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REVIEW ARTICLE

Lactoferrin as an immunomodulatory and iron binding agent: Possible clinical implication in COVID-19

Dalia S. Morsi[1], Hany M. Ibrahim[1], Azza H. Mohamed[1], Gamalat Y. Osman[1], Mohamed L. Salem[2,3]

[1]Zoology Department, Faculty of Science, Menoufia University, Shebin El Kom, Egypt
[2]Zoology Department, Faculty of Science, Tanta University, Tanta
[3]Center of Excellence for Cancer Research, Tanta University, Tanta, Egypt

ABSTRACT

Since December 2019, COVID-19 is a pandemic disease which associates with severe acute respiratory syndrome due to infection with the new corona virus SARS-Cov-2. SARS-Cov-2 infection has been recorded in more than 6.4 million patients and associated with over 380,000 deaths. Several scenarios have been suggested to explain the pathogenesis behind the rapid death of patients who SARS and several treatment option have been presented to minimize the complication and safe the life of patients. The latest scenario behind the pathogenicity caused by SARS-Cov-2 infection is the capability of the virus to bind with iron molecule in hemoglobin, resulting in anemia and thrombosis. This scenario might explain the efficacy of the anti-malaria drug hydroxychloroquine in protection of the patients since it compete with the virus to bind with the iron molecule. Due to the side effects of hydroxychloroquine, it is of paramount significance to explore new drug that can safely block the binding of SARS-Cov-2 to iron molecule. Lactoferrin is an iron-binding glycoprotein related to the transferrin family, which has several activities beside its anti-viral and immunomodulatory effects. Our hypothesis is that lactoferrin can be used as an alternative to hydroxychlroquine in treatment of SARS-Cov-2.

Keywords: COVID-19, Lactoferrin, Therapy, Iron, Immunomodulatory

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Correspondence to:
Dr. Dalia S. Morsi, PhD
Lecturer of Immunology
Zoology Department,
Faculty of Science,
Menoufia University,
Shebin El-kom, Egypt
Tel: +02-048-3849293
Email: daliasami46@yahoo.com

INTRODUCTION

Corona viruses are enveloped positive-stranded RNA viruses with a crown-like appearance under an electron microscope due to the presence of spike glycoproteins on the envelope. There are four genera of CoVs; alphacoronavirus (alphaCoV), betacoronavirus (betaCoV), deltacoronavirus (deltaCoV) and gammacoronavirus (gammaCoV). Furthermore, the betaCoV genus is divided into five sub-genera or lineages (Chan et al., 2013). The new coronavirus (SARS-CoV-2) belongs to betaCoVs genus. It has a round, elliptic or often pleomorphic form, and a diameter of approximately 60–140 nm.

In December 2019, the outbreak of the new coronavirus disease (COVID-19) occurred in China and then spread worldwide and becomes a major international concern. COVID-19 disease associates with clusters of severe respiratory illness similar to those of severe acute respiratory syndrome caused by SARS coronavirus. Human-to-human transmission via droplets, contaminated hands or surfaces has been recorded with incubation times of 2-14 days (Zhai et al., 2020). COVID-19 treatments include antiviral agents (Elfekky, 2020; Yao et al., 2020), chloroquine and hydroxychloroquine (Colson et al., 2020; Gao et al., 2020), corticosteroids (Huang et al., 2020), antibodies

(Tian et al., 2020; Wrapp et al., 2020), convalescent plasma transfusion (Zhou et al., 2020b). So far, there has no single drug with specific anti-viral for SARS-Cov-2. Therefore, adjuvant or alternative therapies are needed in order to improve the efficiency or the quality of COVID-19 treatment.

Lactoferrin (LF) is a glycosylated globular protein with molecular weight of 78 kDa that nearly consists of 690 amino acid residues (Baker and Baker 2005). It was first known as the "red protein" of milk, which was subsequently defined as an iron-binding protein due to its sequestration of Fe^{2+} and Fe^{3+} free ions and is therefore categorized as a metalloprotein (González-Chávez et al., 2009). LF is found in milk of many mammalian animals as camels and goats but it is more prominent in bovine milk as well as in humans. In the latter, it is found in secretions such as breast milk (especially in the colostrum), seminal fluid, uterine secretions, tears, and saliva and synthetized by different cell populations, including neutrophils, macrophages, and glandular epithelial cells, and it is mainly secreted in response to inflammatory processes (Baker and Baker 2005; Legrand et al., 2008; Actor et al., 2009; González-Chávez et al., 2009). Breast milk represents the main source of LF found in the gut of infants, where high levels of fecal LF in the first days of life represents the initiation development and/or composition of the neonatal gut microbiota (Mastromarino et al., 2014).

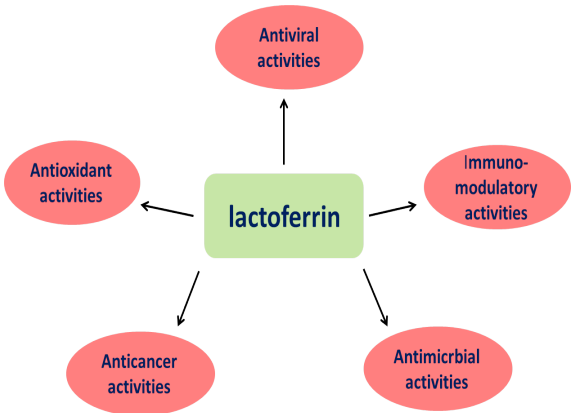

Figure 1. Suggested physiological activities of LF.

LF consists of a single polypeptide chain which is folded into two lobes (N & C lobes) with 33-41% homology (González-Chávez et al., 2009). Both lobes are linked by a α-helical residue, making LF a flexible molecule. The two lobes of LF are made of α-helix and β-sheet and each lobe can bind to Fe^{+2} or Fe^{+3} ions in synergy with the carbonate ion (CO_3^{2-}) (Iafisco et al., 2011). The major role of LF in human is the transportation of iron in blood plasma (Iafisco et al., 2011). LF in nature is partially saturated with iron and hence can be fully saturated with iron from the external environment (Tsuda et al., 2004; Kanwar et al., 2008). The iron binding affinity of LF is the maximum amongst transferrin family. There are two forms of LF, namely the iron-free form (Apo-LF) and the iron containing (holo-LF) (Baker and Baker 2005). LF is considered to be an important host defense molecule and has a diverse range of physiological functions (Fig. 1), including anti-microbial properties, antitumor, immunomodulatory and antioxidant activities (Burrow et al., 2011; Parhi et al., 2012; Ibrahim et al., 2019; Ibrahim et al., 2020).

LF exerts an immunomodulatory function on antigen presenting cells in general, producing their activation, maturation, and migration to inflamed areas (Legrand et al., 2008; Actor et al., 2009; González-Chávez et al., 2009; Puddu et al., 2009). LF was found to bind to receptors on enterocytes, dendritic cells, and lymphocytes in murine model. Upon binding to its receptor, LF induces the release of cytokines and increasing the number of natural killer (NK) cells as well as $CD4^+$ helper and $CD8^+$ cytotoxic T cells (Tomita et al., 2009). 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 (Actor et al., 2009). Moreover, it can change the balance between Th1 type and Th2 type cells of $CD4^+$ T cells by promoting Th1 cytokine (IL-2 and IFN-γ) synthesis and inhibiting Th2 cytokine (IL-4, IL-5, and IL-13) synthesis, activating cell responses and reducing the release of inflammatory factors (Drago-Serrano et al., 2017).

LF has been reported to express antiviral activity against enveloped RNA and DNA viruses (Siqueiros-cendón et al., 2014). It has also been found to both directly and indirectly control several viruses that cause disease in humans. It can directly inhibit viruses by binding to the viral receptor sites, thus preventing the virus from infecting healthy cells. For example, *in vitro* studies have found that LF strongly binds to the V3 loop of the gp120 receptor on HIV-1 and HIV-2, resulting in inhibition of virus-cell fusion and entry of the virus into cells (Swart et al., 1998). In addition, LF indirectly kills or inhibits viruses by augmenting the systemic immune response to a viral invasion. LF was also found to have potent anti-viral effects against the replication of both human HIV and cytomegalovirus (CMV) in several in vitro studies with no cytopathic effects on healthy cells. In addition, several studies have reported that LF can inhibit herpes simplex type 1 infection of healthy cells by preventing viral attachment to healthy cells via the blocking of viral proteins and direct immune interactions with natural killer cells, lymphocytes and phagocytes (Harmsen et al., 1995; Superti et al., 1997; Puddu et al., 1998; Swart et al., 1998).

Recently SARS-Cov-2 has been found to bind to the host blood hemoglobin, where its glycoproteins bind to the heme, resulting in dissociation of toxic oxidative iron ion to become free in the patient's blood (Liu and Li, 2020). These free iron ions is considerably toxic as it cause a rapid oxidative damage to the lungs. Indeed, these events would explain the bilateral- and always bilateral-ground glass opacities which have been seen on the chest CT of COVID-19 patients, which were previously mistakenly treated as bilateral pneumonia. Meanwhile, the damaged hemoglobin cannot bind to O_2, causing resistant hypoxia and as a consequence it ends with micro thrombosis, multi-organ failure and eventually death.

Given the high affinity of binding LF to iron ions, we hypothesize that administration of LF into COVID-19 patients will make Apo-LF to bind and sequesters iron ions released by SARS-Cov-2, thereby limiting the binding of the viral particles to the released iron ions and thus prevent vascular thrombosis.

Amount of iron available to support pathogen growth, and react with oxygen-dependent immune effectors, such as hydrogen peroxide and superoxide (Appelmelk et al., 1994; Flores-Villasenor et al., 2010). Hence, it will minimize the side effects of the toxic oxidative iron ion disassociation by COVID-19.

Hydroxychloroquine (HCQ), a less toxic derivative of chloroquine used as anti-malarial drug, has been recommended to treat COVID-19 patients (Colson et al., 2020). The antimalarial effect of HCQ is known to be mediated by its protection of hemoglobin against invasion by malaria parasite. Similarly, the recorded beneficial effect of HCQ toward COVID-19 has been suggested to be mediated by a similar mechanism. Treatment with HCQ, however, associates with serious adverse effects as bone marrow failure and cardiac arrhythmia, limiting its application. Given the same mechanistic effect of LF and HCQ with regard to binding with free iron ions, we hypothesize that LF, which has no adverse effects can be used as an alternative or adjuvant therapy to COVID patients.

Severe COVID-19 infection was associated with increased mortality in patients as the disease resulted in hyper-inflammatory status and cytokine storm through the elevation of IL-6 (Zhou et al., 2020a). Indeed, LF has been reported to be secreted during the acute-phase protein in response to IL-6, IFN-γ, and TNF-α (Masson et al., 1969; Topham et al., 1998). This LF secretion in response to inflammation has been found to induce anti-inflammatory effects including prevention of sepsis by controlling TNF-α (Drago-Serrano et al., 2017).

In conclusion, we hypothesize that LF could be a promising alternative or adjuvant treatment for COVID-19 patients by prevention of thrombosis, anti-inflammatory effects as wells as anti-viral and immunoenhancing effects (Fig. 2).

AUTHOR CONTRIBUTION

MLS designed the study, DSM and HMI contributed to the writing of the manuscript. HMI designed the figures, MLS, DSM, HMI reviewed and approved the final version of the manuscript.

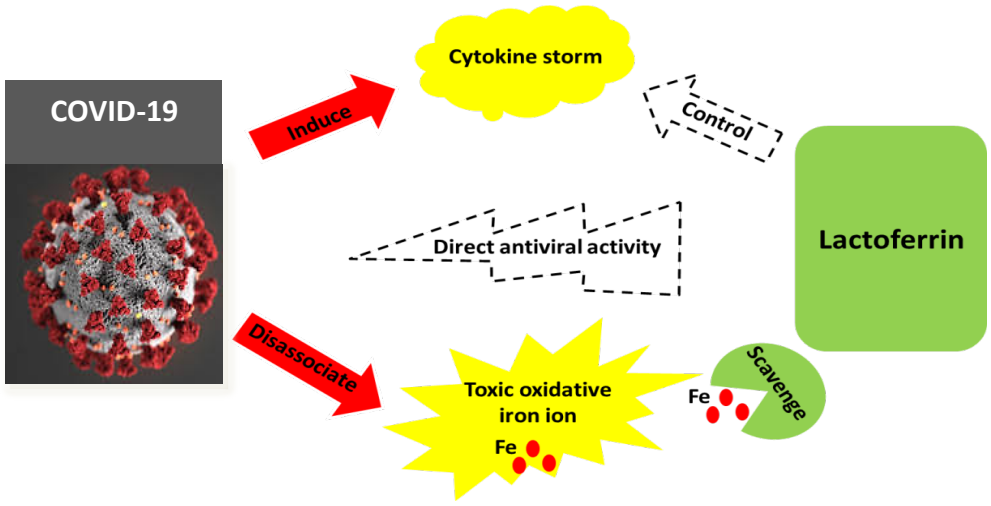

Figure 2. The hypnotized possible response of lactoferrin against COVID-19 infection.

CONFLICT OF INTEREST

The authors declare that they have no competing interests

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EACR **Chairman,**
Prof. Mohamed Labib Salem, PhD
Professor of Immunology
Faculty of Science, Tanta Universiy, Egypt

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For more information, contact

Hamdi Kandil
Tanta University, Egypt
Email: Ijcbr100@gmail.com