BioMetals **17:** 225–229, 2004.

Lactoferrin and host defence: an overview of its immuno-modulating and anti-inflammatory properties

Dominique Legrand*, Elisabeth Elass, Annick Pierce & Joël Mazurier
Unité de Glycobiologie Structurale et Fonctionnelle et Unité Mixte de Recherche n°8576 du Centre National de la Recherche Scientifique, Institut Fédératif de Recherche n°118, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq cedex, France.
**Author for correspondence (Tel: 33 3 20 33 72 38; Fax.: 33 3 20 43 65 55; E-mail: dominique.legrand@univ-lille1.fr)*

Key words: immunity, inflammation, lactoferrin

Abstract

Lactoferrin is a member of the transferrin family of iron-binding glycoproteins that is abundantly expressed and secreted from glandular epithelial cells. In secretions, such as milk and fluids of the intestinal tract, lactoferrin is an important component of the first line of host defence. During the inflammatory process, lactoferrin, a prominent component of the secondary granules of neutrophils (PMNs), is released in infected tissues and in blood and then it is rapidly cleared by the liver. In addition to the antimicrobial properties of lactoferrin, a set of studies has focused on its ability to modulate the inflammatory process and the overall immune response. Though many *in vitro* and *in vivo* studies report clear regulation of the immune response and protective effect against infection and septic shock by lactoferrin, elucidation of all the cellular and molecular mechanisms of action is far from being achieved. 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.

Introduction

Recent sequencing of the entire human genome has revealed that the number of genes is considerably inferior to the number of cell functions. Hence, while some proteins have very pronounced specificity and activity, others are multifunctional molecules. Lactoferrin (Lf), a widespread iron-binding glycoprotein, is definitely one of the latter. When it was first discovered in milk (Montreuil *et al.* 1960), Lf was called lactotransferrin, as it was formerly considered to be a functionally-related transferrin variant. Iron binding is, without any doubt, a key property of Lf and accounts for some of its many biological roles in host defence such as bacteriostasis and protection against oxygen radicals catalyzed by free iron. The possibility of Lf having other functions than just simple iron sequestration emerged immediately it was reported that Lf binds to cells and components of the immune system. *In vivo* and *in vitro* studies further confirmed the ability of Lf to modulate the immune response. This paper reviews our current knowledge of the still controversial mechanisms governing the regulatory functions of Lf in the immune system.

Lf is in the front line of the innate immune system

Lf is widely distributed all over the entire body. It is indeed found in large amounts in most secretions, particularly in milk where its concentration in humans may vary from 1 g/l (mature milk) to 7 g/l (colostrum) (Houghton *et al.* 1985), and in the secondary granules of neutrophils (PMNs) (Masson *et al.* 1969). During inflammation and in some pathologies, Lf levels of

biological fluids may greatly increase. This is particularly noticeable in plasma where Lf concentration can be as low as 0.4–2 mg/l under normal conditions but increases to up 200 mg/l in septicaemia (Bennett & Kokocinski 1978, Maaks *et al.* 1989). Furthermore, since maximal Lf release from PMNs occurs in inflamed tissues, plasma Lf only represents the tip of the iceberg. Lastly, cationic Lf can bind in large quantities to glycosaminoglycans (Mann *et al.* 1994), so that cells may provide high local concentrations of still functional Lf on their surfaces. Interestingly, Lf immobilized to airway epithelium, but not soluble Lf, may activate eosinophils (Thomas *et al.* 2002), thus underlining the importance of Lf bound to epithelia.

In vivo evidence for lactoferrin regulation of the immune system

Evidence for lactoferrin regulation of the immune system has been provided by a number of *in vivo* experiments on Lf-supplemented models revealing host-protecting effects, not only against microbial infections (review: van Hooijdonk *et al.* 2000), but also lactoferrin itself, thus preventing septic shock, allergy or cancer. In particular, a protective effect during lethal bacteraemia in mice was reported (Zagulski *et al.* 1989) and orally-administered Lf was shown to protect piglets against septic shock (Lee *et al.* 1998). On a molecular basis, altered expressions of cytokines, mostly pro-inflammatory interleukin-1 β (IL-1β), IL-6 and tumor necrosis factor α (TNF-α), and granulocyte-macrophage colony-stimulating factor were detected (Sawatzki & Rich 1989, Broxmeyer *et al.* 1987, Machnicki *et al.* 1993, Kruzel *et al.* 2002). Additionally, up-regulation of anti-inflammatory IL-4 and IL-10 was found after oral Lf administration in rats with colitis (Togawa *et al.* 2002). On a cellular basis, there seems to be an increased number of NK cells (Shimizu *et al.* 1996, Yamauchi *et al.* 1998), increased phagocytosis-enhancing effect (Szuster-Ciesielska *et al.* 1995, Wakabayashi *et al.* 2003) and modulation of myelopoiesis (Broxmeyer *et al.* 1987). Lastly, a recent study on human Lf-transgenic mice showed enhanced Th1 response to *Staphylococcus aureus (S. aureus)* infection (Guillen *et al.* 2002).

Mechanisms accounting for up-regulation of the immune system by lactoferrin

In a few instances, the iron-binding Lf capacity was shown to promote *in vitro* cell proliferation and maturation, as it could act as an alternative iron donor for T-cells (Mincheva-Nilsson *et al.* 1997, Mazurier *et al.* 1989) and enhance Th1 response by modulating iron supply to the spleen (Guillen *et al.* 2002). In fact, most mechanisms through which Lf up-regulates 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 signalling, casual endocytosis and/or nuclear targeting (review: Suzuki & Lönnerdal 2002). Unfortunately, data on these putative receptors and pathways is disparate and sometimes contradictory.

Firstly, Lf is likely to regulate lymphocyte maturation and activation. In T-lymphocytes, a 105 kDa receptor was described (Mazurier *et al.* 1989) and it was shown that Lf interactions with Jurkat T-cells up-regulate the expression of CD4 antigen through the stimulation of the mitogen-activated protein kinase (Dhennin-Duthille *et al.* 2000). In this connection, Lf differentiation effects were previously described on isolated thymocytes and splenic B-cells (Zimecki *et al.* 1995, 1991). Furthermore, in cervical cancer patients, a recent finding indicates that Lf can regulate the expression of the zeta chain of the T-cell receptor (Frydecka *et al.* 2002).

Promotion of lytic cell activity seems another important aspect of the Lf function. Lf is already expressed on resting PMNs where it could participate in the binding of micro-organisms (Deriy *et al.* 2000). It is then massively released from PMNs upon TNF-α and phorbols stimuli and binds to PMN membrane (Maneva *et al.* 1983, Afeltra *et al.* 1997). 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 (Gahr *et al.* 1991, Shinoda *et al.* 1996, Sorimachi *et al.* 1997) and a recent study indeed demonstrates enhanced phagocytosis against *S. aureus* (Kai *et al.* 2002). The molecular mechanisms explaining 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 (Kijlstra & Jeurissen 1982) or to activate (Kai *et al.* 2002, Rainard 1993) the classical and alternate pathways of complement. Direct Lf binding to PMNs and opsonin-like activity could also be involved (Miyauchi *et al.* 1998). Lastly, an increased number and activity of natural killer (NK) cells by Lf was reported *in vitro* (Shau *et al.* 1992) and *in vivo* in mice infected by cytomegalovirus (Shimizu *et al.* 1996) and in humans (Yamauchi *et al.*, 1998). We demonstrated in the laboratory that the Lf activating effect is due both to the modulation of NK cell cytotoxicity and an increased sensitivity of target cells to lysis (Damiens *et al.* 1998).

The latest data supporting the immunotropic activity of Lf is a recent report showing its adjuvant effect in the generation of delayed-type hypersensitivity in mice due to Lf binding on the mannose receptor of immature antigen-presenting skin cells (Zimecki *et al.* 2002).

Mechanisms governing the anti-inflammatory properties of Lf

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 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, an overexpression of Lf was reported in some specific areas of the brain (Fillebeen *et al.* 2001). This event, together with transcytosis of plasma Lf through the blood-brain barrier during inflammation (Fillebeen *et al.* 1999), could contribute to limit oxidative stress in the brain.

The last decade has shed light on several molecular mechanisms governing the iron-independent anti-inflammatory properties of Lf. As pointed out before, altered expressions of pro-inflammatory cytokines, mainly TNF-α, IL-1β, IL-6 and IL-8, were reported through *in vivo* studies and confirmed *in vitro*. It is freely admitted now that this unpaired cytokine production is mostly mediated by the neutralizing effect of Lf against exogenous pro-inflammatory molecules such as bacterial lipopolysaccharides (LPS) (Miyazawa *et al.* 1991) but also bacterial unmethylated CpG-containing oligonucleotides (Britigan *et al.* 2001). Lf was indeed found to bind to the lipid-A of bacterial lipopolysaccharides (LPS) with high affinity through the lactoferricin domain of Lf (Appelmelk *et al.* 1994, Elass-Rochard *et al.* 1995). *In vivo* and *in vitro* neutralization of LPS by lactoferricin itself was also demonstrated (Zhang *et al.* 1999). This interaction prevents LPS from binding to the main actors of LPS signalling, such as the serum LPS-binding protein (LBP) and soluble CD14 (sCD14), membrane CD14 (mCD14) on monocytes and L-selectin on PMNs (Elass-Rochard *et al.* 1998, Baveye *et al.* 2000b). Other mechanisms of inhibition of LPS-induced cytokine release have also been described. We have indeed reported high-affinity interactions between Lf and sCD14 and the sCD14-LPS complex abolishing their activating functions (Baveye *et al.* 2000a). Furthermore, it was recently demonstrated that Lf may down-regulate LPS-induced cytokines in THP1 through a mechanism involving Lf internalization, nuclear localization and interference with NF-kappaB activation (Haversen *et al.* 2002).

Interestingly, the LPS-neutralizing effect of Lf could have consequences not only for the activation of immune cells but also for endothelial cells. In fact, endothelial cells induced by LPS and the sCD14-LPS complex express adhesion molecules, selectins and integrin ligands, and IL-8 necessary for the local recruitment of immune cell at inflammatory sites. We demontrated that Lf inhibits the LPS-induced expression of E-selectin, ICAM-1 and IL-8 by endothelial cells (Baveye *et al.* 2000a, Elass *et al.* 2002). These studies also pointed out the ability of Lf to compete with chemokines such as IL-8 for their binding to proteoglycans and their further presentation to leukocytes.

In vivo studies showed Lf protection against skin and lung allergies (Elrod *et al.* 1997, Griffiths *et al.* 2001). Lf is overexpressed in patients with allergies (Zweiman *et al.* 1990), a process which involves the activation of mast cells and basophils and IL-1β and TNF-α-triggered migration of antigen-presenting cells. In skin allergies, a mechanism by which Lf binds to keratinocytes and inhibits the release of TNF-α from these cells has been proposed (Kimber *et al.* 2002). Another explanation has been found in the ability of Lf to destabilize tryptase, a potent pro-inflammatory protease released from mast cells (Elrod *et al.* 1997). 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

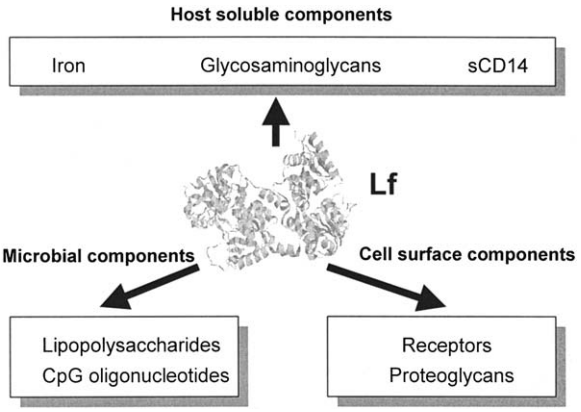

Fig. 1. Known microbial and host components that interact with Lf and account for its regulatory effect on the immune system.

with tryptase but also with chymase and cathepsin G (He *et al.* 2003).

Conclusions

Prophylactic and therapeutic effects of exogenous Lf have aroused keen interest from the scientific community, thus prompting the elucidation of the mechanisms accounting, in addition to iron sequestration, for the immune regulatory properties of Lf. 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. As summarized in Figure 1, most of these clearly depend upon the great ability of Lf to bind endotoxins and glycosaminoglycans. Others involve direct Lf interactions with immune cells and receptors through pathways whose understanding, still patchy at the moment, represents a true and exciting challenge for the future.

References

Afeltra A, Caccavo D, Ferri GM *et al.* 1997 Expression of lactoferrin on human granulocytes: analysis with polyclonal and monoclonal antibodies. *Clin Exp Immunol* **109**, 279–285.

Appelmelk BJ, An YQ, Geerts M *et al.* 1994 Lactoferrin is a lipid A-binding protein. *Infect Immun* **62**, 2628–2632.

Baveye S, Elass E, Fernig DG, Blanquart C, Mazurier J, Legrand D. 2000a Human lactoferrin interacts with soluble CD14 and inhibits expression of endothelial adhesion molecules, E-selectin and ICAM-1, induced by the CD14-lipopolysaccharide complex. *Infect Immun* **68**, 6519–6525

Baveye S, Elass E, Mazurier J, Legrand D. 2000b Lactoferrin inhibits the binding of lipopolysaccharides to L-selectin and subsequent production of reactive oxygen species by neutrophils. *FEBS Lett* **469**, 5–8.

Bennett RM, Kokocinski T. 1978 Lactoferrin content of peripheral blood cells. *Br J Haematol* **39**, 509–521.

Britigan BE, Lewis TS, Waldschmidt M, McCormick ML, Krieg AM. 2001 Lactoferrin binds CpG-containing oligonucleotides and inhibits their immunostimulatory effects on human B cells. *J Immunol* **167**, 2921–2928.

Broxmeyer HE, Williams DE, Hangoc G *et al.* 1987 The opposing actions in vivo on murine myelopoiesis of purified preparations of lactoferrin and the colony stimulating factors. *Blood Cells* **13**, 31–48.

Damiens E, Mazurier J, el Yazidi I *et al.* 1998 Effects of human lactoferrin on NK cell cytotoxicity against haematopoietic and epithelial tumour cells. *Biochim Biophys Acta* **1402**, 277–287.

Deriy LV, Chor J, Thomas LL. 2000 Surface expression of lactoferrin by resting neutrophils. *Biochem Biophys Res Commun* **275**, 241–246.

Dhennin-Duthille I, Masson M, Damiens E, Fillebeen C, Spik G, Mazurier J. 2000 Lactoferrin upregulates the expression of CD4 antigen through the stimulation of the mitogen-activated protein kinase in the human lymphoblastic T Jurkat cell line. *J Cell Biochem* **79**, 583–593.

Elass E, Masson M, Mazurier J, Legrand D. 2002 Lactoferrin inhibits the lipopolysaccharide-induced expression and proteoglycan-binding ability of interleukin-8 in human endothelial cells. *Infect Immun* **70**, 1860–1866.

Elass-Rochard E, Legrand D, Salmon V *et al.* 1998 Lactoferrin inhibits the endotoxin interaction with CD14 by competition with the lipopolysaccharide-binding protein. *Infect Immun* **66**, 486–491.

Elass-Rochard E, Roseanu A, Legrand D *et al.* 1995 Lactoferrin-lipopolysaccharide interaction: involvement of the 28–34 loop region of human lactoferrin in the high-affinity binding to *E. coli* 055B5 lipopolysaccharide. *Biochem J* **312**, 839–845.

Elrod KC, Moore WR, Abraham WM, Tanaka RD. 1997 Lactoferrin, a potent tryptase inhibitor, abolishes late-phase airway responses in allergic sheep. *Am J Respir Crit Care Med* **156**, 375–381.

Fillebeen C, Dehouck B, Benaïssa M, Dhennin-Duthille I, Cecchelli R, Pierce A. 1999 Tumor necrosis factor-alpha increases lactoferrin transcytosis through the blood-brain barrier. *J Neurochem* **73**, 2491–2500.

Fillebeen C, Ruchoux MM, Mitchell V, Vincent S, Benaïssa M, Pierce A. 2001 Lactoferrin is synthesized by activated microglia in the human substantia nigra and its synthesis by the human microglial CHME cell line is upregulated by tumor necrosis factor alpha or 1-methyl-4-phenylpyridinium treatment. *Brain Res Mol Brain Res* **96**, 103–113.

Frydecka I, Zimecki M, Bocko D *et al.* 2002 Lactoferrin-induced up-regulation of zeta (zeta) chain expression in peripheral blood T lymphocytes from cervical cancer patients. *Anticancer Res* **22**, 1897–1901.

Gahr M, Speer CP, Damerau B, Sawatzki G. 1991 Influence of lactoferrin on the function of human polymorphonuclear leukocytes and monocytes. *J Leukoc Biol* **49**, 427–433.

Griffiths CE, Cumberbatch M, Tucker SC *et al.* 2001 Exogenous topical lactoferrin inhibits allergen-induced Langerhans cell migration and cutaneous inflammation in humans. *Br J Dermatol* **144**, 715–725.

Guillen C, McInnes IB, Vaughan DM *et al.* 2002 Enhanced Th1 response to *Staphylococcus aureus* infection in human lactoferrin-transgenic mice. *J Immunol* **168**, 3950–3957.

Haversen L, Ohlsson BG, Hahn-Zoric M, Hanson LA, Mattsby-Baltzer I. 2002 Lactoferrin down-regulates the LPS-induced

cytokine production in monocytic cells via NF-kappaB. *Cell Immunol* **220**, 83–95.

He S, McEuen AR, Blewett SA *et al.* 2003 The inhibition of mast cell activation by neutrophil lactoferrin: uptake by mast cells and interaction with tryptase, chymase and cathepsin G. *Biochem Pharmacol* **65**, 1007–1015.

van Hooijdonk AC, Kussendrager KD, Steijns JM. 2000 *In vivo* antimicrobial and antiviral activity of components in bovine milk and colostrum involved in non-specific defence. *Br J Nutr* **84**, S127–134.

Houghton MR, Gracey M, Burke V, Bottrell C, Spargo RM. 1985 Breast milk lactoferrin levels in relation to maternal nutritional status. *J Pediatr Gastroenterol Nutr* **4**, 230–233.

Kai K, Komine K, Komine Y *et al.* 2002 Lactoferrin stimulates A *Staphylococcus aureus* killing activity of bovine phagocytes in the mammary gland. *Microbiol Immunol* **46**, 187–194.

Kijlstra A, Jeurissen SH. 1982 Modulation of classical C3 convertase of complement by tear lactoferrin. *Immunology* **47**, 263–270.

Kimber I, Cumberbatch M, Dearman RJ, Headon DR, Bhushan M, Griffiths CE. 2002 Lactoferrin: influences on Langerhans cells, epidermal cytokines, and cutaneous inflammation. *Biochem Cell Biol* **80**, 103–107.

Kruzel ML, Harari Y, Mailman D, Actor JK, Zimecki M. 2002 Differential effects of prophylactic, concurrent and therapeutic lactoferrin treatment on LPS-induced inflammatory responses in mice. *Clin Exp Immunol* **130**, 25–31.

Lee WJ, Farmer JL, Hilty M, Kim YB. 1998 The protective effects of lactoferrin feeding against endotoxin lethal shock in germfree piglets. *Infect Immun* **66**, 1421–1426.

Maaks S, Yan HZ, Wood WG. 1989 Development and evaluation of luminescence based sandwich assay for plasma lactoferrin as a marker for sepsis and bacterial infections in pediatric medicine. *J Biolumines Chemilumines* **3**, 221–226.

Machnicki M, Zimecki M, Zagulski T. 1993 Lactoferrin regulates the release of tumour necrosis factor alpha and interleukin 6 *in vivo*. *Int J Exp Pathol* **74**, 433–439.

Maneva AI, Sirakov LM, Manev VV. 1983 Lactoferrin binding to neutrophilic polymorphonuclear leucocytes. *Int J Biochem* **15**, 981–984.

Mann DM, Romm E, Migliorini M. 1994 Delineation of the glycosaminoglycan-binding site in the human inflammatory response protein lactoferrin. *J Biol Chem* **269**, 23661–23667.

Masson PL, Heremans JF, Schonne E. 1969 Lactoferrin, an iron-binding protein in neutrophilic leukocytes. *J Exp Med* **130**, 643–658.

Mazurier J, Legrand D, Hu WL, Montreuil J, Spik G. 1989 Expression of human lactotransferrin receptors in phytohemagglutinin-stimulated human peripheral blood lymphocytes. *Eur J Biochem* **179**, 481–487.

Mincheva-Nilsson L, Hammarstrom S, Hammarstrom ML. 1997 Activated human gamma delta T lymphocytes express functional lactoferrin receptors. *Scand J Immunol* **46**, 609–618.

Miyauchi H, Hashimoto S, Nakajima M, Shinoda I, Fukuwatari Y, Hayasawa H. 1998 Bovine lactoferrin stimulates the phagocytic activity of human neutrophils: identification of its active domain. *Cell Immunol* **187**, 34–37.

Miyazawa K, Mantel C, Lu L, Morrison DC, Broxmeyer HE. 1991 Lactoferrin-lipopolysaccharide interactions. Effect on lactoferrin binding to monocyte/macrophage-differentiated HL-60 cells. *J Immunol* **146**, 723–729.

Montreuil J, Tonnelat J, Mullet S. 1960 Préparation et propriétés de la lactosidérophiline (lactotransferrine) du lait de femme. *Biochim Biophys Acta* **45**, 413–421.

Rainard P. 1993 Activation of the classical pathway of complement by binding of bovine lactoferrin to unencapsulated *Streptococcus agalactiae*. *Immunology* **79**, 648–652.

Sawatzki G, Rich IN. 1989 Lactoferrin stimulates colony stimulating factor production *in vitro* and *in vivo*. *Blood Cells* **15**, 371–385.

Shau H, Kim A, Golub SH. 1992 Modulation of natural killer and lymphokine-activated killer cell cytotoxicity by lactoferrin. *J Leukoc Biol* **51**, 343–349.

Shimizu K, Matsuzawa H, Okada K *et al.* 1996 Lactoferrin-mediated protection of the host from murine cytomegalovirus infection by a T-cell-dependent augmentation of natural killer cell activity. *Arch Virol* **141**, 1875–1889.

Shinoda I, Takase M, Fukuwatari Y, Shimamura S, Koller M, Konig W. 1996 Effects of lactoferrin and lactoferricin on the release of interleukin 8 from human polymorphonuclear leukocytes. *Biosci Biotechnol Biochem* **60**, 521–523.

Sorimachi K, Akimoto K, Hattori Y, Ieiri T, Niwa A. 1997 Activation of macrophages by lactoferrin: secretion of TNF-α, IL-8 and NO. *Biochem Mol Biol Int* **43**, 79–87.

Suzuki YA, Lönnerdal B. 2002 Characterization of mammalian receptors for lactoferrin. *Biochem Cell Biol* **80**, 75–80.

Szuster-Ciesielska A, Kaminska T, Kandefer-Szerszen M. 1995 Phagocytosis-enhancing effect of lactoferrin on bovine peripheral blood monocytes *in vitro* and *in vivo*. *Arch Vet Pol* **35**, 63–71.

Thomas LL, Xu W, Ardon TT. 2002 Immobilized lactoferrin is a stimulus for eosinophil activation. *J Immunol* **169**, 993–999.

Togawa J, Nagase H, Tanaka K *et al.* 2002 Oral administration of lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. *J Gastroenterol Hepatol* **17**, 1291–1298.

Wakabayashi H, Takakura N, Teraguchi S, Tamura Y. 2003 Lactoferrin feeding augments peritoneal macrophage activities in mice intraperitoneally injected with inactivated *Candida albicans*. *Microbiol Immunol* **47**, 37–43.

Yamauchi K, Wakabayashi H, Hashimoto S, Teraguchi S, Hayasawa H, Tomita M. 1998 Effects of orally administered bovine lactoferrin on the immune system of healthy volunteers. *Adv Exp Med Biol* **443**, 261–265.

Zagulski T, Lipinski P, Zagulska A, Broniek S, Jarzabek Z. 1989 Lactoferrin can protect mice against a lethal dose of *E. coli* in experimental infection in vivo. *Br J Exp Pathol* **70**, 697–704.

Zhang GH, Mann DM, Tsai CM. 1999 Neutralization of endotoxin *in vitro* and *in vivo* by a human lactoferrin-derived peptide. *Infect Immun* **67**, 1353–1358.

Zimecki M, Kocieba M, Kruzel M. 2002 Immunoregulatory activities of lactoferrin in the delayed type hypersensitivity in mice are mediated by a receptor with affinity to mannose. *Immunobiology* **205**, 120–131.

Zimecki M, Mazurier J, Machnicki M, Wieczorek Z, Montreuil J, Spik G. 1991 Immunostimulatory activity of lactotransferrin and maturation of CD4- CD8-murine thymocytes. *Immunol Lett* **30**, 119–123.

Zimecki M, Mazurier J, Spik G, Kapp JA. 1995 Human lactoferrin induces phenotypic and functional changes in murine splenic B cells. *Immunology* **86**, 122–127.

Zweiman B, Kucich U, Shalit M *et al.* 1990 Release of lactoferrin and elastase in human allergic skin reactions. *J Immunol* **144**, 3953–3960.