The love and death relationship between lactoferrin and bacteria.

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Introduction

Lactoferrin (Lf) is a non-haem iron-chelating glycoprotein of 70-80 kDa that is part of the innate immune system and is present in body secretions of mammals, like bile, pancreatic juice, intestinal secretions, bronchoalveolar fluid, semen, vaginal secretions, tears, and saliva [1]. In bovine colostrum, lactoferrin is present in high concentration (1 a 2 mg/mL) and in mature milk in lower concentration (0.1-0.3 mg/mL) [2,3]; Lf is part of the protection to the newborn. In addition, Lf is produced by the secondary granules of polymorphonuclear neutrophils, from which is released following activation in infection sites.

The molecule consists of two lobes, each one with two domains, and each lobe can bind one ferric-iron ion reversibly; the apo-form is iron-free (apo-Lf) whereas the holo-form binds one or two iron ions (holo-Lf) [4]. Interestingly, Lf has multiple functions: it is microbiostatic, by chelating the iron necessary for pathogens in fluids and mucosae; it possesses serine protease activity, affecting adhesins and the secretion system type III; Lf avoids biofilm formation; it is immunomodulator by decreasing the release of some interleukins and enhancing monocyte and natural killer cell cytotoxicity; Lf has microbicidal effect against bacteria, fungi and viruses; and it has been tested in combination with antibiotics to treat some infections in humans and animals [5-7].

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The Love

Iron is an essential micronutrient for virtually all organisms. However, it is not free inside the organism; specific systems for transport, utilization, and storage of this element have been developed by cells. Most of iron in mammals is associated to proteins, one of them is Lf [8,9]. Several pathogens are able to use holo-Lf as an iron source, such as species of the Neisseriaceae and Moraxellaceae families; for example, the veterinary pathogen Moraxella bovis [9,10]. The bacterial iron uptake is through a receptor complex, consisting in two outer membrane proteins (OMPs): a TonB-dependent integral membrane protein (LbpA) and a peripheral lipidated protein (LbpB) (Figure 1) [11-13]. LbpA is predicted to have large surface loops to bind Lf, forcing the separation of the domains surrounding the iron-binding sites to release iron, whereas LbpB is attached to the outer membrane with an N-terminal lipid anchor; it is possible that LbpB may act as initial binding site for holo-Lf [9,13]. In this case, the relationship between Lf and pathogens is beneficial to the bacteria that use this glycoprotein as an iron source for growth.

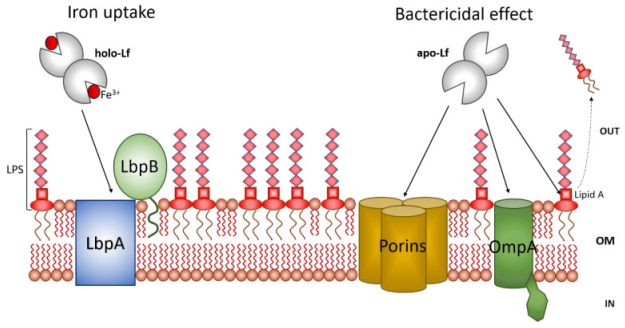

Figure 1. *Binding of lactoferrin (Lf) by Gram-negative bacteria, depending on apo- or holo-form of Lf.*

LPS: Lipopolysaccharide; Lbp: Lactoferrin binding protein; OM: Outer membrane; OmpA: Outer membrane protein A

The Death

In other cases, the binding between Lf and pathogens results in a fatal relationship. Apo-Lf can be bactericidal in certain Gram-negative species by altering the outer membrane, this is due to Lf binds to the lipid A portion of lipopolysaccharide (LPS) causing its release (Figure 1) [14]. Lf also binds to porins affecting its permeability to ions and antibiotics [15,16]. Lf is bactericidal for Gram-positive bacteria by binding to teichoic acid [7]. Our research group has demonstrated that bovine apo-Lf has bactericidal effects against the veterinary pathogens Actinobacillus pleuropneumoniae and Mannheimia haemolytica; the MIC found were 10-14 µM and 4-7 µM, respectively. Also, we have reported the binding between bovine Lf and OMPs in M. haemolytica [17,18]. Interestingly, both apo-Lf and holo-Lf were bound to two OMPs of 32.9 and 34.2 kDa with estimated IP of 8.18 and 9.35, which were identified as OmpA (heat-modifiable protein) and a membrane protein (porin), respectively [18]. In other pathogens as Escherichia coli and Salmonella enterica serovar Typhimurium the correlation of antibacterial activity of apo-Lf and binding to porins has been demonstrated [15,19]. In addition, Erdei et al. [16] evidenced the interaction of Lf with OmpF and OmpC porins.

As we describe above, the binding to Lf could be beneficial or harmful to bacteria. Holo-Lf can be used as an iron source for some pathogens, whereas apo-Lf can kill them.

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