X. Du. 2011. Bovine lactoferrin can be taken up by the human intestinal lactoferrin receptor and exert bioactivities. *Journal of Pediatric Gastroenterology and Nutrition* 53 (6): 1. http://journals.lww.com/00005176-900000000-99269.

Machnicki, M., M. Zimecki, and T. Zagulski. 1993. Lactoferrin regulates the release of tumour necrosis factor alpha and interleukin 6 in vivo. *International Journal of Experimental Pathology* 74 (5): 433–9.

Mader, J. S., J. Salsman, D. M. Conrad, and D. W. Hoskin. 2005. Bovine lactoferricin selectively induces apoptosis in human leukemia and carcinoma cell lines. *Molecular Cancer Therapeutics* 4 (4): 612–24. doi: 10.1158/1535-7163.MCT-04-0077.

Mahalhal, A., J. M. Williams, S. Johnson, N. Ellaby, C. A. Duckworth, M. D. Burkitt, X. Liu, G. L. Hold, B. J. Campbell, and P. D. Mark. 2018. Oral iron exacerbates colitis and influences the intestinal microbiome. *PLoS One* 13 (10):e0202460.

Mancinelli, R., F. Olivero, G. Carpino, D. Overi, L. Rosa, M. S. Lepanto, A. Cutone, A. Franchitto, G. Alpini, P. Onori, et al. 2018. Role of lactoferrin and its receptors on biliary epithelium. *Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine* 31 (3):369–79.

Marek, A., M. Zagierski, A. Liberek, E. Aleksandrowicz, M. Korzon, G. Krzykowski, B. Kamińska, and A. Szlagatys-Sidorkiewicz. 2009. TGF-β1, IL-10 and IL-4 in colostrum of allergic and nonallergic mothers. *Acta Biochimica Polonica* 56 (3):411–4.

Meilinger, M., M. Haumer, K. A. Szakmary, F. Steinböck, B. Scheiber, H. Goldenberg, and M. Huettinger. 1995. Removal of lactoferrin from plasma is mediated by binding to low density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor and transport to endosomes. *FEBS Letters* 360 (1):70–4. doi: 10.1016/0014-5793(95)00082-K.

Mikulic, N., M. A. Uyoga, E. Mwasi, N. U. Stoffel, C. Zeder, S. Karanja, and M. B. Zimmermann. 2020. Iron absorption is greater from apo-lactoferrin and is similar between holo-lactoferrin and ferrous sulfate: stable iron isotope studies in Kenyan infants. *The Journal of Nutrition* 150:3200–7.

Milewska, A., M. Zarebski, P. Nowak, K. Stozek, J. Potempa, and K. Pyrc. 2014. Human coronavirus NL63 utilizes heparan sulfate proteoglycans for attachment to target cells. *Journal of Virology* 88 (22): 13221–30. doi: 10.1128/JVI.02078-14.

Miller, J. L. 2013. Iron deficiency anemia: A common and curable disease. *Cold Spring Harbor Perspectives Medicine* 3 (7):a011866.

Mizuki, M., T. Tsukahara, H. Oda, M. Tanaka, K. Yamauchi, F. Abe, and T. Nomiyama. 2020. Effects of lactoferrin on prevention of acute gastrointestinal symptoms in winter: A randomized, double-blinded, placebo-controlled trial for staff of kindergartens and nursery schools in Japan. *International Journal of Environmental Research and Public Health* 17 (24):9582. doi: 10.3390/ijerph17249582.

Mohanraj, R., and S. Sivasankar. 2014. Sweet potato (Ipomoea batatas [L.] Lam)-a valuable medicinal food: A review. *Journal of Medicinal Food* 17 (7):733–41. doi: 10.1089/jmf.2013.2818.

Moore, M. J., T. Dorfman, W. Li, S. K. Wong, Y. Li, J. H. Kuhn, J. Coderre, N. Vasilieva, Z. Han, T. C. Greenough, et al. 2004. Retroviruses pseudotyped with the severe acute respiratory syndrome coronavirus spike protein efficiently infect cells expressing angiotensin-converting enzyme 2. *Journal of Virology* 78 (19): 10628–35.

Morgenthau, A., A. Beddek, and A. B. Schryvers. 2014. The negatively charged regions of lactoferrin binding protein B, an adaptation against anti-microbial peptides. *PLoS One* 9 (1):e86243. doi: 10.1371/journal.pone.0086243.

Nakano, M., H. Wakabayashi, H. Sugahara, T. Odamaki, K. Yamauchi, F. Abe, J. Z. Xiao, K. Murakami, K. Ishikawa, and S. Hironaka. 2017. Effects of lactoferrin and lactoperoxidase-containing food on the oral microbiota of older individuals. *Microbiology and Immunology* 61 (10):416–26. doi: 10.1111/1348-0421.12537.

Norrby, K., I. Mattsby-Baltzer, M. Innocenti, and S. Tuneberg. 2001. Orally administered bovine lactoferrin systemically inhibits VEGF165-mediated angiogenesis in the rat. *International Journal of Cancer* 91 (2):236–40. doi: 10.1002/1097-0215(200002)9999:9999<::AID-IJC1024>3.3.CO;2-K.

Oh, S. M., C. W. Pyo, Y. Kim, and S. Y. Choi. 2004. Neutrophil lactoferrin upregulates the human p53 gene through induction of NF-kappaB activation cascade . *Oncogene* 23 (50):8282–91. doi: 10.1038/sj.onc.1208021.

Okubo, K., M. Kamiya, Y. Urano, H. Nishi, J. M. Herter, T. Mayadas, D. Hirohama, K. Suzuki, H. Kawakami, M. Tanaka, et al. 2016. Lactoferrin suppresses neutrophil extracellular traps release in inflammation. *EBioMedicine* 10:204–15. doi: 10.1016/j.ebiom.2016.07.012.

Oria, R., L. Sanchez, M. Calvo, M. Ismail, and J. H. Brock. 1993. Effect of heat treatment and other milk proteins on the interaction of lactoferrin with monocytes. *The Journal of Dairy Research* 60 (3): 363–9. https://www.cambridge.org/core/journals/journal-of-dairy-research/article/effect-of-heat-treatment-and-other-milk-proteins-on-the-interaction-of-lactoferrin-with-monocytes/F6CE9B3D32F50FF06EE749EBFFC25727.

Ostan, N., A. Morgenthau, R. H. Yu, S. D. Gray-Owen, and A. B. Schryvers. 2017. A comparative, cross-species investigation of the properties and roles of transferrin- and lactoferrin-binding protein B from pathogenic bacteria. *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire* 95 (1):5–11. doi: 10.1139/bcb-2016-0055.

Ostan, N. K. H., R.-H. Yu, D. Ng, C. C.-L. Lai, A. K. Pogoutse, V. Sarpe, M. Hepburn, J. Sheff, S. Raval, D. C. Schriemer, et al. 2017. Lactoferrin binding protein B - a bi-functional bacterial receptor protein. *PLoS Pathogens* 13 (3):e1006244. doi: 10.1371/journal.ppat.1006244.

Paesano, R., F. Berlutti, M. Pietropaoli, W. Goolsbee, E. Pacifici, and P. Valenti. 2010. Lactoferrin efficacy versus ferrous sulfate in curing iron disorders in pregnant and non-pregnant women. *International Journal of Immunopathology and Pharmacology* 23 (2):577–87. doi: 10.1177/039463201002300220.

Paesano, R., F. Berlutti, M. Pietropaoli, F. Pantanella, E. Pacifici, W. Goolsbee, and P. Valenti. 2010. Lactoferrin efficacy versus ferrous sulfate in curing iron deficiency and iron deficiency anemia in pregnant women. *Biometals* 23 (3):411–7. doi: 10.1007/s10534-010-9335-z.

Paesano, R., T. Natalizi, F. Berlutti, and P. Valenti. 2012. Body iron delocalization: The serious drawback in iron disorders in both developing and developed countries. *Pathogens and Global Health* 106 (4):200–16.

Paesano, R., E. Pacifici, S. Benedetti, F. Berlutti, A. Frioni, A. Polimeni, and P. Valenti. 2014. Safety and efficacy of lactoferrin versus ferrous

sulphate in curing iron deficiency and iron deficiency anaemia in hereditary thrombophilia pregnant women: An interventional study. *Biometals* 27 (5):999–1006. doi: 10.1007/s10534-014-9723-x.

Paesano, R., M. Pietropaoli, S. Gessani, and P. Valenti. 2009. The influence of lactoferrin, orally administered, on systemic iron homeostasis in pregnant women suffering of iron deficiency and iron deficiency anaemia. *Biochimie* 91 (1):44–51. doi: 10.1016/j.biochi.2008.06.004.

Pan, W. R., P. W. Chen, Y. L. S. Chen, H. C. Hsu, C. C. Lin, and W. J. Chen. 2013. Bovine lactoferricin B induces apoptosis of human gastric cancer cell line AGS by inhibition of autophagy at a late stage. *Journal of Dairy Science* 96 (12):7511–20. doi: 10.3168/jds.2013-7285.

Paulsson, M. A., U. Svensson, A. R. Kishore, and A. S. Naidu. 1993. Thermal behavior of bovine lactoferrin in water and its relation to bacterial interaction and antibacterial activity. *Journal of Dairy Science* 76 (12):3711–20. doi: 10.3168/jds.S0022-0302(93)77713-9.

Paz-Ares, L., V. Hirsh, L. Zhang, F. De Marinis, C.-H. Yang, H. Wakelee, T. Seto, T. Schmelter, T. J. Ong, T. S. K. Mok, et al. 2012. Fortis-M, a randomized, double-blind, placebo-controlled phase 3 study of oral talactoferrin alfa with best supportive care in patients with advanced non-small cell lung cancer following two or more prior regimens- by the Fortis-M Study Group. *Annals of Oncology* 23:ixe23.

Peen, E., A. Johansson, M. Engquist, and T. Skogh. 1998. Hepatic and extrahepatic clearance of circulating human lactoferrin: An experimental study in rat. *European Journal of Haematology* 61 (3):151–9. doi: 10.1111/j.1600-0609.1998.tb01078.x.

Perdijk, O., R. J. J. van Neerven, E. van den Brink, H. F. J. Savelkoul, and S. Brugman. 2018. Bovine lactoferrin modulates dendritic cell differentiation and function. *Nutrients* 10 (7):848. doi: 10.3390/nu10070848.

Peterson, L. W., and D. Artis. 2014. Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nature Reviews. Immunology* 14 (3):141–53. doi: 10.1038/nri3608.

Pfefferkorn, M. D., J. H. Boone, J. T. Nguyen, B. E. Juliar, M. A. Davis, and K. K. Parker. 2010. Utility of fecal lactoferrin in identifying crohn disease activity in children. *Journal of Pediatric Gastroenterology & Nutrition* 51 (4):425–8. doi: 10.1097/MPG.0b013e3181d67e8f.

Pietrantoni, A., A. M. Di Biase, A. Tinari, M. Marchetti, P. Valenti, L. Seganti, and F. Superti. 2003. Bovine lactoferrin inhibits adenovirus infection by interacting with viral structural polypeptides. *Antimicrobial Agents and Chemotherapy* 47 (8):2688–91. doi: 10.1128/AAC.47.8.2688-2691.2003.

Pietrantoni, A., C. Fortuna, M. E. Remoli, M. G. Ciufolini, and F. Superti. 2015. Bovine lactoferrin inhibits toscana virus infection by binding to heparan sulphate. *Viruses* 7 (2):480–95. doi: 10.3390/v7020480.

Pires, L. A., R. Hegg, F. R. Freitas, E. R. Tavares, C. P. Almeida, E. C. Baracat, and R. C. Maranhão. 2012. Effect of neoadjuvant chemotherapy on low-density lipoprotein (LDL) receptor and LDL receptor-related protein 1 (LRP-1) receptor in locally advanced breast cancer. *Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Medicas e Biologicas* 45 (6):557–64. doi: 10.1590/s0100-879x2012007500068.

Prieels, J. P., S. V. Pizzo, L. R. Glasgow, J. C. Paulson, and R. L. Hill. 1978. Hepatic receptor that specifically binds oligosaccharides containing fucosyl alpha1 leads to 3 N-acetylglucosamine linkages. *Proceedings of the National Academy of Sciences of the United States of America* 75 (5):2215–9. doi: 10.1073/pnas.75.5.2215.

Puddu, P., M. G. Carollo, F. Belardelli, P. Valenti, and S. Gessani. 2007. Role of endogenous interferon and LPS in the immunomodulatory effects of bovine lactoferrin in murine peritoneal macrophages. *Journal of Leukocyte Biology* 82 (2):347–53. doi: 10.1189/jlb.1106688.

Puddu, P., P. Valenti, and S. Gessani. 2009. Immunomodulatory effects of lactoferrin on antigen presenting cells. *Biochimie* 91 (1):11–8. doi: 10.1016/j.biochi.2008.05.005.

Rao, S. S., Y. Hu, P. L. Xie, J. Cao, Z. X. Wang, J. H. Liu, H. Yin, J. Huang, Y. J. Tan, J. Luo, et al. 2018. Omentin-1 prevents inflammation-induced osteoporosis by downregulating the pro-inflammatory cytokines. *Bone Research* 6 (1):9. doi: 10.1038/s41413-018-0012-0.

Rastogi, N., A. Singh, P. K. Singh, T. K. Tyagi, S. Pandey, K. Shin, P. Kaur, S. Sharma, and T. P. Singh. 2016. Structure of iron saturated C-lobe of bovine lactoferrin at pH 6.8 indicates a weakening of iron coordination. *Proteins Struct Proteins* 84 (5):591–9. http://doi.wiley.com/10.1002/prot.25004. doi: 10.1002/prot.25004.

Rawat, P., S. Kumar, N. Sheokand, C. I. Raje, and M. Raje. 2012. The multifunctional glycolytic protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a novel macrophage lactoferrin receptor. *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire* 90 (3):329–38. doi: 10.1139/o11-058.

Rezk, M., R. Dawood, M. Abo-Elnasr, A. Al Halaby, and H. Marawan. 2016. Lactoferrin versus ferrous sulphate for the treatment of iron deficiency anemia during pregnancy: A randomized clinical trial. *The Journal of Maternal-Fetal & Neonatal Medicine* 29 (9):1387–90. doi: 10.3109/14767058.2015.1049149.

Rosa, L., A. Cutone, M. S. Lepanto, R. Paesano, and P. Valenti. 2017. Lactoferrin: A natural glycoprotein involved in iron and inflammatory homeostasis. *International Journal of Molecular Sciences* 18: 1985.

Roussel-Jazédé, V., I. Jongerius, M. P. Bos, J. Tommassen, and P. van Ulsen. 2010. NalP-mediated proteolytic release of lactoferrin-binding protein B from the meningococcal cell surface. *Infection and Immunity* 78 (7):3083–9. doi: 10.1128/IAI.01193-09.

Rubio, M. G., K. Amo-Mensah, J. M. Gray, V. Q. Nguyen, S. Nakat, D. Grider, K. Love, J. H. Boone, and D. Sorrentino. 2019. Fecal lactoferrin accurately reflects mucosal inflammation in inflammatory bowel disease. *World Journal of Gastrointestinal Pathophysiology* 10 (5):54–63. doi: 10.4291/wjgp.v10.i5.54.

Sabra, S., and M. M. Agwa. 2020. Lactoferrin, a unique molecule with diverse therapeutical and nanotechnological applications. *International Journal of Biological Macromolecules* 164:1046–60. doi: 10.1016/j.ijbiomac.2020.07.167.

Saidi, H., J. Eslahpazir, J. Eslaphazir, C. Carbonneil, L. Carthagena, M. Requena, N. Nassreddine, and L. Belec. 2006. Differential modulation of human lactoferrin activity against both R5 and X4-HIV-1 adsorption on epithelial cells and dendritic cells by natural antibodies. *Journal of Immunology* 177 (8):5540–9. doi: 10.4049/jimmunol.177.8.5540.

Saito, H., M. Takase, Y. Tamura, S. Shimamura, and M. Tomita. 1994. Physicochemical and antibacterial properties of lactoferrin and its hydrolysate produced by heat treatment at acidic pH. In *Advances in experimental medicine and biology*, 219–26. New York: Springer LLC.

Sano, H., K. Nagai, H. Tsutsumi, and Y. Kuroki. 2003. Lactoferrin and surfactant protein A exhibit distinct binding specificity to F protein and differently modulate respiratory syncytial virus infection. *European Journal of Immunology* 33 (10):2894–902. doi: 10.1002/eji.200324218.

Sanui, T., M. Takeshita, T. Fukuda, A. Haraguchi, Y. Aida, and F. Nishimura. 2017. Anti-CD14 antibody-treated neutrophils respond to LPS: Possible involvement of CD14 upregulated by anti-CD14 antibody binding. *Immunological Investigations* 46 (2):190–200. doi: 10.1080/08820139.2016.1238925.

Seo, Y. D., X. Jiang, K. M. Sullivan, F. G. Jalikis, K. S. Smythe, A. Abbasi, M. Vignali, J. O. Park, S. K. Daniel, S. M. Pollack, et al. 2019. Mobilization of CD8+ T cells via CXCR4 blockade facilitates PD-1 checkpoint therapy in human pancreatic cancer. *Clinical Cancer Research: Research* 25 (13):3934–45. doi: 10.1158/1078-0432.CCR-19-0081.

Seril, D. N., J. Liao, K. L. K. Ho, A. Warsi, C. S. Yang, and G. Y. Yang. 2002. Dietary iron supplementation enhances DSS-induced colitis and associated colorectal carcinoma development in mice. *Digestive Diseases and Sciences* 47 (6):1266–78. doi: 10.1023/A:1015362228659.

Serrano, G., I. Kochergina, A. Albors, E. Diaz, G. Hueso, and J. M. Serrano. 2020. Liposomal lactoferrin as potential preventative and cure for COVID-19. *International Journal of Health Sciences and Research* 8 (1):8–15.

Sessa, R., M. Di Pietro, S. Filardo, A. Bressan, P. Mastromarino, A. V. Biasucci, L. Rosa, A. Cutone, F. Berlutti, R. Paesano, et al. 2017. Lactobacilli-lactoferrin interplay in Chlamydia trachomatis infection. *Pathogens and Disease* 75 (5): ftx054. https://pubmed.ncbi.nlm.nih.gov/28505248/.

Shau, H., A. Kim, and S. H. Golub. 1992. Modulation of natural killer and lymphokine-activated killer cell cytotoxicity by lactoferrin. *Journal of Leukocyte Biology* 51 (4):343–9. doi: 10.1002/jlb.51.4.343.

Shin, K., H. Wakabayashi, K. Yamauchi, T. Yaeshima, and K. Iwatsuki. 2008. Recombinant human intelectin binds bovine lactoferrin and its peptides. *Biological & Pharmaceutical Bulletin* 31 (8):1605–8. doi: 10.1248/bpb.31.1605.

Singh, P. K., M. R. Parsek, E. P. Greenberg, and M. J. Welsh. 2002. A component of innate immunity prevents bacterial biofilm development. *Nature* 417 (6888):552–5. doi: 10.1038/417552a.

Siqueiros-Cendón, T., S. Arévalo-Gallegos, B. F. Iglesias-Figueroa, I. A. García-Montoya, J. Salazar-Martínez, and Q. Rascón-Cruz. 2014. Immunomodulatory effects of lactoferrin. *Acta Pharmacologica Sinica* 35 (5):557–66. doi: 10.1038/aps.2013.200.

Sorimachi, K., K. Akimoto, Y. Hattori, T. Ieiri, and A. Niwa. 1997. Activation of macrophages by lactoferrin: Secretion of TNF-alpha, IL-8 and NO. *Biochemistry and Molecular Biology International* 43 (1):79–87. doi: 10.1080/15216549700203841.

Spik, G., B. Brunet, C. Mazurier-Dehaine, G. Fontaine, and J. Montreuil. 1982. Characterization and properties of the human and bovine lactotransferrins extracted from the faeces of newborn infants. *Acta Paediatrica Scandinavica* 71 (6):979–85. doi: 10.1111/j.1651-2227.1982.tb09560.x.

Sreedhara, A., R. Flengsrud, V. Prakash, D. Krowarsch, T. Langsrud, P. Kaul, T. G. Devold, and G. E. Vegarud. 2010. A comparison of effects of pH on the thermal stability and conformation of caprine and bovine lactoferrin. *International Dairy Journal* 20 (7):487–94. doi: 10.1016/j.idairyj.2010.02.003.

Steijns, J. M., and A. C. M. Van Hooijdonk. 2000. Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. *British Journal of Nutrition* 84:11–17.

Strunk, T., J. E. Hibbert, D. Doherty, E. Nathan, K. Simmer, S. K. Patole, S. Trend, P. Richmond, D. Burgner, and A. Currie. 2020. Lactoferrin expression is not associated with late-onset sepsis in very preterm infants. *Neonatology* 117:606–611.

Sungnak, W., N. Huang, C. Bécavin, M. Berg, R. Queen, M. Litvinukova, C. Talavera-López, H. Maatz, D. Reichart, F. Sampaziotis, et al. 2020. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nature Medicine* 26 (5):681–7. doi: 10.1038/s41591-020-0868-6.

Superti, F. 2020. Lactoferrin from bovine milk: A protective companion for life. *Nutrients* 12:1–26.

Superti, F., and F. De Seta. 2020. Warding off recurrent yeast and bacterial vaginal infections: Lactoferrin and lactobacilli. *Microorganisms* 8:130.

Suzuki, Y. A., and B. Lönnerdal. 2002. Characterization of mammalian receptors for lactoferrin. *Biochemistry and Cell Biology* 80:75–80.

Suzuki, Y. A., and B. Lönnerdal. 2004. Baculovirus expression of mouse lactoferrin receptor and tissue distribution in the mouse. *Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine* 17 (3):301–9.

Suzuki, Y. A., V. Lopez, and B. Lönnerdal. 2005. Mammalian lactoferrin receptors: Structure and function. *Cellular and Molecular Life Sciences: CMLS* 62 (22):2560–75. doi: 10.1007/s00018-005-5371-1.

Tarnow-Mordi, W. O., M. E. Abdel-Latif, A. Martin, M. Pammi, K. Robledo, P. Manzoni, D. Osborn, K. Lui, A. Keech, W. Hague, et al. 2020. The effect of lactoferrin supplementation on death or major morbidity in very low birthweight infants (LIFT): A multicentre, double-blind, randomised controlled trial. *The Lancet Child & Adolescent Health* 4 (6):444–54. doi: 10.1016/S2352-4642(20)30093-6.

Tavassoly, O., F. Safavi, and I. Tavassoly. 2020. Heparin-binding peptides as novel therapies to stop SARS-CoV-2 cellular entry and infection. *Molecular Pharmacology* 98 (5):612–9. doi: 10.1124/molpharm.120.000098.

Tiwari, V., J. C. Beer, N. V. Sankaranarayanan, M. Swanson-Mungerson, and U. R. Desai. 2020. Discovering small-molecule therapeutics against SARS-CoV-2. *Drug Discovery Today* 25 (8):1535–44. doi: 10.1016/j.drudis.2020.06.017.

Togawa, J. I., H. Nagase, K. Tanaka, M. Inamori, A. Nakajima, N. Ueno, T. Saito, and H. Sekihara. 2002. Oral administration of lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. *Journal of Gastroenterology and Hepatology* 17 (12):1291–8. doi: 10.1046/j.1440-1746.2002.02868.x.

Togawa, J. I., H. Nagase, K. Tanaka, M. Inamori, T. Umezawa, A. Nakajima, M. Naito, S. Sato, T. Saito, and H. Sekihara. 2002. Lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 283 (1):46–1.

Troost, F. J., W. H. M. Saris, and R. J. M. Brummer. 2002. Orally ingested human lactoferrin is digested and secreted in the upper gastrointestinal tract in vivo in women with ileostomies. *The Journal of Nutrition* 132 (9):2597–600. doi: 10.1093/jn/132.9.2597.

Troost, F. J., J. Steijns, W. H. M. Saris, and R. J. M. Brummer. 2001. Gastric digestion of bovine lactoferrin in vivo in adults. *The Journal of Nutrition* 131 (8):2101–4. doi: 10.1093/jn/131.8.2101.

Tung, Y. T., H. L. Chen, C. C. Yen, P. Y. Lee, H. C. Tsai, M. F. Lin, and C. M. Chen. 2013. Bovine lactoferrin inhibits lung cancer growth through suppression of both inflammation and expression of vascular endothelial growth factor. *Journal of Dairy Science* 96 (4):2095–106. doi: 10.3168/jds.2012-6153.

Van Hooijdonk, A. C. M., K. D. Kussendrager, and J. M. Steijns. 2000. In vivo antimicrobial and antiviral activity of components in bovine milk and colostrum involved in non-specific defence. *British Journal of Nutrition* 84 (S1):127–34. doi: 10.1017/S000711450000235X.

Van Veen, H. A., M. E. J. Geerts, P. H. C. Van Berkel, and J. H. Nuijens. 2004. The role of N-linked glycosylation in the protection of human and bovine lactoferrin against tryptic proteolysis. *European Journal of Biochemistry* 271 (4):678–84. doi: 10.1111/j.1432-1033.2003.03965.x.

Vega-Bautista, A., M. de la Garza, J. C. Carrero, R. Campos-Rodríguez, M. Godínez-Victoria, and M. E. Drago-Serrano. 2019. The impact of lactoferrin on the growth of intestinal inhabitant bacteria. *International Journal of Molecular Sciences* 20 (19):4707. doi: 10.3390/ijms20194707.

Velliyagounder, K., W. Alsaedi, W. Alabdulmohsen, K. Markowitz, and D. H. Fine. 2015. Oral lactoferrin protects against experimental candidiasis in mice. *Journal of Applied Microbiology* 118 (1):212–21. doi: 10.1111/jam.12666.

Venkatesh, M. P., and L. Rong. 2008. Human recombinant lactoferrin acts synergistically with antimicrobials commonly used in neonatal practice against coagulase-negative staphylococci and Candida albicans causing neonatal sepsis. *Journal of Medical Microbiology* 57 (Pt 9):1113–21. doi: 10.1099/jmm.0.2008/001263-0.

Vergara, D., O. López, M. Bustamante, and C. Shene. 2020. An in vitro digestion study of encapsulated lactoferrin in rapeseed phospholipid-based liposomes. *Food Chemistry* 321:126717. doi: 10.1016/j.foodchem.2020.126717.

Vincent, J.-L., J. C. Marshall, R. P. Dellinger, S. G. Simonson, K. Guntupalli, M. M. Levy, M. Singer, and R. Malik. 2015. Talactoferrin in severe sepsis: Results from the phase II/III oral talactoferrin in severe sepsis trial. *Critical Care Medicine* 43 (9):1832–8. doi: 10.1097/CCM.0000000000001090.

Voswinkel, L., T. Vogel, and U. Kulozik. 2016. Impact of the iron saturation of bovine lactoferrin on adsorption to a strong cation exchanger membrane. *International Dairy Journal* 56:134–40. doi: 10.1016/j.idairyj.2016.01.008.

Wakabayashi, H., H. Oda, K. Yamauchi, and F. Abe. 2014. Lactoferrin for prevention of common viral infections. *Journal of Infection and Chemotherapy* 20 (11):666–71. doi: 10.1016/j.jiac.2014.08.003.

Walker, T. R., M. L. Land, A. Kartashov, T. M. Saslowsky, D. M. Lyerly, J. H. Boone, and P. A. Rufo. 2007. Fecal lactoferrin is a sensitive and specific marker of disease activity in children and young adults with inflammatory bowel disease. *Journal of Pediatric*

Gastroenterology & Nutrition 44 (4):414–22. doi: 10.1097/MPG.0b013e3180308d8e.

Wally, J., and S. K. Buchanan. A structural comparison of human serum transferrin and human lactoferrin. In *BioMetals*, 249–62. Springer; 2007. /pmc/articles/PMC2547852/?report = abstract

Wang, B., Y. P. Timilsena, E. Blanch, and B. Adhikari. 2019. Lactoferrin: Structure, function, denaturation and digestion. *Critical Reviews in Food Science and Nutrition* 59 (4):580–96. doi: 10.1080/10408398.2017.1381583.

Ward, P. P., S. Uribe-Luna, and O. M. Conneely. 2002. Lactoferrin and host defense. *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire* 80 (1):95–102. doi: 10.1139/o01-214.

Weng, T. Y., L. C. Chen, H. W. Shyu, S. H. Chen, J. R. Wang, C. K. Yu, H. Y. Lei, and T. M. Yeh. 2005. Lactoferrin inhibits enterovirus 71 infection by binding to VP1 protein and host cells. *Antiviral Research* 67 (1):31–7. doi: 10.1016/j.antiviral.2005.03.005.

Wu, J., J. Chen, W. Wu, J. Shi, Y. Zhong, E. A. F. Van Tol, Q. Tang, and W. Cai. 2014. Enteral supplementation of bovine lactoferrin improves gut barrier function in rats after massive bowel resection. *British Journal of Nutrition* 112 (4):486–92. doi: 10.1017/S000711451400107X.

Yao, X., C. Bunt, J. Cornish, S. Y. Quek, and J. Wen. 2013. Oral delivery of lactoferrin: A Review. *International Journal of Peptide Research and Therapeutics* 19 (2):125–34. doi: 10.1007/s10989-012-9326-8.

Zaczyńska, E., M. Kocięba, E. Liwińska, and M. Zimecki. 2014. Bovine lactoferrin enhances proliferation of human peripheral blood lymphocytes and induces cytokine production in whole blood cultures. *Advances in Clinical and Experimental Medicine* 23 (6):871–6. doi: 10.17219/acem/30168.

Żelechowska, P., J. Agier, and E. Brzezińska-Błaszczyk. 2016. Endogenous antimicrobial factors in the treatment of infectious diseases. *Central-European Journal of Immunology* 41 (4):419–25. doi: 10.5114/ceji.2016.65141.

Zhang, G., J. Han, E. J. Welch, R. D. Ye, T. A. Voyno-Yasenetskaya, A. B. Malik, X. Du, and Z. Li. 2009. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. *Journal of Immunology* 182 (12):7997–8004. doi: 10.4049/jimmunol.0802884.

Zhang, Q., C. Chen, M. Swaroop, M. Xu, L. Wang, J. Lee, M. Pradhan, M. Shen, Z. Luo, Y. Xu, et al. 2020. Targeting heparan sulfate proteoglycan-assisted endocytosis as a COVID-19 therapeutic option. bioRxiv.

Zhao, X., X. X. Xu, Y. Liu, E. Z. Xi, J. J. An, D. Tabys, and N. Liu. 2019. The in vitro protective role of bovine lactoferrin on intestinal epithelial barrier. *Molecules* 24 (1):148.

Zhou, C., J. Wang, N. Sun, J. Tian, J. Wang, Y. Lv, P. Wang, K. Huang, and H. Che. 2014. Allergenicity of recombinant human lactoferrin to an animal model Brown Norway rats. *Food and Agricultural Immunology* 25 (1):34–48. doi: 10.1080/09540105.2012.733352.

Zielińska, A., M. Sałaga, M. Włodarczyk, and J. Fichna. 2019. Focus on current and future management possibilities in inflammatory bowel disease-related chronic pain. *International Journal of Colorectal Disease* 34 (2):217–27. doi: 10.1007/s00384-018-3218-0.

Zimecki, M., and J. Artym. 2005. Właściwości terapeutyczne białek i peptydów z siary i mleka Therapeutic properties of proteins and peptides from colostrum and milk. *Postepy Higieny I Medycyny Doswiadczalnej* 59:309–23.

Zimecki, M., J. Dawiskiba, B. Zawirska, Z. Krawczyk, and M. Kruzel. 2003. Bovine lactoferrin decreases histopathological changes in the liver and regulates cytokine production by splenocytes of obstructive jaundiced rats. *Inflammation Research* 52 (7):305–10. doi: 10.1007/s00011-003-1178-4.

Zimecki, M., D. Stepniak, A. Szynol, and M. L. Krzuel. 2001. Lactoferrin regulates proliferative response of human peripheral blood mononuclear cells to phytohemagglutinin and mixed lymphocyte reaction. *Archivum immunologiae et therapiae experimentalis* 49 (2):147–54.

Zimecki, M. W. 2001. Lactoferrin regulates the immune responses in post-surgical patients. *Archivum immunologiae et therapiae experimentalis* 49 (4):325–33.

Zimmermann, M. B., C. Chassard, F. Rohner, E. K. N'Goran, C. Nindjin, A. Dostal, J. Utzinger, H. Ghattas, C. Lacroix, and R. F. Hurrell. 2010. The effects of iron fortification on the gut microbiota in African children: A randomized controlled trial in Côte d'Ivoire. *The American Journal of Clinical Nutrition* 92 (6):1406–15. doi: 10.3945/ajcn.110.004564.