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Review article

# Multifunctional capacity and therapeutic potential of lactoferrin

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ABSTRACT

Lactoferrin (LF) is a glycoprotein with high functional versatility that is found in most body fluids. The objective of this study was to gather and update information on the properties attributed to LF.

According to this review, LF is a good immunomodulatory agent that acts on both innate and adaptive immune responses. It possesses antimicrobial activity against parasites, fungi, and viruses and also has regenerative properties at tissue level and anti-carcinogenic activity. All of these properties endow LF with major therapeutic potential of which little advantage has been taken to date.

## 1. Introduction

Lactoferrin (LF) is a glycosylated globular protein first known as the "red protein" of milk. It was subsequently defined as an iron-binding protein due to its sequestration of $Fe^{2+}$ and $Fe^{3+}$ free ions and is therefore included in the group of metalloproteins [1]. It is found in human secretions such as breast milk (especially in the colostrum), seminal fluid, uterine secretions, tears, and saliva. LF is synthetized by different cell populations, including neutrophils (polymorphonuclear lymphocytes), macrophages, and glandular epithelial cells, and it is mainly secreted in response to inflammatory processes [1–4]. This biomolecule has a multifunctional capacity, including: immunomodulatory properties in relation to innate and adaptive immune responses [1–4]; antimicrobial capacity against bacteria, parasites, fungi, and viruses [5]; antioxidant and anti-inflammatory activity, contributing to its tissue regeneration capacity [6]; and anti-carcinogenic activity, by direct effect on transformed cells or by indirect effect *via* the immune system [7].

The broad functional capacity attributed to LF suggests that it may have major therapeutic potential, and this review of the literature was designed to provide an update of scientific knowledge on this biomolecule and its properties.

## 2. Immunological Properties of Lactoferrin

LF is considered to be capable of modifying innate and adaptive immune responses by inducing or suppressing immune system components [3,4,8].

LF plays an important role during the first stages of life, and human colostrum contains high concentrations (7 g/L), while breast milk has a lower concentration (1 g/L). A key function of colostrum is to provide neonates with essential components for the development of their immune system, and it therefore exerts a protective function [9,10]. This is provided at systemic and local level in the neonate intestine by supporting postprandial pH and favoring the protection and development of the immature intestine. LF modulates the immune response of lymphoid tissue associated with intestinal tissue and also promotes the concentration-dependent proliferation and differentiation of small epithelial cells, thereby affecting the mass, length, and expression of small intestine digestive enzymes [1,8,11]. These effects explain why LF is given to children as a dietary supplement in various countries. No adverse effects of its use have been reported to date [12].

One of the main functions of LF at immune system level is to interact with antigen-presenting cells, including macrophages, dendritic cells, and B lymphocytes [13]. Macrophages are phagocytic cells *par excellence*, with an essential role in controlling infection by the

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intracellular destruction of microorganisms or by inhibition of their replication through the secretion of cytokines or mediators such as nitric oxide (NO). They are also involved in inflammation, secreting inflammation mediators and proinflammatory cytokines, which participate in tissue repair as a final stage of the inflammatory process [13,14]. Macrophages are activated by interaction of LF with their LF receptors, increasing their phagocytic capacity and their synthesis of IL-12, a molecule that attracts more macrophages to the inflamed area and activates T $CD4^+$ lymphocytes [2,13].

Dendritic cells are a heterogeneous cell population highly specialized in antigen recognition, and they are considered to play a key role in the immune system by controlling the induction of immunity and tolerance. The interaction of LF with dendritic cells *via* specific receptors on their surface induces their maturation and therefore functional activity. Dendritic cells maturation by Talactoferrin, a recombinant human LF, displayed an enhanced release of IL-8 and chemokine CXCL10, as well as a significantly reduced production of IL-6, IL-10, and chemokine CCL20 [15].

LF acts on B lymphocytes and other antigen-presenting cells by activating and accelerating antigen processing, enabling the interaction and activation of T cells. The presence of LF can also stimulate B lymphocyte differentiation and maturation, increasing their capacity to present T lymphocytes [2]. The oral administration of LF was reported to increase IgA and IgG secretion in mice [2,16] and was even found to produce humoral recovery in immunosuppressed mice, suggesting that it can stimulate the proliferation and differentiation of activated B lymphocytes [17].

In summary, LF exerts an immunomodulatory function on antigen-presenting cells in general, producing their activation, maturation, and migration to inflamed areas [1,2,4,13].

In a murine study, Tomita M et al. [18] found that LF bound to receptors on enterocytes, dendritic cells, and lymphocytes, inducing the release of cytokines and increasing the number of NK, $CD4^+$, and $CD8^+$ cells. This response favors the systemic immune response, with an increase in immune cells, humoral factors, and cytokines in lymph nodes and spleen that then migrate to the whole organism. These effects are largely attributable to the immunomodulatory role of LF, with increases in cytokines of the TH1 response, maturation of dendritic cells, a greater activation of macrophages, and a higher cytotoxicity of peritoneal NK cells.

LF can also modulate the functional capacity of T lymphocytes by acting on the maturation process, inducing CD4 expression and therefore directing differentiation of immature T lymphocytes toward the $CD4^+$ T lymphocyte subpopulation [2,14]. It can also change the balance between TH1 and TH2 subpopulations of $CD4^+$ cells by promoting TH1 responses (IL-2 and IFN-γ synthesis) and inhibiting TH2 responses (IL-4, IL-5, and IL-13 synthesis), activating cell responses and reducing the release of inflammatory factors. Thus, LF controls allergic rhinitis by inhibiting the response of TH2 and TH17 lymphocyte subpopulations, which are directly involved in allergic responses [19].

However, LF may possess anti-inflammatory activity, and various clinical trials found that LF may prevent sepsis by controlling TNF-α, a cytokine directly implicated in septic shock [20].

## 3. Antimicrobial Activity of LF

The antibacterial activity of Lf has been documented in the past, both *in vitro* and *in vivo* for Gram-positive and Gram-negative bacteria and some acid-alcohol resistant bacteria. Differents studies shown that LF structure plays an essential role in this activity [2,4,21].

### 3.1. Antibacterial activity

Various highly diverse microorganisms (*Haemophillus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Legionella pneumophila*, *or Mycobacterium tuberculosis*) have proven susceptible to the action of LF, which can have bactericide and/or bacteriostatic effects [21].

One of the mechanisms underlying the bacteriostatic activity of LF is its capacity to bind to large amounts of iron, impeding its utilization by microorganisms for growth [22].

The bactericidal activity of LF mainly occurs by direct interaction with bacterial surfaces. Thus, the permeability of the bacterial membrane of Gram-negative bacteria can be damaged by interaction of the protein fraction of LF (its structure has cationic areas) with the A lipid of the lipopolysaccharide (LPS) (anionic character) and its subsequent neutralization [22]. LF can interfere with bacterial adhesion by binding with LPS, thereby inhibiting of one of the most important virulence factors of these microorganisms [4,21,23].

The interaction of LF with LPS or other bacterial membrane proteins is known to enhance the effect of natural bactericides such as lysozymes, which are secreted in high concentrations alongside LF in mucosa. LF acts against Gram-positive bacteria by binding to anionic molecules (*e.g.*, lipoteichoic acid) on the bacterial surface, reducing the negative load on the bacterial cellular wall. This favors contact between lysozyme and peptidoglycan, facilitating the enzymatic effect [1]. LF can also enhance the effect of certain bactericide drugs, such as Rifampicin [21,22].

Furthermore, the interaction of LF with fractions of microbial origin promotes the release of proinflammatory mediators, including cytokines (IL-1, IL-6, IL-8, IL-12 and TNF-α), lipid mediators, and reactive oxygen species. Its ability to bind to bacterial oligonucleotides means that it impacts on B cells, probably by interacting with Toll-like receptors (TLRs) such as TLR-9, mainly present in dendritic cells, macrophages, and B lymphocytes, which play a key role in the onset of the inflammatory response and pathogen detection [8,21].

### 3.2. Antiviral activity

The antiviral activity of LF against DNA and RNA viruses with or without viral envelope has been known since 1994 [24]. LF can protect the host from viral infections by inhibiting the binding of the virus to target cells, thereby hindering its subsequent intracellular replication, and by improving systemic immune functions [25].

Various action mechanisms have been proposed to underlie antiviral effects of LF, and one of the most widely accepted is based on its ability to bind to and block viral receptors such as glycosaminoglycans, especially heparan sulfate (HS). Thus, the binding of LF to HS avoids the first contact between host cell and virus, preventing viral infection [26]. Immune system modulation is another proposed mechanism, and LF was found to increase the phagocytic activity of macrophages in infections by vesicular stomatitis virus infections [2]. LF administration also enhances Natural Killer (NK) cell activity and the response of TH1 lymphocytes, which secrete cytokines that protect against viral infection [25].

In an *in vitro* study on the HepG2 cell line of hepatocytes infected with hepatitis B virus (HBV), Li et al. [27] found that replication of the virus was significantly inhibited by treatment with LF or iron- and zinc-saturated LF, suggesting that LF may be a potential resource in anti-hepatitis B therapy.

*In vitro* studies and clinical trials have demonstrated that treatment with complete LF or previously purified fractions can inhibit hepatitis C virus (HCV) replication at intracellular level, but contradictory data have been published on their capacity to prevent entry of the virus into the target cell [24].

LF has demonstrated a powerful inhibitory activity against human immunodeficiency virus (HIV), while some LF fragments, such as lactoferricin, were found to exert a mild inhibitory action on HIV-1 reverse transcriptase and HIV-1 integrase [26]. According to Carthagena et al. [28], LF can interfere with HIV-1 transmission at mucosal level, blocking its binding to epithelial cells, and with its transmission from dendritic cells to T $CD4^+$ cells, two crucial steps in HIV diffusion from