Overview of Lactoferrin as a Natural Immune Modulator

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Lactoferrin is thought to be the most polyvalent protein present in host defense against tissue injuries and infections in vertebrates. Owing to the propensity of its basic N-terminal domain to interact with various microbial and host targets, lactoferrin not only has antimicrobial properties, but also modulates the innate and adaptive immune responses. Lactoferrin may indeed up- and downregulate immune cell activation, migration, and growth. Whereas the immunomodulatory properties of lactoferrin are evidenced from in vivo studies using either lactoferrin-knockout, lactoferrin-overexpressing transgenic models, and dietary lactoferrin, few mechanisms from in vitro studies have been proposed to explain these properties. The best characterized lactoferrin targets are negatively charged molecules. They encompass pro-inflammatory microbial molecules, such as pathogen-associated molecular patterns (eg, lipopolysaccharide), but also host components such as DNA, the glycosaminoglycan chains of proteoglycans, and surface cell receptors. Signaling through these receptors is thought to be the main lever used by lactoferrin to influence immune cells and cytokine-balance-controlling cell activity. This article aims to review our current understanding, though incomplete, of the many ways lactoferrin influences the complex immune machinery and the known and putative mechanisms that may explain its properties. *(J Pediatr 2016;173S:S10-5)*.

Lactoferrin is thought to be the most polyvalent protein found in vertebrates. Since its discovery more than 50 years ago,[1] lactoferrin has been the object of more than 3000 studies reporting its activities in host defense as well as the mechanisms to explain them. The immune system is a highly complex and potentially harmful biological system in which lactoferrin not only plays antimicrobial roles, but also seems to actuate strategic levers to modulate host defense. In other words, lactoferrin appears to have the ability to stimulate the immune system to counteract pathogenic invaders and injuries, while preventing overreactions, which are harmful to the host.[2] Systemic immune modulation effects may be gained by oral lactoferrin supplementation,[3] which raises numerous questions as to how dietary lactoferrin could mimic and even enhance the activities of naturally occurring host lactoferrin. A better understanding of the mechanisms of immune regulation by host lactoferrin is needed. In this article, we review our current knowledge of these proven and putative mechanisms.

Lactoferrin is Distributed at Key Positions in the Organism

Lactoferrin is expressed by epithelial cells in most exocrine secretions such as seminal fluid, pancreatic exocrine secretions, tears, saliva, uterine secretions, and in milk where its concentration in humans may vary between 1 and 7 g/L (colostrum).[4] Lactoferrin lines all mucosa and epithelia where, together with secreted IgA and other defensins and owing to its innate antimicrobial action, it may influence microbial homeostasis.[5]

Lactoferrin is also expressed by cells of the innate immune system, which may locally deliver the molecule at inflammatory sites.[2] Neutrophil leukocytes (polymorphonuclear neutrophils [PMNs]), which represent more than one-half of total white blood cells, express lactoferrin and store the molecule in secondary granules. Upon activation of PMNs, which begins at the very first steps of adhesion to the activated endothelium, lactoferrin is released in the blood, where its concentration may rise up to 200 mg/L (from about 1 mg/mL under normal conditions), especially in inflamed tissues.[6] In addition, microglial cells, which act as the resident macrophages in the brain, also release lactoferrin upon inflammation.[7]

Lactoferrin is a Mediator of Host Defense

Most direct evidence for the role of lactoferrin in host defense comes from both lactoferrin deficiency in human and mice models and lactoferrin supplementation in vivo models, indicating protective effects against various threats, such as infection, allergic inflammation, and cancer.[8] From these studies, it appears that lactoferrin

APC	Antigen-presenting cell
IL	Interleukin
Lfc	Lactoferricin
LPS	Lipopolysaccharide
PAMP	Pathogen-associated microbial pattern
PMN	Polymorphonuclear neutrophil
Tf	Transferrin
TLR	Toll-like receptor

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carries on activities at different levels and on different targets, oscillating between pro- and anti-inflammatory activities, in accordance with the threat and the host immune status.

In mucosal fluids, lactoferrin, together with secreted IgA and defensins, is part of the arsenal of the innate system designed to achieve microbial homeostasis.[5] Lactoferrin is a multifunctional molecule by nature of its high affinity for ferric iron, which deprives microbes from the free iron necessary for their growth and of its propensity to interact with microbial and target host cell surfaces.[9] The same properties are used by neutrophilic lactoferrin, which is released at high concentration in infected tissues, likely bound to the chromatin fibril matrix released from neutrophils (neutrophil extracellular traps),[8] thus, providing a powerful antimicrobial environment. In addition, lactoferrin could participate to the opsonization of microbes either by serving as an opsonin-like molecule bound to phagocytes, or by activating complement cascades, although this latter mechanism is controversial.[2]

Interestingly, besides its antimicrobial activities, lactoferrin is a mediator of both innate and adaptive responses.[2] This mediation results in local and systemic changes in the expression of the signaling molecules that govern the balance between pro- and anti-inflammatory, as well as humoral and cellular immune responses. Major molecular levers of the immune system are cytokines, either anti-inflammatory (eg, interleukin [IL]-4, IL-10) or pro-inflammatory (eg, tumor necrosis factor-α, IL-1, IL-6, IL-12) ILs and chemokines (eg, IL-8), for which control of expression by lactoferrin was reported through many in vitro and in vivo studies.[2] This results in effects on the growth, differentiation, activation, and functions of immune cells. Until now, only a few molecular mechanisms have been characterized in in vitro studies, whereas may other potential mechanisms are still under investigation. All of these and the corresponding lactoferrin molecular targets will be reviewed in the following paragraphs, and summarized in the **Figure**.

Specific Molecular Features Account for Lactoferrin Properties in Host Defense

Lactoferrin is similar to serum transferrin (Tf), as they are both 70-80 kDa bilobed glycoproteins with 2 domains in each lobe (N1, N2 and C1, C2) delimiting an iron-binding site, but with 2 majors differences.[10] First, lactoferrin binds 2 ferric ions with very high affinity, making the protein a powerful iron scavenger, not an iron transporter. Second, lactoferrin is a very basic protein (pi 9) with a high propensity to interact with negatively-charged molecules, either in solution or at the surface of cells. The N-lobe contains the highest density of basic amino acids and particularly 2 peptide sequences, lactoferricin ([Lfc] residues 1-47 in human) and lactoferrampin (residues 269-285 in human), which have been identified as the major binding sites to lactoferrin molecular targets. Lastly, the type, the number, and the location of glycans linked to the molecule differentiate Tfs and lactoferrins.[10] Regarding human lactoferrin, the molecule possesses 2 main sialylated and fucosylated N-acetyl-lactosaminic type glycans linked to domains N2 and C2.

Iron Binding by Lactoferrin Downregulates Inflammation

Although chelation of iron is essential to the antimicrobial activities of lactoferrin, it also accounts for its antioxidant properties. At inflammatory sites, phagocytes produce and release reactive oxygen species, which are very efficient against microbes but also cause apoptosis and necrosis of tissues. Wound tissues, therefore, release ferric and ferrous iron, which participate to the Haber-Weiss reaction generating new free radicals. In addition, hydrogen peroxide is used by PMN myeloperoxidase to produce toxic hypochlorous acid. Thus, iron scavenging by PMN lactoferrin strongly contributes to the mitigation of oxidative stress. Such scavenging of iron deposits in the brains of patients with Alzheimer or Parkinson disease likely also occurs as a result of lactoferrin, originating from the activated microglia or from blood.[7]

Lactoferrin Modulates Innate Immunity by Interacting with Pathogen-Associated Microbial Patterns and Their Receptors

Many lactoferrin immune-modulatory properties are related to its ability to bind to several pro-inflammatory pathogen-associated microbial patterns (PAMPs), chiefly recognized by specific receptors of innate immune cells, the toll-like receptors (TLRs). Such binding has been reported for Gram negative bacterial lipopolysaccharide (LPS)[11] and unmethylated CpG-containing DNA.[12] LPS is the prototypical PAMP in that the Lfc domain of lactoferrin binds with high affinity through the lipid-A moiety.[13] Such binding was reported in vitro to impede LPS signaling by LPS-receptors, such as TLR-4 and L-selectin, not only in a number of immune cells, including monocytes-macrophages, neutrophils, and lymphocytes, but also in endothelial cells.[2] In addition, high-affinity binding of lactoferrin to soluble CD14 (sCD14), a co-receptor of TLR-4, alone or in complex with LPS, was demonstrated.[14] All of these interactions were shown to inhibit LPS-induced activation of cells at inflammatory sites, resulting in lower expression by immune cells of powerful pro-inflammatory cytokines, such as tumor necrosis factor-α, IL-1, and IL-6. The same is true of expression of adhesion molecules (E-selectin and intercellular adhesion molecule-1) and IL-8 (a key component of the chemokine gradient in inflamed tissues) by endothelial cells.[2] As a consequence, PMN-released lactoferrin could mitigate responses and later recruit immune cells at the very heart of inflammatory sites, thus, reducing further damages to tissues. Interestingly, binding of lactoferrin to TLR-4 has been reported, suggesting that the molecule can moderately activate immune cells through the TLR-4 pathway.[15] Such duality of promotion and inhibition of immune cell activation by lactoferrin also could be applied to the lactoferrin-LPS complex. Indeed, because the Lfc domain