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Review

Lactoferrin as a nutraceutical protein from milk, an overview

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A B S T R A C T

Lactoferrin, a non-haeme iron-binding glycoprotein, known as the red fraction of milk, has been recently called a nutraceutical protein. Lactoferrin protein participates in some immunological processes and has an antimicrobial response against a broad spectrum of pathogens: it is also proposed to play a role in mechanisms of both neurodevelopment and neuropathy, and recently, the impact of lactoferrin against the metabolic syndrome was suggested. Other properties like anticancer, antioxidant and enzymatic activity have been reported. Because of this, the potential use of lactoferrin as a nutraceutical in the food industry makes it especially attractive to study and characterise. This review focuses on the description of lactoferrin as an important nutraceutical in science and food industry.

Contents

1. Introduction

The term “nutraceutical” dates almost 30 years ago. Stephen DeFelice (Foundation for Innovation in Medicine, New York, US), coined this word to include all the food and/or their chemical components, that provide a health benefit to the consumer. Nutraceuticals are currently classified within the nutritional supplements containing active ingredients in concentrations greater than those found naturally in foods. Examples include: polyunsaturated fatty acids, minerals, antioxidants, dietary fibre and proteins, glycopeptides and amino acids (Andlauer & Fürst, 2002; Espín, García-Conesa, & Tomás-Barberán, 2007; Kalra, 2003).

Dietary components may play beneficial roles above nutrition, driving to the development of the functional food concept and nutraceuticals (Cencic & Chingwaru, 2010). The proteins present in food, as well as being an important primary nutrition component, are a source of bioactive peptides with functions that can promote health and prevent disease (de Mejia & Dia, 2010). Currently, the consumption of food with nutraceutical properties has increased in the world. Foods like milk, chia seeds (*Salvia hispanica* L.), soybean (*Glycine max*), canary seeds (*Phalaris canariensis* L), among others, have been described by their high nutraceutical content; however,

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the availability of these last two is limited to regions of the world due to climatic conditions necessary for its production (Pandey et al., 2016; Sandoval-Oliveros & Paredes-López, 2012; Valverde, Orona-Tamayo, Nieto-Rendón, & Paredes-López, 2017; Wada & Lönnerdal, 2014).

Milk is one of the most consumed foods in the world; it is an excellent source of nutritional content and contains all the nutrients needed for the healthy development of infants (Lönnerdal, 2013). There are several reasons causes why breastfeeding is not an option for mothers at times, so the use of infant formulas is common practice for the development of the newborn (Figueroa-Lozano, Valk-Weeber, van Leeuwen, Dijkhuizen, & de Vos, 2018). In this respect, Lactoferrin (Lf), identified in milk in 1939 (Sorensen & Sorensen, 1940), has a wide spectrum of functions and currently is recognised as a nutraceutical protein. Currently, multiple activities, such as immunomodulator (Siqueiros-Cendón et al., 2014), antimicrobial (Legrand & Mazurier, 2010), antioxidant (Kruzel et al., 2013), anticancer (Tsuda et al., 2010) have been ascribed to lactoferrin, and recently its roll in metabolic disorders as obesity, diabetes and cardiovascular diseases has been described (Mayeur, Spahis, Pouliot, & Levy, 2016). In the present review, we will describe the most important properties of Lf and its potential use as nutraceutical and also perspectives on infant nutrition.

2. Lactoferrin characteristics

Lactoferrin is a ubiquitous glycoprotein of mammals. It has a molecular mass of approximately 80 kDa, and its structure is formed by two symmetrical lobes (N-lobe and C-lobe) connected with a α-helix. The simple polypeptide chain includes approximately 700 amino acids, according to the species; however, there is a high homology among species. Lf presents high affinity for iron; each lobe can bind one ferric ion; moreover, it can bind Cu^{+2}, Zn^{+2} and Mn^{+2} ions (Adlerova, Bartoskova, & Faldyna, 2008; García-Montoya, Cendón, Arévalo-Gallegos, & Rascón-Cruz, 2012). The highest levels of lactoferrin production are attributed to milk and colostrum (1 g L^{-1} and 7 g L^{-1}, respectively); the latter has a significant impact in the proportion of essential components in the immune system of infants (Moreno-Expósito et al., 2018). Moreover, lactoferrin is secreted by several body fluids as seminal and vaginal fluids, tears and saliva; it is present in neutrophils at a concentration of approximately 0.4 mg L^{-1} in human plasma under normal conditions, increasing up to 5000-fold in the infection process (Embleton, Berrington, McGuire, Stewart, & Cummings, 2013).

3. The role of lactoferrin in the immune system

Since the discovery (Sorensen & Sorensen, 1940), and subsequent characterisation of lactoferrin (Groves, 1960), many properties of this protein have been documented. The role that this molecule plays during the immune response has been extensively studied and is one of the main features that have made it extremely attractive to both the pharmaceutical industry and research.

Lactoferrin is strongly involved in immunological processes response to pathogens, as well as inflammatory disorders, including allergy, arthritis, and cancer (Legrand et al., 2008). In response to an inflammatory and/or neurodegenerative process, secretion levels of Lf increase significantly (Zucca et al., 2017); Lf can mediate the actions of the innate and adaptive immune responses (Siqueiros-Cendón et al., 2014). Although the molecular mechanisms of immunomodulatory action have not been fully understood, it is known that Lf can regulate the production of inflammatory mediators both up and downwards (Giansanti, Panella, Leboffe, & Antonini, 2016).

Several authors have suggested that a fundamental part of the immunomodulatory mechanism of action of Lf is related with the glycosylation of the molecule. The degree of Lf glycosylation could affect its ability to modulate the immune response (Choi et al., 2008; Zimecki, Artym, Kocięba, Duk, & Kruzel, 2014); in this context, three potential glycosylation sites have been reported in human lactoferrin (hLf) (Asn138, Asn479, and Asn624; Spik et al., 1994) and five potential sites in bovine lactoferrin (bLf) (Asn233, Asn281, Asn368, Asn476 and Asn545; Wei, Nishimura, & Yoshida, 2000). These sites are mostly exposed on the external surface of the molecule and are involved in recognition of certain cell receptors of the immune system (Latorre, Puddu, Valenti, & Gessani, 2010). In vivo and in vitro studies have shown that expression levels of inflammatory cytokines like IL-4 and IL-10, pro-inflammatory cytokines like TNF-α, IL-1, IL-6 and IL-12, as well as chemokines as IL-8, are altered in the presence of lactoferrin, which affects the natural processes of growth, differentiation, and activation of immune cells (Legrand, 2016). An interesting feature of the immunomodulatory effect of Lf, is the fact that is not only limited to the alteration in the expression levels of cells: its ability to chelate iron ions, enables lactoferrin to act as a natural antioxidant (Sharma, Chakraborty, & Gupta, 2015); also, Lf prevents peroxidation caused by free radicals (Yuksel, Yigit, Cinar, Atmaca, & Onaran, 2015). Table 1 summarises the most significant effects of Lf as an immunomodulator that have been reported in recent years.

Table 1
Cell-based studies demonstrating immune responses mediated by lactoferrin.

Source	Effect	Model	Reference
Human lactoferrin	↓Proinflammatory response in monocyte derived macrophages	Monocyte-derived macrophages	(Wisgrill et al., 2018)
Native lactoferrin	We first confirmed that oral lactoferrin application could modulate rat immune responses. Oral lactoferrin administration significantly elevated the plasma IFN-γ while suppressed IgA levels We first confirmed that oral lactoferrin application could modulate rat immune responses. Oral lactoferrin administration significantly elevated the plasma IFN-γ while suppressed IgA levels ↑Plasma IFNγ; ↓IgA	Rats	(Wen et al., 2017)
Recombinant human Lf	↑Expression of CD86 and CD16 stimulated with BCG or LPS.	Human macrophages	(Hwang, Kruzel, & Actor, 2016)
Lf-derived peptides	Induction of necrosis in leukemia cell lines	Mice	(Lu et al., 2016)
Bovine Lf	↑IgA and IgM levels; regulation of T and B lymphocyte populations at Peyer's patches and Lamina propria.	Mice	(Arciniega-Martínez et al., 2016)
Human and bovine Lf	Inhibition of the formation of neutrophil extracellular traps.	Human neutrophils	(Okubo et al., 2016)
Bovine and buffalo Lf	↓ROS (apo lactoferrin); ↑ROS (holo lactoferrin); ↑Phagocytic capability (holo lactoferrin).	Human red blood cells and macrophages	(Anand et al., 2015)
Bovine Lf	↑Chemokine Ligand 1 (CCL1) secretion.	Human Peripheral blood mononuclear cells	(Latorre et al., 2015).
Bovine Lf	↓Pro inflammatory cytokine mRNA expression.	Human Caco-2 cells	(Bertuccini et al., 2014)
Recombinant human and mouse Lf	Several alterations in the production patterns of some inflammatory cells were observed during MSRA infection.	MSRA- infected mice	(Hwang, Kruzel, & Actor, 2014)

4. Antimicrobial activity of lactoferrin

Lf is capable of exerting antimicrobial activity against a broad spectrum of pathogens including bacteria, fungi, parasites, and viruses (González-Chávez, Arévalo-Gallegos, & Rascón-Cruz, 2009). Its ability to sequester iron ions generates a shortage of nutrients essential to growth and division of siderophores (Kanwar et al., 2015). Moreover, Lf has the capability to bind to the bacterial surface causing the release of lipopolysaccharide (LPS) from the outer membrane, causing cell damage and death (Jenssen & Hancock, 2009).

On the other hand, when Lf is subjected to proteolytic digestion with pepsin, lactoferricin (Lfcin), located in the N-terminal region, is released from the entire molecule and is a bioactive peptide that exerts a higher antimicrobial activity than Lf (Arias et al., 2014; Bellamy et al., 1992).

In the last five years, several authors have reported the antimicrobial effects of Lf, mostly in bacteria; in our previous study, we reported the antibacterial activity of recombinant bLf from yeast against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and the higher antibacterial activity was achieved using Lfcin. (Iglesias-Figueroa et al., 2016). Also, Lf has tested against *Streptococcus mutans* (Velusamy, Markowitz, Fine, & Velliyagounder, 2016), *Salmonella enterica* sv *Typhimurium* (Wu et al., 2016), *Helicobacter pylori* (Yuan et al., 2015), *Aggregatibacter actinomycetemcomitans* (Velusamy et al., 2014) and *Mycobacterium tuberculosis* (Welsh et al., 2011). Also, the potential antimicrobial effect of Lf and its bioactive peptides has been documented for over two decades (Jones, Smart, Bloomberg, Burgess, & Millar, 1994).

Finally, the use of Lf and Lfcin against microorganisms has not been limited to human pathogens. Fukuta et al. (2012) expressed lactoferricin in transgenic tobacco plants. The plants were subjected to infection with *Pseudomonas syringae* pv. tabaci and *Botrytis cinereal*; the results of this experiment demonstrated that transgenic plants survived 30 days after infection, in contrast to wild-type plants that died as a consequence of pathogen damage. Bovine Lf has also been shown to clear an *E. coli* infection in cattle (Kieckens, Rybarczyk, Cox, & Vanrompay, 2018).

5. Lactoferrin in neurodevelopment and neuronal diseases

Lf, being a protein rich in sialic acid, intervenes in neurological processes, because this carbohydrate moiety contributes to the neural development (Jahan, Wynn, & Wang, 2016). Studies in animal models, predominantly in piglets, showed that the administration of Lf contributes to neurodevelopment, cognitive functions, and memory (Wang, 2016). It has also been suggested that diets rich in Lf promotes an increase in lactation and a decrease in impulsivity and anxiety in young piglets (Mudd et al., 2016).

On the other hand, iron metabolism is involved in neurological disorders such as Parkinson's and Alzheimer's diseases (Talebi, Ahmadi, Afraz, & Abdoli, 2016). Alzheimer's patients accumulate significant amounts of iron and other metals in amyloid plaques, so the development of new therapies to treat Alzheimer's could be based on the use of non-toxic chelators that can cross the blood–brain barrier (Liu, Fan, Yang, Wang, & Guo, 2018). We suggest that Lf, being a metal chelator molecule and able to cross the barrier (Ji et al., 2006), could be potential therapy for this pathology; however, it is necessary to perform a series of studies to support this theory (Robert, Liu, Nguyen, & Meunier, 2015).

6. Lactoferrin implication in metabolic disorders

Recently, a novel application for Lf has been discovered. Its recent use as a potential treatment for metabolic disorders such as obesity, diabetes, and hypertension has been gaining ground in clinical research. Although the mechanisms of action of Lf against these disorders are still unknown, the interaction of Lf with mammalian receptors like Omentin-1, implicated in the obesity and type-2-diabetes, and low-density lipoprotein receptor-related proteins, involved in lipid metabolism has been reported (Mayeur et al., 2016).

The use of lactoferrin in weight loss studies was reported when Shi et al. (2012) showed that a diet supplemented with Lf contributed to weight loss and decreased body fat in murine models. More recent studies demonstrated that the oral administration of bLf in mice reduces the body weight gain, and decreases total cholesterol levels, blood glucose levels, and TNF-α and IL-Iβ levels, proinflammatory cytokines implicated in obesity (Sun, Ren, Xiong, Zhao, & Guo, 2016). On the other hand, the administration of bLf in micro mini pigs also reduces fat accumulation and low-density lipoproteins levels. These findings suggest that the administration of bovine lactoferrin in humans is a viable pathway for the prevention and treatment of metabolic disorders (Morishita et al., 2016).

7. Other physiological implications of lactoferrin

The versatility of Lf extends throughout the body; because it has the ability to inhibit osteoblastic apoptosis, bone regeneration is potentiated in the presence of the molecule (Görmez et al., 2015; Li, Zhu, & Hu, 2015). Other relevant functions such as the anti-carcinogenic activity of Lf has been reported in tumour cells developed in the colon, lungs, bladder and mammary gland, which has been inhibited in the presence of Lf (Gibbons, Kanwar, & Kanwar, 2015; Patel, 2015; Vogel, 2012). On other hand, Lf was recently used as anti-venom against *Naja nigricollis* snake, the adjuvant effect on the production of antibodies in response to venom in horses was demonstrated (Elaraby et al., 2016).

8. Future considerations

The widely studied diversity of functions of lactoferrin makes it attractive for the generation of new nutritional supplements. Currently, the use of infant formulas supplemented with bovine Lf has been reported (Chen et al., 2016). A problem associated with oral delivery of bovine Lf is low bioavailability due to enzymatic degradation in the gastrointestinal tract. These phenomena could be diminished by loading bovine Lf into liposomes (Yao, Bunt, Cornish, Quek, & Wen, 2015). Extraction methods can be inconvenient regarding cost and yield of the final product. Also, thermal processing of milk to obtain Lf may increase the presence of contaminants such as LPS, whose presence diminishes the biological activity of Lf due to the high affinity of the protein for the LPS (Lönnerdal, 2014).

We currently have recombinant DNA-technologies that allow us to obtain recombinant bovine Lf expressed in GRAS microorganisms such as *Pichia pastoris*. Previous reports have led us to the study of molecular mechanisms of Lf in murine models to approach the design of a product that in the future may be potentially used in children's formulas (García-Montoya, Salazar-Martínez, Arévalo-Gallegos, Sinagawa-García, & Rascón-Cruz, 2013; Iglesias-Figueroa et al., 2016).

9. Concluding remarks

Lactoferrin, an important milk protein and cell secretory mediator, has a biological participation in practically all metabolic systems of the body. Even though multiple biological effects have been reported, there is still a lack of knowledge about the mechanisms of action that this protein exerts. Recent studies in our research group have led us to believe that the glycosylation profile present in this protein plays a fundamental role in establishing its mechanism of action (unpublished data). Additionally, the antimicrobial activity of lactoferrin, including its derived-peptides, is well established that. Despite the molecular basis of action of lactoferrin not being fully clarified, its application as a nutraceutical compound will have a substantial impact on health and disease prevention.

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