Protective effect of human lactoferrin in the gastrointestinal tract

Efeito protetor da lactoferrina humana no trato gastrintestinal

Efecto protector de la lactoferrina humana en el sistema gastrointestinal

Valterlinda Alves de O. Queiroz[1], Ana Marlúcia O. Assis[2], Hugo da Costa R. Júnior[3]

ABSTRACT

Objective: To describe mechanisms of action of human lactoferrin to protect gastrointestinal morbidities.

Data sources: Nonsystematic literature review using the following databases: SciELO, Lilacs and Medline from 1990 to 2011. The key-words used were lactoferrin, human milk/breastfeeding, gastrointestinal, and immunity, in Portuguese and English.

Data synthesis: Lactoferrin is the second predominant protein in the human milk, with higher concentrations in the colostrum (5.0 to 6.7mg/mL) if compared to mature milk (0.2 to 2.6mg/mL.) In contrast, cow's milk has lower levels, with 0.83mg/mL in the colostrum and 0.09mg/mL in the mature milk. Lactoferrin has several physiological functions to protect the gastrointestinal tract. The antimicrobial activity is related to the ability to sequester iron from biological fluids and/or to destruct the membrane of microorganisms. Lactoferrin also has the ability to stimulate cell proliferation. The anti-inflammatory action exercised by lactoferrin is associated with its ability to penetrate the core of the leukocyte and to block the Kappa B nuclear factor transcription. Given the importance of lactoferrin to prevent infectious diseases for breastfed children, the industry is using genetic engineering techniques to develop the expression of recombinant human lactoferrin in animals and plants, attempting to adjust the composition of infant formulas to that of human milk.

Conclusions: Human lactoferrin is a peptide with great potential for preventing morbidity, especially in the gastrointestinal tract. Scientific evidence of the protective effects of human lactoferrin strengthens even more the recommendation for breastfeeding.

Key-words: lactoferrin; milk, human; morbidity; gastrointestinal tract.

RESUMO

Objetivo: Descrever os mecanismos de ação da lactoferrina humana na proteção de morbidades gastrintestinais.

Fontes de dados: Revisão não sistemática da literatura utilizando como estratégia de busca pesquisa bibliográfica em bases de dados, as quais incluíram SciELO, Lilacs e MedLine entre 1990 e 2011. Os descritores utilizados foram: lactoferrina, leite materno/humano, gastrintestinal e imunidade, nos idiomas português e inglês.

Síntese dos dados: A lactoferrina é a segunda proteína predominante no leite humano, com concentrações mais elevadas no colostro (5,0 a 6,7mg/mL) em relação ao leite maduro (0,2 a 2,6mg/mL). Em contraste, o leite de vaca contém teores inferiores, 0,83mg/mL no colostro e 0,09mg/mL no leite maduro. A lactoferrina desempenha diversas funções fisiológicas na proteção do trato gastrintestinal. A atividade antimicrobiana está relacionada à capacidade de sequestrar ferro dos fluidos biológicos e/ou de desestruturar a membrana de micro-organismos. A lactoferrina possui também a capacidade de estimular a proliferação celular. A ação anti-inflamatória desempenhada pela lactoferrina está associada à capacidade de penetrar no núcleo do leucócito e bloquear a transcrição do fator nuclear Kappa B. Diante da importância da lactoferrina na prevenção de doenças infecciosas em crianças aleitadas ao peito, a indústria vem, por meio da engenharia genética, desenvolvendo tecnologias para expressar esta proteína recombinante humana em plantas e animais, na tentativa de adequar a composição das fórmulas infantis àquela do leite humano.

Instituição: Escola de Nutrição da Universidade Federal da Bahia (UFBA), Salvador, BA, Brasil
[1]Doutora em Medicina e Saúde pela UFBA; Professora Adjunta da Escola de Nutrição da UFBA, Salvador, BA, Brasil
[2]Doutora em Saúde Pública pela UFBA; Professora Titular da Escola de Nutrição da UFBA, Salvador, BA, Brasil
[3]Doutor em Medicina Interna pela UFBA; Professor Adjunto da UFBA e Diretor Geral do Complexo Hospitalar Universitário Professor Edgard Santos, UFBA, Salvador, BA, Brasil

Endereço para correspondência:
Valterlinda Alves de O. Queiroz
Rua Araújo Pinho, 32 – Canela
CEP 40110-150 – Salvador/BA
E-mail: valterlinda.oliveira@gmail.com

Conflito de interesse: nada a declarar

Recebido em: 12/1/2012
Aprovado em: 29/5/2012

Conclusões: A lactoferrina humana é um peptídeo com potencial para prevenir morbidades, especialmente às gastrintestinais. Evidências científicas dos efeitos protetores da lactoferrina humana fortalecem ainda mais a recomendação para prática do aleitamento materno.

Palavras-chave: lactoferrina; leite humano; morbidade; trato gastrintestinal.

RESUMEN

Objetivo: Describir los mecanismos de acción de la lactoferrina humana en la protección de morbilidades gastrointestinales.

Fuentes de datos: Revisión no sistemática de la literatura utilizando como estrategia de búsqueda investigación bibliográfica en bases de datos, que incluyeron SciELO, Lilacs y MedLine entre 1990 y 2011. Los descriptores utilizados fueron: lactoferrina, leche materna/humana, gastrointestinal e inmunidad, en los idiomas portugués e inglés.

Síntesis de los datos: La lactoferrina es la segunda proteína predominante en la leche humana, con concentraciones más elevadas en el calostro (5,0 a 6,7mg/mL) respecto a la leche madura (0,2 a 2,6mg/mL). En contraste, la leche de vaca contiene tenores inferiores, 0,83mg/mL en el calostro y 0,09mg/mL en la leche madura. La lactoferrina desempeña diversas funciones fisiológicas en la protección del sistema gastrointestinal. La actividad antimicrobiana está relacionada a la capacidad de secuestrar hierro de los fluidos biológicos y/o de desestructurar la membrana de microorganismos. La lactoferrina posee además la capacidad de estimular la proliferación celular. La acción antiinflamatoria desempeñada por la lactoferrina está asociada a la capacidad de penetrar en el núcleo del leucocito y bloquear la transcripción del nuclear factor Kappa B. Frente a la importancia de la lactoferrina en la prevención de enfermedades infecciosas en niños amamantados al pecho, la industria viene, por medio de ingeniería genética, desarrollando tecnologías para expresar esta proteína recombinante humana en plantas y animales en el intento de adecuar la composición de las fórmulas infantiles a aquella de la leche humana.

Conclusiones: La lactoferrina humana es un péptido con potencial para prevenir morbilidades, especialmente las gastrointestinales. Evidencias científicas de los efectos protectores de la lactoferrina humana fortalecen todavía más la recomendación para la práctica de la lactancia materna.

Palabras clave: lactoferrina; leche humana; morbilidad; sistema gastrointestinal.

Introduction

At birth, the immune system of the child is still immature, the stomach has less ability to eliminate pathogens, and the intestine lacks the gut microbiota. Based on these considerations, it is reasonable to assume that the child is dependent on exogenous protection. In that sense, the human milk represents the ideal food as it has immunological, nutritive and digestive components that favor the maturity process of the intestinal mucosa and counterbalance the little competence for adaptive response of the digestive system, as well as the immaturity of other body systems, strongly contributing with the body defenses in the first two years of life[1].

In the neonatal period, the unfavorable effects of the immunological immaturity are compensated by physiologic mechanisms, such as the transplacentary passage of immunoglobulin G (IgG) from to mother to the fetus during pregnancy and the intake of immunological components of the human milk. Breastfeeding keeps the maternal-fetal link of protection after birth, favoring the transfer of maternal factors that modulate the immune system. This contributes for the child´s immune competence during a period that is crucial for the development of its own immunity[2].

The protection provided by the human milk is due the presence of a variety of functional proteins, including the immunoglobulin A (IgA), lactoferrin, growth factors and cytokines, which play an important role in the maturation of the child´s gastrointestinal tract[3].

Among the milk components, lactoferrin has great importance in the defense line against diseases, in particular the gastrointestinal ones[4-5]. Lactoferrin is a glycoprotein from the transferrin family that has been attracting growing scientific interest since the late 1950´s, due to its high concentration in human milk[6] and its physiological functions, specially the antimicrobial, anti-inflammatory and immunomodulating functions on the gastrointestinal tract[7]. Thus, the objective of this review is to report, using the analysis of the literature, the mechanisms of action of the human lactoferrin in the protection of the gastrointestinal tract.

Data collection

The search of the references was performed using the electronic databases *Scientific Electronic Library On-line* (SciELO), Literatura Latino-Americana e do Caribe em Ciências da Saúde (Lilacs) and *Medical Literature Analysis and Retrieval System Online* (MedLine) of the *National Library of Medicine*, in the period from 1990 to 201. The references of the selected articles were also consulted to identify classical studies on the subject.

Therefore, because of the relevance of the contributions, six studies that were published outside the predetermined search period were detected and included in this review. The keywords used in Portuguese, and their correspondents in English, were: lactoferrin (*lactoferrina*), breast milk/ human milk (*leite materno/humano*), gastrointestinal (*gastrintestinal*), and immunity (*imunidade*). The search was performed using the keywords both alone and in combination.

Data synthesis

The structure of lactoferrin

Lactoferrin is the second predominant protein of the human milk[4], whose concentration is higher in the colostrum. Each lactoferrin molecule consists of one unique polypeptide chain with two globular lobes located in each of the terminals, named lobe C (acetyl) and lobe N (amino), which are linked by an α-helix, with each lobe containing one binding site[7-8]. Each one of them consists of two sub-lobes named N1, N2, C1 and C2[8].

Each lobe can bind to a metal in synergy with the bicarbonate ion, which is essential for the binding of the iron ion with the lactoferrin. The metals that usually bind to lactoferrin are Fe^{+2} or Fe^{+3}, but others (Cu^{+2}, Zn^{+2}, Mn^{+2}) can also bind to the lactoferrin lobes[9].

Concentration of lactoferrin in the human milk

Lactoferrin is widely distributed in the body fluids, especially in the human milk[5]. Its concentration varies among the animal species, with the human and other primates' milk showing the highest concentrations in comparison with cow´s milk[6].

The concentration of lactoferrin varies widely[10], from 5.0–6.7mg/mL in the colostrum and 0.1–2.6mg/mL in the human mature milk[11-12]. In contrast, cow´s milk shows lower concentrations of lactoferrin, with 0.83mg/mL in the colostrum and 0.09mg/mL in the mature milk[13].

Digestion of the lactoferrin

Studies have shown that not only the active form of lactoferrin has biological activity. Digestion of lactoferrin produces lactoferricin, a peptide that derives from the N-terminal of lactoferrin and has strong activity against Gram-positive and Gram-negative pathogenic bacteria[14]. Therefore, both lactoferrin and the product derived from its digestion show antimicrobial activity. It is probable that lactoferricin has bactericidal effect stronger than lactoferrin due to its size, which facilitates its direct action against the bacterial cell membrane[15].

Antimicrobial properties

Diarrhea is, usually, an infection caused by bacteria, viruses or parasites, and represents the second cause of death in children[16]. The infections caused by rotavirus are the main cause of severe diarrhea in children under five years old worldwide[17]. Rotavirus is responsible for about 527.000 deaths and 2 million hospital admissions due to dehydration per year[18].

Among the well-recognized preventive measures against diarrhea is the adoption of the breastfeeding practice, exclusive in the six first months, and complementary for at least two years[16]. In addition to avoiding the contamination from other foods, the efficacy of the breastfeeding as a preventive measure can be attributed to multiple anti-inflammatory, anti-infectious and immunomodulating factors, which are present in the human milk[19,20] and act in the protection of the child[21]. Among these protective factors, lactoferrin is well known by its antimicrobial properties, mainly related to its ability to sequester iron from biological fluids, and/or disrupt the membrane of the microorganisms, acting on the body´s defense against bacteria, viruses and protozoa[22]. The effect of lactoferrin on the intestinal cells may be one of the mechanisms against enteric infections[23].

Antibacterial effect

The bactericidal action of lactoferrin is attributed to two distinct protection mechanisms. The first one is the bacteriostatic action related to the affinity of the protein for the iron molecules, which inhibit the growth of the bacteria that need this nutrient[24], including several Gram-positive and Gram-negative bacteria[25].

The bacteriostatic effect of lactoferrin is also dependent of its degree of saturation[24]: the lesser the iron saturation, the greater its ability to sequester iron[26]. Bearing this is mind, the importance of the lactoferrin that is present in the human milk must be emphasized, as its saturation ranges from 5 to 8% and, therefore, has greater bacteriostatic action[23] in comparison with cow´s milk lactoferrin, whose saturation ranges from 15 to 20%[8]. The lactoferrin with less than 5% iron saturation is known as apolactoferrin (apo), while the lactoferrin with higher saturation is known as hololactoferrin (holo)[8]. In the human milk, lactoferrin predominates in the form of apolactoferrin (90%)[27].

The second protective mechanism against bacterial overgrowth that can be attributed to lactoferrin is related to its interaction with the bacterial surface[28]. Studies on the molecular structure of lactoferrin have shown that it interacts directly with the anionic lipid A, which is a component of the lipopolysaccharides that, in their turn, constitute part of the Gram-negative cell wall[29]. The enhanced affinity of lactoferrin with the lipopolysaccharides is related to its N-terminal, differently from the C-terminal, which shows low affinity[30].

Such interaction can damage the bacterial membrane, affecting its permeability and promoting the release of lipopolysaccharides[31,32]. These alterations facilitate the action of lactoperoxidase and other proteins of defense against the bacteria. The interaction of lactoferrin with the lipopolysaccharides also potentiates the action of other natural antibacterial factors, such as lysozyme[28].

According to *in vivo* studies conducted by Kruzel *et al*, the administration of human lactoferrin to mice one hour before the injection of lipopolysaccharides increased significantly the survival, reducing the mortality from 83.3 to 16.7% in the animals who were pre-treated with lactoferrin. The histopathologic analysis of the intestine of the pre-treated animals showed greater resistance to the damages caused by the lipopolysaccharides. In the animals not treated with human lactoferrin, severe atrophy and edema of the intestinal vilosities, in addition to epithelial vacuolization, were observed[30].

Recent studies have shown that the action of the lipopolysaccharides on the activation of the nuclear factor Kappa B (NF-kB) is insignificant in the presence of human lactoferrin. The NF-kB plays an essential role in the regulation of the immune system and the inflammatory response. In the same experiment, it was also observed that human lactoferrin can induce the NF-kB activation in concentrations much lower than those found in the human milk. According to the authors, it is probable that human lactoferrin acts as a trigger of the tool-like receptor 4 (TLR4) in the intestine of breastfed babies. The TLR4 can detect the lipopolysaccharides, and is important on the activation of children´s innate immune system[33].

The mechanism of action of lactoferrin against Gram-positive bacteria is similar to that described for Gram-negative bacteria, but acting on the lipoteichoic acid, which is a component of the cell wall of the Gram-positive bacteria[34]. According to Leitch and Willcox, lactoferrin and lysozyme exert combined effect against Gram-positive bacteria[34].

Antiviral effect

Lactoferrin can inhibit the replication of several viruses. The antiviral mechanisms of action of lactoferrin have not been elucidated yet. One of the most accepted theories is that lactoferrin prevents viral invasion of the host cells by either blocking the viral receptors or by directly binding to the virus, thus avoiding the infection[9].

Lactoferrin is effective in the control of infections caused by rotavirus, a double helix RNA virus from the *Reoviridae* family which infects mature erythrocytes. These infections are the most frequent cause of gastroenteritis in children worldwide[35].

The apo form of lactoferrin has been shown to be more potent than the holo form, but the reasons for this have not been elucidated yet. It is speculated that most enzymes need metallic ions to exert their actions, and that apolactoferrin is more efficient to capture metallic ions from the environment in comparison with the saturated forms of lactoferrin[9], which are found in greater concentrations in cow´s milk.

Antiparasite effect

The action of lactoferrin against parasitic diseases is still unclear. Studies have attributed its effects to the action of lactoferrin on the integrity of the protozoa membrane[36]. *In vitro* studies have shown that apolactoferrin is the milk protein with greater activity against *Entamoeba histolytica*, as the binding of the protein to the trophozoite´s membrane causes its rupture, and subsequent damage to the protozoan[37].

Estimulation of the healthy microbiota

The development of the intestinal microbiota in children who are breastfed is quite different from those on artificial feeding. Exclusively breastfed babies present the intestinal flora pattern consisting of high percentages of lactobacilli, especially *Lactobacillus bifidus*, while those who are fed with cow´s milk or formulas have their microbiota similar to that of the adults[1].

The human milk contains substances that have probiotic action and stimulate the growth of beneficial bacteria. These substances, originally named *bifidus* factor, can promote the growth of bifidobacterium and lactobacilli, protecting the intestine by limiting the multiplication of several different pathogens due to the decrease of the intestinal pH[38].

Evidences indicate that the oligosaccharides present in the human milk have bifidogenic activity[39], that act together with the proteins, peptides and nucleotides in the development of lactobacilli and bifidobacterium in the gastrointestinal tract of children[40].

The antimicrobial activity of lactoferrin exerts beneficial effect on the intestinal microbiota, because its bacteriostatic action does not impair the growth of bacteria that produce lactic acid, as they have low iron requirements[41,42].

In vitro studies conducted by Liepke *et al* showed the presence of peptides after the digestion of human milk with pepsin. Two of them originated from lactoferrin and from the secretory IgA. The bifidogenic effect of these peptides were superior to that of N-acetyl-glucosamine, a well know bifidogenic factor[43].

Promotion of cell proliferation

The epithelial growth factor is a polypeptide found in the human colostrum (200μg/L) and in the mature milk (30–50μg/L); it is observed in much lower concentrations in cow´s milk[44].

According to Playford *et al*, the growth factor of the human milk stimulates the proliferation and the differentiation of the child´s intestinal cells[45]. Studies from Corps *et al* have demonstrated that the presence of the (purified) growth factor *per se* cannot explain its mitogenic action. The authors concluded that the growth factor present in the human milk, together with other compounds, has greater mitogenic activity than the purified factor[46], pointing to a synergic action of the growth factor and other milk compounds.

The experiments conducted by Hagiwara *et al* showed that lactoferrin can promote the proliferation of cells from the gastrointestinal tract. According to the authors, there is a synergic action between lactoferrin and the epithelial growth factor on cell proliferation[47].

Anti-inflammatory activity

Lactoferrin presents great ability to penetrate the leucocyte nucleus and to block the transcription of the NF-kB, which in its turn induces the release of the pro-inflammatory cytokines interleukin 1 beta (IL1β), tumor necrosis factor alpha (TNFα), interleukin 6 (IL6) and interleukin 8 (IL8)[48].

Furthermore, lactoferrin is part of the immune system homeostasis that can diminish the molecular oxidative stress, and control the excessive inflammatory response. The oxidative stress develops when the production of the potentially destructive oxygen reactive species exceeds the body´s natural anti-oxidant defenses, resulting in cell damage[49].

One study performed *in vivo* using lactoferrin concentrations similar to those of the human colostrum showed that lactoferrin blocked the development of the inflammatory process induced by *Shigella flexnery* in the intestine of rabbits[50].

The *in vivo* experiments of Haversen *et al*[51] confirmed the anti-inflammatory activity of lactoferrin. Laboratory mice with colitis induced by dextran sulfate and treated with human lactoferrin showed lesser faecal occult blood, as well as lesser damage to the rectal mucosa, less evident colon shortening, reduced plasma levels of IL1β, and less amount of TNFα producing cells[51].

Genetic engineering and the synthesis of lactoferrin

The human milk is protective to the child´s health, and there is a consensus that it represents the ideal food, that it must be the only source of feeding in the first six months of life and offered in complementation for at least two years. Nevertheless, many children do not benefit from breastfeeding, either totally or partially, for different reasons. Thus, the industry has been developing technologies trying to adjust the composition of the infant formulas to that of the human milk, not only regarding the nutrients composition, but also the bioactive compounds.

The evolution of the genomic sciences and the consequent disclosing of the human genome have favored the development of technologies that use gene sequences which codify the proteins of the human milk. Such technological progress favors the expression of bioactive proteins of plants and animals, and allows the large scale production of these compounds[52].

Genetic engineering has allowed the expression of recombinant proteins of the human milk in fruits (banana), cereals (rice and barley) e tubercles (potatoes). Among these studies, the rice lactoferrin takes greater importance. The use of this cereal is justified because it has no toxic compounds, in addition to the low allergenic potential, and its intake can be easily incorporated by children all around the world. *In vitro* studies have shown that the recombinant human lactoferrin of the rice has activity and stability properties that are similar to those of the native proteins[53].

Transgenic animals represent another alternative for the expression of the bioactive compounds of the human milk. In this sense, studies have shown that the recombinant human lactoferrin obtained from transgenic cows has identical ability to bind to iron when compared with the human lactoferrin, and showed the same efficacy in three different models of *in vivo* infection[54].

Considering the possibility of obtaining large scale recombinant biological compounds of the human milk by using plants and genetically modified animals for the enrichment of infants' formulas, further studies to evaluate the efficacy and safety of these compounds both *in vitro* and *in vivo* are necessary.

Conclusions

Human lactoferrin is a natural peptide that shows great potential to prevent morbidities, particularly the gastrointestinal diseases, as they present antimicrobial, anti-inflammatory, and cell proliferative activities, and favors the development of the bifidus intestinal flora.

The scientific evidences of the protective effects of the human lactoferrin suggest that this molecule can be involved in the protection of the gastrointestinal tract of breastfed infants, which reinforces the recommendations for the practice of breastfeeding, particularly in the first years of life. This life period is more vulnerable to harm due to the fragility of the adaptive response of the digestive system, and the immaturity of other body systems.

Efforts must be done to develop technologies that allow the expression of the recombinant human protein in plants and animals, with the aim of favoring children that, for various reasons, cannot totally or partially consume human milk.

References

1. Newburg DS. Innate immunity and human milk. J Nutr 2005;135:1308-12.
2. Chirico G, Marzollo R, Cortinovis S, Fonte C, Gasparoni A. Antiinfective properties of human milk. J Nutr 2008;138:1801S-6.
3. Donovan SM. Role of human milk components in gastrointestinal development: current knowledge and future needs. J Pediatr 2006;149 (Suppl 1):S49-61.
4. Chierici R, Vigi V. Lactoferrin in infant formulae. Acta Paediatr 1994;s402:83-8.
5. Lönnerdal B. Nutritional roles of lactoferrin. Curr Opin Clin Nutr Metab Care 2009;12:293-7.
6. Lönnerdal B, Iyer S. Lactoferrin: molecular structure and biological function. Ann Rev Nutr 1995;15:93-110.
7. Ochoa TJ, Cleary TG. Effect of lactoferrin on enteric pathogens. Biochimie 2009;91:30-4.
8. Steijns JM, van Hooijdonk AC. Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. Br J Nutr 2000;84 (Suppl 1):S11-7.
9. Van der Strate BW, Beljaars L, Molema G, Harmsen MC, Meijer DK. Antiviral activities of lactoferrin. Antiviral Res 2001;52:225-39.
10. Gifford JL, Hunter HN, Vogel HJ. Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. Cell Mol Life Sci 2005;62:2588-98.
11. Hirai Y, Kawakata N, Satoh K, Ikeda Y, Hisayasu S, Orimo H *et al*. Concentrations of lactoferrin and iron in human milk at different stages of lactation. J Nutr Sci Vitaminol (Tokyo) 1990;36:531-44.
12. Ronayne de Ferrer PA, Baroni A, Sambucetti ME, López NE, Ceriani Cernadas JM. Lactoferrin levels in term and preterm milk. J Am Coll Nutr 2000;19:370-3.
13. Sánchez L, Aranda P, Pérez MD, Calvo M. Concentration of lactoferrin and transferrin throughout lactation in cow's colostrum and milk. Biol Chem Hoppe Seyler 1988;369:1005-8.
14. Newburg DS, Walker WA. Protection of the neonate by the innate immune system of developing gut and of human milk. Pediatr Res 2007;61:2-8.
15. Rodríguez-Franco DA, Vásquez-Moreno L, Montfort GR. Actividad antimicrobiana de la lactoferrina: mecanismos y aplicaciones clínicas potenciales. Rev Latino-am Microbiol 2005;47:101-11.
16. The United Nations Children's Fund; World Health Organization. Diarrhoea: why children are still dying and what can be done. Geneva: UNICEF/WHO; 2009.
17. Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis 2006;12:304-6.
18. Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D *et al*. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis 2009;200 (Suppl 1):S9-15.
19. Goldman AS. The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties. Pediatr Infect Dis J 1993;12:664-71.
20. Hamosh M. Bioactive factors in human milk. Pediatr Clin North Am 2001;48:69-86.
21. Field CJ. The immunological components of human milk and their effect on immune development in infants. J Nutr 2005;135:1-4.
22. Puddu P, Latorre D, Valenti P, Gessani S. Immunoregulatory role of lactoferrin-lipopolysaccharide interactions. Biometals 2010;23:387-97.
23. Yen CC, Shen CJ, Hsu WH, Chang YH, Lin HT, Chen HL *et al*. Lactoferrin: an iron-binding antimicrobial protein against Escherichia coli infection. Biometals 2011;24:585-94.
24. Bullen JJ, Rogers HJ, Leigh L. Iron-binding proteins in milk and resistance to Escherichia coli infection in infants. Br Med J 1972;1:69-75.
25. Levy O. Antibiotic proteins of polymorphonuclear leukocytes. Eur J Haematol 1996;56:263-77.
26. Conneely OM. Antiinflammatory activities of lactoferrin. J Am Coll Nutr 2001;20 (Suppl 5):389S-95.
27. Lönnerdal B. Bioactive proteins in human milk: mechanisms of action. J Pediatr 2010;156 (Suppl 2):S26-30.
28. González-Chávez SA, Arévallo-Gallegos S, Rascón-Cruz Q. Lactoferrin: structure, function and applications. Int J Antimicrob Agents 2009;33:301.
29. Brandenburg K, Jürgens G, Müller M, Fukuoka S, Koch MH. Biophysical characterization of lipopolysaccharide and lipid A inactivation by lactoferrin. Biol Chem 2001;382:1215-25.
30. Kruzel ML, Harari Y, Chen CY, Castro GA. Lactoferrin protects gut mucosal integrity during endotoxemia induced by lipopolysaccharide in mice. Inflammation 2000;24:33-44.
31. Coughlin RT, Tonsager S, McGroarty EJ. Quantitation of metal cations bound to membranes and extracted lipopolysaccharide of Escherichia coli. Biochemistry 1983;22:2002-7.
32. Ellison RT, Giehl TJ, Laforce FM. Damage of the outer membrane of enteric gram-negative bacteria by lactoferrin and transferrin. Infect Immun 1988;56:2774-81.
33. Ando K, Hasegawa K, Shindo K, Furusawa T, Fujino T, Kikugawa K *et al*. Human lactoferrin activates NF-kappaB through the Toll-like receptor 4 pathway while it interferes with the lipopolysaccharide-stimulated TLR4 signaling. FEBS J 2010;9:2051-66.
34. Leitch EC, Willcox MD. Elucidation of the antistaphylococcal action of lactoferrin and lysozyme. J Med Microbiol 1999;48:867-71.
35. Arnold D, Di Biase AM, Marchetti M, Pietrantoni A, Valenti P, Seganti L *et al*. Antiadenovirus activity of milk proteins: lactoferrin prevents viral infection. Antiviral Res 2002;53:153-8.
36. Farnaud S, Evans RW. Lactoferrin – a multifunctional protein with antimicrobial properties. Mol Immunol 2003;40:395-405.
37. León-Sicairos N, López-Soto F, Reyes-López M, Godínez-Vargas D, Ordaz-Pichardo C, de la Garza M. Amoebicidal activity of milk, apo-lactoferrin, sIgA and lysozyme. Clin Med Res 2006;4:106-13.
38. Lönnerdal B. Nutritional and physiologic significance of human milk proteins. Am J Clin Nutr 2003;77:1537S-43.
39. Newburg DS. Do the binding properties of oligosaccharides in milk protect human infants from gastrointestinal bacteria? J Nutr 1997;127 (Suppl 5):980S-4.
40. Lawrence RM, Pane CA. Human breast milk: current concepts of immunology and infectious diseases. Curr Probl Pediatr Adolesc Health Care 2007;37:7-36.
41. Petschow BW, Talbott RD, Batema RP. Ability of lactoferrin to promote the growth of Bifidobacterium spp. in vitro is independent of receptor binding capacity and iron saturation level. J Med Microbiol 1999;48:541-9.
42. Coppa GV, Zampini L, Galeazzi T, Gabrielli O. Prebiotics in human milk: a review. Dig Liver Dis 2006;38 (Suppl 2):S291-4.
43. Liepke C, Adermann K, Raida M, Mägert HJ, Forssmann WG, Zucht HD. Human milk provides peptides highly stimulating the growth of bifidobacteria. Eur J Biochem 2002;269:712-8.
44. Read LC, Francis GL, Wallace JC, Ballard FJ. Growth factor concentrations and growth-promoting activity in human milk following premature birth. J Dev Physiol 1985;7:135-45.
45. Playford RJ, MacDonald CE, Johnson WS. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. Am J Clin Nutr 2000;72:5-14.
46. Corps AN, Blakeley DM, Carr J, Rees LH, Brown KD. Synergistic stimulation of Swiss mouse 3T3 fibroblasts by epidermal growth factor and other factors in human mammary secretions. J Endocrinol 1987;112:151-9.
47. Hagiwara T, Shinoda I, Fukuwatari Y, Shimamura S. Effects of lactoferrin and its peptides on proliferation of rat intestinal epithelial cell line, IEC-18, in the presence of epidermal growth factor. Biosci Biotechnol Biochem 1995;59:1875-81.
48. Hanson LA. Symposium on 'Nutrition in early life: new horizons in a new century'. Session 1: feeding and infant development breast-feeding and immune function. Proc Nutr Soc 2007;66:384-96.
49. Actor JK, Hwang SA, Kruzel ML. Lactoferrin as a natural immune modulator. Curr Pharm Des 2009;15:1956-73.
50. Gomez HF, Ochoa TJ, Herrera-Insua I, Carlin LG, Cleary TG. Lactoferrin protects rabbits from shigella flexneri-induced inflammatory enteritis. Infec Immun 2002;70:7050-3.
51. Haversen LA, Baltzer L, Dolphin G, Hanson LA, Mattsby-Baltzer I. Anti-inflammatory activities of human lactoferrin in acute dextran sulphate-induced colitis in mice. Scand J Immunol 2003;57:2-10.
52. Camelo Jr JJ, Martinez FE. Lactoengenharia do leite humano. In: Pereira GR, Leone CR, Alves Filho N, Trindade Filho O, editors. Nutrição do recém-nascido pré-termo. Rio de Janeiro: Medbook; 2008. p. 11-29.
53. Lönnerdal B. Expression of human milk proteins in plants. J Am Coll Nutr 2002;21 (Suppl 3):218S-21.
54. Van Berkel PH, Welling MM, Geerts M, van Veen HA, Ravensbergen B, Salaheddine M *et al*. Large scale production of recombinant human lactoferrin in the milk of transgenic cows. Nature Biotechnol 2002;20:484-7.