

# Lactoferrin may inhibit the development of cancer via its immunostimulatory and immunomodulatory activities (Review)

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**Abstract.** Lactoferrin (Lf) is secreted by ectodermal tissue and has a structure similar to that of transferrin. Although Lf seems to be multifunctional, its main function is related to the natural defense system of mammals. The present review aims to highlight the major actions of Lf, including the regulation of cell growth, the inhibition of toxic compound formation, the removal of harmful free radicals and its important role in immune response regulation. Moreover, Lf has antibacterial, antiviral, antioxidant, anticancer and anti-inflammatory activities. In addition, the use of Lf for functionalization of drug nanocarriers, with emphasis on tumor-targeted drug delivery, is illustrated. Such effects serve as an important theoretical basis for its future development and application. In neurodegenerative diseases and the brains of elderly people, Lf expression is markedly upregulated. Lf may exert an anti-inflammatory effect

by inhibiting the formation of hydroxyl free radicals. Through its antioxidant properties, Lf can prevent DNA damage, thereby preventing tumor formation in the central nervous system. In addition, Lf specifically activates the p53 tumor suppressor gene.

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*Abbreviations:* Lf, lactoferrin; LPS, lipopolysaccharide; HCV, hepatitis C virus; HS, heparan sulfate; CdKs, cyclin-dependent kinases; NK, natural killer

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## 1. Introduction

Lactoferrin (Lf), which has a molecular weight of 77-80 kDa, is an iron-binding glycoprotein that has multiple functions in the body; it is involved in the apoptosis of cancer cells and can regulate various immune responses (1). Lf was first discovered in 1939 and is a 'red protein' found in milk; it can be separated and purified from human milk and cow's milk. The isolated protein structure of Lf is similar to that of serum transferrin, with a 60% sequence homology, and it reversibly binds to iron (Fe<sup>3+</sup>) ions (2). Therefore, Lf is classified as a member of the transferrin family, together with serum transferrin, melanotransferrin and ovotransferrin (3). This multifunctional protein is present in mucosal secretions, including tears,

saliva, vaginal secretions, semen, nasal secretions, bronchial secretions, bile, gastrointestinal secretions, urine, cow's milk and human milk (4). Lf is also present on the mucosal surface and granules of white blood cells. Human milk and cow's milk are the most abundant sources of Lf (5). Lf is very similar between different species. In fact, the homology between the Lf of humans and cattle is 77% (6).

Lf promotes the absorption of iron by the body; it regulates cell growth, removes harmful free radicals and inhibits the formation of toxic compounds. In the regulation of immune responses, Lf exerts antibacterial, antiviral, anti-oxidation, anticancer and anti-inflammatory activities (5). Accordingly, Lf is added to a number of commercial products, including infant formula, fermented milk, cosmetics, therapeutic drinks, toothpaste and other products used in daily life (7).

In human endometrial stromal cells (8) and human embryonic kidney cells, Lf can enhance DNA synthesis in normal cells in a dose-dependent manner (9). In addition, another form of Lf,  $\Delta$ Lf, can be expressed in glandular epithelium cells such as those of the prostate and salivary glands.  $\Delta$ Lf is a protein subtype of Lf that lacks the leader sequence and the first 25 residues of the original protein (10). The truncated protein mRNA is detected in all normal tissues, but not in some tumor-derived cell lines. Several studies have shown that the chromosomal region encoding Lf is deleted in various tumors, which is a spontaneous process that may occur during cancer (11).  $\Delta$ Lf plays a role as a transcription factor in cells, participates in the regulation of specific gene expression and plays a role in cancer (12). A number of *in vivo* studies have indicated the potential antitumor effect of Lf, suggesting that the oral bovine Lf (bLf) administration could decrease chemically induced carcinogenesis in rodents, along with marked cytotoxic and anti-metastatic activity against numerous cancer cell lines (13,14). The antitumor effect of Lf acts via a number of different mechanisms, including the induction of apoptosis in tumor tissues (7).

Owing to the overexpression of a number of cell surface receptors, Lf has a positive targeting effect; therefore, it is considered to be an ideal nanocarrier for certain hydrophobic therapeutic agents. In addition, as Lf can cross the blood-brain barrier (BBB), it has proven to be a good candidate for manufacturing nanocarriers to specifically deliver drugs for brain tumors. Therefore, Lf appears as a promising molecule with multiple applications in the fields of cancer treatment and nanomedicine. Lf has numerous advantages in terms of its ability to actively participate in the manufacture of nanocarriers. Furthermore, it is one of the few proteins that have a net positive charge under physiological conditions [isoelectric point (pI) 8.0-8.5]. Owing to its high pI value, Lf is positively charged over a wide range of pH values (15), is fairly stable in the gastrointestinal tract and possesses a number of intestinal receptors that facilitate the oral absorption and bioavailability of Lf-based nanocarriers within the circulation.

## 2. Lf exerts both immunostimulatory and immunomodulatory activities

Lf is a natural immune modulator that plays roles in the innate and acquired immune systems, which regulate antibody formation, T- and B-cell maturation, and increase the percentage of

natural killer cells in the lymphocyte population (16). The ability of Lf to regulate the activity of the immune response may be due to its ability to bind endotoxins [lipopolysaccharides (LPSs)] (17,18). In a previous study, Lf was found to alleviate the cellular inflammation induced by LPS by attenuating the nuclear factor- $\kappa$ B/mitogen-activated protein kinase pathways, mitigating oxidative stress and maintaining cellular barrier integrity. Such a finding implies that Lf plays an important role in immune regulation (19). When gram-negative bacteria try to invade the human host, the bacteria come into contact with various proteins of the innate immune system. Part of the bacterial outer membrane contains LPS. When this 'pathogen-related molecular pattern' is recognized by Toll-like receptor 4, it triggers a number of immune responses in various white blood cells and platelets (20,21). The combination of Lf and endotoxins released by bacteria can decrease the degree of stimulation of the immune system. This process can prevent overstimulation, which sometimes occurs in diseases such as sepsis. The hLf1-11 peptide derived from human lactoferrin (hLf) can inhibit myeloperoxidase, which is a major host defense enzyme found in a variety of white blood cells, which may further decrease the innate immune response (22). Furthermore, hLf has been shown to stimulate the maturation of dendritic cells and recruit various white blood cells (23). Therefore, Lf plays an activating role in innate and adaptive immune responses.

Lactoferrin is an allosteric enhancer of the proteolytic activity of cathepsin G, thereby affecting the function of adaptive immune cells (24). Lf has a positive charge that enables it to bind to the negatively charged surface molecules of various immune system cells, and this connection is believed to trigger signaling pathways that result in cellular responses such as activation, differentiation and proliferation. Lf can be transported to the nucleus in order to bind DNA and activate various signaling pathways (25). Lactoferrin can bind to DNA, and through its highly positively charged N-terminal region, which remains associated with the extruded DNA in the neutrophil extracellular traps, can still contribute to the bacterial killing in this process. As the granules also secrete a variety of proteolytic enzymes, Lf or other polypeptides may also be locally released from intact Lf (26).

As well as the induction of systemic immunity, skin immunity is promoted and allergic reactions are suppressed by Lf (27). The immune system is activated against skin allergens, resulting in the dose-dependent inhibition of Langerhans cell migration and dendritic cell accumulation in lymph nodes. The exposure of leukocyte Lf to cytokines, pro-inflammatory cytokines, TNF- $\alpha$ , IL-6 and IL-1 $\beta$  may be adjusted to increase and decrease. The production of these factors depends on the type of signal that is recognized by the immune system. At the cellular level, Lf can increase the number of CD4<sup>+</sup> and CD8<sup>+</sup> cells in natural killer (NK) cells and T cells (28), promote the recruitment of leukocytes in the blood, induce phagocytosis and regulate the process of bone marrow formation (29). Lf also increases the expression of hyaluronic acid, which is required for the formation of granulation tissue, upregulates platelet-derived growth factors and promotes the proliferation and migration of keratinocytes; this is a necessary condition for the re-epithelialization of wounds. Lf also protects cells from apoptosis (30).

Table I. Anti-carcinogenic activity of Lf against various tumor types.

Cancers associated with Lf	Protein	Outcome	(Refs.)
Breast cancer	hLf	Arrest cancer cells in the G <sub>0</sub> /G <sub>1</sub> phase, induction of apoptosis, and modulation of Bcl-2 and Bax expression	(100)
	bLf	Suppression of V-H <sup>+</sup> ATPase and decrease of the acidity of the tumor microenvironment	(101)
Colorectal cancer	hLf, bLf	Increased expression of TGF-β1, stimulation of IL-18 secretion in Caco-2 cells	(102)
	bLf	Enhanced infiltration of CD4 <sup>+</sup> and CD8 <sup>+</sup> cells, increased production of IL-18	(103)
GBM	hLf	Suppression of the proliferation of NMD and FN primary cells by a decrease in the expression of cyclin D1 and D4	(104)
Lung cancer	hLf	Antiproliferative effects attributed to the elevated levels of hypophosphorylated Rb in H1299 cells	(105)
	bLf	Decreased levels of TNF-α, IL-4, IL-6 and IL-10 cytokines, limiting inflammation and restricting tumor proliferation	(106)
NPC	hLf	Downregulation of PDPK1 via the MAPK/c-Jun pathway and suppression of K18-facilitated AKT stimulation	(107)
OSCC	bLf	Selective suppression of growth through mTOR/S6K and JAK/STAT3 signaling pathways and triggering of apoptosis in OSCC	(108)
Prostate cancer	bLf	Inhibition of the plasma membrane V-ATPase, suppressing tumor progression and metastasis in PC-3 cells	(14)

hLf, human lactoferrin; bLf, bovine lactoferrin; NPC, nasopharyngeal carcinoma; OSCC, oral squamous cell carcinoma; GBM, glioblastoma.

### 3. Lf is a natural immune modulator involved in the antitumor response

Lf is considered as a key component of the first line of defense for the human body (15) and has a variety of biological effects, including regulation of the immune response, iron absorption, and anti-inflammatory and antioxidant activities. Lf exerts antitumor effects through a variety of mechanisms. Oral bLf can decrease chemically induced carcinogenesis in rodents and has significant cytotoxicity and anti-metastatic activity in numerous cancer cell lines, such as breast cancer and stomach cancer cell lines (31,32). Table I summarizes some of the anti-carcinogenic mechanisms of Lf.

Lf is a survival factor of rheumatoid synovial neutrophils, an iron-binding protein that is released from activated neutrophils in inflammatory sites, and has anti-inflammatory and antibacterial properties (33). Although the isolation of iron by Lf and the direct effect on reactive oxygen intermediates are major factors in decreasing excessive inflammatory response damage by directly controlling the development of higher-order immune functions, Lf can regulate injury and pathology caused by injury. This ultimately leads to a decrease in the pathological damage during inflammation (34). The mechanism of action of Lf involves a component that differentially regulates the cellular immune response in sepsis models *in vivo*. Apoptotic cells can release Lf and combine with neutrophils to inhibit the chemotaxis of neutrophils, enabling macrophages to swallow apoptotic cells, thereby exerting anti-inflammatory effects (Fig. 1) (35).

Bezault *et al* (36) confirmed, for the first time, the antitumor activity of Lf in fibrosarcoma and melanoma mouse

models. Furthermore, an intraperitoneal injection of hLf could inhibit the growth of solid tumors and lung metastasis, independent of the iron saturation of the protein. Notably, the anticancer ability of LF is related to the presence of NK cells. To further prove the relevance of Lf in anticancer activities, Damiens *et al* (37) studied the role of Lf in cancer progression under inflammatory conditions. The experimental results showed that Lf regulates NK cell cytotoxicity and the sensitivity of target cells to lysis. Similar results were also obtained in experiments by Shi and Li (38). However, Iyer *et al* (39) pointed out that the antitumor properties of Lf may partly be due to its iron-binding properties. Free iron may act as a mutagenic promoter via the induction of oxidative damage to the nucleic acid structure (40), thereby decreasing the risk of tumors induced by oxidation (Fig. 2) (41,42).

Numerous studies (43-45) have shown that exogenous treatment with Lf and its derivatives can effectively inhibit tumor growth and decrease tumor susceptibility. Specifically, the downregulation or silencing of Lf and its derivatives can lead to an increased chance of developing a tumor (46). Conversely, the proliferation of cancer cells is prevented after the restoration of the Lf gene (47). However, these studies did not definitively conclude the mechanism underlying the anticancer effect of Lf. This review discusses the potential applications of Lf gene expression in cancer treatment and the association between Lf and cancer. To date, it has been indicated that the cytotoxicity of Lf to several cancers occurs via three methods under different conditions: i) Destruction of cell membranes; ii) induction of cell apoptosis; and iii) cell cycle arrest and cellular immune response.

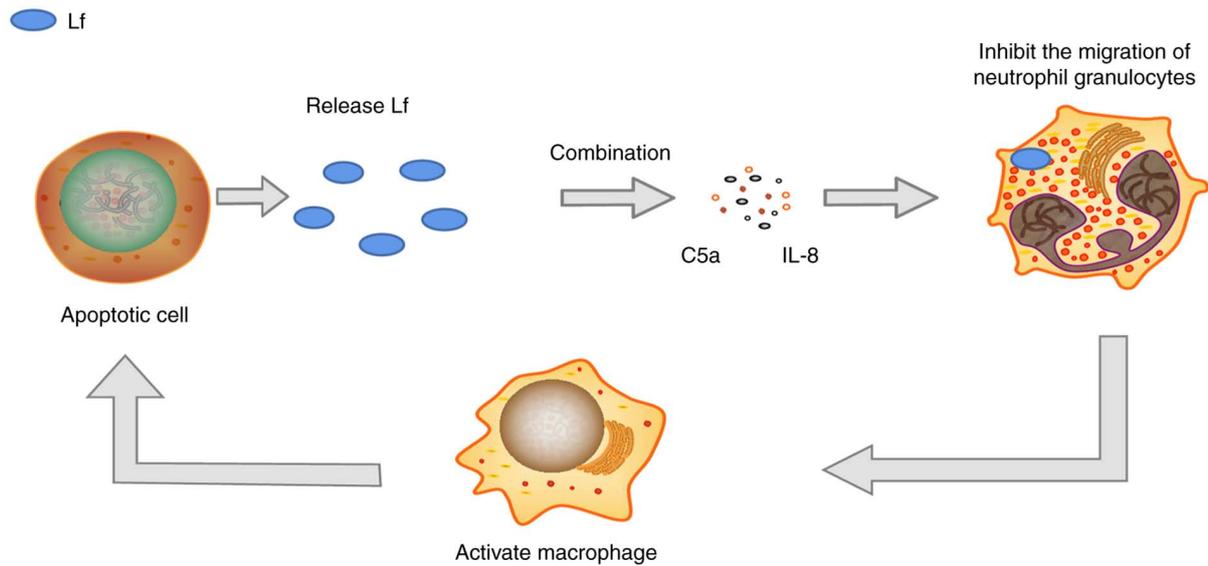


Figure 1. Inhibition of neutrophil chemotaxis via active production of factor(s) by apoptotic cells. Apoptotic cells can release Lf and combine with neutrophils to inhibit the chemotaxis of neutrophils, which enable macrophages to engulf apoptotic cells, thereby exerting anti-inflammatory effects. Lf, lactoferrin.

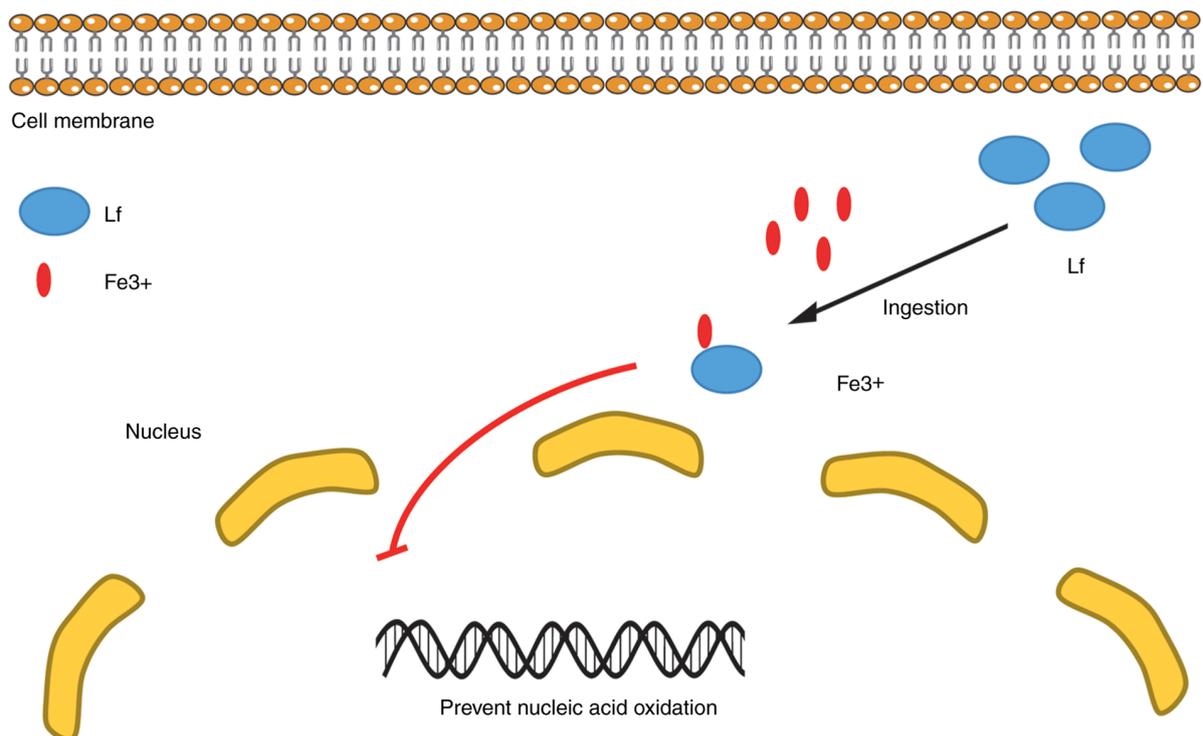


Figure 2. Lactoferrin is classified as an iron binding protein. Iron is formed of metal ions, which have a catalytic effect on the formation of hydroxyl radicals, suppressing the activity of host defense cells. Due to its iron-binding properties, Lf has been proposed to play a role in iron uptake by the intestinal mucosa and to act as a bacteriostatic agent by withholding iron from iron-requiring bacteria. The iron-binding properties of Lf may contribute to its antitumor properties. Lf, lactoferrin.

**Destruction of cell membranes.** Lf and its derived peptides are easily endocytosed by Jurkat cells. Cutone *et al* (48) found that in T lymphocytes, Lf enters cells through receptor-mediated endocytosis and is almost completely degraded in lysosomes. The Lf-active peptide, Lf-B, also exhibits conformation-dependent uptake efficiency (49). The increase in membrane permeability may change the barrier function of the membrane and promote cell death. At low concentrations, Lf and its derivative

peptides can increase cell lysis, and at high concentrations, Lf can regulate cell lysis, depending on the phenotype of the target cells (50). Most cancer cells contain a large amount of proteoglycans, aminoglycans and sialic acid, which all interact with Lf (51,52). This recognition may be the basis of the specificity and selectivity of Lf anticancer drugs. For example, Riedl *et al* (50) found that phosphatidylserine, a component of the cytoplasmic membrane mainly found in tumor cells, is a

key target for the specific anticancer activity of hLf derivatives. This selective interaction through cell surface receptors is actually a cytotoxic reaction. Particularly at high concentrations, hLf and bLf and the peptides derived from them have been shown to promote cytotoxicity and cell death in prokaryotic and eukaryotic pathogens and cancer cells (53,54). This is mainly related to the cationic charge of Lf. Lf can promote electrostatic interactions with negatively charged cell surface receptors. Cationic peptides derived from LF have a low mass ratio and can enter the cell membrane and destroy its stability, thereby easily inducing cell membrane dissolution (55).

**Apoptosis induction.** Cell apoptosis induced by Lf has been described as the pivotal pathway whereby peptides exert their cytotoxic effects against various cancer cells. However, the apoptotic pathway that they trigger depends on the cell type (56,57).

In a previous study, stomach cancer SGC-7901 cells were treated with Lf, and phosphorylated Akt and numerous key proteins involved in the Akt signaling pathway were decreased as a result. However, the expression levels of phosphorylated caspase-9 and phosphorylated glycogen synthase kinase-3 $\beta$  were increased, indicating that, in stomach cancer SGC-7901 cells, Lf-induced apoptosis may be regulated via the Akt pathway (32). Lf was also found to induce a stress-related mitogen-activated protein kinase pathway in Jurkat T cells, where c-Jun N-terminal kinase (JNK) associated with Bcl-2 was hypothesized to be the pathway responsible for the apoptosis induced by Lf (58). Lf treatment induced caspase-9 and -3 activation and increased the level of Bcl-2 phosphorylation. Following the abolition of JNK activation, cell death did not occur in Lf-treated Jurkat cells. Additionally, BLf was demonstrated to induce the apoptotic extrinsic pathway by upregulating Fas signaling in the colon mucosa of azoxymethane-treated rats (59).

**Cell cycle arrest and cellular immune responses.** Mammalian cell cycles are usually strictly controlled by hormones and growth factors, and abnormal regulation may lead to tumors. Cyclins, cyclin-dependent kinases (CdKs) and their antagonists, CdK inhibitors, are key factors that regulate cell cycle progression. In breast cancer MDA-MB-231 cells, hLF was found to inhibit cell growth during the transition phase from G<sub>1</sub> to S of the cell cycle. At the molecular level, hLF induces a significant decrease in the protein level and activity of Cdk2 and Cdk4, activates cyclins D and E, and plays a key role in the transition from the G<sub>1</sub> to the S phase (60). Similar hLf effects have also been reported in four head and neck cancer cell models, with blockage of the G<sub>1</sub> to S phase after hLf treatment (61). Lf was reported to induce cell growth arrest by reducing phospho-Akt resulting in increased expression and activity of p21Cip1 and p27Kip1 (62).

Lf has been confirmed to enhance the adaptive immune response and is an effective anti-inflammatory drug (63). Although its molecular mechanism still needs to be revealed, researchers have found that both hLf and bLf can enter the host cell nucleus and bind to DNA to regulate gene expression, thereby exerting their anti-inflammatory activity (64). hLf has significantly increased NK cell-mediated cytotoxicity in breast cancer and colon cancer cell lines (65).

#### **4. Lf has anticancer effects on tumors of the central nervous system**

Tammam *et al* (66) revealed that the cytotoxicity of Lf to gliomas can be attributed to its cytoplasmic distribution. The nuclear transmission of Lf induces cell proliferation rather than cytotoxicity, suggesting that the mode of action of Lf in glioma is related to cell location.

It is well known that tumor cells overexpress Lf receptors in order to fulfill the increased nutritional demands of these highly proliferative cells (67). Lf is an ideal nanocarrier for certain hydrophobic therapeutics due to its active targeting potential as a result of its receptor being overexpressed on the surface of a number of cells. Moreover, Lf is good potential candidate for fabricating nanocarriers that specifically deliver drugs to brain tumors, as Lf can cross the BBB. Consequently, Lf appears as a promising molecule with multiple applications in the fields of cancer therapy and nanomedicine (68). Song *et al* (69) demonstrated the potential utility of Lf-conjugated GO@Fe<sub>3</sub>O<sub>4</sub> nanocomposites for therapeutic applications in the treatment of gliomas (69). Lf-conjugated iron oxide nanoparticles can be used as tracers for targeted brain glioma imaging using magnetic particle imaging (70). Lf/phenylboronic acid-functionalized hyaluronic acid (HA) nanogel crosslinked with a disulfide bond crosslinker was generated as a reduction-sensitive dual-targeting glioma therapeutic platform for doxorubicin hydrochloride (DOX) delivery (71). Lf-HA-DOX significantly increased drug delivery to the glioma and may thus serve as a promising anti-glioma therapy (72).

#### **5. Lf affects tumor progression by exerting anti-bacterial and anti-viral activities**

*Lf has a broad inhibitory effect on anti-bacterial infections.* Inflammatory bowel disease is a chronic inflammatory and relapsing condition of the gastrointestinal tract (73). Normally, the gut microbiota is composed of 90% Bacteroidetes and Firmicutes, with rare phyla, such as Proteobacteria and Actinobacteria, as well as fungi, viruses, and protists, composing the remaining 10%. Anti-microbial activity has been described as the first Lf function linked to the ancestral host defense-linked mechanisms to target pathogen infections. This activity, evaluated in several *in vitro* (74,75) and *in vivo* (76) models, can be both independent and dependent of the iron-binding ability of Lf. The anticancer activity of Lf via host immunomodulation has been widely reported, particularly in colorectal cancer (43).

Lf is a multifunctional natural defense protein with significant antibacterial activity (77); its function is mainly reflected in the absorption of Fe<sup>3+</sup>, which limits the use of Fe<sup>3+</sup> by bacteria in the infected site, and inhibits the growth and reproduction of these microorganisms and the expression of their virulence factors. The bactericidal effect of Lf is mainly mediated by its interaction with the bacterial surface. *In vivo* (78) and *in vitro* (79) studies have shown that Lf prevents certain bacteria from adhering to host cells (15) (Fig. 3).

Lf destroys the outer membrane of gram-negative bacteria by interacting with LPS. The positively charged n-terminus of Lf prevents LPS and bacterial cations (Ca<sup>2+</sup> and Mg<sup>2+</sup>) from

interacting, resulting in the release of LPS from the cell wall, increasing membrane permeability and subsequently causing damage to the bacteria. The interaction between Lf and LPS also enhances the effect of natural antibacterial agents, such as lysozyme, which are secreted from the mucosa at high concentrations together with Lf via the BBB (15). Dialysis chamber research indicates that bacterial killing requires direct contact with Lf, and work with purified LPS suggests that this relates to direct LPS-binding by the protein. As Lf and lysozyme are both present in mucosal secretions and neutrophil granules, their interaction may help the host defense (80).

The ability of *Helicobacter pylori* to use hLf as a source of iron depends on the contact between cells and proteins. As Lf is abundant in gastrectomy specimens of patients with superficial or atrophic gastritis, the uptake of iron by *H. pylori* through specific hLf receptors may play a major role in the virulence of *H. pylori* infection (Fig. 4) (81). The combination of bovine Lf and *Streptococcus pneumoniae* surface protein is poor, and human transferrin does not bind to *S. pneumoniae* surface protein (82). Breast milk Lf inactivates two putative colonization factors expressed by *Haemophilus influenzae*. Breast milk Lf may decrease the pathogenic potential of *H. influenzae* by the selective inactivation of iga1 protease and hap, thereby interfering with the colonization of the bacteria (83).

*Lf has a broad inhibitory effect on DNA and RNA viruses.* Lf is an iron-binding glycoprotein found in some mucosal secretions and with antiviral activity against DNA and RNA viruses, such as HIV and rotavirus. The antiviral effect of LF occurs in the early stages of infection. Lf prevents the virus from entering the host cell by blocking cell receptors (84). In particular, Lf has antiviral and immune responses, such as demonstrated by Lf against SARS-CoV, which is closely related to SARS-CoV-2 that causes COVID-19 (85).

Lf can prevent the internalization of certain viruses into host cells, such as poliovirus type 1, which causes human polio, herpes simplex virus types I and II, and giant cell viruses. For other viruses, such as hepatitis C virus (HCV) and rotavirus, instead of preventing their entry, Lf inhibits virus replication in the host cell. At present, the specific inhibitory mechanism remains to be studied; however, a widely accepted hypothesis is the binding and blockage of glycosaminoglycan virus receptors, particularly heparan sulfate (HS), by Lf. LF and HS in combination stops the first contact between the host and virus cells, thereby preventing infection. *In vitro* studies have shown that in human plasma and milk proteins, LF exerts a strong activity on HIV, and this effect is due to the inhibition of virus replication in the host cells (15). The HCV envelope protein binds to Lf. hLf and bLf, a multifunctional immunomodulator, combines two HCV envelope proteins. Based on western blotting with milk separated by sodium lauryl sulfate-polyacrylamide gel electrophoresis or immunopurification, bacteria expressing e1 and e2 can bind to Lf (86-88).

## 6. Lf has enzyme activity functions

The effect of Lf on gram-positive bacteria is mainly based on the combination of cations on the surface of Lf and anions

on the surface of the bacteria, thereby neutralizing the negative charges on the surface of the gram-positive bacteria. For example, lipoteichoic acid decreases the negative charge on the cell wall, thereby facilitating the contact between lysozyme and the peptidoglycan under the cell wall, ultimately exerting an enzymatic effect (15).

Lf functions as an enzyme in certain reactions. Lf is the milk protein with the highest activity of DNase, RNase, ATPase and amylase. However, these activities are not the only enzymatic activities of Lf. bLf binds two HCV envelope proteins (88). Lf has DNA-binding properties (89) and can participate in the transcriptional activation of specific DNA sequences (90) or as a signal transduction mediator (91). Celiac disease has the highest amylase and ATP activities (92). The discovery of the properties of the Lf enzyme helps to clarify a number of its physiological functions.

## 7. Lf plays an extensive role in nanotechnology

Lf is used as a nanocarrier of DOX, as its receptor is highly expressed on the surface of highly proliferating cells (such as cancer cells). DOX is an effective cytotoxic anticancer drug, but has been reported to exhibit extensive toxicity to the heart and spleen, in addition to its limited oral absorption (93,94). Drug-loaded preparations have shown good physical stability, indicating that damage to the red blood cell membrane is negligible. Drug delivery through nano-formulations not only minimizes the cardiotoxicity of DOX, but also improves the efficacy and bioavailability of the drug in a targeted-specific manner (95).

A study has also shown that Lf nanoparticles are used to encapsulate antiviral drugs. Zidovudine nano-encapsulation into Lf nanoparticles has been achieved through sol-oil chemistry. Zidovudine is an effective antiviral drug with good bioavailability (50-75%); however, it can cause bone marrow suppression, neutropenia and organ toxicity. In the study by Kumar *et al* (96), the size of the prepared nanoparticles was 50-60 nm, the drug encapsulation efficiency was 67% and good physical stability was observed at room temperature and at 4°C. Furthermore, there was no significant change in particle size or drug content. Oral administration of efavirenz-loaded Lf nanoparticles resulted in anti-HIV-1 effects comparable to those of the free drug. In addition, compared with free efavirenz, drug-loaded nanoparticles showed improved pharmacokinetic characteristics and lower organ toxicity, indicating that this nanoformulation is a safe nanoplatform that can enhance drug delivery (96).

The cationic nature of Lf can be used to form complexes with negatively charged DNA through electrostatic complexation. In this context, in a previous study, plasmid pGFPC1, which encodes the green fluorescent protein, was used as a cargo gene (97). Lf nanoparticles loaded with plasmids were prepared using the sol-oil method. The diameter of the prepared Lf nanoparticles was 60 nm and the PDI was low, indicating the uniformity of the preparation. The prepared Lf nanoparticles also showed enhanced physical stability at a temperature of 4°C for ≤10 weeks without the particle size exhibiting significant changes. Incubation in DMEM containing 10% serum at 37°C for 8 h also did not result in changes to the particle size, which would result in longer

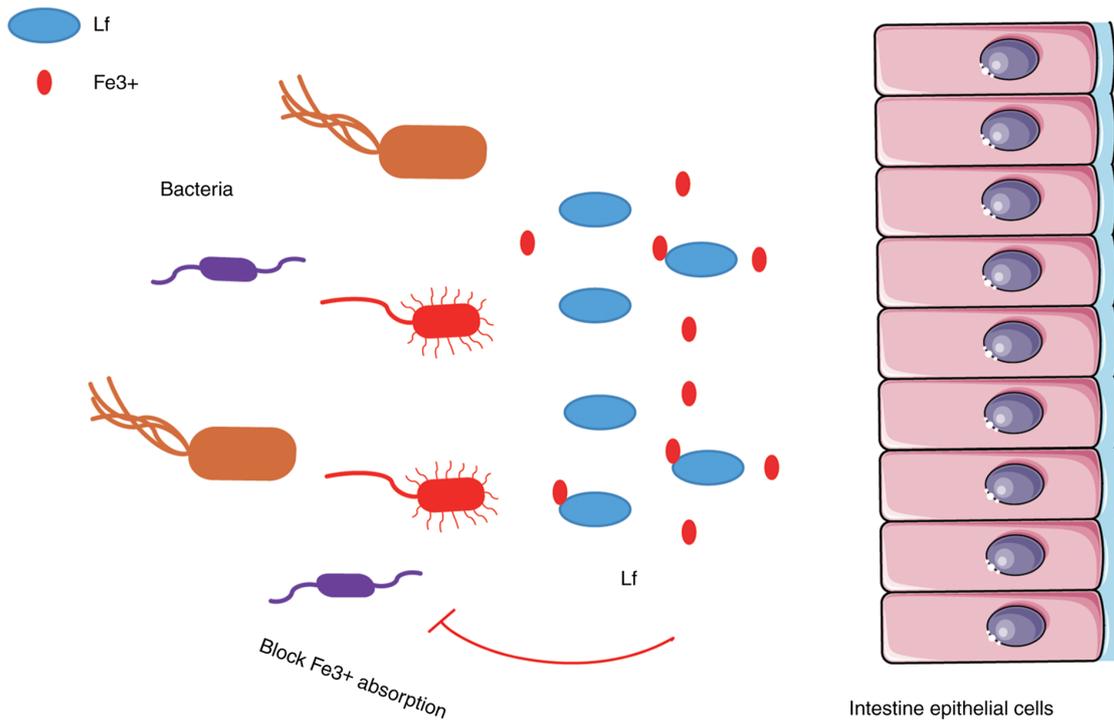


Figure 3. Human and bovine Lf exhibits a wide antimicrobial spectrum against Gram-positive and Gram-negative bacteria, fungi and a number of viruses. Lf interacts with the surface of bacteria to inhibit the bacterial absorption of  $Fe^{3+}$ . The bactericidal effect of Lf is mainly mediated by its interaction with the bacterial surface. *In vivo* and *in vitro* studies have shown that Lf prevents certain bacteria from adhering to host cells. Lf, lactoferrin.

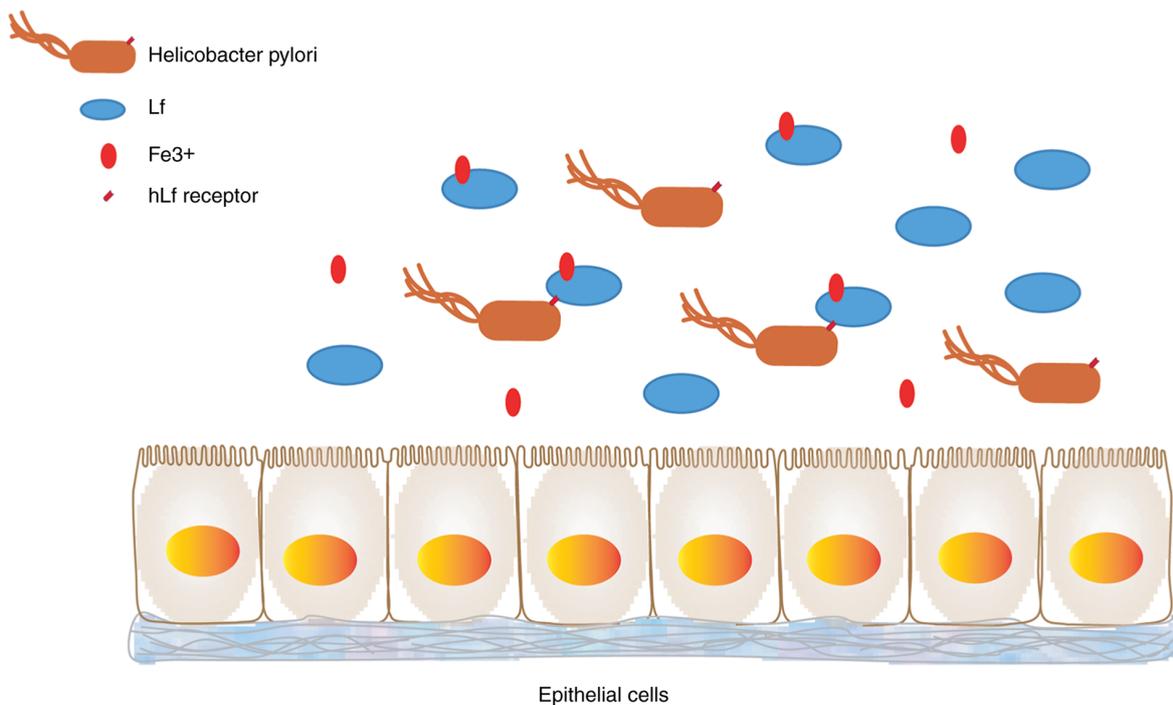


Figure 4. Ability of *Helicobacter pylori* to use hLf as a source of iron depends on the contact between cells and protein. As significant amounts of lactoferrin have been recorded in resection specimens of the human stomach in patients with atrophic gastritis, the iron uptake of *H. pylori* via a specific hLf receptor may serve a crucial role in *H. pylori* infection virulence. hLf, human lactoferrin.

plasma levels. This improved stability may be related to the strong electrostatic interactions between the positively charged Lf and negatively charged DNA. According to reports, Lf has a DNA-binding domain, which may help to

further promote DNA binding and the formation of a tighter DNA-Lf nanocomplex (98).

The methods used to prepare Lf nanoparticles include nanoparticle albumin-bound (NAB) and thermal denaturation

Table II. Lf-based nanocarriers for drug delivery applications.

Drug	Indication	Outcome	(Refs.)
Rapamycin and wogonin, or dasatinib and Fe <sub>3</sub> O <sub>4</sub> NPs	Breast cancer	Enhanced synergistic cytotoxicity and suppression of MCF-7 cells and EAT tumor growth	(109,110)
DOX and Ellagic acid	NSCLC	Higher cytotoxic effect and uptake into A549 cancer cells triggered by Tf and CD44 receptors	(111)
TMZ	Glioma	Significant decrease of tumor volume and improved median survival time	(112)
DOX	HCC	Minimized cardiotoxicity of DOX and enhanced its efficacy and bioavailability	(95)
5-FU	Melanoma	Higher intracellular uptake and 2.7-fold improved cytotoxicity against B16F10 melanoma cells	(113)
Shikonin JQ1	Colorectal cancer	NPs elicited immunogenic cell death and repolarized TAMs to M1 macrophages	(114)
EFV	HIV	Two-fold increased anti-HIV-1 activity in comparison with free drug	(115)
Gambogic acid	HCC	Enhanced oral bioavailability and anticancer effect of gambogic acid, thus decreasing its toxicity	(116)
DOX	Prostate cancer	Oral Fe-bLf-DOX inhibited tumor growth, prolonged survival and decreased DOX toxicity	(117)

DOX, doxorubicin; 5-FU, 5-fluorouracil; EFV, efavirenz; TMZ, temozolomide; EAT, Ehrlich ascites tumor; NP, nanoparticle; HIV-1, human immunodeficiency virus; Fe-bLf-DOX, Dox conjugated to iron-saturated bovine Lf.

methods. The NAB technology is primarily dependent on the presence of an oily phase, which is slowly added to the aqueous phase containing Lf. As a nanoparticle, Lf forms a gel when exposed to heat treatment, ionic strength or changes in pH. Generally, the thermal gelation of a protein starts with a heating step to denature the protein, followed by the addition of salt to induce protein aggregation (87).

Lf can participate in the production of polyelectrolyte complex nanocarriers. The synthesis of such nanocarriers is based on the use of two oppositely charged molecules, such as a positively charged protein and a negatively charged natural polysaccharide. Nanocarriers based on polyelectrolyte complexes are likely stabilized by strong electrostatic interactions between cationic proteins and anionic polysaccharides. This stabilization can also enhance the stability of the encapsulated active ingredients (99). Table II summarizes representative examples of Lf-based nanocarriers for drug delivery applications.

## 8. Conclusion and perspectives

This review provides an in-depth summary of the biological characteristics of Lf, including the structure of Lf biomolecules, its binding affinity to iron, and the interaction between Lf and the host. Lf plays an important role in the regulation of the immune response, and has immune stimulation, immune activation, anti-inflammatory activity, antibacterial, anti-viral effects and, in particular, anticancer activity. Based on the results of several studies, it is known that Lf-based nanocarriers can be easily prepared by simple methods and have excellent active targeting potential for tumor tissues, particularly brain

tumors. The manufacture of Lf-based nanocarriers can broadly enhance the therapeutic potential of encapsulating active molecules. The present review discussed the latest preparation methods for Lf-based nanocarriers prepared by the sol-oil method, NAB technology and thermal denaturation method.

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## Availability of data and materials

Not applicable.

## Authors' contributions

HW, YG, XZ, GH, LL and SP collected the related papers and completed the manuscript and figures. YG and XZ provided constructive guidance and performed critical revisions. XY, SW, TZ, HW and SP participated in the design of this review. All authors read and approved the final manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

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Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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