Lactoferrin: a potential candidate to fight respiratory infections in the pandemic COVID-19 era

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Respiratory infections are a significant burden at any age, but especially in childhood and aging. The COVID-19 pandemic has worsened the issue since there is no specific treatment and vaccine is not available. Moreover, respiratory symptoms cause social stigma in subjects suffering from an infection of any kind. As new drugs require a very long time to be marketed, a natural compound's interest is growing. In this regard, lactoferrin is a multifunctional protein present in secretions, mainly in breast milk. Lactoferrin has marked antimicrobial activity, including antibacterial, antiviral, antiparasitic, and antifungal. Moreover, lactoferrin strongly affects immune response and cellular control activity. Therefore, this natural component could provide a promising effect in preventing respiratory infections and potentially also for COVID-19.

Background

A new coronavirus (SARS-CoV-2) caused the coronavirus disease, which emerged in late 2019 (COVID-19). COVID-19 started in China and rapidly spread worldwide, so becoming a pandemic. Being a virus new to humankind, everyone is potentially susceptible to this infection. Consequently, the situation has evolved so rapidly that over 10 million people have been infected worldwide, and hundreds of thousands have died until now. Moreover, there is no specific treatment for COVID-19 at present, and a traditional drug requires 10-15 years to be available for clinical use. Active immunization depends on a safe and effective vaccination, but the SARS-CoV-2 vaccine is still under investigation. Therefore, there is a need to identify a potential candidate among the various therapeutic options available to date (1). In this regard, it is, above all, fundamental to know in detail the immunological response to SARS-CoV-2. This virus belongs to the *Coronaviridae* family (2). It has been reported that the genome of SARS-CoV-2 corresponds to 80% of SARS-CoV-1 (3), the etiologic agent for the severe acute respiratory syndrome (SARS). Therefore, the available information for the pathogenesis SARS-CoV-1 infection could help define potential treatments for COVID-19.

The SARS emerged in 2003 (4), and the genome sequence has been described in detail (5). Reghunathan and colleagues investigated the expression profile of immune response genes in patients suffering from SARS (6). Surprisingly, there was no expression of genes coding for cytokines nor a specific adaptive immune response against CoV, but several genes, involved in innate immunity, were overexpressed, including the genes coding for lactoferrin (6). Lactoferrin (LF) expression was elevated by approximately 150 fold in SARS patients compared with healthy controls. That study also demonstrated that LF enhanced NK cell activity and stimulated neutrophil

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aggregation and adhesion. Therefore, it has been speculated that Lactoferrin (LF) could be a candidate in the fight against COVID-19 (7, 8).

On the other hand, there is growing concern about the possibility of getting other respiratory infections. Furthermore, another source of concern is the stigma of those who complain of respiratory symptoms such as sneezing, coughing, and fever potentially attributable to COVID-19. Also, contracting a respiratory infection during this period can severely affect the work and school presence. Fever (>37.5°) and respiratory symptoms are factors that preclude access to public places pending diagnostic tests. As a result, never as now is the need to be can prevent respiratory infections. So even in this case, the use of substances that modulate the immune response appears attractive.

Based on this background, lactoferrin could be a potential biological agent able to modify the immune response.

Lactoferrin

LF is a multifunctional protein present in external secretions, including saliva, tears, milk, nasal, and bronchial secretions, gastrointestinal fluids, and urine mucosal secretions, and is an essential constituent of the neutrophilic granules of leukocytes (9). In particular, it has been believed that LF is the most polyvalent protein in vertebrates (10). LF was initially identified in 1939 as a "red protein" in the whey (11). The most abundant source of LF is human and bovine milk (12). The concentration varies with the lactation stage as colostrum contains up to 8 mg/mL, whereas mature breast milk about 2-3 mg/mL.

LF is a glycosylated globular protein and binds iron due to its sequestration of Fe^{2+} and Fe^{3+} free ions; therefore, it is included in the metalloproteins family (13). LF is produced by different cell populations, including glandular epithelial cells, neutrophils, lymphocytes, and macrophages (14). LF is rapidly and abundantly secreted during an inflammatory response (15).

LF has an extraordinary multitasking ability, such as has metabolic activity, modulates innate and adaptive immunity, has antimicrobial activity against bacteria, viruses, parasites, and fungi, exerts antioxidant and anti-inflammatory effects, and repairs damaged tissues (16).

LF and immune response

LF is a relevant modifier of innate and adaptive immunity and significantly affects the immune system's maturation during the first stages of life (13). Consistently, the breast milk and mostly colostrum are plenty of LF. Adequate maternal LF intake guarantees the immune response's physiological plasticity and defends from infections (9). LF supplementation provided beneficial results (17), but conflicting outcomes were reported, probably influenced by methodological bias, including timing and patients' selection (18).

LF activates antigen-presenting cells (APC), namely dendritic cells, macrophages, and B cells, so increasing their phagocytosis and release of interleukin(IL)-12 that amplifies APC activity (19). LF stimulates dendritic cells to release IL-8, but reduces IL-6 and IL-10, modulating the immune response (20). LF promotes B lymphocyte differentiation and maturation, antigen presentation to T cells, and IgG and IgA (19). Moreover, LF advances Type 1 response and dampen type 2 inflammation, typical of allergic disorders, balancing a physiologic immune response (21).

LF and inflammatory response

LF down-regulates pro-inflammatory production, dampening acute inflammation and facilitates inflammation resolution (22). LF blocks the detrimental persistence of inflammation leading to chronic inflammation. LF exerts a pivotal anti-inflammatory activity in several aseptic inflammation diseases, including iron-deficient chronic anemia, type diabetes mellitus, Alzheimer's disease, atherosclerosis, and septic inflammation which experience patients with cystic fibrosis, inflammatory bowel disease, and bacterial infections (22). LF supplementation provided beneficial anti-inflammatory effects, as recently reviewed (15).

LF and infections

LF displays antimicrobial activity against bacteria, viruses, fungi, and parasites. It possesses a dual antibacterial activity, such as bacteriostatic, chelating Fe^{3+}, limiting bacterial growth, and bactericidal, disrupting bacterial cell wall and increasing membrane permeability, and so causing bacterial death (10). Moreover, LF interferes with bacterial adhesion to mucosal tissues; consequently, LF reduces virulence (23). LF, interacting with fractions of microbial origin, such as pathogen-associated molecular patterns (PAMPS), promotes the release of pro-inflammatory cytokines (IL-1, IL-6, IL-

8, IL-12, TNF-α), lipid-derived mediators, and reactive oxygen molecules, that antagonize bacteria. Finally, LF interacts with toll-like receptors amplifying the immune response against microbes.

LF plays different antiviral activity, mainly inhibiting the viral binding to host cells, hindering the intracellular replication, and enhancing immune response. In particular, LF blocks the glycosaminoglycans, mainly heparan sulfate, which are initial viral receptors. LF also stimulates NK activity and type 1 cytokines, that fight virus infection.

LF has a wide-spectrum activity against fungi and antiparasitic activity, modulating the immune response and increasing T $CD4^+$ cell effects.

LF and COVID-19

LF, as mentioned above, interacts with the virus in the early stages of exposure. LF interferes with the first anchoring of CoV acting on heparin sulfate glycosaminoglycan (HSPG) cell receptor. HSPG molecules provide the preliminary docking sites on the cellular surface. In other words, HSPG functions as a storage site for CoV, mediating an "in trans" infection and presenting it to the target cells. CoV, after initial anchoring to HPSG, is accumulating on the cell surface where can recognizes specific receptors, namely angiotensin-converting enzyme 2 (ACE2): a metallopeptidase hooking the virus to spike proteins and so allowing virus penetration and internalization into host cells, the so-called "viral surfing" (24). In this regard, Lang performed an elegant study that provided evidence concerning the LF ability to inhibit SARS-CoV binding to HSPG (25). LF protects against coronavirus, indirectly enhancing NK activity and neutrophil aggregation, and directly blocking intracellular entry.

In this way, LF may protect the host against coronavirus invasion. Therefore, based on this evidence, LF could be an intriguing candidate to fight respiratory infections in the COVID-19 era. A proof of concept has been recently provided to support this hypothesis by a preliminary study conducted in Spain (26). Serrano and colleagues enrolled 75 patients with typical COVID-19 symptoms. Patients were treated with a liposomal bovine lactoferrin nutritional syrup food supplement, containing 32 mg of LF/10 mL and 12 mg of vitamin C, 4-6 times/day per 10 days. In some patients, an additional zinc solution was administered. Family members, cohabitant with patients, were also treated with a half dose. This treatment ultimately resolved the COVID-19 in all patients within the first 4-5 days. Equally, preventive treatment in family members was effective. The authors proposed different mechanisms of actions, including LF anti-inflammatory effects by balancing digestive microbiota, increasing "good" cytokines (IL-4, IL-10), reducing pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), and downregulating transcription factors (NFkB). Of course, the open design and the lack of robust methodology require further rigorous studies to confirm these exciting outcomes.

LF future applications

As discussed, LF could represent a promising biological agent potentially able to prevent and cure a respiratory infection, mainly of viral origin. LF properties ensure a multifunctional activity carrying out the immune response, inflammatory reaction, and microbial infection.

Conclusions

Lactoferrin could be a promising candidate to prevent and cure respiratory infections, mainly in the pandemic COVID-19 era. Lactoferrin is a multifunctional agent, providing anti-inflammatory, antimicrobial, and immunomodulatory effects. Moreover, block virus docking and enhances immune response. In particular, severe COVID-19 is characterized by hyper-inflammation and high virulence (27). In this regard, lactoferrin effectively counteracts both inflammation and infection (28).

On the other hand, lactoferrin is a natural component, so it is usually safe and well-tolerated at any age, mainly in children. In particular, it seems to be one of the essential physiologic immunomodulant in early life. Lactoferrin could be, therefore, able to prevent and fight a respiratory infection. In the absence of specific vaccines and medications, this new therapeutic strategy could also be useful from an emotional point of view, as people are looking for valid preventive options. Of course, there is a need to provide adequate evidence to support this opportunity.

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