The biology of lactoferrin, an iron-binding protein that defends against viruses and bacteria

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ABSTRACT

Lactoferrin is a nutrient classically found in mammalian milk. It binds iron and is transferred into and between cells, serum, bile and cerebrospinal fluid, via a variety of receptors. It has important immunological properties, and is both antibacterial and antiviral. In particular, there is evidence that it can bind to at least some of the receptors used by coronaviruses and thereby block their entry. It may consequently be of preventive and therapeutic value during the present COVID-19 pandemic.

KEYWORDS

Lactoferrin; Coronaviruses; Iron, Membrane Receptors

ABBREVIATIONS

LF: Lactoferrin; lactotransferrin
SARS-CoV: acute respiratory syndrome coronavirus
LRP-1/CD91: LDL receptor-related protein-1
TLR2 and 4: Toll-like receptor 2 and 4
CXCR4: cytokine receptor 4
GAG: glycosaminoglycan
AP-1: activator protein 1
NF-κB: NF-kappa beta
IRF: Interferon regulatory factor
MAPK: Mitogen-activated protein kinase
HSPG: Heparan sulfate proteoglycans
ACE2: Angiotensin-converting enzyme 2
IL: Interleukin
G-CSF: Granulocyte colony-stimulating factor
GM-CSF: Granulocyte-Macrophage Colony Stimulating Factor
IFN: Interferon
TNFα: Tumour necrosis factor alpha
IP10: Interferon gamma-induced protein 10
MCP1: Monocyte Chemoattractant Protein-1
(MIP1) A and B: Macrophage inflammatory protein 1 (A and B)
LMWH: Low molecular weight heparin
vWF: von Willebrand Factor
PAD4: protein arginine deiminase 4
NETS: Neutrophil extracellular traps

INTRODUCTION

Lactoferrin (LF) or lactotransferrin has recently come under the spotlight, particularly with regards to the new coronavirus pandemic that started in 2019 (COVID-19). Diet and supplements support a well-functioning immune system, and favourably influence the body's ability to fight infection. Although LF is produced by the body itself, as a secretion by exocrine glands (such as maternal milk or tears) and secondary granules of human neutrophils (Okubo et al., 2016), it can also be taken as a supplement, where it then acts as nutraceutical or functional food. Here we collate some of the evidence that shows how LF may be an important nutrient to support host immunity, including as an antibacterial and antiviral agent, but particularly with the current COVID-19 pandemic in mind. Our particular focus is on its role as an oral supplement. We summarise the layout of this paper in Figure 1.

DISCOVERY AND STRUCTURE

Human LF is a cationic glycosylated protein consisting of 691 amino acids (Anderson et al., 1990) folded into two globular lobes (80 kDa bi-lobal glycoprotein) (Vogel, 2012), that are connected by an α–helix (Karav et al., 2017, Karav, 2018). Bovine LF contains 689 amino acids (Moore et al., 1997). LF was first discovered and isolated from bovine milk in 1939 (Sorensen and Sorensen, 1939), and is a member of the transferrin family (60% amino acid sequence identity with serum transferrin) (Karav et al., 2017). LF and transferrin have similar amino acid compositions, secondary structures (including their disulphide linkages), and tertiary structures, although they differ in terms of biological functions (Karav et al., 2017, Querinjean et al., 1971, Bluard-Deconinck et al., 1974) (see Figure 2). There are also 3 different isoforms: LF-α is the iron-binding isoform, while LF- β and LF-γ both have ribonuclease activity but do not bind iron (Karav et al., 2017, Furmanski et al., 1989). When it is iron-rich it is referred to hololactoferrin and when iron-free apolactoferrin (Jameson et al., 1998). The tertiary structures of the two forms are significantly different: apolactoferrin is characterized by an open conformation of the N-lobe and a closed conformation of the C-lobe, while both lobes are closed in the hololactoferrin (Jameson et al., 1998). Human LF and bovine LF possess high sequence homology and have very similar antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory and immunomodulatory activities (Rosa et al., 2017, Teraguchi et al., 2004, Togawa et al., 2002). Consequently, it is common to give the bovine form rather than say a recombinant human form as a supplement. Bovine LF is also deemed a "generally recognized as safe" substance by the Food and Drug Administration (FDA, USA), and is commercially available in large quantities (Rosa et al., 2017).

Figure 1: Overview of this review of lactoferrin (LF). We discuss **1)** discovery and structure of LF; **2)** LF membrane receptors and some of the bacteria, their products and viruses that might also bind to these receptors, **3)** including how acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (causing COVID-19) may interact with host cells (see Figure 6 and Conclusion for a detailed discussion); **4)** and how LF assists with host immunity. Diagram created with BioRender (www.biorender.com).

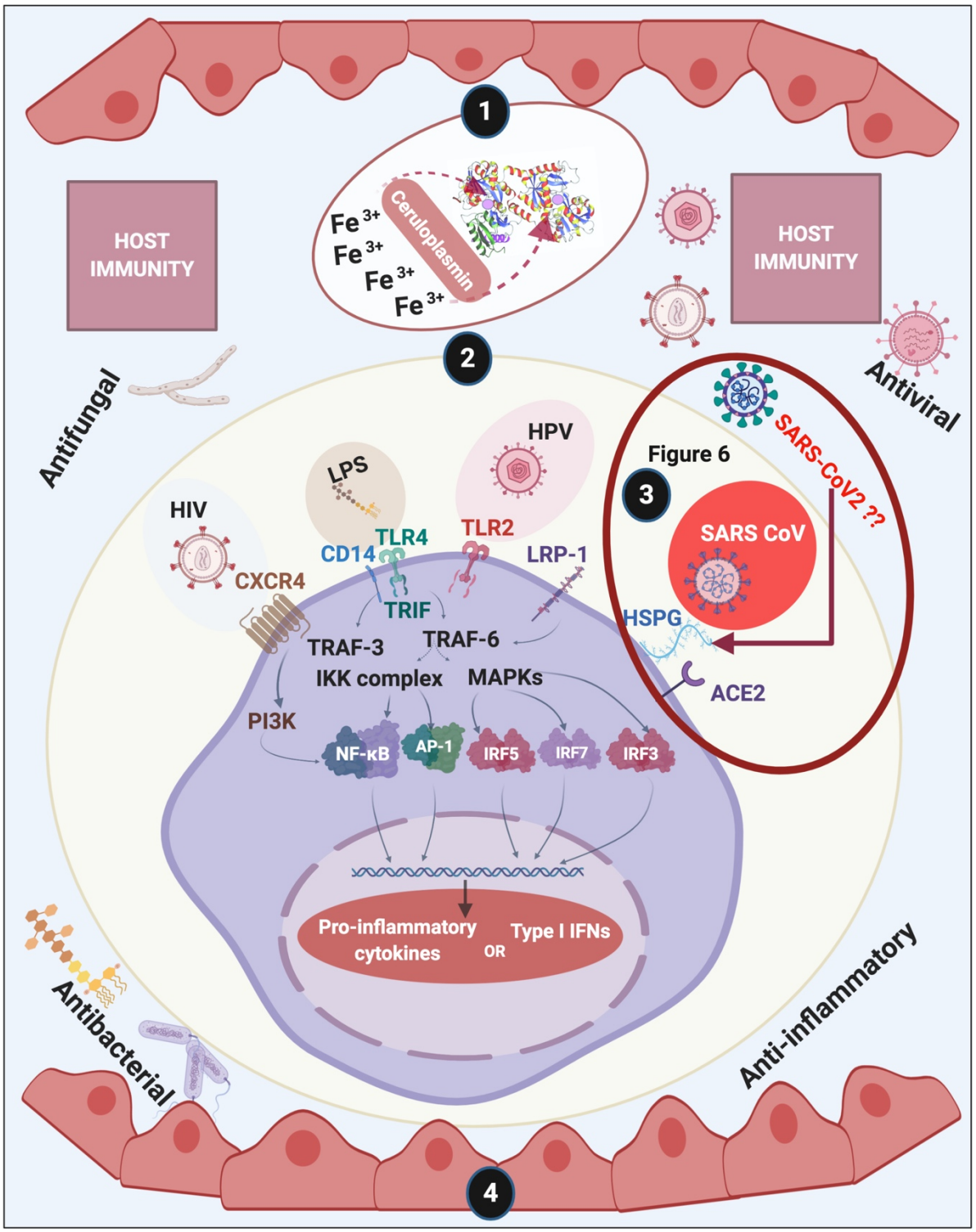
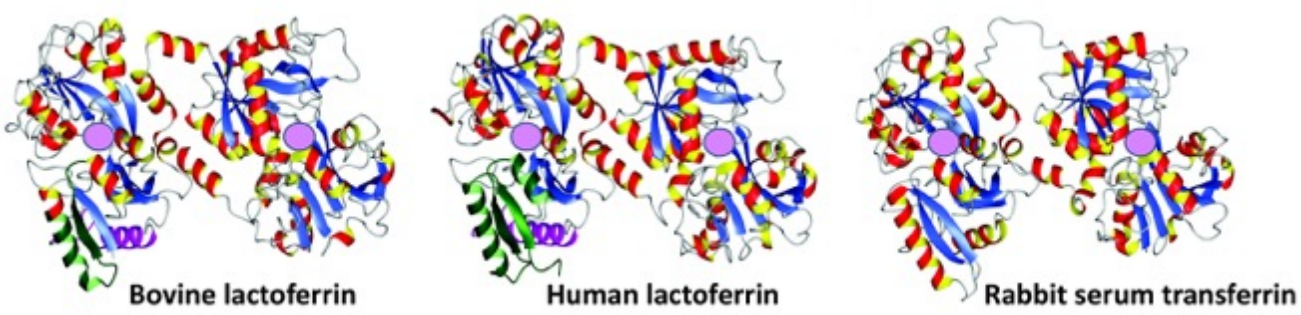


Figure 2: Crystal structures of bovine lactoferrin (PDB code = 1BLF), human lactoferrin (1B0L), and rabbit serum transferrin (1JNF). Adapted from (Vogel, 2012). Pink spheres represent ferric iron (Fe^{3+}) binding sites.



Due to its similarities to transferrin, which is the main iron transporting molecule in serum (Ashall et al., 2009, Anderberg et al., 2015), α-LF possesses iron binding capabilities (Brock, 2002, Brock, 2012), and it can chelate two ferric irons (Fe^{3+}) (Lepanto et al., 2019). LF binds one ferric iron atom in each of its two lobes; however, an important attribute is that it does not release its iron, even at pH 3.5. This is of importance as this property assures iron sequestration in infected tissues where the pH is commonly acidic (Ganz, 2018). In the context of its iron-binding capabilities, it means that when it binds ferric and siderophore-bound iron, it limits the availability of essential iron to microbes (Ganz, 2018).

In healthy individuals, iron is largely intracellular and sequestered within ferritin or as a co-factor of cytochromes and FeS proteins, and as haem complexed to haemoglobin within erythrocytes. Circulating iron is rapidly bound by transferrin (Kell, 2009, Kell and Pretorius, 2014). When erythrocytes lyse and haemoglobin or haem is released into the circulation, their haemoglobin is captured by haptoglobin, and haem by hemopexin (Skaar, 2010). Here, circulating serum ferroxidase ceruloplasmin is of importance, as LF can bind to ceruloplasmin, such that a direct transfer of ferric iron between the two proteins is possible (White et al., 2012). A direct transfer of ferric iron from ceruloplasmin to lactoferrin prevents both the formation of potentially toxic hydroxyl radicals (Kell and Pretorius, 2018) and the utilization of iron by pathogenic bacteria. LF is therefore an important player in preventing bacteria from acquiring and sequestering iron, which (with the possible exception of *Borrelia burgdorferi* (Posey and Gherardini, 2000)); they require for growth and virulence. LF also acts as biomarker, as it is commonly upregulated when the host is suffering from various kinds of disease. See Table 1 for selected references.

Table 1: Lactoferrin as a major player in host defence and iron binding, and its use as biomarker for various diseases.

Area of action	References
Protecting neonates via breast milk	(Telang, 2018, Chow et al., 2016, Hettinga et al., 2011, Ballard and Morrow, 2013, Woodman et al., 2018, Czosnykowska-Łukacka et al., 2019, Lönnerdal, 2016, Cai et al., 2018)
LF in cervicovaginal mucosa and female reproductive tract; antibacterial, antifungal antiparasitic, antiviral	(Valenti et al., 2018, Cole, 2006, Bard et al., 2003, Boesch et al., 2013)
LF in the airways	(Laube et al., 2006, Vargas Buonfiglio et al., 2018)
Mucosal surfaces, allergen-induces skin infections	(Ward et al., 2002)
Neutrophil extracellular trap (NET) production	(Delgado-Rizo et al., 2017)
Saliva and its antimicrobial activities and iron binding.	(Lynge Pedersen and Belstrøm, 2019, van Leeuwen et al., 2019, Wang et al., 2018)
Saliva as biomarker for neurological diseases	(Farah et al., 2018, Gleerup et al., 2019, Carro et al., 2017)
Saliva as biomarker for periodontal disease and oral dryness	(Koshi et al., 2018, Mizuhashi et al., 2015, Glimvall et al., 2012, Jalil et al., 1993)

LACTOFERRIN AND ITS MEMBRANE RECEPTORS

LF is thought to exert its main biological activities following interaction with receptors on target cells. There are in fact many LF receptors, though sometimes one is referred to as 'the' lactoferrin receptor. They have been detected in multiple tissues and cell types including intestinal epithelial cells and lymphocytes (Jiang et al., 2011, Suzuki et al., 2005). Receptors that bind LF include CD14 (Rawat et al., 2012), LDL receptor-related protein-1 (LRP-1/CD91) (Fillebeen et al., 1999, Grey et al., 2004, Ikoma-Seki et al., 2015) intelectin-1 (omentin-1) (Shin et al., 2008), Toll-like receptor 2 and 4 (TLR4)(Gao et al., 2018) and cytokine receptor 4 (CXCR4) (Takayama et al., 2017) (see Table 2). Importantly, LF also binds to heparan sulfate proteoglycans (HSPGs), which are cell-surface and extracellular matrix macromolecules that are composed of a core protein decorated with covalently linked

glycosaminoglycan (GAG) chains (Sarrazin et al., 2011, Frankel and Pabo, 1988, Lang et al., 2011, Milewska et al., 2014). See Table 2. Different receptors express at vastly different levels in different tissues; thus intelectin-1 is really expressed only in the intestine (https://www.proteinatlas.org/ENSG00000179914-ITLN1/tissue), while LRP1 is far more widely distributed https://www.proteinatlas.org/ENSG00000123384-LRP1/tissue. These multiple receptors arguably underpin the substantial and widespread effects that LF can induce, since only when multiple targets are hit simultaneously can one normally have major effects (Cornish-Bowden et al., 1995, Kell and Knowles, 2006).

The entry of bacteria, bacterial products or viruses into host cells may also occur via some of these receptors. Such binding evokes signalling systems and pathways involving, amongst others, mitogen-activated protein kinase (MAPK) (Liu et al., 2019), NF-κB (Zhou et al., 2018), activator protein 1 (AP-1) (Srivastava et al., 2019), and various interferon regulatory factors (IRFs) (for a comprehensive review see (Futosi et al., 2013)). During infection, activation of these signalling pathways results in a cellular response that shares multiple cytoplasmic components, leading ultimately to the activation of a complex biomolecular network. Phosphorylation of relevant substrates (e.g. enzymes, microtubules, histones, and transcription factors) plays a crucial role in determining the host's cellular response (Dreyfuss et al., 2009). Viruses (Christianson and Belting, 2014, Milewska et al., 2018), as well as bacteria (Xu et al., 2015), interact with and bind to HSPGs, using this proteoglycan as entry into the cell (see also Figure 1). LF acts as an important element in host defence mechanisms by binding to these receptors, but also binding to HSPG on cells, since these are locations where binding to bacteria and their cell wall products as well as viruses occur. The membrane-penetrating peptide HIV-tat, released from HIV-infected cells, also enters surrounding cells using HSPGs (Sarrazin et al., 2011, Frankel and Pabo, 1988). This binding capacity allows LF to compete with such molecules for receptor occupancy (Elass-Rochard et al., 1998, Baveye et al., 1999), and therefore plays a vital role in host immunity (Teraguchi et al., 2004). LF can also serve to prevent nephrotoxicity, e.g. of cisplatin (Kimoto et al., 2013).

Table 2: Receptors for lactoferrin, cells where these receptors are present, and other molecules and/or components that might bind to these receptors.

Receptor for lactoferrin	Cell types where receptor are present	Selected references
Lactoferrin receptor/ LRP-1/CD91/	Multiple tissues and cell types including intestinal epithelial cell	(Rawat et al., 2012)

apoE receptor or the chyclomicron remnant receptor	lymphocytes, fibroblasts, neurons, hepatocytes, endothelial cells	(Patel and Shah, 2017, Tamaki et al., 2007, Yan et al., 2008, Jiang et al., 2011)
Intelectin-1 (omentin-1)	Visceral (omental and epicardial) fat, mesothelial cells, vascular cells, airway goblet cells, small intestine, colon, ovary, and plasma	(Watanabe et al., 2017, Shin et al., 2008)
TLR2 and TLR4	Endothelial cells, platelets, neutrophils	(Tang et al., 2017, Vogel and Thein, 2018, Olumuyiwa-Akeredolu et al., 2019, Pretorius, 2019, García-Culebras et al., 2019, Page and Pretorius, 2020, Assinger et al., 2012, He et al., 2016)
CXCR4	Platelets, endothelial cells, neutrophils, T-cells	(Page and Pretorius, 2020, Zhang et al., 2009, De Filippo and Rankin, 2018, Seo et al., 2019)
CD14	Macrophages, neutrophils	(Rawat et al., 2012, Sanui et al., 2017, Palipane et al., 2019)
Heparan sulfate proteoglycans (HSPGs),	Epithelial cells, endothelial cells, fibroblasts, lymphocytes	(Sarrazin et al., 2011, Milewska et al., 2014)
Interleukin 1	Various cells	
Selected molecules and entities that bind to these receptors, other than lactoferrin		
Receptor	Molecule or cellular entity	Reference
Lactoferrin receptor	bacteria	(Skaar, 2010)
LRP-1	Amyloid beta (Aβ)	(Kim et al., 2016, Patel and Shah, 2017, Liu et al., 2007, Kanekiyo et al., 2013)
Intelectin-1 (omentin-1)	Microbial sugars, including β-D-galactofuranose (β-Gal*f*), D-glycerol 1-phosphate, d-*glycero*-D-*talo*-oct-2-ulosonic acid (KO),	(McMahon et al., 2020)

	and 3-deoxy-d-*manno*-oct-2-ulosonic acid (KDO)	
TLR4	Bacterial lipopolysaccharides (LPSs) Herpex simplex,	(Kell and Pretorius, 2015, Singer-Englar et al., 2019, Lv et al., 2018, Page and Pretorius, 2020)
CXCR4	Viruses (including HIV)	(Page and Pretorius, 2020, Chen, 2019, Mehrbod et al., 2019)
CD14	LPS, H7N9 Influenza virus	(Kell and Pretorius, 2015, Lee et al., 2019)
Heparan sulfate proteoglycans (HSPGs)	Various viruses, including HIV and SARS-CoV	(Sarrazin et al., 2011, Frankel and Pabo, 1988, Lang et al., 2011, Milewska et al., 2014, Naskalska et al., 2019, Cagno et al., 2019, Szczepanski et al., 2019)

LACTOFERRIN TRANSPORT

Small molecules, including pharmaceutical drugs, require solute carriers of the SLC family (Hediger et al., 2013) to effect their uptake (Dobson and Kell, 2008, Kell, 2015b, Kell, 2015a, Kell et al., 2013, Kell et al., 2011, Kell and Oliver, 2014, Superti-Furga et al., 2020, Girardi et al., 2020). Lactoferrin, as a protein, is far too large to exploit such a route, and instead passes from the stomach via epithelial cells and into the blood via endocytosis (Harada et al., 1999, Matsuzaki et al., 2019), especially via Peyer's patches (Talukder et al., 2003a), and especially when it is encapsulated ('enterically formulated') in liposomes (Takeuchi et al., 2006, Ishikado et al., 2005, Roseanu et al., 2010). This uptake then occurs mostly via the lymphatic rather than the portal circulation (Takeuchi et al., 2004, Wakabayashi et al., 2004). LF can also enter, and be reabsorbed from, the bile (Harada et al., 1999). Blood LF can further be transported to the CNS via cerebrospinal fluid (Kamemori et al., 2008, Talukder et al., 2003b) and via the Blood Brain Barrier (Kamemori et al., 2008, Fillebeen et al., 1999).

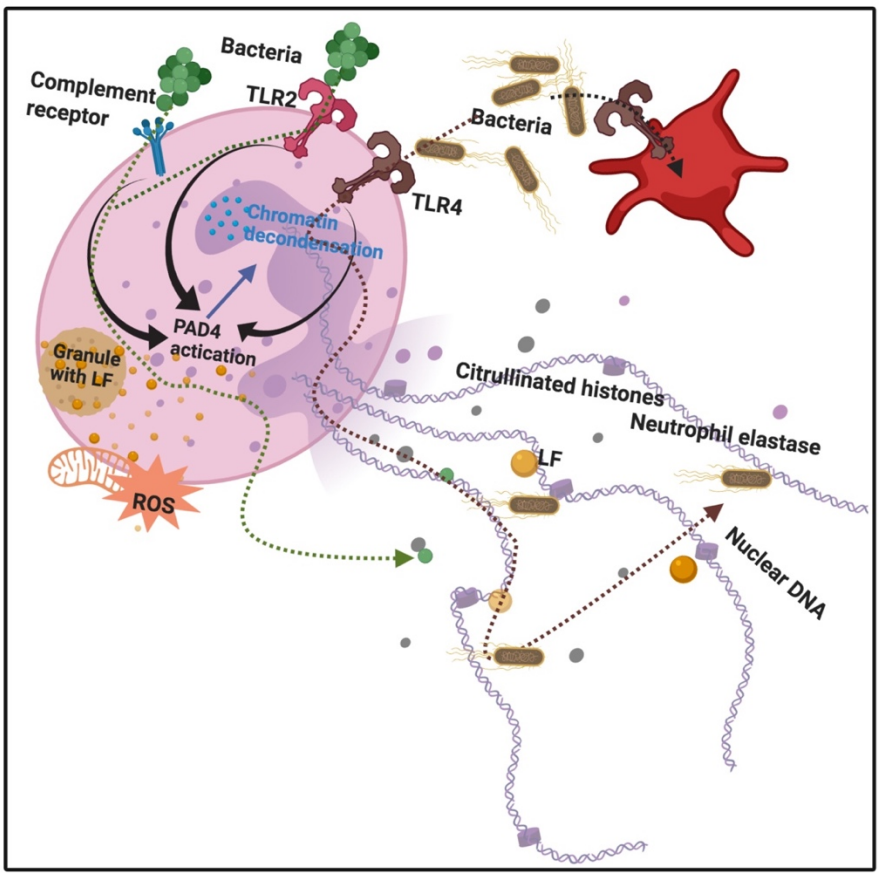
LACTOFERRIN: AN IMPORTANT ELEMENT IN HOST DEFENCE

Neutrophils and lactoferrin

LF plays an important role in host defence, upon its release from the neutrophil (Lepanto et al., 2019). As part of the host's inflammatory response, leucocytes, including neutrophils,

release LF from their granules, where it is normally stored. Activated neutrophils also release chromatin fibres, known as neutrophil extracellular traps (NETs), which trap and kill, amongst others, bacteria (Okubo et al., 2016, Brinkmann et al., 2004). These NETs likewise modulate both acute and chronic inflammation (Castanheira and Kubes, 2019, Hahn et al., 2016). NETs are also found in various autoimmune conditions such as rheumatoid arthritis, systemic lupus erythematosus (Lee et al., 2017, Papayannopoulos, 2018). Interestingly, 10^6 human neutrophils can release 15 µg of LF (Lepanto et al., 2019). In addition to DNA and histones, NET fibers contain extranuclear proteins and proteins such as elastase, myeloperoxidase (MPO), and LF (Urban et al., 2009). LF may also serve as an intrinsic inhibitor of NETs release into the circulation, and may therefore be a central in controlling NETs release (Okubo et al., 2016). See Figure 3.

Figure 3: Bacterial binding to various receptors, e.g. Toll-like receptors 2 and 4 (TLR2 and 4), as well as complement receptors, leads to protein arginine deiminase 4 (PAD4) activation, followed by chromatin decondensation, hypercitrullination of histones 3 and 4 in the nucleus, and nuclear membrane disruption. Granules also release lactoferrin. Neutrophil Extracellular Traps (NETs) and their protein constituents (including lactoferrin) are released from the neutrophil. Adapted from (Jorch and Kubes, 2017, Law and Gray, 2017). Bacteria are expelled and trapped in the NETs. Diagram created with BioRender (https://biorender.com/).



Bacteria and lactoferrin

One of the most well-known characteristics of LF is that it is antibacterial (Rosa et al., 2017, Petrik et al., 2017, Beddek and Schryvers, 2010, Pogoutse and Moraes, 2017, Wandersman and Stojiljkovic, 2000, Huang and Wilks, 2017), antiviral (Redwan et al., 2014, Lang et al., 2011, Chen et al., 2017, Carvalho et al., 2017), antifungal (Fernandes and Carter, 2017, Liao et al., 2019, Andrés et al., 2016), anti-inflammatory (Lepanto et al., 2019) and anti-carcinogenic (Wang et al., 2019). Its ability to of limit iron availability to microbes is one of its crucial amicrobial properties. Bacteria have, however, developed various ways to sequester iron (Nairz et al., 2010). Figure 4 shows how bacteria acquire iron through receptor-mediated recognition of transferrin, hemopexin, haemoglobin or haemoglobin-haptoglobin complexes and also LF (Skaar, 2010). As well as binding it directly from the environment, bacterial siderophores can obtain iron by removing it from transferrin, lactoferrin or ferritin(Kell and Pretorius, 2018). These siderophore-iron complexes are then recognized by receptors on the bacterium (Skaar, 2010). Host innate immune functions are supported by the circulating protein, siderocalin, also known as Neutrophil gelatinase-associated lipocalin (NGAL), lipocalin2 or Lcn2 as it inhibits siderophore-mediated iron acquisition and release (Skaar, 2010).

Figure 4: Ways by which bacteria acquire iron (adapted from (Skaar, 2010, Rosa et al., 2017)). Transferrin receptor, lactoferrin receptor, hemophore (Hp), hemophore receptor and hemopexin. Siderophores remove iron from lactoferrin, ferritin and transferrin, and also from the environment. Stealth siderophores are modified in such a way as to prevent siderocalin binding. A primary bacterial defence against siderocalin involves the production of stealth siderophores. Modified from (Skaar, 2010, Rosa et al., 2017). Diagram created with BioRender (https://biorender.com/).

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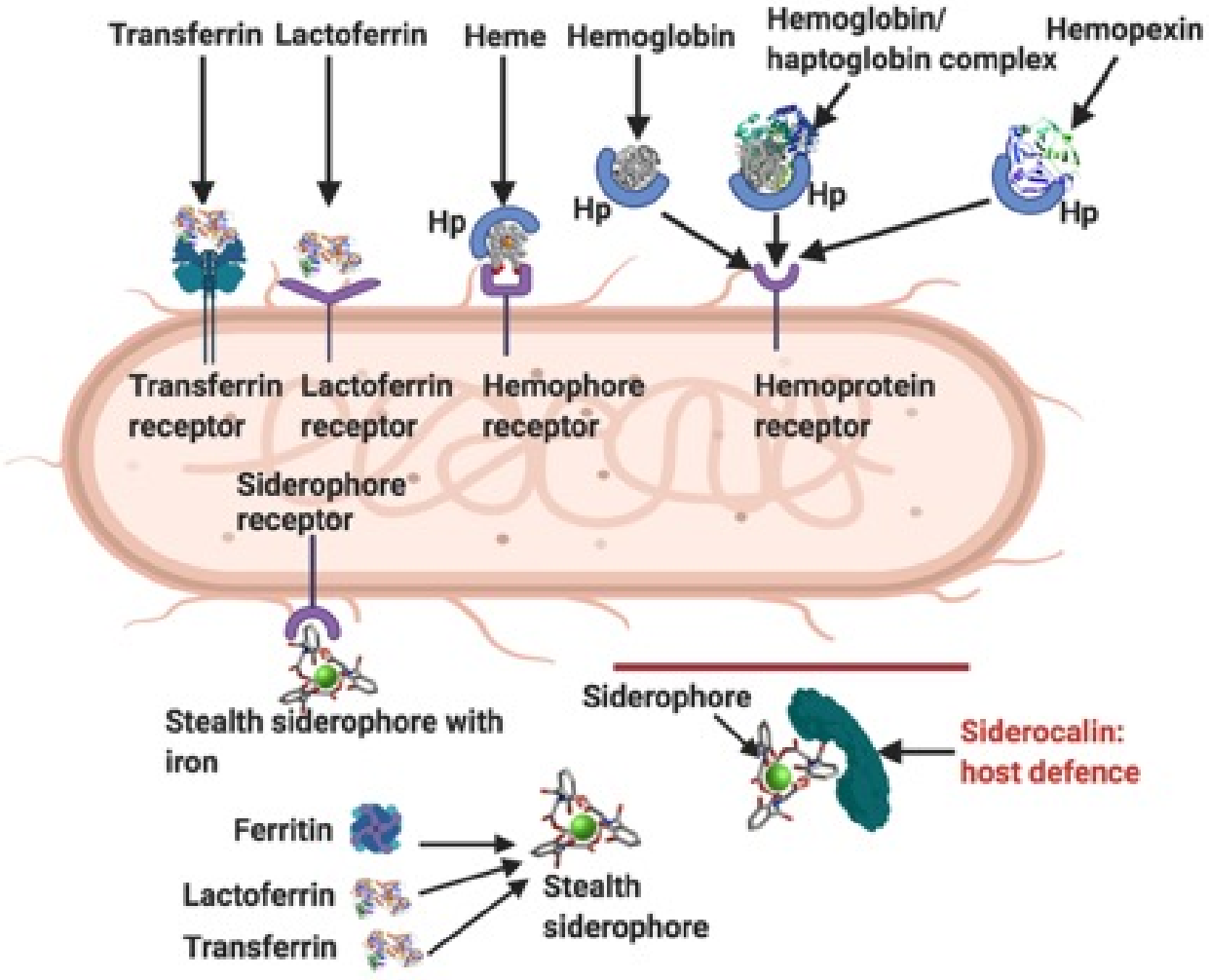


Although LF has various means to counteract bacteria as part of its immune function (Takeuchi et al., 2004), it is also capable of being hijacked to benefit the activities of bacteria. Thus, bacteria can also exploit LF by removing its bound ferric iron (Skaar, 2010, Rosa et al., 2017). This process involves 1) synthesis of high-affinity ferric ion chelators by bacteria, 2) iron acquisition through LF or transferrin binding, mediated by bacterial-specific surface bacterial receptors, 3) or iron acquisition through bacterial reductases, which are able to reduce ferric

to ferrous ions (Rosa et al., 2017, Petrik et al., 2017, Beddek and Schryvers, 2010, Pogoutse and Moraes, 2017, Wandersman and Stojiljkovic, 2000, Huang and Wilks, 2017).

Several Gram-negative pathogens including members of the genera *Neisseria* and *Moraxella* have evolved two-component systems that can extract iron from the host LF and transferrin (Brooks et al., 2014). *N. meningitidis* is a principal cause of bacterial meningitis in children. While the majority of pathogenic bacteria employ siderophores to chelate and scavenge iron (Weinberg, 2009), *Neisseria* has evolved a series of protein transporters that directly hijack iron sequestered in host transferrin, lactoferrin and haemoglobin (Schryvers and Stojiljkovic, 1999). The system consists of a membrane-bound transporter that extracts and transports iron across the outer membrane (TbpA for transferrin and LbpA for lactoferrin), and a lipoprotein that delivers iron-loaded lactoferrin/transferrin to the transporter (TbpB for transferrin and LbpB for lactoferrin) (Brooks et al., 2014). LbpB binds the N-lobe of lactoferrin, whereas TbpB binds the C-lobe of transferrin (Brooks et al., 2014). However, more than 90% of LF in human milk is in the form of apolactoferrin (Fransson and Lönnerdal, 1980), which competes with siderophilic bacteria for ferric iron, and disrupts the proliferation of these microbial pathogens. Similarly LF supplements may play an important role to counteract bacterial processes. LF is consequently a significant element of host defence (Rosa et al., 2017), and its levels may vary in health and during disease. It is hence known to be a modulator of innate and adaptive immune responses (Legrand, 2016).

Viruses and lactoferrin

LF has strong antiviral activity against a broad spectrum of both naked and enveloped DNA and RNA viruses (Redwan et al., 2014, Lang et al., 2011, Chen et al., 2017, Carvalho et al., 2017). LF inhibits the entry of viral particles into host cells, either by direct attachment to the viral particles or by blocking their cellular receptors (discussed in previous paragraphs) (Redwan et al., 2014). Some of the viruses that LF prevents from entering host cells e.g. *Herpes simplex* virus (Belting, 2003), human papillomavirus (Drobni et al., 2004), human immunodeficiency virus (HIV) (Puddu et al., 1998) and rotavirus (Superti et al., 2001). These viruses typically utilize common molecules on the cell membrane to facilitate their invasion into cells, including HSPGs (Figure 1). HSPGs provide the first anchoring sites on the host cell surface, and help the virus make primary contact with these cells (Belting, 2003, Lang et al., 2011). HSPGs can be either membrane bound, or in secretory vesicles and in the extracellular matrix (Sarrazin et al., 2011). It has been shown that LF is able to prevent the internalization of some viruses by binding to HSPGs (Sarrazin et al., 2011).

COVID-19 and lactoferrin

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Many COVID-19 patients develop acute respiratory distress syndrome (ARDS), which leads to pulmonary edema and lung failure, and have liver, heart, and kidney damages. These symptoms are associated with a cytokine storm (Mehta et al., 2020, Kell and Pretorius, 2016) manifesting elevated serum levels of interleukin (IL) IL-1β, IL-2, IL-7, IL-8, IL-9, IL-10, IL-17, granulocyte colony-stimulating factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), interferon (IFN)γ, tumour necrosis factor (TNF)α, Interferon gamma-induced protein 10 (IP10), Monocyte Chemoattractant Protein-1 (MCP1), macrophage inflammatory protein 1(MIP1)A and MIP1B (Wu and Yang, 2020). IL-22, in collaboration with IL-17 and TNFα, induces antimicrobial peptides in the mucosal organs. IL-22 also upregulates mucins, fibrinogen, anti-apoptotic proteins, serum amyloid A, and LPS binding protein (Zenewicz, 2018); therefore, IL-22 may contribute to the formation of life-threatening oedema with mucins and fibrin (Tse et al., 2004), seen in SARS-CoV-22 and SARS-CoV patients (Wu and Yang, 2020).

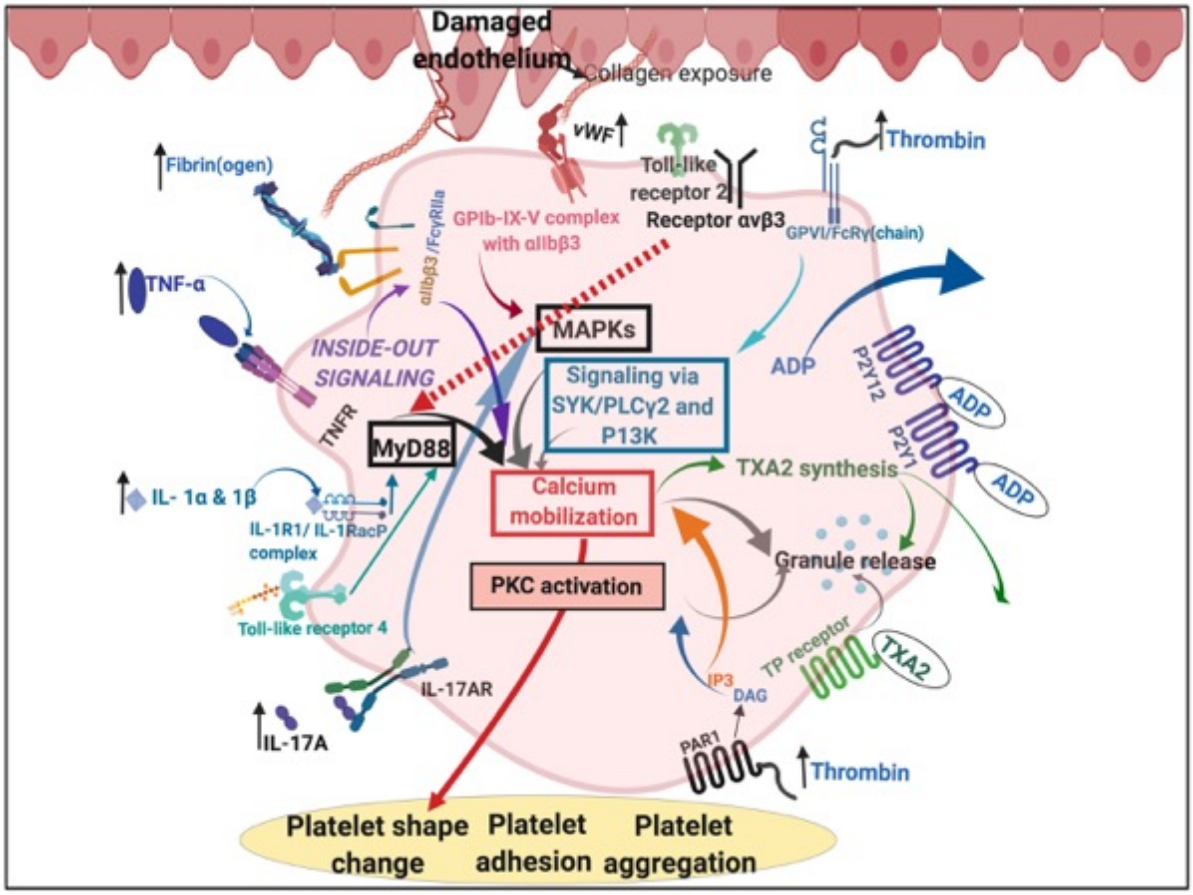
The 2003 SARS-CoV strain, that also causes severe acute respiratory syndrome, attaches to host cells via host receptor angiotensin-converting enzyme 2 (ACE2) (Wan et al., 2020). This type I integral membrane protein receptor is a well-known receptor for respiratory viruses, and is abundantly expressed in tissues lining the respiratory tract (Milewska et al., 2018). During COVID-19 infection, SARS-CoV-2 also enters host cells via the ACE2 receptor (Baig et al., 2020). ACE2 is highly expressed on human lung alveolar epithelial cells, enterocytes of the small intestine, and the brush border of the proximal tubular cells of the kidney (Lang et al., 2011). HSPGs are also one of the preliminary docking sites on the host cell surface and play an important role in the process of SARS-CoV cell entry (Lang et al., 2011). There is no current confirmed information that SARS-CoV-2 binds to HSPGs; however, LF blocks the infection of SARS-CoV by binding to HSPGs (Lang et al., 2011). It is not presently known whether LF binds to ACE2, but it does bind to HSPGs (Lang et al., 2011). Whether SARS-CoV-2 also enters host cells via HPSGs in the same way, as does (the 2003) SARS-CoV clearly warrants further investigation.

Of particular interest, and in the context of this paper, is the set of interactions between SARS-CoV-2 and host platelets. This is of importance, as COVID-19 infection, can cause hyperinflammation due to a cytokine storm (Mehta et al., 2020).

Pathogens like the influenza virus and *Francisella tularensis*, do trigger life-threatening cytokine storms (D'Elia et al., 2013). Such a cytokine storm will significantly affect platelets, as platelets have many receptors where these inflammatory molecules may bind (D'Elia et al., 2013) (see Figure 5). Circulating cytokines and inflammagens will hyperactivate platelets, causing low platelet count (thrombocytopenia), and a significant chance of hypercoagulation. Thrombocytopenia is associated with increased risk of severe disease and mortality in patients with COVID-19, and thus serves as clinical indicator of worsening illness during hospitalization (Lippi et al., 2020, Zhang et al., 2020). Patients with type 2 diabetes are also particularly prone to increased levels of circulating inflammatory cytokines and hypercoagulation (Pretorius, 2019). COVID-19 patients without other comorbidities but with diabetes are at higher risk of severe pneumonia, excessive uncontrolled inflammatory responses and a hypercoagulable state (Guo et al., 2020). Guo and co-workers in 2020 also found that serum levels of IL-6, C-reactive protein, serum ferritin, and D-dimer, were significantly higher in diabetic patients compared with those without, suggesting that patients with diabetes are more susceptible to an inflammatory storm eventually leading to rapid deterioration of the patient with COVID-19 (Papayannopoulos, 2018). Acute pulmonary embolism has also been reported in COVID-19 infection (Danzi et al., 2020). Focal accumulation of activated platelets within the oedematous area *ex vivo* correlated well with the size of the pulmonary embolism (Heidt et al., 2016). Interestingly, anticoagulant therapy, mainly with (intravenous) heparin (and mainly with low molecular weight heparin, LMWH), appears to be associated with better prognosis in severe COVID-19 patients (Tang et al., 2020).

In COVID-19 infection, LF may have a role to play in not only sequestering iron and inflammatory molecules that are severely increased during the cytokine burst, but also possibly in assisting in occupying receptors and HSPGs to prevent virus binding. Receptor occupancy is an important characteristic of LF, when taken as supplement. Furthermore, it may assist in preventing thrombocytopenia, and hypercoagulation, both prominent features of COVID-19 infection.

Figure 5: Simplified platelet signalling and receptor activation during disease with main dysregulated molecules thrombin, fibrin(ogen), von Willebrand Factor (vWF) interleukins (IL) like IL-1α, IL-1β, and IL17A and cytokines like TNF-α. Diagram created with BioRender (https://biorender.com/).



LACTOFERRIN AS A NUTRACEUTICAL

There is little doubt that oral LF can be of health benefits to the host, and while it is not considered to be absolutely necessary for mammalian life (so it is not a vitamin), it is reasonable to class it as a nutraceutical along with a variety of other molecules such as those in (Ames, 2018, Borodina et al., 2020).

Nutritional sources, availability and uses for lactoferrin as supplement

There is considerable LF availability in various forms and sources. Table 3 shows some of the sources and the references to research where it has been used to treat various conditions.

Table 3: Lactoferrin sources as supplements, and examples where it has been used to treat various conditions.

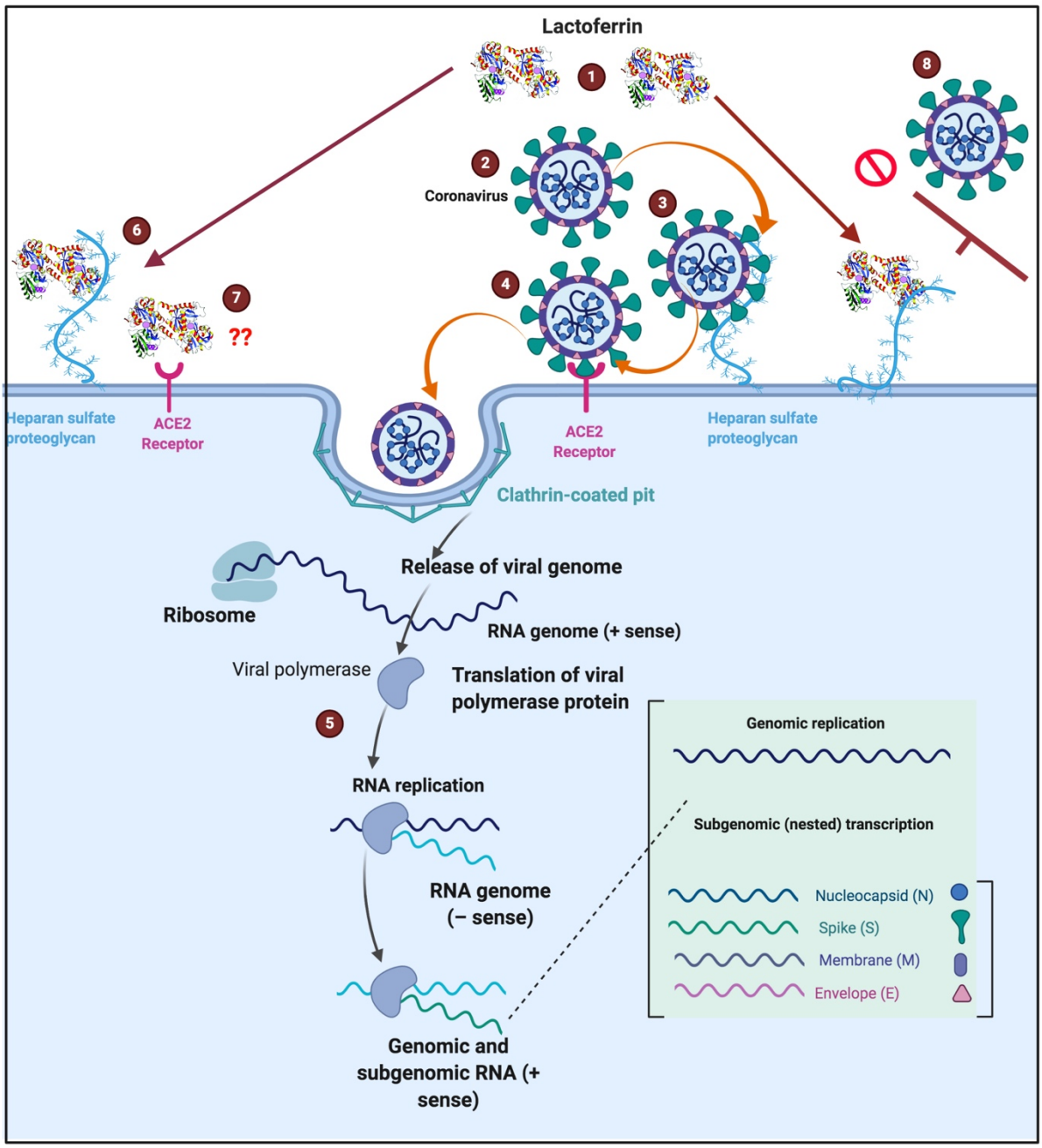
Lactoferrin sources as supplements	
Product	References
Bovine and human milk	Morinaga Industries in Japan (Bellamy et al., 1992) DoMO Food Ingredients, a subsidiary of Friesland Dairy Foods, in the Netherlands (Pammi and Suresh, 2017)
Human recombinant lactoferrin	Talactoferrin from Agennix, Inc., Houston, Texas, USA (Pammi and Suresh, 2017)
Lactoferrin expression in transgenic rice	Ventrus Biosciences, New York City, New York, USA (Pammi and Suresh, 2017)
Transgenic cattle expressing human lactoferrin	(Cooper et al., 2013, Wang et al., 2017)
Transgenic maize	Meristem Therapeutics, Clermont-Ferrand, France (Pammi and Suresh, 2017)
Lactoferrin supplementation in treatment of various diseases	
Might be useful in treating sepsis or necrotizing enterocolitis in preterm neonates	(Pammi and Suresh, 2017)
Support for vaginal healthy	(Russo et al., 2018)
LF may play a protective role in host defence against SARS-CoV infection through binding to HSPGs and blocking the preliminary interaction between SARS-CoV and host cells (cell culture study)	(Lang et al., 2011)
LF is a modulator of innate immune responses in the urinary tract and has potential application in novel therapeutic design for urinary tract infection (animal study)	(Patras et al., 2019)

Possible therapy against *Candida albicans* in the oral cavity (a hypothesis	(Chanda et al., 2017)
Protection against *Chlamydia trachomatis* (cell culture study)	(Sessa et al., 2017)
Treatment of taste and smell abnormalities after chemotherapy	(Wang et al., 2018)
LF supplements and food with high levels of LF for oral health	(Lang et al., 2011, Morita et al., 2017)
LF treatment of black stain associated with of iron metabolism disorders with lactoferrin	(Sangermano et al., 2019)
Aerosolized bovine LF counteracts infection, inflammation and iron dysbalance in a cystic fibrosis mouse model of P*seudomonas aeruginosa* chronic lung infection	(Cutone et al., 2019)
LF inhalations for lung health	(Marshall et al., 2016)
LF for optimal skin moisture	(Oda et al., 2019)

CONCLUSIONS

Lactoferrin clearly has immunological benefits, as well as having an important antibacterial and antiviral role. Because it is known to interfere with some of the receptors used by coronaviruses, it may contribute usefully to the prevention and treatment of coronavirus infections. Figure 6 shows a possible scheme on how LF might interfere with SARS-CoV-2 binding. The binding of LF to HSPGs prevents the first contact between virus and host cells and thus prevents subsequent infection (Lang et al., 2011). HSPGs themselves are not sufficient for SARS-CoV entry. However, in SARS-CoV infections, the HSPGs play an important role in the process of cell entry (Lang et al., 2011). The anchoring sites provided by HSPGs permit initial contact between the virus and host cells and the concentration of virus particles on cell surface. SARS-CoV bound to HSPGs then rolls onto the cell membrane and scans for specific entry receptors, which leads to subsequent cell entry (Lang et al., 2011). LF enhances natural killer cell activity and stimulates neutrophil aggregation and adhesion in immune defence (Reghunathan et al., 2005) can restrict the entry of the virus into host cells during infection. We suggest that this process might be the same for COVID-19 (see Figure 6 for a visual representation), thereby offering useful strategies for prevention and treatment.

Figure 6: Possible action of **1)** lactoferrin by occupying binding sites of **2)** SARS-CoV-2 that causes COVID-19. **3)** Entry into host cells occur when SARS-CoV-2 first attaches to Heparan sulfate proteoglycans (HSPGs). This attachment initiates the first contact between the cell and the virus, concentrating the virus on the cell surface, **4)** followed attaching of the virus to the host receptor (ACE2) and association and entering are then facilitated via clathrin-coated pits **5)** Virus replication can then happens inside the cell. **6)** One of the characteristics of Lactoferrin, is that it attaches to HSPGs. **7)** Currently we do not know if ACE2 is also a receptor for lactoferrin. **8)** Lactoferrin may block the entry of SARS-CoV-2 into the host cell, by occupying HPSGs, thereby preventing SARS-CoV-2 initial attachment and accumulation on the host cell membrane. COVID-19 infection template adjusted from www. biorender.com.



DECLARATIONS

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors approved submission of the paper.

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TABLE AND FIGURE LEGENDS

Table 1: Lactoferrin as a key player in host defence and iron binding, and its use as biomarker for various diseases.

Table 2: Receptors for lactoferrin, cells where these receptors are present, and other molecules and/or components that might bind to these receptors.

Table 3: Lactoferrin sources as supplements, and examples where it has been used to treat various conditions.

Figure 1: Overview of this review of lactoferrin (LF). We discuss **1)** discovery and structure of LF; **2)** LF membrane receptors and some of the bacteria, their products and viruses that might also bind to these receptors, **3)** including how acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (causing COVID-19) may interact with host cells (see Figure 6 and Conclusion for a detailed discussion); **4)** and how LF assists with host immunity. Diagram created with BioRender (www.biorender.com).

Figure 2: Crystal structures of bovine lactoferrin (PDB code = 1BLF), human lactoferrin (1B0L), and rabbit serum transferrin (1JNF); adapted from (Vogel, 2012). Pink areas represent ferric iron (Fe^{3+}) binding sites.

Figure 3: Bacterial binding to various receptors, e.g. Toll-like receptors 2 and 4 (TLR2 and 4), as well as complement receptors, leads to protein arginine deiminase 4 (PAD4) activation, followed by chromatin decondensation, hypercitrullination of histones 3 and 4 in the nucleus, and nuclear membrane disruption. Granules also release lactoferrin. Neutrophil Extracellular

Traps (NETs) and its protein constituents (including lactoferrin) are released from the neutrophil. Adapted from (Jorch and Kubes, 2017, Law and Gray, 2017). Bacteria are expelled and trapped in the NETs. Diagram created with BioRender (https://biorender.com/).

Figure 4: Ways by which bacteria acquire iron (adapted from (Skaar, 2010, Rosa et al., 2017)). Transferrin receptor, lactoferrin receptor, hemophore (Hp), hemophore receptor and hemopexin. Siderophores remove iron from lactoferrin, ferritin and transferrin, and also from the environment. Stealth siderophores are modified in such a way as to prevent siderocalin binding. A primary bacterial defence against siderocalin involves the production of stealth siderophores. Modified from (Skaar, 2010, Rosa et al., 2017). Diagram created with BioRender (https://biorender.com/).

Figure 5: Simplified platelet signalling and receptor activation during disease with main dysregulated molecules thrombin, fibrin(ogen), von Willebrand Factor (vWF) interleukins (IL) like IL-1α, IL-1β, and IL17A and cytokines like TNF-α. Diagram created with BioRender (https://biorender.com/).

Figure 6: Possible action of **1)** lactoferrin by occupying binding sites of **2)** SARS-CoV-2 that causes COVID-19. **3)** Entry into host cells occur when SARS-CoV-2 first attaches to Heparan sulfate proteoglycans (HSPGs). This attachment initiates the first contact between the cell and the virus, concentrating the virus on the cell surface, **4)** followed attaching of the virus to the host receptor (ACE2) and association and entering are then facilitated via clathrin-coated pits **5)** Virus replication can then happens inside the cell. **6)** One of the characteristics of Lactoferrin, is that it attaches to HSPGs. **7)** Currently we do not know if ACE2 is also a receptor for lactoferrin. **8)** Lactoferrin may block the entry of SARS-CoV-2 into the host cell, by occupying HPSGs, thereby preventing SARS-CoV-2 initial attachment and accumulation on the host cell membrane. COVID-19 infection template adjusted from www. biorender.com.

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