INTRODUCTION / INTRODUCTION

# Lactoferrin, a bird's eye view

**Hans J. Vogel**



**Abstract:** Lactoferrin is an abundant iron-binding protein in milk. This 80 kDa bilobal glycoprotein is also present in several other secreted bodily fluids, as well as in the secondary granules of neutrophils. The potent iron-binding properties of lactoferrin can locally create iron deficiency, and this is an important factor in host defense as it prevents bacteria from growing and forming biofilms. In addition to having antibacterial activity, lactoferrin is now known to have a long list of other beneficial biological properties. It has direct antiviral, antifungal, and even some anticancer activities. It can also promote wound healing and bone growth, or it can act as an iron carrier. Moreover, lactoferrin displays a cytokine-like "alarmin" activity, and it activates the immune system. Simultaneously, it can bind endotoxin (lipopolysaccharide), and in doing so, it modulates the activity of the host immune response. The majority of these intriguing biological activities reside in the unique positively charged N-terminal region of the protein. Interestingly, several peptides, which retain many of the beneficial activities, can be released from this region of lactoferrin. An isoform of the human protein, known as delta-lactoferrin, is expressed inside many cells, where it acts as a transcription factor. Lactoferrin purified from human and bovine milk have very similar but not completely identical properties. Lactoferrin receptors have been identified on the surface of various cells, and some of these can bind both the human and the bovine protein. Because of the extensive health-promoting effects of lactoferrin, there has been considerable interest in the use of bovine or human lactoferrin as a "protein nutraceutical" or as a therapeutic protein. When lactoferrin is used as a "biologic drug", it seems to be orally active in contrast to most other therapeutic proteins.

*Key words:* antimicrobial, anticancer, endotoxin, iron-binding protein, immuno-modulator, transcription factor.

**Résumé :** La lactoferrine est une protéine de liaison du fer abondante dans le lait. Cette glycoprotéine bilobée de 80 kDa se retrouve aussi dans plusieurs autres liquides biologiques sécrétés ainsi que dans les granules secondaires des neutrophiles. Les fortes propriétés de liaison du fer de la lactoferrine peuvent créer une déficience locale en fer, constituant ainsi un facteur de défense de l'hôte important en empêchant les bactéries de croître et de former des biofilms. En plus de son activité antibactérienne, la lactoferrine est connue maintenant pour posséder de nombreuses autres propriétés bénéfiques. Elle exerce des activités antivirales, antifongiques et même quelques activités anticancéreuses directes. Elle peut aussi promouvoir la cicatrisation et la croissance osseuse, ou elle peut agir comme transporteur de fer. En outre, la lactoferrine montre une activité « alarmine » semblable à celle des cytokines et elle active le système immunitaire. Parallèlement, elle peut lier une endotoxine (lipopolysaccharide) et ce faisant, elle module l'activité de la réponse immune de l'hôte. La majorité de ces activités biologiques fascinantes reposent sur la région N-terminale de la protéine chargée positivement. Fait intéressant, plusieurs peptides, qui conservent plusieurs des activités bénéfiques, peuvent être libérés de cette région de la lactoferrine. Une isoforme de la protéine humaine, connue sous le nom de delta-lactoferrine, est exprimée à l'intérieur de plusieurs cellules où elle agit comme facteur de transcription. La lactoferrine purifiée du lait humain et celle du bovin ont des propriétés très similaires mais pas tout à fait identiques. Les récepteurs de lactoferrine ont été identifiés à la surface de différentes cellules et certains d'entre eux peuvent lier tant la lactoferrine humaine que la lactoferrine bovine. Compte tenu des effets bénéfiques considérables de la lactoferrine pour la santé humaine, l'utilisation de la lactoferrine bovine ou humaine comme nutraceutique ou comme protéine thérapeutique a suscité un grand intérêt. Lorsque la lactoferrine est utilisée comme « médicament biologique », elle semble active oralement contrairement aux autres protéines thérapeutiques.

*Mots-clés :* antimicrobien, anticancéreux, endotoxine, protéine de liaison du fer, immunomodulateur, facteur de transcription.

[Traduit par la Rédaction]

Received 12 April 2012. Revision received 18 April 2012. Accepted 12 April 2012. Published at www.nrcresearchpress.com/bcb on 27 April 2012.

**H.J. Vogel.** Biochemistry Research Group, Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada.

**E-mail for correspondence:** vogel@ucalgary.ca.

## Introduction

Lactoferrin (also known as lactotransferrin) was first identified in 1939 as a red protein that was present in bovine milk (Sorensen and Sorensen 1939). It was subsequently found that the protein could bind two ferric irons ($Fe^{3+}$), and that the binding of this metal ion gave rise to the reddish color (Groves 1960; Johansson 1960; Montreuil et al. 1960). As such, lactoferrin was quickly recognized as a member of the transferrin family, a group of iron-binding proteins that are found in many eukaryotic organisms (Lambert et al. 2005). It is now well-known that all living organisms, with the exception of a few microbes, all need iron for growth (Chu et al. 2010). Several important intracellular enzymes rely on the unique oxidation–reduction properties of this metal ion to carry out their function; as such, a dedicated protein-based iron-handling system is in place that takes care of iron uptake, transport, and storage in our bodies (Crichton 2009). In humans and other mammals, the main function of the serum transferrin protein is to transport iron through our bloodstream from sites of storage, such as the liver, to the bone marrow, where the red blood cells are produced. It is important that $Fe^{3+}$ and $Fe^{2+}$ are both carefully handled inside our bodies, as these ions can become spontaneously involved in so-called Fenton chemistry, which would lead to the generation of highly reactive radicals that in turn could cause serious damage. Consequently, iron in biological systems is almost always bound to proteins (or other compounds), where it is held in a stable nonreactive form. The largest pool of iron in our bodies is in the erythrocytes, which contain large amounts of the iron-binding oxygen-carrying hemoglobin protein. Once the red blood cells are recycled at the end of their 120-day lifespan, the macrophages consume the hemoglobin protein and break it down into its amino acid constituents, while at the same time they carefully recycle and store the iron, so that it can be reutilized (Ganz and Nemeth 2012).

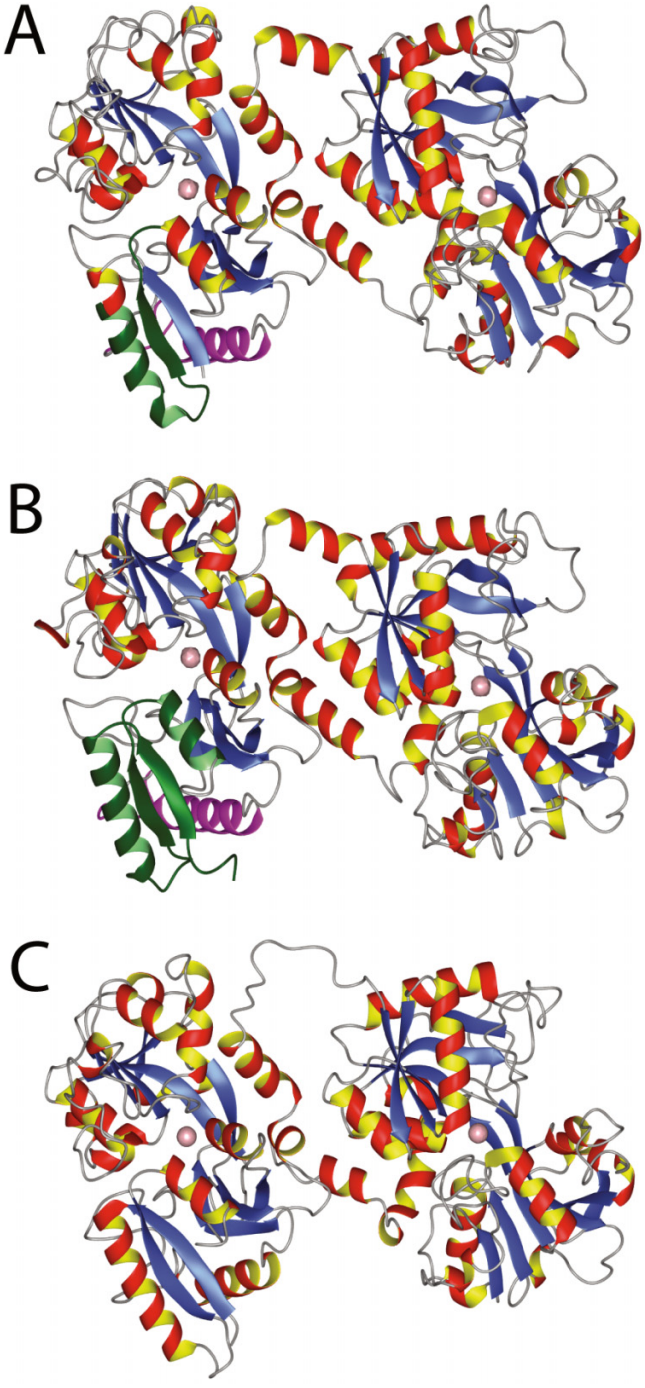
The question arises that if transferrin is used for iron transport through the bloodstream, what is the function of lactoferrin? The two proteins share similar iron-binding properties, yet lactoferrin does not appear to be important for iron transport in our bodies. Original ideas focused on the notion that lactoferrin, being present in large quantities in human milk, played a role in providing iron to nursing newborns. Considerable research has been done in this area, and some evidence for such a role has emerged. This notion gained further credibility when lactoferrin receptor proteins were discovered by Lonnerdal and colleagues in the colon that are capable of binding human as well as bovine lactoferrin (Davidson and Lonnerdal 1988; Lonnerdal et al. 2011). However, the structure of these lactoferrin receptors, also known as "intelectins", was very different from the transferrin receptors that are known to internalize serum transferrin into cells (Cheng et al. 2004). Studies with knockout mice have shown that lactoferrin is not absolutely required as an iron source for infants (Ward et al. 2003). Consequently, the extent to which this process occurs is still a matter of debate, as alternative pathways for iron acquisition seem to exist.

Further insight into the biological role of lactoferrin arose when researchers found that lactoferrin was abundant in neutrophils (Masson et al. 1969), where the protein is stored inside the secondary granules together with various other host defense proteins (Levy 2004). Neutrophils, which are the most abundant white blood cells, are an important part of our host defense system. They attack and kill invading bacteria by phagocytosis, a process in which they engulf invading pathogenic bacteria, or by releasing a series of antimicrobial proteins at the site of infection (Borregaard et al. 2007). Thus, the presence of lactoferrin in the granules of neutrophils strongly indicates that it plays an important role as a host defense protein. Indeed, it is now known that lactoferrin is present in various other secreted biofluids, such as tears, saliva, as well as nasal and genital secretions, where it could help protect us from invading bacteria. Recently, the protein was also discovered in sweat (Park et al. 2011). Evidence has been presented that the biosynthesis of lactoferrin can increase during certain bacterial infections, and these discoveries provided further evidence that support the idea of an important role in host defense. Consequently, it is now widely accepted that lactoferrin is an important part of our innate immune system (Legrand and Mazurier 2010; Valenti and Antonini 2005; Ward et al. 2005). The related ovotransferrin protein, found in abundance in egg whites, seems to play a major role in host defense as well (Giansanti et al. 2012). This now begs the question of what is the role of lactoferrin in host defense, and how does the protein do this? Before discussing this, we should first take a look at the protein structure.

## Protein structure: iron-binding sites and the basic N-terminal region

The full length, 692-residue, human lactoferrin protein is normally secreted from the cell after the 19-residue leader sequence, which can be identified in the gene sequence for the protein, is cleaved off. During its passage through the endoplasmic reticulum and the golgi, the protein becomes glycosylated at a few positions; the glycoforms that become attached are typical N-linked branched oligosaccharides, similar to those seen in other secreted proteins. To understand the multiple actions of this protein, it is useful to take a look at its three-dimensional structure. The crystal structure of human lactoferrin was first reported for the iron-bound form in 1987 by Baker and colleagues (Anderson et al. 1987). The protein was shown to have a very similar overall fold as the rabbit serum transferrin protein, whose 3D structure was solved around the same time (Bailey et al. 1988). Later, a very similar structure for chicken ovotransferrin was also reported (Kurokawa et al. 1995), illustrating strong structural relationships between the proteins that comprise the transferrin family. Today, crystal structures are available for lactoferrin proteins from many different species, and proteins can be either in the "closed" iron-bound or in the "open" apo-state (Baker and Baker 2012; Lambert et al. 2005). A prominent feature of the lactoferrin and transferrin proteins is that they have a bilobal structure, where the two lobes of the protein have the same overall fold (see Fig. 1). The full-length ~80 kDa protein therefore seems to have arisen through gene duplication. In each lobe, the ferric ion is bound at the bottom of a deep cleft, where the metal ion is surrounded by the side chains of two Tyr residues, one Asp and one His residue. Furthermore, $Fe^{3+}$ normally prefers a six-coordinate

**Fig. 1.** Crystal structures of (A) bovine lactoferrin (PDB code = 1BLF), (B) human lactoferrin (1B0L), and (C) rabbit serum transferrin (1JNF). Clearly, lactoferrins and transferrins have very similar overall folds, which allow them to bind two $Fe^{3+}$ atoms (pink spheres, light grey in the print version), one in each lobe of the protein. The lactoferricin regions in bovine and human lactoferrin that are cleaved by the action of pepsin in the gut are highlighted in green (dark grey in print). Intact bovine lactoferricin corresponds to residues 17–41 of bovine lactoferrin, while full-length human lactoferricin encompasses residues 1–49 of the human protein. The lactoferrampin regions are highlighted in purple (medium grey in print, residues 265–284 in bovine lactoferrin and 266–286 in human lactoferrin).



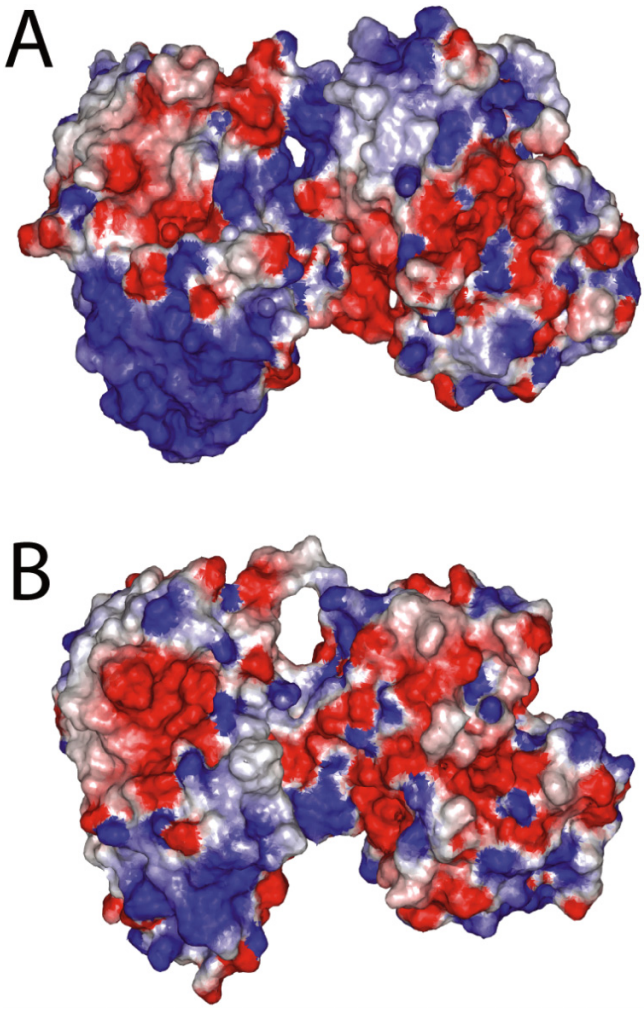

octahedral surrounding, and this requirement is fulfilled by the presence of a carbonate ion ($CO_3^{2-}$), which provides two oxygen ligands and acts as a "synergistic anion" (Saponja and Vogel 2011). The carbonate ion can be protonated in certain biological compartments that maintain a low pH — for example, the endosome — and this helps to promote the release of iron, which is otherwise bound extremely tightly to the protein ($K_d \sim 10^{-20}$ mol/L). Upon removal of the iron, the cleft of each lobe can open up substantially; the results obtained with various apo-proteins indicate that the binding of $Fe^{3+}$ bridges between the two domains that make up each lobe. While lactoferrin and transferrin both have the same global structure and use the same ligands for binding iron, lactoferrin actually binds iron slightly better than transferrin at low pH values (Baker and Baker 2004). The strong iron-binding potency of these two proteins creates local iron deprivation, and this is an important factor in preventing bacteria from growing (Arnold et al. 1977). Moreover, the low iron conditions also prevent the formation of bacterial biofilms (Singh et al. 2002).

In terms of its functional properties, while human serum transferrin avidly binds iron, this protein does not share all the other host defense properties displayed by human lactoferrin. Thus a simple inspection of the protein surfaces of both proteins should allow one to identify regions on lactoferrin that may be responsible for these effects. As can be seen in Fig. 2, when compared with transferrin, the N-terminal region of lactoferrin is highly basic, containing many positively charged Arg and Lys sidechains. Several studies have indicated that this region of the protein can interact with lipopolysaccharide (Appelmelk et al. 1994), glycosaminoglycans (El Yazidi-Belkoura et al. 2001), and with DNA (Kanyshkova et al. 1999). It should be noted that a major portion of this region is actually deleted in the delta-lactoferrin isoform of the protein that is expressed intracellularly (vide infra). Interestingly, the native human protein starts with the sequence Gly-Arg-Arg-Arg-Arg-, which resembles a nuclear localization sequence. Intriguingly, many studies have shown that lactoferrin can spontaneously enter various eukaryotic cells, where it can influence gene transcription (He and Furmanski 1995). It is not known if these N-terminal residues play a role like a "penetratin" in this spontaneous uptake (Deshayes et al. 2005; Duchardt et al. 2009), or if the uptake is mediated via specific receptors. Regardless, the notion that lactoferrin can enter cells and influence various intracellular pathways is now widely accepted by most workers in the field.

## Peptides derived from lactoferrin

In 1992, a group of researchers from the Japanese dairy company, Morinaga, reported that an antimicrobial peptide could be derived through pepsin cleavage from intact bovine lactoferrin (Bellamy et al. 1992). This peptide, termed lactoferricin, encompassed residues 17–41 of the intact protein (see highlighted region in Fig. 1A). Lactoferricin is naturally occurring, it has actually been identified as a breakdown product in the gut of humans (Kuwata et al. 1998*a*, 1998*b*). The peptide is extremely basic, and it contains two Trp residues that are important for its antimicrobial activity (Strøm et al. 2002). The single disulfide linkage in bovine lactoferricin

**Fig. 2.** Charge distribution on the surface of (A) bovine lactoferrin compared with (B) rabbit transferrin with anionic regions shown in red (medium grey in the print version), cationic regions in blue (dark grey in print), and neutral regions in white. Much of the biological activity of lactoferrin has been attributed to the cationic N-terminal lobe of the protein (bottom left of the protein). This strong cationic region is considerably less pronounced in the transferrin structure. Note that the orientation of the proteins in Figs. 1 and 2 is the same.




is not essential to maintain the antimicrobial activity (Bellamy et al. 1992), yet it proved to be a necessary requirement for the anticancer activity of this peptide (Eliassen et al. 2006). Interestingly, during in vitro testing, the lactoferricin peptide was much more potent as an antimicrobial than the intact lactoferrin protein (Bellamy et al. 1992). This observation could be explained when the solution structure of the peptide was determined by NMR spectroscopy (Hwang et al. 1998); it turns out that upon release from the protein, the peptide loses its α-helical structure, and instead it adopts an amphipathic beta hairpin structure that seems ideally designed to bind to bacterial membranes. The 25-residue peptide displays better potency than intact lactoferrin, and it possesses numerous physiological activities (for a review see Gifford et al. (2005)). A portion of bovine lactoferricin with the amino acid sequence -Arg-Arg-Trp-Gln-Trp-Arg- seems to play a major role in the antibacterial properties of bovine lactoferricin. This tryptophan- and arginine-rich hexapeptide has been used as a starting point for developing novel classes of antibiotics containing unnatural amino acids (Haug et al. 2007). Interestingly, the same region of bovine lactoferricin can also kill specific cancer cell lines (Richardson et al. 2009). Unfortunately, however, the linear hexapeptide is not all that stable in human serum, although its serum stability and its antimicrobial activity can be significantly improved upon cyclization (Nguyen et al. 2010). As such, the head-to-tail cyclized bovine lactoferricin hexapeptide may have considerable clinical potential when administered intravenously.

When the human lactoferrin protein is treated with pepsin, a much longer "human lactoferricin" peptide, now known to encompass residues 1–49, is released (see highlighted region in Fig. 1B). This peptide seems to be unstructured in aqueous solution, but it retains some of its original α-helical structure when studied in membrane mimetic solvents (Hunter et al. 2005). The full-length human lactoferricin peptide does not appear to have strong direct antimicrobial activity (Bellamy et al. 1992), but shorter peptides derived from this sequence are active in vitro (Stallmann et al. 2005) and in animal model systems (Dijkshoorn et al. 2004), perhaps acting indirectly through stimulating immune regulatory activities. In addition, human lactoferricin is an excellent binder of lipopolysaccharide (Elass-Rochard et al. 1995). It is noteworthy that a short N-terminal 11-residue peptide derived from lactoferricin still possesses the endotoxin binding properties of human lactoferricin (Andrä et al. 2005). The same region has also been shown to have beneficial host defense properties in humans and in various animal cell systems (van der Does et al. 2010; van der Does et al. 2012*a*), and it also displays antifungal activity in a mouse model (Lupetti et al. 2007). Finally, the technetium-99 labeled hLf1–11 region of human lactoferricin has been used to localize bacterial infections in imaging experiments (Nibbering et al. 2004; Welling et al. 2001).

More recently, a group of Dutch researchers have shown that a synthetic peptide encompassing the region 265–284 from bovine lactoferrin also has antimicrobial and antifungal activities (see highlighted region in Figs. 1A and 1B). This linear peptide, which they called lactoferrampin (van der Kraan et al. 2004, 2005), has no structure in aqueous solution, but it adopts an amphipathic partially helical conformation when bound to membrane mimetic micelles (Haney et al. 2007). It does not appear that bovine lactoferrampin actually occurs as such in vivo. The same region of the human lactoferrin protein does not have detectable antimicrobial activity, but it can be mutated to increase its potency (Haney et al. 2009). In the crystal structure of the intact bovine protein, the lactoferricin and lactoferrampin regions are spatially close (see Fig. 1). Hence, a covalently linked complex of the bovine lactoferricin and lactoferrampin peptides has also been reported and this "chimera" (Haney et al. 2012; Bolscher et al. 2012) has increased antimicrobial potency and is now being used in various animal model testing studies.

Taken together, the studies with the different lactoferrin-derived antibacterial, antifungal, antitumor, and host defense peptides provide further evidence that many of the intriguing properties of lactoferrin originate from its highly basic N-terminal region. Since serum transferrin does not have

the same highly basic region, it is easy to understand why this protein is devoid of these activities.

## Delta-lactoferrin, an intracellular isoform

Studies have shown that an alternative form of lactoferrin can be expressed intracellularly in many cells (Siebert and Huang 1997). This isoform of the protein, called delta-lactoferrin, lacks the leader sequence and the first 25 residues of the native protein. The mRNA for this truncated protein was detected in all normal tissues, but it was not found in several tumor-derived cell lines. The latter observation seems consistent with other reports that have indicated that the chromosomal region that codes for lactoferrin is deleted in various tumors, a spontaneous process that can occur during carcinogenesis (Klein et al. 2007; Yang et al. 1999). Several studies have indicated that delta-lactoferrin acts as a transcription factor inside cells (Mariller et al. 2012), and that it is involved in the regulation of the expression of specific genes. Clearly, taken together these data indicate that lactoferrin can play a role in cancer, and this area of research deserves more attention in the future.

## Antimicrobial activities

The beneficial effects of intact lactoferrin and of peptides derived from both the bovine and human protein to combat various infections have received considerable attention. As mentioned above, lactoferrin can act as an antimicrobial by lowering the iron concentration locally or by acting directly on bacteria through its cationic N-terminal region. Additionally, the protein can activate the immune system (vide infra). Numerous papers describe the effects measured in vivo, and the results may depend on whether one uses the bovine or the human protein, or one of the peptides derived from the larger protein. In addition, investigators have used different doses in their experiments, looked at widely different bacterial or fungal strains, and in some cases intravenous rather than oral administration was used. As such, the literature on this topic is hard to summarize in a mini-review paper such as this. The reader is therefore directed to a number of reviews on the topic that may help in analyzing the voluminous literature on the topic (Brouwer et al. 2011; Gifford et al. 2005; Jenssen and Hancock 2009; Yen et al. 2011). Overall, many beneficial effects for the intact proteins as well as for some of its peptides have been reported. Some of these seem to fall in line with the "direct" antibacterial and antifungal effects that can be measured in bacterial or fungal cultures during simple minimal inhibitory concentration (MIC) testing. However, in many animal studies the engagement of the immune system was also demonstrated, and these "indirect" effects may have had an overriding effect on the antibacterial and antifungal activities.

Similar animal model experiments have recently been done in the area of protozoal infections (León-Sicairos et al. 2012). Again, many beneficial effects have been reported in this area, and conveniently, the literature on this topic was recently reviewed (Ortíz-Estrada et al. 2012). Studies on this particular topic are complicated by the fact that some protozoa seem to be able to utilize iron-saturated lactoferrin as a source of iron for their own growth; consequently, administration of lactoferrin has not always had the desired effects.

The antiviral effects of lactoferrin and its peptide derivatives have also been studied extensively. Again, the literature on this topic is complicated to summarize in this mini-review because of differences between the test animals used, the different lactoferrin preparations under study, and other variations in the study design. In general, it has been found that most enveloped and naked viruses can be combatted by lactoferrin and some of its peptide derivatives, as these block entry of the viral particles into cells (Berlutti et al. 2011; Jenssen 2005; Jenssen 2009; Valenti and Antonini 2005). This process involves the glycosaminoglycans on the cell surface, and indeed it has been known for some time that lactoferrin binds to glycosaminglycans through its unique N-terminal region (El Yazidi-Belkoura et al. 2001). Additionally, lactoferrin may even have beneficial effects in the treatment of human immunodeficiency virus type 1 (Berkhout et al. 2004). Recent studies have shown that lactoferrin-derived peptides can be more efficient at treating infections of herpes simplex virus than the intact protein (Shestakov et al. 2012).

Interestingly, some pathogenic bacteria are able to use lactoferrin itself as a source of iron. To this end, they rely on lactoferrin receptors on their cell surface that specifically recognize the lactoferrin protein. The receptors comprise two interacting proteins, which together mediate the extraction and uptake of iron (Beddek and Schryvers 2010). Structural information for this class of proteins, as well as the related bacterial transferrin binding proteins, is now becoming available (Calmettes et al. 2012; Noinaj et al. 2012). This is an example of how pathogenic bacteria can gain access to iron in the host and sometimes overcome the effects of the host defense system.

## Anticancer activities

There are a growing number of papers that indicate that bovine and human lactoferrin can have beneficial effects for the treatment of cancer (Gibbons et al. 2011). Studies with various cancer cell lines and animal models have been reported, all showing beneficial effects; this area of research has recently been reviewed by Tsuda and co-workers (Tsuda et al. 2010). Also, some of the antimicrobial lactoferricin peptides have been used as antitumor agents, and in some cases this strategy has been successful (Eliassen et al. 2006). At the time of writing, one company, Agennix, is testing a recombinant form of human lactoferrin, in phase II and phase III clinical trials for the treatment of non-small cell lung cancer (Digumarti et al. 2011; Parikh et al. 2011). Administration of bovine lactoferrin in a randomized placebo-controlled clinical trial setting has also been reported to have beneficial effects for blocking the growth of polyps that are often thought to lead to colon cancer (Kozu et al. 2009). Clearly much more research is needed in this area. Of particular interest is the notion that oral administration may be effective; this is different from essentially all other therapeutic proteins, which typically require other more invasive routes of administration (Leader et al. 2008).

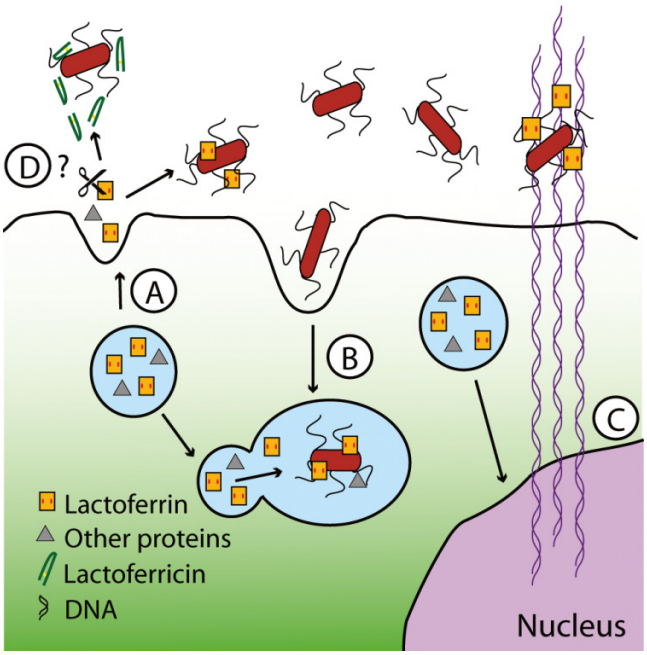
## Immunoregulatory activities of lactoferrin

Many studies have examined the effects of bovine and human lactoferrin on the immune system. The literature on the

topic is continually expanding, and it has been reviewed on a number of occasions. While there are many conflicting reports, it seems that lactoferrin can have both immunostimulatory effects as well as immunomodulatory activities (Latorre et al. 2012; Legrand 2012). The ability of lactoferrin to bind endotoxin (Appelmelk et al. 1994; Elass-Rochard et al. 1995) probably plays a major role in the immunomodulatory process. When Gram-negative bacteria attempt to invade the human host, the bacteria will become exposed to various proteins of the innate immune system. Part of the bacterial outer membrane contains lipopolysaccharide (also known as endotoxin), and as this "pathogen-associated molecular pattern" is recognized by the Toll-like receptor 4, it triggers an assortment of immune responses in various leukocytes and platelets (Beutler 2002; Beutler and Moresco 2008; Clark et al. 2007). Through the binding of bacterially-released endotoxin by lactoferrin, the extent of stimulation of the immune system is reduced, and this process could prevent an overstimulation, which occurs sometimes during a disease such as sepsis. A recent study indicates that the human lactoferricin derived hLf1-11 peptide can inhibit myeloperoxidase, a major host-defense enzyme found in various leukocytes, and this could further reduce the innate immune response (van der Does et al. 2012*b*). On the other hand, human lactoferrin was recently shown to stimulate the maturation of dendritic cells and to recruit various leukocytes (de la Rosa et al. 2008; Spadaro et al. 2008). As such, the protein plays an activating role in the innate as well as the adaptive immune responses.

The lactoferrin that is located in the secondary granules of neutrophils plays a major role in host defense (Levy 2004). Neutrophils can respond to invading pathogenic bacteria in various ways (see Fig. 3). First, at sites of infection neutrophils can degranulate, a process that releases the cocktail of host defense proteins that are present in the secondary and other secretory granules. Together these can create an important local response to the bacterial infection (Amulic et al. 2012; Borregaard et al. 2007). Alternatively, in the phagocytosis process, the neutrophils engulf the invading bacteria; once a bacterium is entrapped inside the neutrophil, merging of the phagocytic vacuole with the granules occurs, and the bacteria are destroyed locally. A third process relies on neutrophil extracellular traps (NETs) that are created by the release of DNA from the nuclei of the neutrophils. In this "kamikazi-like" process, the intracellular granules merge with the nucleus and then the host defense proteins, together with the DNA and the nuclear proteins, are all secreted to the extracellular space (Brinkmann and Zychlinsky 2007; Ma and Kubes 2008). Bacteria then become entrapped in the NETs, where the host defense proteins can act on them. Lactoferrin can bind to DNA (Kanyshkova et al. 1999) and through its highly positively charged N-terminal region, lactoferrin will remain associated with the extruded DNA in the NETs, where it can still contribute to the bacterial killing in this process. The three processes are schematically depicted in Fig. 3. Since several proteolytic enzymes are secreted from the granules as well, it is possible that lactoferricin or other peptides are also locally released from the intact lactoferrin protein, but this possibility does not appear to have been studied yet.

**Fig. 3.** Schematic representation of the response of neutrophils to invading pathogenic bacteria. A number of antimicrobial proteins, including lactoferrin, are contained within the various granules of the neutrophils. (A) The neutrophil can release the contents of the granules to the extracellular space, where the proteins can exert their antimicrobial effect. (B) Alternatively, the bacteria may be internalized through phagocytosis, and the granules can then fuse to the phagocytic vacuole and deliver the cocktail of potent antimicrobials. (C) Neutrophil extracellular traps (NETs) are also formed when the intracellular granules merge with the nucleus. This causes the ejection of the DNA from the cell. The polyanionic DNA attracts cationic proteins, like lactoferrin and other antimicrobial proteins, and traps the bacteria within the NET, which exposes the pathogen to a locally high concentration of these antimicrobials. (D) Finally, a number of proteolytic enzymes are released during granule secretion, and it is possible that these proteases may generate short antimicrobial peptides, like lactoferricin, which would further enhance the local concentration of antimicrobial molecules.




## Other effects on human health

A number of studies have shown that lactoferrin can stimulate bone growth. The protein is capable of promoting the proliferation and differentiation of osteoblasts, the cells that produce bone. At the same time, lactoferrin can also inhibit the formation of osteoclasts, which are highly specialized cells responsible for bone resorption. Bone is constantly being remodeled by the opposing action of the osteoclasts and osteoblasts. Therefore, the overall net result in the presence of physiological levels of lactoferrin is the growth of bone (Cornish et al. 2004; Naot et al. 2005). It is possible that lactoferrin acts together with osteopontin, another protein known to be involved in bone formation, as the two proteins can form a complex (Yamniuk et al. 2009). Other studies have also indicated that lactoferrin can prevent bone loss in ovariectomized mice, suggesting that the protein could have beneficial effects to prevent post menopausal bone loss (Blais et al. 2009; Malet et al. 2011).

Lactoferrin has demonstrated a positive effect on wound healing. Studies with various animal models indicate that recombinant human lactoferrin can stimulate the closure of wounds in vivo (Engelmayer et al. 2008; Tang et al. 2010*b*). Some studies have indicated that the protein does this by modulating an inflammatory response. Other studies found that human lactoferrin stimulated skin keratinocyte function and wound re-epithelialization (Tang et al. 2010*b*), as well as promoted fibroblast proliferation and migration (Tang et al. 2010*a*). There is some in vitro evidence that bovine lactoferrin may have similar effects (Ashby et al. 2011).

Another beneficial effect of lactoferrin may be in the area of myelopoiesis, the process of the formation of leukocytes. However, conflicting reports have been published, which indicate that lactoferrin promotes, reduces, or does not influence this process. This controversy was recently reviewed and the reader is encouraged to read the article by Artym and Zimecki (2007) on this topic for further details.

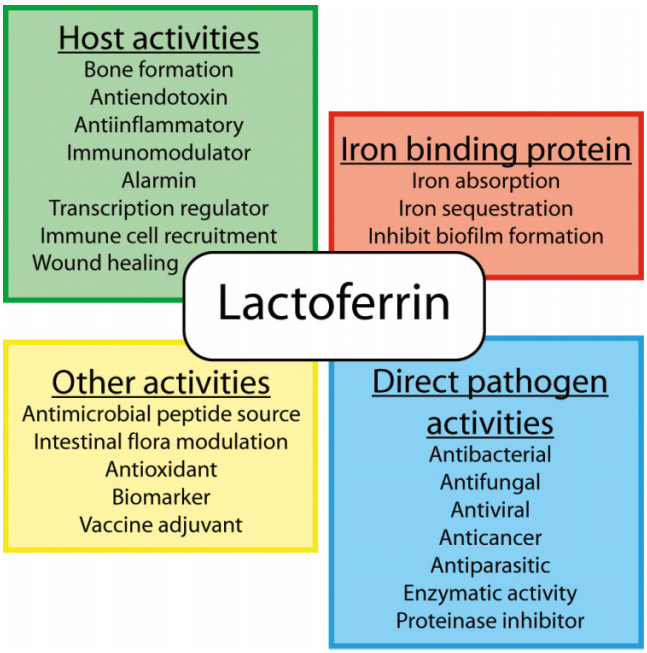
## Lactoferrin as a biomarker of disease

In patients suffering from inflammatory bowel disease, neutrophils usually enter the gut to combat the inflammation. Here they release many of the host defense factors that are stored in their granules, including lactoferrin, to help reduce the chronic inflammation. Since lactoferrin is stable in the gut, the amount of lactoferrin that is released by neutrophils can be detected in fecal stool samples (Beniwal and Harrell 2010; Tibble and Bjarnason 2001). Hence, lactoferrin in stool samples can be used as a quantitative biomarker for inflammatory diseases, such as ulcerative colitis and Crohn's disease. Lactoferrin is often measured in stool samples together with calprotectin, a calcium- and zinc-binding protein that is highly abundant in the cytoplasm of neutrophils and that can be released during the process of NET formation (Voganatsi et al. 2001). Like lactoferrin, calprotectin (also known as S100A8/S100A9 or as MRP8/MRP14) is an antimicrobial protein, which is believed to act by binding zinc and making it unavailable for bacteria (Sohnle et al. 2000). These two protein biomarkers can be used to distinguish inflammatory bowel diseases from celiac disease and irritable bowel syndrome, which clinically present with similar symptoms. Hence, it may be possible in the future to avoid the use of invasive diagnostic procedures such as endoscopy, which are currently used for diagnosis (Dave and Loftus 2012; Langhorst and Boone 2012). Finally, measurements of these biomarkers in stool samples can also be useful to measure recurrence of the disease.

Interestingly, lactoferrin levels are also increased in the synovial fluid of inflamed knee joints, likely because of neutrophil infiltration. The level of lactoferrin was found to be significantly higher in rheumatoid arthritis patients than in osteoarthritis patients. The effect was highly localized as the lactoferrin levels in synovial fluid were increased, but the levels in serum were indistinguishable from healthy controls (Caccavo et al. 1999).

Lactoferrin is also an important component of the mix of antimicrobial peptides and host-defense proteins that provide protection against infectious agents in saliva. Recently it has been found that lactoferrin levels are markedly increased during chronic periodontitis, an infectious chronic inflammatory condition, that ultimately gives rise to destruction of the bone structures underneath the teeth (Glimvall et al. 2012). Perhaps future visits to the dentist will include a simple saliva test for lactoferrin levels!

**Fig. 4.** The wide range of beneficial functional properties described for lactoferrin.




## Health promotion and potential clinical use of lactoferrin

The various biological actions of lactoferrin are schematically summarized in Fig. 4. Clearly, apart from its intrinsic iron-binding ability, there are several "direct" modes of action where the protein can act directly on invading pathogens. In addition, there are several "indirect" actions where the protein acts via specific receptors on specialized cells, or where it acts through the immune system. Many of these effects can be achieved by using the protein as a dietary supplement. Indeed, in Japan bovine lactoferrin has been used for several years as a health promoting additive to various commercial food products (Tomita et al. 2009). Bovine lactoferrin is produced from skim milk or whey, while human recombinant lactoferrin can be produced in Aspergillus niger and in transgenic cows, goats, and rice (Wakabayashi et al. 2006).

Because of the plethora of beneficial effects of lactoferrin, it is perhaps not surprising that a burgeoning nutraceutical industry has started to develop, where lactoferrin supplements are actively marketed as health promoting substances. Many producers suggest that the protein has general immunostimulating properties. Most of these products contain bovine lactoferrin, which is commercially available on a large scale. Some of the health claims made for these products are perhaps exaggerated, particularly in view of the fact that very high levels of lactoferrin may be required to achieve the desired health promoting effects. Since there have never been any studies reported that indicate that high doses of bovine lactoferrin can be toxic, the current interest

in this use of lactoferrin does not seem to pose any immediate health concerns and may in fact be beneficial. However, consumers should be careful that they receive a product of high purity. Some of the commercial products may contain additional proteins or other contaminants that can potentially lead to undesired side effects. For example, some preparations of bovine lactoferrin may contain lipopolysaccharide (endotoxin), and this contaminant may lead to an undesired immune response. In some cases, angiogenin (part of the ribonuclease family of proteins) is present in protein preparations with lactoferrin; again, such contaminants may give rise to responses that are not desired (Shcheglovitova et al. 2003). On the other hand, ribonuclease-enriched lactoferrin preparations have been claimed to have beneficial effects for bone turnover and control of inflammation in postmenopausal women (Bharadwaj et al. 2009, 2010). Perhaps this is because many ribonucleases have antimicrobial properties themselves (Rosenberg 2008). Moreover, cow milk angiogenin has been found to inhibit bone-resorption by osteoclasts (Morita et al. 2008).

Beyond the poorly regulated nutraceutical market however, there are several bona fide clinical trials ongoing at the moment where human or bovine lactoferrin are being tested for various diseases under more rigorous and carefully controlled conditions. Some diseases currently under investigation in clinical trials are lung cancer and sepsis. Oral lactoferrin may also have direct effects on diseases of the colon, such as inflammatory bowel disease or diarrhea (Ha and Kornbluth 2010; Ochoa et al. 2012). Indeed, human lactoferrin has now been shown to modulate the intestinal flora in piglets (Hu et al. 2012), and similar effects would likely be obtained in humans. Additionally, some of the lactoferrin-based peptides are in clinical trials as potential treatments for various diseases. The progress of many of the trials can be followed on the web site administered by the National Institutes of Health (www.clinicaltrials.gov/ct2/results?term=lactoferrin). Given the current interest and the ongoing clinical trials with lactoferrin, it only seems a matter of time until this multifunctional protein becomes useful in clinical practice.

## Acknowledgements

The author would like to thank Dr. Evan Haney for help with the figures and for editing and proofreading the manuscript. Funding from the Canadian Institutes of Health Research, Alberta Cancer Foundation, and from Alberta Innovates Health Solutions has facilitated ongoing research on lactoferrin structure and function in the author's laboratory.

## References

Amulic, B., Cazalet, C., Hayes, G.L., Metzler, K.D., and Zychlinsky, A. 2012. Neutrophil function: from mechanisms to disease. Annu. Rev. Immunol. **30**(1): 459–489. doi:10.1146/annurev-immunol-020711-074942. PMID:22224774.

Anderson, B.F., Baker, H.M., Dodson, E.J., Norris, G.E., Rumball, S.V., Waters, J.M., and Baker, E.N. 1987. Structure of human lactoferrin at 3.2-A resolution. Proc. Natl. Acad. Sci. U.S.A. **84**(7): 1769–1773. doi:10.1073/pnas.84.7.1769. PMID:3470756.

Andrä, J., Lohner, K., Blondelle, S.E., Jerala, R., Moriyon, I., Koch, M.H.J., et al. 2005. Enhancement of endotoxin neutralization by coupling of a C12-alkyl chain to a lactoferricin-derived peptide. Biochem. J. **385**(1): 135–143. doi:10.1042/BJ20041270. PMID:15344905.

Appelmelk, B.J., An, Y.Q., Geerts, M., Thijs, B.G., de Boer, H.A., MacLaren, D.M., et al. 1994. Lactoferrin is a lipid A-binding protein. Infect. Immun. **62**(6): 2628–2632. PMID:8188389.

Arnold, R.R., Cole, M.F., and McGhee, J.R. 1977. A bactericidal effect for human lactoferrin. Science, **197**(4300): 263–265. doi:10.1126/science.327545. PMID:327545.

Artym, J., and Zimecki, M. 2007. The effects of lactoferrin on myelopoiesis: can we resolve the controversy? Postepy Hig. Med. Dosw. (Online), **61**: 129–150. PMID:17410054.

Ashby, B., Garrett, Q., and Willcox, M. 2011. Bovine lactoferrin structures promoting corneal epithelial wound healing in vitro. Invest. Ophthalmol. Vis. Sci. **52**(5): 2719–2726. doi:10.1167/iovs.10-6352. PMID:21282581.

Bailey, S., Evans, R.W., Garratt, R.C., Gorinsky, B., Hasnain, S., Horsburgh, C., et al. 1988. Molecular structure of serum transferrin at 3.3-A resolution. Biochemistry, **27**(15): 5804–5812. doi:10.1021/bi00415a061. PMID:3179277.

Baker, H.M., and Baker, E.N. 2004. Lactoferrin and iron: structural and dynamic aspects of binding and release. Biometals, **17**(3): 209–216. doi:10.1023/B:BIOM.0000027694.40260.70. PMID:15222467.

Baker, H.M., and Baker, E.N. 2012. A structural perspective on lactoferrin function. Biochem. Cell Biol., This issue. doi:10.1139/o11-071. PMID:22292559.

Beddek, A.J., and Schryvers, A.B. 2010. The lactoferrin receptor complex in Gram negative bacteria. Biometals, **23**(3): 377–386. doi:10.1007/s10534-010-9299-z. PMID:20155302.

Bellamy, W., Takase, M., Yamauchi, K., Wakabayashi, H., Kawase, K., and Tomita, M. 1992. Identification of the bactericidal domain of lactoferrin. Biochim. Biophys. Acta, **1121**(1–2): 130–136. doi:10.1016/0167-4838(92)90346-F. PMID:1599934.

Beniwal, P., and Harrell, L. 2010. The status of diagnostic markers for inflammatory bowel disease. Curr. Gastroenterol. Rep. **12**(6): 479–484. doi:10.1007/s11894-010-0145-9. PMID:20890737.

Berkhout, B., Floris, R., Recio, I., and Visser, S. 2004. The antiviral activity of the milk protein lactoferrin against the human immunodeficiency virus type 1. Biometals, **17**(3): 291–294. doi:10.1023/B:BIOM.0000027707.82911.be. PMID:15222480.

Berlutti, F., Pantanella, F., Natalizi, T., Frioni, A., Paesano, R., Polimeni, A., and Valenti, P. 2011. Antiviral properties of lactoferrin–a natural immunity molecule. Molecules, **16**(8): 6992–7018. doi:10.3390/molecules16086992. PMID:21847071.

Beutler, B. 2002. LPS in microbial pathogenesis: promise and fulfilment. J. Endotoxin Res. **8**(5): 329–335. PMID:12537691.

Beutler, B., and Moresco, E.M. 2008. The forward genetic dissection of afferent innate immunity. Curr. Top. Microbiol. Immunol. **321**: 3–26. doi:10.1007/978-3-540-75203-5_1. PMID:18727485.

Bharadwaj, S., Naidu, A.G., Betageri, G.V., Prasadarao, N.V., and Naidu, A.S. 2009. Milk ribonuclease-enriched lactoferrin induces positive effects on bone turnover markers in postmenopausal women. Osteoporos. Int. **20**(9): 1603–1611. doi:10.1007/s00198-009-0839-8. PMID:19172341.

Bharadwaj, S., Naidu, T.A., Betageri, G.V., Prasadarao, N.V., and Naidu, A.S. 2010. Inflammatory responses improve with milk ribonuclease-enriched lactoferrin supplementation in postmenopausal women. Inflamm. Res. **59**(11): 971–978. doi:10.1007/s00011-010-0211-7. PMID:20473630.

Blais, A., Malet, A., Mikogami, T., Martin-Rouas, C., and Tome, D. 2009. Oral bovine lactoferrin improves bone status of ovariectomized mice. Am. J. Physiol. Endocrinol. Metab. **296**(6): E1281–E1288. doi:10.1152/ajpendo.90938.2008. PMID:19336659.

Bolscher, J., Nazmi, K., van Marle, J., van 't Hof, W., and Veerman, E. 2012. Chimerization of lactoferricin and lactoferrampin peptides strongly potentiates the killing activity against Candida albicans. Biochem. Cell Biol.This issue. doi:10.1139/o11-085. PMID:22364313.

Borregaard, N., Sorensen, O.E., and Theilgaard-Monch, K. 2007. Neutrophil granules: a library of innate immunity proteins. Trends Immunol. **28**(8): 340–345. doi:10.1016/j.it.2007.06.002. PMID: 17627888.

Brinkmann, V., and Zychlinsky, A. 2007. Beneficial suicide: why neutrophils die to make NETs. Nat. Rev. Microbiol. **5**(8): 577–582. doi:10.1038/nrmicro1710. PMID:17632569.

Brouwer, C.P., Rahman, M., and Welling, M.M. 2011. Discovery and development of a synthetic peptide derived from lactoferrin for clinical use. Peptides, **32**(9): 1953–1963. doi:10.1016/j.peptides.2011.07.017. PMID:21827807.

Caccavo, D., Sebastiani, G.D., Di Monaco, C., Guido, F., Galeazzi, M., Ferri, G.M., et al. 1999. Increased levels of lactoferrin in synovial fluid but not in serum from patients with rheumatoid arthritis. Int. J. Clin. Lab. Res. **29**(1): 30–35. doi:10.1007/s005990050059. PMID:10356661.

Calmettes, C., Alcantara, J., Yu, R.H., Schryvers, A.B., and Moraes, T.F. 2012. The structural basis of transferrin sequestration by transferrin-binding protein B. Nat. Struct. Mol. Biol. **19**(3): 358–360. doi:10.1038/nsmb.2251. PMID:22343719.

Cheng, Y., Zak, O., Aisen, P., Harrison, S.C., and Walz, T. 2004. Structure of the human transferrin receptor-transferrin complex. Cell, **116**(4): 565–576. doi:10.1016/S0092-8674(04)00130-8. PMID:14980223.

Chu, B.C., Garcia-Herrero, A., Johanson, T.H., Krewulak, K.D., Lau, C.K., Peacock, R.S., et al. 2010. Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. Biometals, **23**(4): 601–611. doi:10.1007/s10534-010-9361-x. PMID: 20596754.

Clark, S.R., Ma, A.C., Tavener, S.A., McDonald, B., Goodarzi, Z., Kelly, M.M., et al. 2007. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat. Med. **13**(4): 463–469. doi:10.1038/nm1565. PMID:17384648.

Cornish, J., Callon, K.E., Naot, D., Palmano, K.P., Banovic, T., Bava, U., et al. 2004. Lactoferrin is a potent regulator of bone cell activity and increases bone formation in vivo. Endocrinology, **145**(9): 4366–4374. doi:10.1210/en.2003-1307. PMID:15166119.

Crichton, R.R. 2009. Iron Metabolism: From Molecular Mechanisms to Clinical Consequences. John Wiley & Sons Ltd., Chichester, West Sussex.

Dave, M., and Loftus, E.V., Jr. 2012. Mucosal healing in inflammatory bowel disease-a true paradigm of success? Gastroenterol. Hepatol. (N.Y.), **8**(1): 29–38. PMID:22347830.

Davidson, L.A., and Lonnerdal, B. 1988. Specific binding of lactoferrin to brush-border membrane: ontogeny and effect of glycan chain. Am. J. Physiol. **254**(4): G580–G585. PMID: 2833117.

de la Rosa, G., Yang, D., Tewary, P., Varadhachary, A., and Oppenheim, J.J. 2008. Lactoferrin acts as an alarmin to promote the recruitment and activation of APCs and antigen-specific immune responses. J. Immunol. **180**(10): 6868–6876. PMID: 18453607.

Deshayes, S., Morris, M.C., Divita, G., and Heitz, F. 2005. Cell-penetrating peptides: tools for intracellular delivery of therapeutics. Cell. Mol. Life Sci. **62**(16): 1839–1849. doi:10.1007/s00018-005-5109-0. PMID:15968462.

Digumarti, R., Wang, Y., Raman, G., Doval, D.C., Advani, S.H., Julka, P.K., et al. 2011. A randomized, double-blind, placebo-controlled, phase II study of oral talactoferrin in combination with carboplatin and paclitaxel in previously untreated locally advanced or metastatic non-small cell lung cancer. J. Thorac. Oncol. **6**(6): 1098–1103. doi:10.1097/JTO.0b013e3182156250. PMID: 21532506.

Dijkshoorn, L., Brouwer, C.P., Bogaards, S.J., Nemec, A., van den Broek, P.J., and Nibbering, P.H. 2004. The synthetic N-terminal peptide of human lactoferrin, hLF(1–11), is highly effective against experimental infection caused by multidrug-resistant Acinetobacter baumannii. Antimicrob. Agents Chemother. **48**(12): 4919–4921. doi:10.1128/AAC.48.12.4919-4921.2004. PMID: 15561882.

Duchardt, F., Ruttekolk, I.R., Verdurmen, W.P., Lortat-Jacob, H., Burck, J., Hufnagel, H., et al. 2009. A cell-penetrating peptide derived from human lactoferrin with conformation-dependent uptake efficiency. J. Biol. Chem. **284**(52): 36099–36108. doi:10.1074/jbc.M109.036426. PMID:19858187.

El Yazidi-Belkoura, I., Legrand, D., Nuijens, J., Slomianny, M.C., van Berkel, P., and Spik, G. 2001. The binding of lactoferrin to glycosaminoglycans on enterocyte-like HT29–18–C1 cells is mediated through basic residues located in the N-terminus. Biochim. Biophys. Acta, **1568**(3): 197–204. doi:10.1016/S0304-4165(01)00222-7. PMID:11786226.

Elass-Rochard, E., Roseanu, A., Legrand, D., Trif, M., Salmon, V., Motas, C., et al. 1995. Lactoferrin-lipopolysaccharide interaction: involvement of the 28–34 loop region of human lactoferrin in the high-affinity binding to Escherichia coli 055B5 lipopolysaccharide. Biochem. J. **312**(3): 839–845. PMID:8554529.

Eliassen, L.T., Berge, G., Leknessund, A., Wikman, M., Lindin, I., Lokke, C., et al. 2006. The antimicrobial peptide, lactoferricin B, is cytotoxic to neuroblastoma cells in vitro and inhibits xenograft growth in vivo. Int. J. Cancer, **119**(3): 493–500. doi:10.1002/ijc.21886. PMID:16572423.

Engelmayer, J., Blezinger, P., and Varadhachary, A. 2008. Talactoferrin stimulates wound healing with modulation of inflammation. J. Surg. Res. **149**(2): 278–286. doi:10.1016/j.jss.2007.12.754. PMID:18619616.

Ganz, T., and Nemeth, E. 2012. Hepcidin and iron homeostasis. Biochim. Biophys. Acta, In press. doi:10.1016/j.bbamcr.2012.01.014. PMID:22306005.

Giansanti, F., Leboffe, L., Pitari, G., Ippoliti, R., and Antonini, G. 2012. Physiological roles of ovotransferrin. Biochim. Biophys. Acta, **1820**(3): 218–225. doi:10.1016/j.bbagen.2011.08.004. PMID:21854833.

Gibbons, J.A., Kanwar, R.K., and Kanwar, J.R. 2011. Lactoferrin and cancer in different cancer models. Front. Biosci. (Schol. Ed.), **3**(1): 1080–1088. doi:10.2741/212. PMID:21622257.

Gifford, J.L., Hunter, H.N., and Vogel, H.J. 2005. Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. Cell. Mol. Life Sci. **62**(22): 2588–2598. doi:10.1007/s00018-005-5373-z. PMID:16261252.

Glimvall, P., Wickström, C., and Jansson, H. 2012. Elevated levels of salivary lactoferrin, a marker for chronic periodontitis? J. Periodontal Res. In press. doi:10.1111/j.1600-0765.2012.01479.x. PMID:22471324.

Groves, M.L. 1960. The isolation of a red protein from milk. J. Am. Chem. Soc. **82**(13): 3345–3350. doi:10.1021/ja01498a029.

Ha, C., and Kornbluth, A. 2010. Mucosal healing in inflammatory bowel disease: where do we stand? Curr. Gastroenterol. Rep. **12**(6): 471–478. doi:10.1007/s11894-010-0146-8. PMID:20886319.

Haney, E.F., Lau, F., and Vogel, H.J. 2007. Solution structures and model membrane interactions of lactoferrampin, an antimicrobial peptide derived from bovine lactoferrin. Biochim. Biophys. Acta, **1768**(10): 2355–2364. doi:10.1016/j.bbamem.2007.04.018. PMID:17560539.

Haney, E.F., Nazmi, K., Lau, F., Bolscher, J.G., and Vogel, H.J. 2009. Novel lactoferrampin antimicrobial peptides derived from human lactoferrin. Biochimie, **91**(1): 141–154. doi:10.1016/j.biochi.2008.04.013. PMID:18534196.

Haney, E.F., Nazmi, K., Bolscher, J.G., and Vogel, H.J. 2012. Structural and biophysical characterization of an antimicrobial peptide chimera comprised of lactoferricin and lactoferrampin. Biochim. Biophys. Acta, **1818**(3): 762–775. doi:10.1016/j.bbamem.2011.11.023. PMID:22155682.

Haug, B.E., Strom, M.B., and Svendsen, J.S. 2007. The medicinal chemistry of short lactoferricin-based antibacterial peptides. Curr. Med. Chem. **14**(1): 1–18. doi:10.2174/092986707779313435. PMID:17266565.

He, J., and Furmanski, P. 1995. Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA. Nature, **373**(6516): 721–724. doi:10.1038/373721a0. PMID:7854459.

Hu, W., Zhao, J., Wang, J., Yu, T., Wang, J., and Li, N. 2012. Transgenic milk containing recombinant human lactoferrin modulates the intestinal flora in piglets. Biochem. Cell Biol. This issue. doi:10.1139/o2012-003. PMID:22400985.

Hunter, H.N., Demcoe, A.R., Jenssen, H., Gutteberg, T.J., and Vogel, H.J. 2005. Human lactoferricin is partially folded in aqueous solution and is better stabilized in a membrane mimetic solvent. Antimicrob. Agents Chemother. **49**(8): 3387–3395. doi:10.1128/AAC.49.8.3387-3395.2005. PMID:16048952.

Hwang, P.M., Zhou, N., Shan, X., Arrowsmith, C.H., and Vogel, H.J. 1998. Three-dimensional solution structure of lactoferricin B, an antimicrobial peptide derived from bovine lactoferrin. Biochemistry, **37**(12): 4288–4298. doi:10.1021/bi972323m. PMID:9521752.

Jenssen, H. 2005. Anti herpes simplex virus activity of lactoferrin/lactoferricin–an example of antiviral activity of antimicrobial protein/peptide. Cell. Mol. Life Sci. **62**(24): 3002–3013. doi:10.1007/s00018-005-5228-7. PMID:16261265.

Jenssen, H. 2009. Therapeutic approaches using host defence peptides to tackle herpes virus infections. Viruses, **1**(3): 939–964. doi:10.3390/v1030939. PMID:21994576.

Jenssen, H., and Hancock, R.E. 2009. Antimicrobial properties of lactoferrin. Biochimie, **91**(1): 19–29. doi:10.1016/j.biochi.2008.05.015. PMID:18573312.

Johansson, B. 1960. Isolation of an iron-containing red protein from human milk. Acta Chem. Scand. **14**(2): 510–512.

Kanyshkova, T.G., Semenov, D.V., Buneva, V.N., and Nevinsky, G.A. 1999. Human milk lactoferrin binds two DNA molecules with different affinities. FEBS Lett. **451**(3): 235–237. doi:10.1016/S0014-5793(99)00579-7. PMID:10371196.

Klein, G., Imreh, S., and Zabarovsky, E.R. 2007. Why do we not all die of cancer at an early age? Adv. Cancer Res. **98**: 1–16. doi:10.1016/S0065-230X(06)98001-4. PMID:17433906.

Kozu, T., Iinuma, G., Ohashi, Y., Saito, Y., Akasu, T., Saito, D., et al. 2009. Effect of orally administered bovine lactoferrin on the growth of adenomatous colorectal polyps in a randomized, placebo-controlled clinical trial. Cancer Prev. Res. (Phila.), **2**(11): 975–983. doi:10.1158/1940-6207.CAPR-08-0208. PMID:19861543.

Kurokawa, H., Mikami, B., and Hirose, M. 1995. Crystal structure of diferric hen ovotransferrin at 2.4 A resolution. J. Mol. Biol. **254**(2): 196–207. doi:10.1006/jmbi.1995.0611. PMID:7490743.

Kuwata, H., Yip, T.T., Tomita, M., and Hutchens, T.W. 1998*a*. Direct evidence of the generation in human stomach of an antimicrobial peptide domain (lactoferricin) from ingested lactoferrin. Biochim. Biophys. Acta, **1429**(1): 129–141. doi:10.1016/S0167-4838(98)00224-6. PMID:9920391.

Kuwata, H., Yip, T.T., Yip, C.L., Tomita, M., and Hutchens, T.W. 1998*b*. Direct detection and quantitative determination of bovine lactoferricin and lactoferrin fragments in human gastric contents by affinity mass spectrometry. Adv. Exp. Med. Biol. **443**: 23–32. PMID:9781339.

Lambert, L.A., Perri, H., Halbrooks, P.J., and Mason, A.B. 2005. Evolution of the transferrin family: conservation of residues associated with iron and anion binding. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **142**(2): 129–141. doi:10.1016/j.cbpb.2005.07.007. PMID:16111909.

Langhorst, J., and Boone, J. 2012. Fecal lactoferrin as a noninvasive biomarker in inflammatory bowel diseases. Drugs Today (Barc), **48**(2): 149–161. PMID:22384454.

Latorre, D., Berlutti, F., Valenti, P., Gessani, S., and Puddu, P. 2012. LF immunomodulatory strategies: mastering bacterial endotoxin. Biochem. Cell Biol., This issue. doi:10.1139/o11-059. PMID:22300429.

Leader, B., Baca, Q.J., and Golan, D.E. 2008. Protein therapeutics: a summary and pharmacological classification. Nat. Rev. Drug Discov. **7**(1): 21–39. doi:10.1038/nrd2399. PMID:18097458.

Legrand, D. 2011. Lactoferrin, a key molecule in immune and inflammatory processes. Biochem. Cell Biol., This issue. doi:10.1139/o11-056. PMID:22136726.

Legrand, D., and Mazurier, J. 2010. A critical review of the roles of host lactoferrin in immunity. Biometals, **23**(3): 365–376. doi:10.1007/s10534-010-9297-1. PMID:20143251.

León-Sicairos, N., Martínez-Pardo, L., Sánchez-Hernández, B., de la Garza, M., and Carrero, C. 2012. Oral lactoferrin treatment resolves amoebic intracecal infection in C3H/HeJ mice. Biochem. Cell Biol. This issue. doi:10.1139/o2012-008. PMID:22452668.

Levy, O. 2004. Antimicrobial proteins and peptides: anti-infective molecules of mammalian leukocytes. J. Leukoc. Biol. **76**(5): 909–925. doi:10.1189/jlb.0604320. PMID:15292276.

Lönnerdal, B., Jiang, R., and Du, X. 2011. Bovine lactoferrin can be taken up by the human intestinal lactoferrin receptor and exert bioactivities. J. Pediatr. Gastroenterol. Nutr. **53**(6): 606–614. PMID:21832946.

Lupetti, A., Brouwer, C.P., Bogaards, S.J., Welling, M.M., de Heer, E., Campa, M., et al. 2007. Human lactoferrin-derived peptide's antifungal activities against disseminated *Candida albicans* infection. J. Infect. Dis. **196**(9): 1416–1424. doi:10.1086/522427. PMID:17922408.

Ma, A.C., and Kubes, P. 2008. Platelets, neutrophils, and neutrophil extracellular traps (NETs) in sepsis. J. Thromb. Haemost. **6**(3): 415–420. doi:10.1111/j.1538-7836.2007.02865.x. PMID:18088344.

Malet, A., Bournaud, E., Lan, A., Mikogami, T., Tome, D., and Blais, A. 2011. Bovine lactoferrin improves bone status of ovariectomized mice via immune function modulation. Bone, **48**(5): 1028–1035. doi:10.1016/j.bone.2011.02.002. PMID:21303707.

Mariller, C., Hardiville, S., Hoedt, E., Huvent, I., Pina-Canseco, S., and Pierce, A. 2012. delta-lactoferrin, an intracellular lactoferrin isoform that acts as a transcription factor. Biochem. Cell Biol. This issue. doi:10.1139/o11-070. PMID:22320386.

Masson, P.L., Heremans, J.F., and Schonne, E. 1969. Lactoferrin, an iron-binding protein in neutrophilic leukocytes. J. Exp. Med. **130**(3): 643–658. doi:10.1084/jem.130.3.643. PMID:4979954.

Montreuil, J., Tonnelat, J., and Mullet, S. 1960. Préparation et propriétés de la lactosidérophiline (lactotransferrine) du lait de femme. Biochim. Biophys. Acta., **45**: 413–421. PMID:13772242.

Morita, Y., Matsuyama, H., Serizawa, A., Takeya, T., and Kawakami, H. 2008. Identification of angiogenin as the osteoclastic bone resorption-inhibitory factor in bovine milk. Bone, **42**(2): 380–387. doi:10.1016/j.bone.2007.10.012. PMID:18055286.

Naot, D., Grey, A., Reid, I.R., and Cornish, J. 2005. Lactoferrin–a

novel bone growth factor. Clin. Med. Res. **3**(2): 93–101. doi:10.3121/cmr.3.2.93. PMID:16012127.

Nguyen, L.T., Chau, J.K., Perry, N.A., de Boer, L., Zaat, S.A., and Vogel, H.J. 2010. Serum stabilities of short tryptophan- and arginine-rich antimicrobial peptide analogs. PLoS ONE, **5**(9): e12684. doi:10.1371/journal.pone.0012684. PMID:20844765.

Nibbering, P.H., Welling, M.M., Paulusma-Annema, A., Brouwer, C. P., Lupetti, A., and Pauwels, E.K. 2004. 99mTc-Labeled UBI 29–41 peptide for monitoring the efficacy of antibacterial agents in mice infected with Staphylococcus aureus. J. Nucl. Med. **45**(2): 321–326. PMID:14960656.

Noinaj, N., Easley, N.C., Oke, M., Mizuno, N., Gumbart, J., Boura, E., et al. 2012. Structural basis for iron piracy by pathogenic Neisseria. Nature, **483**(7387): 53–58. doi:10.1038/nature10823. PMID:22327295.

Ochoa, T.J., Pezo, A., Cruz, K., Chea-Woo, E., and Cleary, T.G. 2012. Clinical studies of lactoferrin in children. Biochem. Cell Biol., This issue. PMID:22380791.

Ortíz-Estrada, G., Luna-Castro, S., Piña-Vázquez, C., Samaniego-Barrón, L., León-Sicairos, N., Serrano-Luna, J., and de la Garza, M. 2012. Iron-saturated lactoferrin and pathogenic protozoa: could this protein be an iron source for their parasitic style of life? Future Microbiol. **7**(1): 149–164. doi:10.2217/fmb.11.140. PMID: 22191452.

Parikh, P.M., Vaid, A., Advani, S.H., Digumarti, R., Madhavan, J., Nag, S., et al. 2011. Randomized, double-blind, placebo-controlled phase II study of single-agent oral talactoferrin in patients with locally advanced or metastatic non-small-cell lung cancer that progressed after chemotherapy. J. Clin. Oncol. **29**(31): 4129–4136. doi:10.1200/JCO.2010.34.4127. PMID:21969509.

Park, J.H., Park, G.T., Cho, I.H., Sim, S.M., Yang, J.M., and Lee, D.Y. 2011. An antimicrobial protein, lactoferrin exists in the sweat: proteomic analysis of sweat. Exp. Dermatol. **20**(4): 369–371. doi:10.1111/j.1600-0625.2010.01218.x. PMID:21366701.

Richardson, A., de Antueno, R., Duncan, R., and Hoskin, D.W. 2009. Intracellular delivery of bovine lactoferricin's antimicrobial core (RRWQWR) kills T-leukemia cells. Biochem. Biophys. Res. Commun. **388**(4): 736–741. doi:10.1016/j.bbrc.2009.08.083. PMID:19699713.

Rosenberg, H.F. 2008. RNase A ribonucleases and host defense: an evolving story. J. Leukoc. Biol. **83**(5): 1079–1087. PMID: 18211964.

Saponja, J., and Vogel, H.J. 2011. Quadrupolar central transition and carbon-13 NMR competition studies of metal ion binding to ovotransferrin. Can. J. Chem. **89**: 779–788. doi:10.1139/v11-019.

Shcheglovitova, O.N., Maksyanina, E.V., Ionova, I.I., Rustam'yan, Y.L., and Komolova, G.S. 2003. Cow milk angiogenin induces cytokine production in human blood leukocytes. Bull. Exp. Biol. Med. **135**(2): 158–160. doi:10.1023/A:1023871931764. PMID:12802423.

Shestakov, A., Jenssen, H., Nordstrom, I., and Eriksson, K. 2012. Lactoferricin but not lactoferrin inhibit herpes simplex virus type 2 infection in mice. Antiviral Res. **93**(3): 340–345. doi:10.1016/j.antiviral.2012.01.003. PMID:22269645.

Siebert, P.D., and Huang, B.C. 1997. Identification of an alternative form of human lactoferrin mRNA that is expressed differentially in normal tissues and tumor-derived cell lines. Proc. Natl. Acad. Sci. U.S.A. **94**(6): 2198–2203. doi:10.1073/pnas.94.6.2198. PMID: 9122171.

Singh, P.K., Parsek, M.R., Greenberg, E.P., and Welsh, M.J. 2002. A component of innate immunity prevents bacterial biofilm development. Nature, **417**(6888): 552–555. doi:10.1038/417552a. PMID:12037568.

Sohnle, P.G., Hunter, M.J., Hahn, B., and Chazin, W.J. 2000. Zinc-reversible antimicrobial activity of recombinant calprotectin (migration inhibitory factor-related proteins 8 and 14). J. Infect. Dis. **182**(4): 1272–1275. doi:10.1086/315810. PMID:10979933.

Sorensen, M., and Sorensen, S.P.L. 1939. The proteins in whey. C.R. Trav. Lab. Carlsberg, **23**: 55–99.

Spadaro, M., Caorsi, C., Ceruti, P., Varadhachary, A., Forni, G., Pericle, F., and Giovarelli, M. 2008. Lactoferrin, a major defense protein of innate immunity, is a novel maturation factor for human dendritic cells. FASEB J. **22**(8): 2747–2757. doi:10.1096/fj.07-098038. PMID:18364398.

Stallmann, H.P., Faber, C., Bronckers, A.L., de Blieck-Hogervorst, J.M., Brouwer, C.P., Amerongen, A.V., and Wuisman, P.I. 2005. Histatin and lactoferrin derived peptides: antimicrobial properties and effects on mammalian cells. Peptides, **26**(12): 2355–2359. doi:10.1016/j.peptides.2005.05.014. PMID:15979203.

Strøm, M.B., Haug, B.E., Rekdal, O., Skar, M.L., Stensen, W., and Svendsen, J.S. 2002. Important structural features of 15-residue lactoferricin derivatives and methods for improvement of antimicrobial activity. Biochem. Cell Biol. **80**(1): 65–74. doi:10.1139/o01-236. PMID:11908644.

Tang, L., Cui, T., Wu, J.J., Liu-Mares, W., Huang, N., and Li, J. 2010*a*. A rice-derived recombinant human lactoferrin stimulates fibroblast proliferation, migration, and sustains cell survival. Wound Repair Regen. **18**(1): 123–131. doi:10.1111/j.1524-475X.2009.00563.x. PMID:20082685.

Tang, L., Wu, J.J., Ma, Q., Cui, T., Andreopoulos, F.M., Gil, J., et al. 2010*b*. Human lactoferrin stimulates skin keratinocyte function and wound re-epithelialization. Br. J. Dermatol. **163**(1): 38–47. PMID:20222924.

Tibble, J.A., and Bjarnason, I. 2001. Non-invasive investigation of inflammatory bowel disease. World J. Gastroenterol. **7**(4): 460–465. PMID:11819811.

Tomita, M., Wakabayashi, H., Shin, K., Yamauchi, K., Yaeshima, T., and Iwatsuki, K. 2009. Twenty-five years of research on bovine lactoferrin applications. Biochimie, **91**(1): 52–57. PMID: 18585434.

Tsuda, H., Kozu, T., Iinuma, G., Ohashi, Y., Saito, Y., Saito, D., et al. 2010. Cancer prevention by bovine lactoferrin: from animal studies to human trial. Biometals, **23**(3): 399–409. doi:10.1007/s10534-010-9331-3. PMID:20407806.

Valenti, P., and Antonini, G. 2005. Lactoferrin: an important host defence against microbial and viral attack. Cell. Mol. Life Sci. **62**(22): 2576–2587. doi:10.1007/s00018-005-5372-0. PMID: 16261253.

van der Does, A.M., Bogaards, S.J., Jonk, L., Wulferink, M., Velders, M.P., and Nibbering, P.H. 2010. The human lactoferrin-derived peptide hLF1–11 primes monocytes for an enhanced TLR-mediated immune response. Biometals, **23**(3): 493–505. doi:10.1007/s10534-010-9322-4. PMID:20238236.

van der Does, A.M., Joosten, S.A., Vroomans, E., Bogaards, S.J., van Meijgaarden, K.E., Ottenhoff, T.H., et al. 2012*a*. The antimicrobial peptide hLF1–11 drives monocyte-dendritic cell differentiation toward dendritic cells that promote antifungal responses and enhance Th17 polarization. J. Innate Immun., **4**(3): 284–292. doi:10.1159/000332941. PMID:22261275.

van der Does, A.M., Hensbergen, P.J., Bogaards, S.J., Cansoy, M., Deelder, A.M., van Leeuwen, H.C., et al. 2012*b*. The human lactoferrin-derived peptide hLF1-11 exerts immunomodulatory effects by specific inhibition of myeloperoxidase activity. J. Immunol. In press. doi:10.4049/jimmunol.1102777. PMID: 22523385.

van der Kraan, M.I.A., Groenink, J., Nazmi, K., Veerman, E.C.I., Bolscher, J.G.M., and Amerongen, A.V.N. 2004. Lactoferrampin: a novel antimicrobial peptide in the N1-domain of bovine

lactoferrin. Peptides, **25**(2): 177–183. doi:10.1016/j.peptides.2003.12.006. PMID:15062998.

van der Kraan, M.I.A., Nazmi, K., Teeken, A., Groenink, J., van't Hof, W., Veerman, E.C.I., et al. 2005. Lactoferrampin, an antimicrobial peptide of bovine lactoferrin, exerts its candidacidal activity by a cluster of positively charged residues at the C-terminus in combination with a helix-facilitating N-terminal part. Biol. Chem. **386**(2): 137–142. doi:10.1515/BC.2005.017. PMID:15843157.

Voganatsi, A., Panyutich, A., Miyasaki, K.T., and Murthy, R.K. 2001. Mechanism of extracellular release of human neutrophil calprotectin complex. J. Leukoc. Biol. **70**(1): 130–134. PMID:11435495.

Wakabayashi, H., Yamauchi, K., and Takase, M. 2006. Lactoferrin research, technology and applications. Int. Dairy J. **16**: 1241–1251. doi:10.1016/j.idairyj.2006.06.013.

Ward, P.P., Mendoza-Meneses, M., Cunningham, G.A., and Conneely, O.M. 2003. Iron status in mice carrying a targeted disruption of lactoferrin. Mol. Cell. Biol. **23**(1): 178–185. doi:10.1128/MCB.23.1.178-185.2003. PMID:12482971.

Ward, P.P., Paz, E., and Conneely, O.M. 2005. Multifunctional roles of lactoferrin: a critical overview. Cell Mol Life Sci. **62**(22): 2540–2548. doi:10.1007/s00018-005-5369-8. PMID:16261256.

Welling, M.M., Lupetti, A., Balter, H.S., Lanzzeri, S., Souto, B., Rey, A.M., et al. 2001. 99mTc-labeled antimicrobial peptides for detection of bacterial and Candida albicans infections. J. Nucl. Med. **42**(5): 788–794. PMID:11337578.

Yamniuk, A.P., Burling, H., and Vogel, H.J. 2009. Thermodynamic characterization of the interactions between the immunoregulatory proteins osteopontin and lactoferrin. Mol. Immunol. **46**(11–12): 2395–2402. doi:10.1016/j.molimm.2009.04.024. PMID:19477017.

Yang, Y., Kiss, H., Kost-Alimova, M., Kedra, D., Fransson, I., Seroussi, E., et al. 1999. A 1-Mb PAC contig spanning the common eliminated region 1 (CER1) in microcell hybrid-derived SCID tumors. Genomics, **62**(2): 147–155. doi:10.1006/geno.1999.5952. PMID:10610706.

Yen, C.C., Shen, C.J., Hsu, W.H., Chang, Y.H., Lin, H.T., Chen, H.L., and Chen, C.M. 2011. Lactoferrin: an iron-binding antimicrobial protein against Escherichia coli infection. Biometals, **24**(4): 585–594. doi:10.1007/s10534-011-9423-8. PMID:21327478.