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Lactoferrin: a modulator of immune and inflammatory responses

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Abstract. Lactoferrin is an iron-binding glycoprotein of the transferrin family. Abundant expression and secretion of lactoferrin, in particular in milk and fluids of the digestive tract, are related to its implication in the first line of host defense. Lactoferrin is also a prominent component of the secondary granules of neutrophils (PMNs) and is released in infected tissues and blood during the inflammatory process. In addition to its direct antimicrobial properties, the abilities of lactoferrin to regulate the immune response and to protect against infection and septic shock have been described in numerous in vitro and in vivo studies. Although the cellular and molecular mechanisms that account for the modulation of the inflammatory and immune responses by lactoferrin are not yet totally elucidated, many are now established. At the cellular level, lactoferrin modulates the migration, maturation and function of immune cells. At the molecular level and in addition to iron binding, interactions of lactoferrin with a plethora of compounds, either soluble or membrane molecules, account for its modulatory properties. This paper reviews our current understanding of the cellular and molecular mechanisms that explain the regulatory properties of lactoferrin in host defence.

Key words. Lactoferrin; inflammation; immunity; ROS; Th1.

Introduction

When lactoferrin (Lf) was first discovered in milk [1], it was named lactotransferrin, suggesting a functionally related variant of transferrin. Since Lf is homologous to serum transferrin (Tf), it was indeed tempting to consider the molecule solely as an iron-binding molecule. The possibility of Lf having functions other than just simple iron sequestration emerged as soon as it was reported that Lf binds to microbes, host cells and components of the immune system. Besides its direct effects in host defense on bacteria, fungus and parasites, possible roles in the modulation of the immune response were reported. These properties were illustrated with many in vitro and in vivo experiments carried out in humans and animals, sometimes controversial but often leading to similar conclusions. Controversial conclusions are not unexpected, since modulation means both positive and negative effects, sometimes neutral effects, depending on the physiological status of the organism. Furthermore, the regulation of the immune system calls for so many factors that determining the exact role of Lf is very difficult. At the moment, however, we are at a stage where more questions are asked than answers are given about the molecular mechanisms governing the modulating properties of Lf in the immune and inflammatory responses. The present review will attempt to detail our current knowledge of these properties.

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Overall positioning of Lf in the host defense system

Spatio-temporal distribution of Lf in the organism

The widespread distribution of the molecule in all the body suggests that Lf is in the front line of host defense. Lf synthesis and secretion can be continuous (exocrine fluids) or under hormonal control (genital tract) [2]. Alternatively, Lf can be synthesized at a well-defined stage of cell differentiation [3] and stored in secretory pathways, awaiting an external signal to be secreted. It seems that the type of Lf secretion is correlated with its function: direct anti-microbial activities in secretions and at the surface of epithelia or regulation of the inflammatory response.

Lf is secreted in the apo-form from epithelial cells in most exocrine fluids, for example, saliva, bile, pancreatic and gastric fluids, tears and, more particularly, milk [1]. In milk, Lf is synthesized by the epithelial cells of mammary glands, mainly by the glandular epithelial cells, and its concentration in humans may vary from 1 g/l (mature milk) to 7 g/l (colostrum) [4]. Furthermore, Lf is synthesized during the transition from promyelocytes to myelocytes and is thus a major component of the secondary granules of neutrophils (PMNs) [3]. During inflammation and in some pathologies, Lf levels in biological fluids may greatly increase and may constitute, as we report hereafter, a marker for inflammatory diseases. This is particularly noticeable in plasma where the Lf concentration can be as low as 0.4–2 mg/l under normal conditions but increases up to 200 mg/l in septicaemia [5, 6]. Lf released in blood from degranulating PMNs is then rapidly cleared by the liver parenchymal cells [7]. In fact, plasma Lf represents only the tip of the iceberg since (i) most of neutrophil Lf is delivered by PMNs at the sites of inflammation and (ii) Lf can bind to glycosaminoglycans of proteoglycans [8-10], so that cells may provide high local concentrations of functional Lf on their surfaces. Interestingly, Lf immobilized to airway epithelium, but not soluble Lf, may activate eosinophils [11], thus underscoring the importance of Lf bound to epithelia.

Overview of the key functions of Lf in host defense

Host defense involves innate and acquired immune systems, the latter being divided into humoral and cell-mediated immunities. Lf clearly belongs to the innate, non-specific immune system. However, several lines of evidence indicate that it may also contribute, at least indirectly, to acquired immunities. Additionally, host defense includes protective systems, of which Lf is an essential element, against deleterious effects of inflammation.

It is now accepted that Lf plays a direct anti-microbial role in secretions and at the surface of epithelia by limiting the proliferation and adhesion of microbes and/or by killing them. These properties are the subject of an accompanying review, however, and will not be developed further here. Briefly, these properties are mainly related to the ability of Lf either to sequester iron in biological fluids or to destabilize the membranes of microbes [12]. The constitutive expression of Lf in large amounts in all secretion fluids and the massive delivery of Lf from PMNs at inflammation sites [13] ensure the efficiency of such a protective effect.

Besides its direct antimicrobial effects, Lf may be responsible for up- and downregulation of immune cells and cells involved in the inflammatory process. These regulatory activities are due to the iron-binding properties of Lf and above all to its ability to interact with target molecules and cells. On the one hand, some in vitro experiments suggest that it may regulate the proliferation, differentiation and activation of immune cells, thus strengthening, either directly or indirectly, the immune response. On the other hand, Lf mediates anti-inflammatory activities that can lower the harmfulness of the response. When tissues are infected, reactive oxygen species are abundantly produced, either generated by free iron released from necrosed tissues or overproduced by activated granulocytes. This oxidative burst, together with the excessive release of pro-inflammatory cytokines, mainly interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) contributes to the pathogenesis of septic shock [14]. The protective anti-inflammatory activity of Lf lies in its ability to bind not only free ferric ion but also exogenous pro-inflammatory bacterial components such as lipopolysaccharides (LPSs) and their receptors. Whereas Lf iron binding has beneficial detoxication effects in infected and pathological tissues, binding to pro-inflammatory molecules has downregulating effects on both the activation and recruitment of immune cells in inflamed tissues. The molecular and cellular bases for these assertions are developed in the following sections.

Structural and functional features of Lf accounting for its immunomodulatory and anti-inflammatory properties

A full member of the transferrin family but with specific features

Lf belongs to the transferrin family, of which Tf is the best known member. Human Lf (hLf) consists of a single 692-residue polypeptide chain [15] whose three-dimensional (3D) structure has been thoroughly defined [16]. Although both Lf and Tf have very similar 3D structures, they differ significantly in their primary structures; hLf is about 60% identical to human Tf. The differences are found especially in surface-exposed sequences, which contribute specifically to physicochemical and biological properties. In contrast to Tf, which is a rather acidic molecule (pHi ~ 6.5), Lf is a strongly basic protein (pHi

8.5–9). Much of the cationic charge of Lf is located on domain N1, especially in the so-called lactoferricin (Lfc) domain (fig. 1A) which is known to be important for many functions of the molecule [17]. Lfc, a peptide isolated following pepsin digestion of hLf (residues 1–19 and 20–37) and bovine Lf (bLf) (residues 19–36), contains basic amino acid residue repeats in a β-sheet-α-helix structure that was shown to be responsible for most of iron-independent activities of Lf, not only bactericidal but also immunoregulatory and inflammatory activities [18]. Recently, an α-helix-containing basic peptide (residues 268–284 in bLf), close to the Lfc domain, called lactoferrampin, was also recently shown to be involved in bactericidal activity against several bacterial strains [19].

Interestingly, a common feature of all Lfs is to retain iron to pH values as low as 3, whereas Tf releases iron at a pH of about 5.5. This difference of stability towards pH can be explained in part by the presence of a hydrogen bond between a pair of lysine residues in Tf that could provide a trigger for iron release on protonation [20, 21], but in particular by cooperative interactions between the two lobes of Lf [22]. The high affinity and stability of iron binding by Lf make the protein not only a powerful bacteriostatic agent but also an antioxidant protective molecule whose properties will be discussed below.

Ligand and receptor binding by Lf

The cationic nature of Lf accounts for its propensity to 'stick' to many anionic molecules and makes identification of specific ligands contributing to the immunoregulatory roles of Lf difficult. At the surface of cells, the sulfated chains of proteoglycans present the main Lf binding sites, responsible for about 80% of total binding [10, 23]. Although the low affinity (Ka ~ 10^6 M) and ionic nature of the interactions may raise questions about their physiological relevance, it is now accepted that binding to proteoglycans is responsible for high-density binding of Lf at the surface of cells (generally several millions of binding sites on many cells) [23]. It is thought that Lf binding to glycosaminoglycans is an important event in the inflammatory process. The binding determinants in hLf for cell surface glycosaminoglycans and soluble heparin have been identified as the basic stretches ^{1}GRRRRS^{6} and ^{28}RKVR^{31} [9, 10, 23]. These two sections of polypeptide (fig. 1B) seem to act as a cationic cradle to bind sulfated chains [9]. Besides proteoglycans, a few cell receptors have been reported that could account for the signaling, endocytosis and nuclear targeting of Lf in cells. A specific 105-kDa receptor was formerly identified on activated lymphocytes, platelets and mammary gland cells that could permit signaling in cells as well as endocytosis of Lf [25–27]. Very recently, we showed binding of Lf to nucleolin expressed at the surface of dividing cells that participates, together with proteoglycans, in the endocytosis and nuclear targeting of Lf [28]. There are many indications that nucleolin could represent the previously reported Lf receptor, though the receptor binding sites on Lf look different. Another important Lf receptor at the surface of cells is the low-density lipoprotein receptor-related protein (LRP), which has been shown to be responsible for Lf endocytosis but also to function as a mitogenic Lf receptor in osteoblastic cells [29]. Since the LRP is also present at the surface of immune cells, its role in the immuno-modulating activity of Lf may be considered. Finally, a specific receptor visualized as a 34-kDa protein under reducing conditions, responsible for Lf endocytosis, has been found at the surface of intestinal cells [30, 31].

Apart from cell surface target molecules, soluble anionic molecules can be bound by Lf, most of them being important actors in the inflammatory response. First of all, the high-affinity interaction of Lf with the lipid A moiety of *Escherichia coli* LPS was reported [32, 33]. Using *E. coli* LPS, two binding sites with dissociation constants (K_d) of 3.6 +/– 1 nM and 390 +/– 20 nM were found on the N- and C-lobes of hLf [34]. It was shown

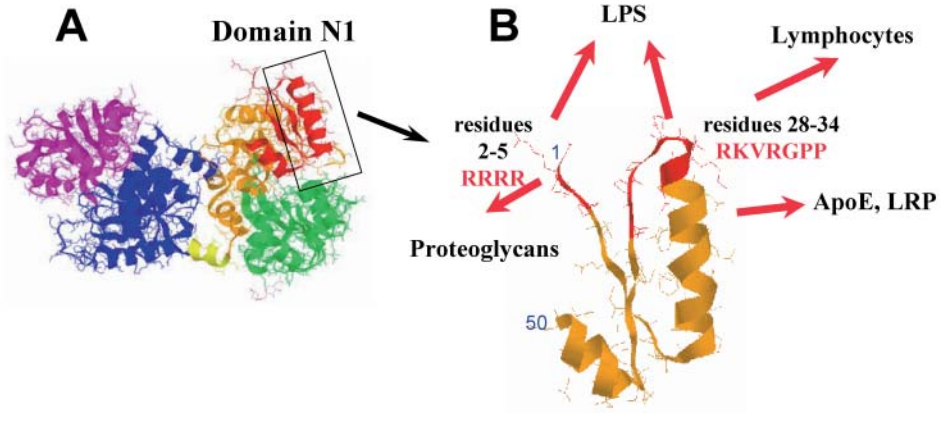

Figure 1. Human Lf binding sites. (A) Localisation of the N1 domain (residues 1–50); (B) Binding sites of LPS, proteoglycans, apoE and lymphocyte receptor.

that both sequences ^{1}GRRRR^{5} and ^{28}RKVRGPP^{34}, the same involved separately or together in the interactions with the 105-kDa Lf receptor, the LRP, glycosaminoglycans and DNA [9, 10, 24] are required for the high-affinity binding of Lf to LPS [34, 35]. Binding of Lf to other bacterial pro-inflammatory components such as unmethylated CpG-containing oligonucleotides was also reported [36]. More important, high-affinity interactions (K_d ~ 16 +/– 7 nM) were reported between Lf and sCD14, a serum-soluble LPS receptor. It was shown that hLf interacts not only with free sCD14 but also, though with different binding properties, with sCD14 complexed to LPS or lipid A-2-keto-3-deoxyoctonic acid-heptose [37]. As for LPS, the cationic N-terminal peptides of Lf are essential for binding [37]. Interestingly, it may be noted that most interactions of Lf with cell receptors and inflammatory molecules also involve the N-terminal Lfc domain of Lf. The Lfc peptide itself was also found to interact with LPS [18,34,38,39]. Recent studies, however, suggest that lipid A is not the main binding site for Lfc but the negative charges present in the inner core [40].

Lf, a signaling molecule in cells?

The binding of Lf to the surface of cells suggests that it might directly trigger cellular responses such as differentiation, activation and proliferation. Several findings, reported below, are in accordance with this postulate. However, it is not clear how exactly Lf could trigger signals in cells. Interestingly, LRP, a Lf receptor at the surface of many cells, has recently been shown to function as a mitogenic Lf receptor in osteoblastic cells, via p42/44 MAP kinase signaling [29]. Such MAP kinase signaling was also observed in Jurkat lymphoblastic T cells owing to the 105-kDa Lf receptor [41]. It is also hypothesized that Lf may enter the cell and be targeted to the nucleus where it can act as a transcriptional activator [42]. Recently, nucleolin ubiquitously expressed on dividing cells was pointed out as a possible Lf carrier between cell surface and nucleus [28]. Interestingly, it has also been shown that Lf may downregulate LPS-induced cytokines in THP1 through a mechanism involving Lf internalization, nuclear localization and interference with nuclear factor-κB (NF-κB) [43]. The mechanisms of interference of Lf with NF-κB, a transcription factor playing a critical role in immune responses and inflammation, are not perfectly clear. However, Oh et al. [44] showed that overexpressed Lf acts as a p53 gene transactivator through the stimulation of the inhibitor of NF-κB (IκB)-kinase activity and NF-κB binding. These authors previously demonstrated a matrix metalloproteinase 1 gene transactivating activity by Lf through stress-activated mitogen-activated protein-kinase (MAPK) signaling modules [42].

Up- and downregulation of the immune system by Lf

In vivo evidence for regulatory roles of Lf

To date there has been no direct in vivo evidence for a regulatory role of Lf in the immune system, although knockout animals for Lf have been produced [45]. Involvement of Lf in the regulation of the immune system was suggested in 1980 [46], when a total absence of Lf in neutrophils, but normal Lf content in glandular secretion [47], was observed for a patient suffering recurrent infections. More recently, hLf-transgenic mice have been shown to clear bacteria significantly better than congenic littermates [48]. This effect is the consequence of direct inhibition of the growth of *Staphylococcus aureus*, and of the enhancement of the T helper (Th) type 1 response due to overexpression and constitutive presence of Lf in animal tissues. Furthermore, the susceptibility to tuberculosis of β-2-microglobulin knockout mice was abolished by Lf treatment [49]. Oral administration of Lf also revealed host-protecting effects against microbial infections [50], during lethal bacteraemia in mice [51, 52] and against oral candidiasis [53]. Finally, orally administered Lf was shown to protect piglets against septic shock [54].

At the molecular level, altered expression of cytokines, mostly pro-inflammatory interferon γ (IFN-γ), interleukin (IL)-1β, IL-6 and TNF-α, and granulocyte-macrophage colony-stimulating factor (GMCSF) have been detected in the presence of exogenous Lf [48, 55–58], with a decrease of IL-5 and IL-10 production. In contrast, upregulation of anti-inflammatory IL-4 and IL-10 was found after oral Lf administration in rats with colitis [59]. At the cellular level, there seems to be an increased number of natural killer (NK) cells [60, 61], increased phagocytosis-enhancing effect [18, 62], an increased recruitment of neutrophils in blood [63] and modulation of myelopoiesis [56].

Effects on both humoral and cellular immune responses

It has previously been shown in vitro that Lf may induce cell proliferation and maturation by acting as an alternative iron donor for T cells [25, 64], but recent in vivo studies establish that Lf mainly acts by scavenging iron [48] and correcting iron overload [49]. In fact, most mechanisms through which Lf upregulates the immune system involve direct Lf interactions with cells. It is assumed that more or less specific receptors bind Lf and are key effectors for cell signaling, casual endocytosis and/or nuclear targeting [65]. Unfortunately, data on these putative receptors and pathways are disparate and sometimes contradictory.

Lf is likely to regulate lymphocyte maturation and activation. Lf differentiation effects were previously described

on isolated thymocytes and splenic B cells [66, 67], and it was shown that Lf interactions with Jurkat T-cells upregulate the expression of CD4 antigen [41]. Furthermore, in cervical cancer patients, a recent finding indicates that Lf can regulate the expression of the ζ chain of the T-cell receptor [68].

Promotion of lytic cell activity seems to be another important aspect of Lf function. Lf is already expressed on resting PMNs where it could participate in the binding of micro-organisms [69]. It is then massively released from PMNs on stimulus by TNF-α and phorbols, and binds to PMN membranes [70, 71]. It was shown in vitro that both release and cell binding promote the activation and phagocytosis of PMNs and monocytes/macrophages. Lf was reported as a promoter of motility, superoxide production and release of pro-inflammatory molecules such as NO, TNF-α and IL-8 [72–74], and a recent study indeed demonstrates enhanced phagocytosis against *S. aureus* [75]. The molecular mechanisms underlying these activities are, however, highly controversial. Phagocytosis by PMNs is enhanced by the interaction of complement activation products, particularly complement factor C3. Nevertheless, it is unclear whether Lf activity is related to complement activation since Lf was shown either to inhibit [76] or to activate [75, 77] the classical and alternate pathways of complement. A recent report shows that the Lfc domain of either hLf or bLf inhibits the classical complement pathway but not the alternative complement pathway [78]. Direct Lf binding to PMNs and opsonin-like activity could also be involved [79].

The latest data supporting the immunotropic activity of Lf are recent reports showing its adjuvant effect in the generation of delayed-type hypersensitivity [80] and in the boost of Bacille Calmette-Guérin (BCG) vaccine efficacy to generate T helper response in mice [81]. This adjuvant effect could be due to bovine Lf binding on the mannose receptor of immature antigen-presenting skin cells [80]. Such binding has been recently confirmed in a study showing that bovine Lf binding to DC-SIGN on dendritic cells blocks its interaction with HIV gp120 and subsequent virus transmission [82].

Modulation of the expression of cytokines by Lf

Anti-inflammatory properties

The anti-inflammatory properties of Lf have been extensively studied for the last decade. Lf modulates the inflammatory process mainly by preventing the release of cytokines which induce recruitment and activation of immune cells at inflammatory sites. Actually, hLf suppresses TNF- , IL-1 and IL-6 production in mononuclear cells in vitro and in vivo, in response to LPS activation [43, 58, 83–85]. Bovine Lf regulates cytokine production by splenocytes of obstructive jaundiced rats [86]. In addition, Lf enhances the secretion of the anti-inflammatory cytokines IL-10 and IL-4, and reduces colitis in rats [59]. The downregulation of pro-inflammatory cytokines can be partly related to the LPS-binding properties of Lf, through its Lfc domain [32-34]. Interestingly, it has been shown that Lfc itself neutralizes LPS activity [85, 87]. Lf competes with serum LPS-binding protein (LBP) for LPS binding and therefore prevents the transfer of endotoxin to mCD14 presented at the surface of macrophages [35]. Lf also suppresses the production of hydrogen peroxide mediated by the binding of LPS to L-selectin of neutrophils [88]. Furthermore, the interaction between Lf and soluble CD14 (sCD14) [37] inhibits the secretion of IL-8, a chemokine induced by the complex sCD14-LPS, by endothelial cells [89].

Apart from LPS and CD14 binding, other mechanisms of inhibition of pro-inflammatory cytokines production have been described. The downregulation of IL-6 secretion induced by TNF-α [85] could result from the inhibition of NF-κB binding to the TNF-α promoter [43] following internalization of Lf in monocytic cells. Furthermore, the inhibition of immunostimulatory effects on human B cells can be correlated to the property of Lf to interact with the bacterial unmethylated CpG-containing oligonucleotides [36]. In collagen-induced and septic arthritis mouse models, peri-articular injection of hLf reduced inflammation [90]. In agreement with this study, oral administration of bLf inhibited TNF-α and increased IL-10 secretion in adjuvant-stimulated arthritis rats [91]. Recombinant hLf and milk bLf also had a preventive effect on LPS-induced preterm delivery in mice through inhibition of IL-6 production [92]. Equally, virus-induced inflammatory responses can be controlled by Lf. Recently, Sano et al. [93] have reported that Lf decreased both the infectivity of respiratory syncytial virus (RSV) and the RSV-induced IL-8 secretion by Hep2 cells through a direct interaction of Lf with a surface protein of RSV.

Pro-inflammatory properties

Several studies have reported that Lf, alone, activates macrophages and induces IL-8, TNF-α and nitric oxide (NO) [74]. The Lf-LPS complex could be under some conditions an inducer of inflammatory mediators in macrophages, through Toll-like receptor 4 [94]. Moreover, after pretreatment with the Lf-LPS complex, cells are rendered tolerant to LPS challenge [94]. Lf restored the humoral immune response and increased production of IL-6 by peritoneal and alveolar cells in cyclophosphamide (CP)-immunocompromised mice [95, 96]. In the same experimental model, it was demonstrated that Lf strongly elevated the pool of CD3+ T cells and CD4+ T cell content. Lf, given orally to CP-immunosuppressed mice, could reconstitute a T cell-mediated immune response by renewal of the T cell pool [97]. A recent study

has demonstrated that oral Lf administration prevents body weight loss and increases cytokine responses during herpes simplex virus type 1 infection of mice [98]. Bovine Lf or its pepsin hydrolysate induces IL-18 in mouse small intestine epithelial cells, which influences expression of a number of genes including IFN-γ and other pro-inflammatory cytokines [99]. According to these authors, this effect of bLf may be a major mechanism by which bLf inhibits carcinogenesis and metastasis. Bovine Lf may also act as an inhibitor of angiogenesis, probably by inducing IL-18 production in serum and blocking endothelial functions [100]. A long-term bLf administration to chronic hepatitis C patients can produce a Th1-cytokine dominant environment in peripheral blood that favors the eradication of chronic hepatitis C virus by IFN therapy [101]. Upregulation of IFN-γ and TNF-α production by cervical lymph node cells stimulated by heat-killed *Candida* a*lbicans* was observed in Lf-treated mice compared with non-treated mice [53].

Role in the recruitment of leukocytes at inflammation sites

The interaction of Lf with LPS and sCD14 interferes not only with the activation of immune cells but also with the expression of adhesion molecules on endothelial cells, necessary for the local recruitment of immune cells at inflammatory sites. In particular Lf inhibits the (sCD14-LPS)-induced expression of E-selectin, intercellular adhesion molecule 1 (ICAM-1) and IL-8 by human umbilical endothelial cells [88, 89]. These studies also pointed to the ability of Lf to compete with chemokines such as IL-8 for their binding to proteoglycans and their further presentation to leukocytes. Interestingly, a recent in vivo study has shown that orally administred recombinant hLf is able to prevent injury by non-steroidal anti-inflammatory drugs in the intestine of rats and mice and that this effect could be linked to attenuation of neutrophil migration to the intestine [102].

Another recent study, however, has reported a role of Lf in the increased recruitment of neutrophils in blood, thus protecting mice from bacteremia [103]. Takakura et al. [53] also showed that alleviation of oral candidiasis by Lf feeding to mice is correlated with the enhancement of the number of leukocytes and their cytokine responses in regional lymph nodes against candida infection.

Role in iron homeostasis and detoxication

Lf is described as a potent molecule in the treatment of common inflammatory diseases. A major anti-inflammatory activity of Lf is related to the scavenging of free iron, which accumulates in inflamed tissues and catalyses the production of tissue-toxic hydroxyl radicals. Apo-Lf is released from PMNs at inflammatory sites and, owing to its iron-binding stability at low pH, participates in iron homeostasis and detoxification. Interestingly, in neurodegenerative diseases, where iron deposits contribute to oxidative stress and neuronal death, overexpression of Lf was reported in some specific areas of the brain [104]. This event, together with transcytosis of plasma Lf through the blood-brain barrier during inflammation [105], could help to limit oxidative stress in the brain.

Lf and allergies

In vivo studies showed Lf protection against skin and lung allergies [106, 107]. Lf is overexpressed in patients with allergies [106], a process which involves the activation of mast cells and basophils, and IL-1β and TNF-α-triggered migration of antigen-presenting cells [108]. In skin allergies, a mechanism by which Lf binds to keratinocytes and inhibits the release of TNF-α from these cells has been proposed [109]. Another explanation has been found in the ability of Lf to destabilize tryptase, a potent pro-inflammatory protease released from mast cells [110]. Lf apparently displaces tryptase from heparin, which is known to maintain enzymatic activity. It was recently shown that inhibition occurs following Lf uptake by mast cells and interaction not only with tryptase but also with chymase and cathepsin G [111]. Recently, these authors also showed an inhibition of anti-immunoglobulin (Ig) E induced histamine and tryptase release from human colon mast cells by Lf [112, 113].

Lf, a marker of inflammatory diseases

Since Lf levels in blood and biological fluids may greatly increase in septicaemia [5, 6], it is tempting to consider Lf as a marker of inflammatory diseases. Increased levels of Lf were measured in synovial fluid but not in serum from patients with rheumatoid arthritis. Lf was thus proposed as a reliable marker of neutrophil activation at sites of inflammation in rheumatoid synovitis, but does not represent a marker of disease activity [114]. The response of severe acute respiratory syndrome (SARS)-affected patients seems to be mainly an innate inflammatory response, rather than a specific immune response. The gene expression of Lf in peripheral blood mononuclear cells is the most strongly increased during SARS (148-fold increase) [115]. This result opens new possibilities for designing new diagnostics and treatments for this disease.

Studies using fecal Lf as a non-invasive diagnostic tool to evaluate the severity of intestinal inflammation in patients presenting abdominal pain and diarrhea [116, 117] are more documented and interesting. Fecal Lf levels quickly increase with the influx of leuko-

cytes into the intestinal lumen during inflammation. This biomarker has been shown to be a sensitive and specific marker of disease activity in chronic inflammatory bowel disease [118]. Increase of fecal Lf is also observed in chronic inflammatory bowel disease proctitis. Patient compliance and stability of the marker make Lf assay a promising method for clinical research [119]. Finally, Buderus et al. [120] identified fecal Lf as a marker of intestinal inflammation and therapeutic response in patients with Crohn's disease. All these studies indicate a possible use of Lf as a clinical marker of inflammatory diseases.

Applications in the prevention of inflammatory diseases and infections

Lf shows effects on non-specific immune responses in fish [121]. The humoral immune response was not influenced by Lf feeding. Lf seems to affect innate immune cellular activity, mainly respiratory burst and natural cytotoxic activity. Lf was proposed as a possible immunostimulant for farmed gilthead seabream [121]. Liposomal Lf was shown to increase IFN-α production and NK activity in healthy volunters [122]. Orally administered Lf lowers the concentration level of endotoxin in the gut of mice. Neither bifidobacteria nor Lf stimulated an increase in B or T cells, or in cytokine production (IL-6, TNF-α, IFN-γ), in Peyer's patches [123].

Interestingly, persorption of bLf from the intestinal lumen into the systemic circulation via the portal vein and the mesenteric lymphatics was demonstrated in growing pigs [124]. The mode of oral bLf administration, however, influences mucosal and systemic immune responses in mice [125]. The addition of Lf to the drinking water had no visible effect on the immune status. Gastric intubation, single buccal doses and continuous doses of Lf in the diet stimulated transient systemic and intestinal antibody responses against Lf. All of these oral modes of Lf exposure biased mucosal and systemic T cell responses toward Th2 types and elevated IgA production by mucosal cells. However, the less natural gastric intubation also promoted Th1-type responses, as evidenced by serum IgG(2a) antibodies and the secretion of Th1 cytokine by mucosal and systemic T cells in vitro. Thus, one should carefully consider the oral mode of administration for understanding regulation of immune responses by food proteins such as Lf.

Conclusion

It has to be recognized that in the last decade important breakthroughs have been made in the field of 'lactobiology', in particular the evidence for neutralizing effects of exogeneous pro-inflammatory molecules. Lf is a glycoprotein able to bind and sequester iron and LPS and thus to interfere in the signaling pathways and biological response induced by them. Additionally, Lf is able to interact with cell surface receptors: proteoglycans, apolipoprotein E/low-density lipoprotein (ApoE/LDL) receptor, nucleolin, lymphocyte and enterocyte receptors. These interactions result in cell capture and the induction of biological responses which, however, until now have been controversial.

The biological properties of Lf depend on the regulation of its synthesis, secretion and presence at a well-defined time period and place. In normal physiological conditions, Lf is only secreted by glandular cells and covers

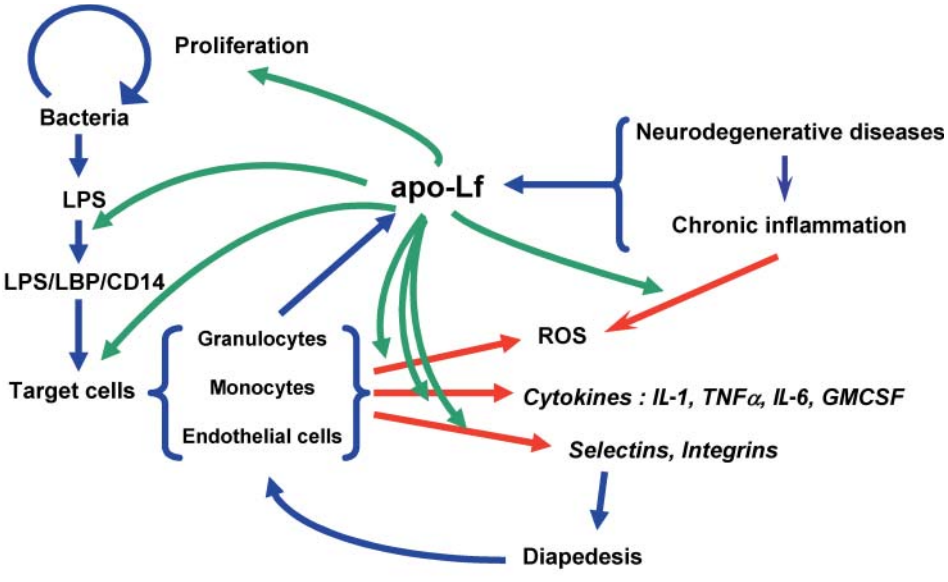

Figure 2. Regulation of the inflammatory response by Lf. Blue arrows indicate physiological processes. Red arrows indicate biological responses induced by infection aggression or neurodegenerative diseases and green arrows indicate downregulation induced by the release of lactoferrin upon inflammatory process.

the surface of mucosa, where it works as a powerful bacteriostatic and bactericidal molecule. During inflammation and neurodegenerative diseases that lead to PMN degranulation at the site of infection and to activation of microglial cells and dopaminergic neurons [104], respectively, the Lf secretion dramatically increases. Consequently, Lf binds to cell surface proteoglycans and receptors which will induce its endocytosis by various cell types. This increased Lf secretion can be monitored by the variation of its plasmatic concentration (fig. 2).

Lf will mainly act as a sequestrator of iron, LPS and CD14, and an activator/modulator of signaling pathways leading to negative feedback of the inflammatory response, as shown by a decrease in production of reactive oxygen species and various pro-inflammatory cytokines. In addition, orally administrated Lf induces interesting and beneficial physiological responses that justify its use in the so-called foods for special health uses (FOSHU). In these applications, special care has to be given to the origin of Lf, whether milk or recombinant Lf from human or bovine species. Indeed, in addition to the biological properties played by the protein itself, one cannot exclude some 'non-specific activity' due to the glycan moiety. For example, the glycan moieties of bLf are of the high-mannose type and thus could allow them to be recognized by the DC-SIGN (dendritic cell-specific intercellular adhesion molecule3-grabbing nonintegrin receptor) and mannose receptor of monocytic cells and to interfere in the immune response.

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