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Review

Antiviral activities of lactoferrin

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Abstract

Lactoferrin (LF) is an iron binding glycoprotein that is present in several mucosal secretions. Many biological functions have been ascribed to LF. One of the functions of LF is the transport of metals, but LF is also an important component of the non-specific immune system, since LF has antimicrobial properties against bacteria, fungi and several viruses. This review gives an overview of the present knowledge about the antiviral activities and, when possible, the antiviral modes of action of this protein. Lactoferrin displays antiviral activity against both DNA- and RNA-viruses, including rotavirus, respiratory syncytial virus, herpes viruses and HIV. The antiviral effect of LF lies in the early phase of infection. Lactoferrin prevents entry of virus in the host cell, either by blocking cellular receptors, or by direct binding to the virus particles. © 2001 Published by Elsevier Science B.V.

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1. Structure and origin of lactoferrin

Lactoferrin (LF) is a member of the transferrin gene family. LF is the product of a 35-kb gene and a high degree of homology of this protein between different species is observed (Metz-Boutigue, 1984; Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995). LF is an 80 kDa glycosylated protein, consisting of 692 amino acids (Metz-Boutigue, 1984; Powell and Ogden, 1990; Rey et al., 1990). LF is a net positively charged protein, with a pI in the range of approximately 8.0–8.5 (Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995). The protein consists of a single polypeptide chain, folded in two-symmetric, globular lobes (N- and C-lobe, Fig. 1 (Baveye et al., 1999)). These two lobes are connected with a 'hinge region', which provides additional flexibility to the molecule (Anderson et al., 1989; Vorland, 1999). Each sep-

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arate lobe is capable of binding one metal atom. Metals that are bound by LF are Fe^{2+} or Fe^{3+}-ions, but also the binding of Cu^{2+}, Zn^{2+} and Mn^{2+}-ions has been described (Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995). Between the separate lobes, an internal amino acid homology of 40% is observed and, therefore, it is assumed that during evolution, a gene duplication has resulted in the current LF-gene (Metz-Boutigue, 1984).

Epithelial cells at the mucosa of many mammalian species (Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995) produce LF. As a result, LF is present in several mucosal secretions such as tears, saliva and seminal and vaginal fluids (Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995; Masson and Heremans, 1966). Furthermore, LF is present in the secondary vesicles of neutrophillic granulocytes (Levy, 1996; Baynes and Bezwoda, 1994; Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995; Borregaard et al., 1993). Lactoferrin is present in low concentrations in plasma, approximately 0.2 μg/ml (van der Strate et al., 2000; Baynes et al., 1986), and it is thought that the plasma concentrations are the net result of the spontaneous release from these granulocytes and clearance from the circulation (van der Strate et al., 2000; Baynes et al., 1986). In fact, a linear correlation between plasma LF concentrations and neutrophil counts has been established (van der Strate et al., 2000).

Breast milk is the major source of LF. It is abundantly excreted in colostrum in a concentration up to 7 g/l (the first breast milk that is

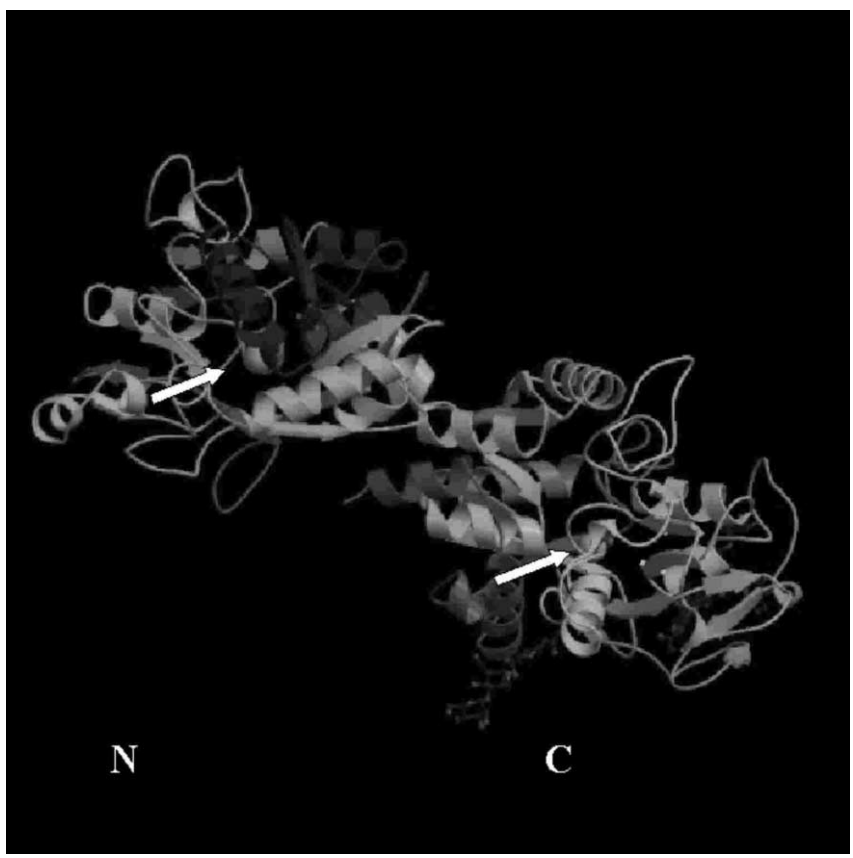

Fig. 1. Chemical structure of LF (adapted from Anderson et al. (Anderson et al., 1989). The N-lobe (N) and C-lobe (C) are connected to each other by the hinge. The white spheres (indicated by arrows) are Fe-ions.

produced post-partum) and the LF concentrations in mature milk decline roughly 7-fold in time during lactation (Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995; Hennart et al., 1991; Montagne et al., 1998; Hirai et al., 1990). LF concentrations in breast milk vary among different mammals, being highest in humans, whereas in rats and dogs no LF has been detected so far (Masson and Heremans, 1971).

2. Pharmacokinetic studies

Pharmacokinetic studies in rats and mice have demonstrated a rapid clearance of LF from the bloodstream by the liver (Ziere et al., 1992; Peen et al., 1998; Regoeczi et al., 1985a; Retegui et al., 1984; Meijer and Ziegler, 1993). Both hepatocytes (Ziere et al., 1992; Mcabee and Esbensen, 1991), as well as Kupffer cells (Peen et al., 1998, 1996) are responsible for uptake of LF. However, higher dosages of LF resulted in prolonged plasma levels (Beljaars et al., 2001). Plasma elimination curves were best described by a two compartment model. The initial plasma half life ($t_{1/2}$) was found to approximately 8 min, while the second component mounted to 220 min. The volume of distribution (V) was found to be 25.1 ml and the initial clearance (Cli) was 0.57 ml/min and an increase in the dosage resulted in an increased plasma $t_{1/2}$ of several hours (Beljaars et al., 2001). In addition, binding to vascular endothelium was observed in vivo. This binding to endothelial cells could be confirmed by in vitro cell binding studies. In addition, LF was found to be associated on membranes of infiltrated leukocytes in various organs and was also detectable in low concentrations in the lymphatic system (Beljaars et al., 2001). LF was also detectable in plasma after i.p. administration. The bioavailability was 0.6%, but could be increased to 3.6% after repeated administration (Beljaars et al., 2001).

Two classes of binding sites for LF on cell membranes have been described. LF can bind with high affinity to a 105 kDa receptor, but binding to low affinity binding sites such as glycosaminoglycans does also occur. The positively charged N-terminus of LF is responsible for the binding to glycosaminoglycans such as heparan sulphate or chondroitin sulphate (Spik et al., 1994; Iyer and Lonnerdal, 1993; Lonnerdal and Iyer, 1995). In addition, the LDL remnant receptor (Ziere et al., 1992; Regoeczi et al., 1985b) and the 45 kDa subunit of the asialoglycoprotein receptor (Bennatt et al., 1997) have been demonstrated to act as receptors for LF.

3. Biological functions of lactoferrin

Since the discovery of LF in bovine (Sorensen and Sorensen, 1939) and human milk (Johansen, 1960), scientists have been intrigued by the function of this protein. First it was thought that LF was a mere iron transporter, since it was able to bind and release metal atoms. Especially during the lactation period, LF may be an important protein for the delivery of essential metals to the newborn (Putman et al., 1999; Brock, 1980; Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995). However, other proteins, like transferrin, are more efficient in the transport of metals and nowadays, it is thought that LF comprises other biological functions.

LF is considered as an important component of the non-specific immune system (Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995; Vorland, 1999). Since the protein is strategically situated at the mucosa, LF plays a role in the first line of defense against microbial infections, since many pathogens tend to enter the body via the mucosa.

LF has bacteriostatic and bacteriocidal activity against both Gram-negative and Gram-positive bacteria (Levy, 1996; Nibbering et al., 2001; Arnold et al., 1977; Hoek et al., 1997; Ellison, 1994; Bellamy et al., 1992). Binding of LF to lipopolysaccharides (LPS) of Gram-negative bacteria may be one of the antibacterial mode of action of LF (Odell et al., 1996; Elass-Rochard et al., 1995; Cohen et al., 1992). In addition, this binding of LF to LPS prevents priming of neutrophils, leading to an inhibition of superoxide anion production (Cohen et al., 1992; Baveye et al., 2000). Furthermore, fungicidal activity, in particular against *Candida* species, has been described (Levay and Viljoen, 1995; Lonnerdal and

Iyer, 1995; Bellamy et al., 1993; Kuipers et al., 1999; Nikawa et al., 1994; Soukka et al., 1992; Kirkpatrick et al., 1971). This antibacterial and antifungal activity is not only achieved by deprivation of iron from the pathogen's micro-environment, but also by binding of the N-terminal region of LF to the cell walls of fungi and bacteria, which causes membrane perturbation and leakage of intracellular components (Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995; Bellamy et al., 1993; Wakabayashi et al., 1996). Plasma LF concentrations are significantly reduced in end-stage AIDS-patients and it is conceivable that, since the specific immune system is already impaired, these lowered LF concentrations, as a component of the non-specific immune system, render these patients more sensitive to opportunistic infections (van der Strate et al., 2000).

4. Antiviral activities of breast milk

For decades it has been generally accepted that breast-feeding is beneficial for the newborn. Comparative studies between bottle-fed and breast-fed children showed that the latter were less confronted with negative sequelae such as diarrhea, that were mediated by bacterial infections. In addition, fewer infections with rotavirus, respiratory syncytial virus (RSV) or vesicular stomatitis virus (VSV) were observed (Lopez Alarcon et al., 1997; Cha et al., 1996; Welsh and May, 1979).

Several constituents in breast milk may have a potentially protective effect. Not only proteins of the non-specific immune system (lysozyme, lactoperoxidase, LF), but also specific immunoglobulins (IgM, IgG and secretory IgA), lipid components, cytokines or prostaglandins help in the protection of the newborn (Hamosh, 1998; el Agamy et al., 1992; Laegreid et al., 1986). Later studies have shown that at least part of the antiviral properties of breast milk can be attributed to a direct antiviral activity of LF. LF comprises antiviral activity against a wide range of human and animal viruses, both RNA- and DNA-viruses. An overview of these antiviral activities and the possible mechanism underlying those activities of LF will be given below.

4.1. Antiviral activities of LF: hepatitis C virus

Hepatitis C virus (HCV) is a member of the *flaviviridae* family (Kato et al., 1996; Tanaka et al., 1995). HCV is an enveloped virus that contains a positive, single strand RNA genome. A unique feature of HCV is its ability to cause a persistent infection. Therefore, HCV is associated with the cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Choo et al., 1989; Kuo et al., 1989).

Little was known about infection and maturation processes of HCV due to the lack of an in vitro culture system. Recently, however, Mituzani et al. (Mizutani et al., 1996) and Ikeda et al. (Ikeda et al., 1998) employed two different human derived cell lines for the replication of HCV. Using these culture systems, an antiviral effect of LF on HCV replication was observed (Ikeda et al., 1998, 2000). The antiviral effect of LF was lost after heat treatment, indicating that the natural conformation of this protein is needed to exert its antiviral effect.

Lactoferricin (LFcin), a tryptic digest obtained from the N-terminal region of the N-lobe, which is strongly bactericidal and fungicidal, proved to be ineffective against HCV. This further illustrates the need for the natural conformation of LF for its antiviral activity (Ikeda et al., 2000). Time of addition assays indicated that LF probably interferes with adsorption of HCV to the target cells: it is most effective if administered before or simultaneous with the viral inoculum. Decrease of incubation times of LF with HCV enhanced viral infection (Ikeda et al., 2000, 1998).

LF can prevent adsorption to target cells by the fact that it binds to the envelope proteins of HCV E1 and E2 (Yi et al., 1997). In addition, it was shown that LF interfered with binding of HCV E2 in vivo, since anti-human LF antibodies, in the presence of LF, were able to co-precipitate secreted and intracellular forms of E2, which were transiently expressed in HepG2 cells. In concordance with others (Ikeda et al., 2000), LFcin did not bind to these envelope proteins E1 or E2 (Yi et al., 1997).

4.2. Antiviral activities of LF: rotavirus

Rotavirus is a member of the *reoviridae*-family (Berkley et al., 1999; Kapikian et al., 1976; Blacklow and Greenberg, 1991). The genome of rotavirus consists of 10 different segments of double stranded RNA, packaged within a three-shelled capsid (Berkley et al., 1999). Rotavirus infections are the most frequent cause of non-bacterial gastroenteritis in neonates and children in the world, causing approximately 1 million death cases world-wide every year (Kapikian et al., 1976; Blacklow and Greenberg, 1991).

LF displays a potent inhibition of a simian rotavirus SA11 in vitro (Superti et al., 1997). In these studies, apo-LF was as potent in inhibiting rotavirus as the metal saturated LF isoforms, but apo-LF had a 600 times higher selectivity index, due to its lack of toxicity. The antiviral mechanism of LF against rotavirus lies in the prevention of adsorption of the virus to the target cell, since LF is capable of binding virus particles, as determined with flow cytometry of virus binding to target cells (Superti et al., 1997). Thus, docking of virus to viral receptors on the target cells is prevented. Since in contrast with many other viruses, rotavirus does not bind to glycosaminoglycans as heparan sulphates (Superti and Donelli, 1995), it is thought that LF cannot compete with rotavirus for binding to its cellular receptors (Superti et al., 1997). Immunohistochemical analysis revealed that LF interfered with antigen synthesis of rotavirus during active infection. Therefore, LF not only prevents infection, but also maintains an antiviral effect after the virus has entered the target cell. The molecular basis for the latter effect is not known at present. Although LF proved to be potent against rotavirus in this study, others (Grover et al., 1997) failed to show any antiviral effect of LF.

4.3. Antiviral activities of LF: friend virus

Friend virus complex (FVC), a murine retrovirus, causes a erythroleukemia in mice within 3 months after infection (Lu et al., 1983). In the early eighties, Lu et al. (Lu et al., 1983) already published an effect of human LF and transferrin on disease progression in mice infected with FVC. Later studies (Lu et al., 1987; Chen et al., 1987; Vorland, 1999) confirmed the antiviral effect of human LF against FVC in their mouse leukemia model. Human LF prolonged survival rates, and decreased viral titres in the spleen of infected mice. For this effect, LF needed to be administered intraperitoneally in the early phase of infection. Even a single bolus injection, if administered within 2 h after infection proved to be effective. Combination of human LF with recombinant murine interferon-γ resulted in synergistic effects.

LF had no direct effect on FVC infection in vitro. Therefore, the antiviral mechanism observed in these animals probably lies in the regulatory effect of LF on the myelopoiesis (Lu et al., 1987). LF was shown to decrease myelopoiesis in bone marrow and the spleen (Gentile and Broxmeyer, 1983; Broxmeyer et al., 1987a,b). Infectivity of FVC is associated with the DNA-synthesis phase of the cycle of the target cell (Schiff and Oliff, 1986). It is postulated that LF is able to accomplish a decrease in cycling status of hemapoietic progenitor cells in vivo. This is confirmed by the regulatory effects of LF in myelopoiesis (Broxmeyer et al., 1986; Broxmeyer and Platzer, 1984; Bagby et al., 1981; Brown et al., 1986; Fletcher and Willars, 1986) and the ability of LF to act as a transcription factor (Fleet, 1995).

4.4. Antiviral activities of LF: poliovirus

Poliovirus is an enterovirus from the *picornaviridae* family (Berkley et al., 1999). Characteristic for picorna viruses is their relatively small genome, consisting of a single stranded positive RNA molecule, which is packaged, in a single capsid without an envelope. The RNA-genome, however, is packaged in a small capsid (Berkley et al., 1999). Infections with poliovirus lead to poliomyelitis, which can cause paralysis of limbs.

Marchetti et al. (Marchetti et al., 1999) have shown antiviral activity of LF against poliovirus in vitro. By addition of LF at various timepoints during infection with poliovirus, the antiviral mechanism of LF against poliovirus was found to be manifest in the early phases of viral infection. Binding of LF to the target cells was confirmed

with immunofluorescent staining, indicating that LF interferes with entry of poliovirus into the target cell. In this study, various LF variants saturated with different metal atoms, such as Fe^{3+}, Zn^{2+} and Mn^{2+}, were tested against poliovirus. Interestingly, Zn^{2+}-LF, which was added after the virus adsorption phase, was still capable of inhibiting viral replication. The authors hypothesised that due to the binding of Zn^{2+}-LF to the target cell, Zn^{2+}-ions were more efficiently delivered to the target cell. The increased availability of Zn^{2+}-ions is a likely cause of impaired poliovirus replication, which was shown as early as in 1976 (Esposito and Obljeski, 1976).

4.5. Antiviral activities of LF: respiratory syncytial virus

Infections with respiratory syncytial virus (RSV), a member of the *paramyxoviridae* family, are the most common cause of acute lower airway infections in infants and children (Berkley et al., 1999). Breast milk has a protective effect against illness from RSV infections (Downham et al., 1976; Pullan et al., 1980). However, little is known about the breast milk components that play a role in the antiviral effect against RSV, although it is thought that immunoglobulins and lipids are the most important components. Nevertheless, breast milk harbours RSV neutralising activity in breast milk that could not be related to presence of immunoglobulins (Laegreid et al., 1986). Moreover, human LF displayed antiviral effect against RSV in concentration ranges well below normal LF levels in breast milk (Grover et al., 1997). The antiviral mode of action of LF against RSV has not been elucidated yet.

4.6. Antiviral activities of LF: HIV

Infection with human immunodeficiency virus (HIV), a member of the *lentiviridae*, causes AIDS. The genome consists of single stranded RNA that is packaged in a capsid. The capsid is surrounded with an envelope, which contains glycoproteins that are involved in the entry of the target cell. Data about LF levels in plasma or saliva of HIV-infected subjects are conflicting. An increase in LF levels (Baynes and Bezwoda, 1994; Mandel et al., 1992) but also decreases in LF levels were observed (Defer et al., 1995; Muller et al., 1992). However, the observed decreases in LF levels were eminent in tears and plasma of symptomatic AIDS patients, who are more often subject to opportunistic infections. Semba et al. (Semba et al., 1998) demonstrated a linear correlation between low maternal serum LF levels and perinatal transmission of HIV to the neonate. All these clinical data demonstrate that LF is involved in the antiviral defense against HIV in vivo.

Bovine, as well as human LF are potent inhibitors of HIV-infection in vitro (Harmsen et al., 1995; Swart et al., 1996; Puddu et al., 1998; Swart et al., 1999). The combination of LF with zidovudine could have synergistic inhibitory effects (Viani et al., 1999).

The antiviral mechanism of LF against HIV takes place in an early phase of infection, probably during adsorption of the virus to target cells (Puddu et al., 1998; Harmsen et al., 1995). The antiviral effect of LF diminishes when LF is administered at increasing time points after infection. LF is capable of binding to the GPGRAF-domain in the V3 loop of the gp120 glycoprotein, albeit to a lesser extent as compared with negatively charged albumins (Swart et al., 1996). The negatively charged hinge region was responsible for binding to the gp120. It is possible that binding to gp120 is responsible for the antiviral effect of LF, since gp120 plays an important role in the adsorption and entry of HIV into target cells by binding to CD4 or chemokinereceptors (Choe et al., 1998; Genoud et al., 1999; Kozak et al., 1999; Siciliano et al., 1999a,b).

In addition, all these studies showed that the iron saturation of LF does not play an important role. Both apo-LF, as well as holo-LF (fully saturated with metal atoms) displays antiviral activity against HIV, although apo-LF remains more potent than holo-LF.

4.7. Antiviral activities of LF: Herpesviridae: herpes simplex viruses

Herpes simplex virus type 1 and 2 (HSV-1 and -2) are members of the α-herpes virus family

(Berkley et al., 1999). The genome of all herpes viruses consists of DNA and infection with HSV can be persistent or latent. Reactivation of HSV-1 and -2 causes mild disease in immunocompetent subjects. However, reactivations in immunocompromised patients such as AIDS-patients, transplant recipients and premature neonates can be quite severe and even life threatening (Hammer et al., 2000). Several groups have reported antiviral effect of bovine and human LF against both HSV-1 and -2. Both apo-LF, as well as holo-LF were capable of inhibiting both viruses (Hasegawa et al., 1994). Later, Fujihara et al. (Fujihara and Hayashi, 1995) reported antiviral activity of LF against HSV-1 in vitro, but also in vivo in a mouse cornea infection model. Topical administration of 1% LF solution significantly decreased infection, however, virus replication was not fully inhibited.

Other groups have confirmed the in vitro antiviral activity of LF against both HSV-1 and -2 (Hammer et al., 2000; Marchetti et al., 1996; Siciliano et al., 1999a,b; Marchetti et al., 1998). The antiviral mechanism lies in the early phase of infection. Using metabolically labelled virions, LF was found to inhibit adsorption of virus to the target cells (Hasegawa et al., 1994; Gross et al., 1994). The metal saturation of LF did not play a significant role in the inhibition of HSV (Marchetti et al., 1998). Furthermore, incubation of target cells with virus, in the presence of LF at 4 °C, followed by a temperature shift to 37 °C prevented internalisation of virus into the target cells. In addition, the observation that virus particles could be bound to latex beads that were coated with LF indicates that entry of virus is at least partially prevented by binding of LF to virus particles (Marchetti et al., 1998, 1996).

Not only intact LF was capable of inhibiting HSV; a tryptic digest of LF was also antivirally active (Hammer et al., 2000; Siciliano et al., 1999a,b). Further purification of the tryptic digest resulted in four different fractions, both from the C- and N-lobe, that displayed antiviral activity (Siciliano et al., 1999a,b). Hammer et al. (Hammer et al., 2000) demonstrated that LFcin, a residue of 24 amino acids derived from the N-lobe, displayed antiviral activity too. Both studies revealed that, although peptide fragments display antiviral activity against HSV, the native protein was more potent (Hammer et al., 2000; Siciliano et al., 1999a,b).

4.7.1. Antiviral activities of LF: Herpesviridae: cytomegalovirus

Cytomegalovirus (CMV) is a member of the β-herpes virus family. Like other herpesviruses, CMV causes a latent and persistent infection (Alford et al., 1990). CMV is often acquired during the early years of life and primary infection is generally unnoticed due to the lack of clinical symptoms. In western countries, up to 60% of the population is carrier of this virus. However, depending on socio-economic status or population density, seropositivity may exceed 90% (Numazaki, 1997; Alford et al., 1990). CMV is able to reactivate under circumstances of immunosuppression. Reactivations in these immunocompromised hosts such as AIDS patients, transplant recipients or pre-term neonates, cause severe morbidity and mortality (Plummer, 1973; Weller, 1971a,b). HIV-infected subjects, who are also seropositive for CMV, progress more rapidly to AIDS (Webster, 1991; Sinicco et al., 1997; Griffiths, 1992, 1998). In fact, symptomatic AIDS patients who suffer frequently from CMV-reactivations with high viral loads are associated with a decreased survival time (Spector et al., 1998; De Jong et al., 1998; Bowen et al., 1996).

The antiviral effect of LF against CMV in vitro was established in 1994 (Hasegawa et al., 1994), later studies confirmed this effect (Harmsen et al., 1995; Clarke and May, 2000; Swart et al., 1999, 1998). LF probably interferes with the entry of virus into the target cell (Andersen et al., 2001), since pre-incubation of target cells with LF is essential for its antiviral effect. Low affinity binding of LF to heparan sulphate proteoglycans (HSPGs) (Damiens et al., 2000; Mann et al., 1994; Van Berkel et al., 1997; Zou et al., 1992) prevents the virus from docking to the target cell (Compton, 1995). The N-terminal region of LF proved to be essential for its antiviral activity. Deletion of the Arg-stretch, which is responsible for binding to HSPGs, gradually diminishes the antiviral activity of LF (Swart et al., 1998, 1999). The po-

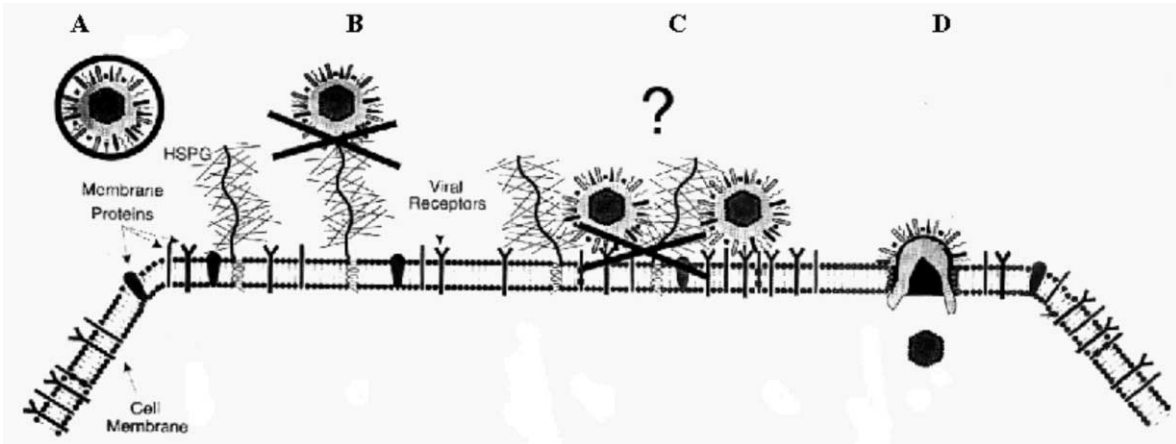

Fig. 2. Schematic representation of different antiviral modes of action of LF against several viruses. LF prevents infection of the host cell by virus particles by either direct binding to virus particles (A), interfering with the docking of virus to cells by binding to HSPGs (B) or by direct binding to viral receptors of the host cell that the virus uses for cell entry (C). Finally, an intracellular activity of LF has been postulated (D).

tency of LF was increased when the positive charge of the protein was increased by chemical modification, whereas addition of negative charge abolished the antiviral effect of LF (Swart et al., 1999).

Although LF has a direct effect on CMV in vitro, an indirect effect of LF against CMV in vivo has been established. In a mouse model for CMV infections, LF protected against a potentially lethal infection with murine CMV (MCMV). The antiviral effect was optimal when LF was administered previous to infection with MCMV (Shimizu et al., 1996). Further studies indicated that the protective effect of LF was due to an upregulation of Natural Killer cells (NK-cells), which eliminated the infection. The stimulation of NK-cells, but also monocytes and granulocytes by LF both in vivo and in vitro has been documented earlier (Crouch et al., 1992; Damiens et al., 1998; Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995).

In vivo studies in transmission of human CMV (HCMV) to neonates by breast feeding indicated that HCMV could hardly be detected in breast milk, the first month post-partum, either by culture or by PCR (Numazaki, 1997; Asanuma et al., 1996). These studies claim a protective effect of LF in the transmission of HCMV to the newborn during the first stage post-partum. However, other studies could not confirm this protective effect (van der Strate et al., 2001).

5. Summary and discussion

Besides a broad antimicrobial spectrum against bacteria and fungi, LF is capable of inhibiting replication of a wide range of viruses. Nearly all studies indicate that LF prevents infection of the host cell, rather than inhibiting virus replication after the target cell has become infected (Fig. 2). Infection of the target cell is prevented by direct binding to virus particles, as described for HCV, polio- and rotavirus, HSV and possibly HIV. Another mechanism for the antiviral activity of LF is binding to host cell molecules that the virus uses as a receptor or co-receptor. For instance binding of LF to HSPGs is a central phenomenon. Many viruses tend to dock on HSPGs of target cells. After this initial contact, the virus particles roll to their specific viral receptor and subsequently enter the host cell, for instance by fusing with the host cell membrane (Laquerre et al., 1998; Sawitzky, 1996; Voigt et al., 1995; Sawitzky et al., 1993; Compton, 1995). Binding of LF to HSPGs prevents this first contact and thus

subsequent infection of the host cell. Interestingly, peptide fragments of LF, such as LFcin do not inhibit most of the viruses tested. Although LFcin is at least partially responsible for the antimicrobial effect against bacteria and fungi, by the formation of pores in the cell wall of fungi and bacteria, this peptide apparently does not seem to be important for the antiviral effect.

For some of the viruses tested it was found that apoLF was more potent than the metal-saturated isoforms of LF. The reason for this is unknown. However, it is speculated that binding of LF to target cells may lead to an increased uptake of metals such as Zn^{2+}, which showed to be antivirally active against poliovirus (Esposito and Obljeski, 1976). Another reason for the increased activity of apo-LF may be that most enzymes, including viral enzymes, require metal ions as a co-factor for their function. It is conceivable that apo-LF is more efficient in the withdrawal of metal ions from the micro-environment, compared with the partially or fully metal saturated isoforms of LF.

Other studies have shown, that LF does not only exert a direct antiviral effect either by binding to target cells or virus particles. An indirect antiviral mode of action of LF is taking place through the upregulation of the antiviral response of the immune system. Administration of LF to cell cultures in vitro, or animals or healthy volunteers led to an upregulation of NK-cells, monocyte/macrophages and granulocytes. These cell types play an important role during the early phases of viral infection, before the specific immune system is upregulated and takes over the antiviral response.

6. Future applications of LF

Currently, the development of severe side effects and the development of antiviral drug resistance complicate antiviral therapy. The selective delivery of antiviral drugs may limit the development of side effects. An advantage of this drug targeting strategy is the fact that fewer side effects may be expected, since the drugs only reach the target cells (in this case, infected cells). Therefore, lower amounts of drugs can be used to gain the same effect compared with conventional therapy. Moreover, more potent, and more often, more toxic drugs can be used.

The intrinsic antiviral activity of LF makes this protein an interesting candidate for application as a drug carrier. In this strategy, conventional drugs are chemically coupled to intrinsically active proteins, which can be modified to specifically home to certain cell types of tissues (Molema et al., 1991; Meijer et al., 1996; Molema and Meijer, 1994). Specific delivery of this drug-carrier-conjugate may prevent the drawbacks that were mentioned earlier. In addition, combination of antiviral drugs with different mechanisms of action may prevent development of drug resistance. Such an approach was taken by us (Jansen et al., 1993) and others for Hepatitis B-virus targeting using lactosaminated proteins and polymer carriers (Di Stefano et al., 1997, 1995; Fiume et al., 1997; Jansen et al., 1993).

Different studies have already demonstrated the synergistic effects of combinations of conventional drugs with LF in vitro. Combination of LF with conventional antifungal drugs led to a synergistic inhibition of *Candida* species (Kuipers et al., 1999; Okutomi et al., 1997). Combination of LF with the anti-CMV drug cidofovir resulted in enhanced inhibition of CMV-infection (van der Strate et al., unpublished research). In concordance, synergistic activity against HIV was suggested on the combination of LF with the nucleoside analogue AZT (Viani et al., 1999).

Therefore, it deserves further studies to combine antivirals with LF, or to couple antiviral drugs to LF. We are presently studying whether covalent binding of cidofovir to LF yields an effective drug targeting preparation for CMV-infected target cells.

References

Alford, C.A., Britt, J.W., 1990. Cytomegalovirus. In: Fields, B.N., Knipe, D.M. (Eds.), Virology, second edition, Raven Press Ltd., New York.

Andersen, J.H., Osbakk, S.A., Vorland, L.H., Traavik, T., Gutteberg, T.J., 2001. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. Antiviral Research 51 (2), 141–149.

Anderson, B.F., Baker, H.M., Norris, G.E., Rice, D.W., Baker, E.N., 1989. Structure of human lactoferrin: Crystallographic structure analysis and refinement at 2.8 Å resolution. Journal of Molecular Biology 209 (4), 711–734.

Arnold, R.R., Cole, M.F., Mcghee, J.R., 1977. A bactericidal effect for human lactoferrin. Science 197, 263–265.

Asanuma, H., Numazaki, K., Nagata, N., Hotsubo, T., Horino, K., Chiba, S., 1996. Role of milk whey in the transmission of human cytomegalovirus infection by breast milk. Microbiology and Immunology 40 (3), 201–204.

Bagby, G.C. Jr, Rigas, V.D., Bennett, R.M., Vandenbark, A.A., Garewal, H.S., 1981. Interaction of lactoferrin, monocytes, and T lymphocyte subsets in the regulation of steady-state granulopoiesis in vitro. Journal of Clinical Investigation 68, 56–63.

Baveye, S., Elass, E., Mazurier, J., Spik, G., Legrand, D., 1999. Lactoferrin: a multifunctional glycoprotein involved in the modulation of the inflammatory process. Clinical Chemistry and Laboratory Medicine 37 (3), 281–286.

Baveye, S., Elass, E., Mazurier, J., Legrand, D., 2000. Lactoferrin inhibits the binding of lipopolysaccharides to L-selectin and subsequent production of reactive oxygen species by neutrophils. FEBS Letters 469 (1), 5–8.

Baynes, R.D., Bezwoda, W.R., 1994. Lactoferrin and the inflammatory response. Advances in Experimental Medicine and Biology 357, 133–141.

Baynes, R.D., Bezwoda, W.R., Khan, Q., Mansoor, N., 1986. Plasma lactoferrin content: differential effect of steroid administration and infective illnesses: lack of effect of ambient temperature at which specimens are collected. Scandinavian Journal of Haematology 37, 353–359.

Beljaars, L., Bakker, H.I., van der Strate, B.W.A., Smit, C., Duijvestijn, A.M., Meijer, D.K.F., Molema, G., 2001. The antiviral protein human lactoferrin distributes in the body to cytomegalovirus infection prone cells and tissues. Pharm. Res., in press.

Bellamy, W., Takase, M., Yamauchi, K., Wakabayashi, H., Kawase, K., Tomita, M., 1992. Identification of the bactericidal domain of lactoferrin. Biochimica et Biophysica Acta 1121, 130–136.

Bellamy, W., Wakabayashi, H., Takase, M., Kawase, K., Shimamura, S., Tomita, M., 1993. Killing of *Candida albicans* by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. Medical Microbiology and Immunology 182 (2), 97–105.

Bennatt, D.J., Ling, Y.Y., Mcabee, D.D., 1997. Isolated rat hepatocytes bind lactoferrins by the RHL-1 subunit of the asialoglycoprotein receptor in a galactose-independent manner. Biochemistry 36 (27), 8367–8376.

Berkley, S.F., Carbon, C.J., Clumeck, N., Durack, D.T., Finch, R.G., Kiehn, T.E., Louria, D.B., McAdam, K.P.W., Norby, S.R., Opal, S.M., Polsky, P., Quie, G., Ronald, A.R., Solberg, C.O., Verhoef, J., 1999. Infectious Diseases. In: Armstrong, D., Cohen, J. (Eds.), Infectious Diseases. Mosby, London.

Blacklow, N.R., Greenberg, H.B., 1991. Viral gastroenteritis. New England Journal of Medicine 325 (4), 252–264.

Borregaard, N., Lollike, K., Kjeldsen, L., Sengelov, H., Bastholm, L., Nielsen, M.H., Bainton, D.F., 1993. Human neutrophil granules and secretory vesicles. European Journal of Haematology 51 (4), 187–198.

Bowen, E.F., Wilson, P., Cope, A., Sabin, C., Griffiths, P., Davey, C., Johnson, M., Emery, V., 1996. Cytomegalovirus retinitis in AIDS patients: influence of cytomegaloviral load on response to ganciclovir, time to recurrence and survival. AIDS 10 (13), 1515–1520.

Brock, J.H., 1980. Lactoferrin in human milk: its role in iron absorption and protection against enteric infection in the newborn infant. Archives of Disease in Childhood 55 (6), 417–421.

Brown, R.D., Yuen, E., Rickard, K.A., Vincent, P.C., Young, G., Kronenberg, H., 1986. Plasma lactoferrin in patients with neutropenia. Blut 52, 289–295.

Broxmeyer, H.E., Platzer, E., 1984. Lactoferrin acts on I-A and I-E/C antigen + subpopulations of mouse peritoneal macrophages in the absence of T lymphocytes and other cell types to inhibit production of granulocyte-macrophage colony stimulatory factors in vitro. Journal of Immunology 133 (1), 306–314.

Broxmeyer, H.E., Bicknell, D.C., Gillis, S., Harris, E.L., Pelus, L.M., Sledge, G.W. Jr, 1986. Lactoferrin: affinity purification from human milk and polymorphonuclear neutrophils using monoclonal antibody (II 2C) to human lactoferrin, development of an immunoradiometric assay using II 2C, and myelopoietic regulation and receptor-binding characteristics. Blood Cells 11, 429–446.

Broxmeyer, H.E., Williams, D.E., Hangoc, G., Cooper, S., Gentile, P., Shen, R.N., Ralph, P., Gillis, S., Bicknell, D.C., 1987a. The opposing actions in vivo on murine myelopoiesis of purified preparations of lactoferrin and the colony stimulating factors. Blood Cells 13 (1-2), 31–48.

Broxmeyer, H.E., Williams, D.E., Cooper, S., Shadduck, R.K., Gillis, S., Waheed, A., Urdal, D.L., Bicknell, D.C., 1987b. Comparative effects in vivo of recombinant murine interleukin 3, natural murine colony-stimulating factor-1, and recombinant murine granulocyte-macrophage colony-stimulating factor on myelopoiesis in mice. Journal of Clinical Investigation 79 (3), 721–730.

Cha, T.A., Tom, E., Kemble, G.W., Duke, G.M., Mocarski, E.S., Spaete, R.R., 1996. Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. Journal of Virology 70 (1), 78–83.

Chen, L.T., Lu, L., Broxmeyer, H.E., 1987. Effects of purified iron-saturated human lactoferrin on spleen morphology in mice infected with Friend virus complex. American Journal of Pathology 126, 285–292.

Choe, H., Martin, K.A., Farzan, M., Sodroski, J., Gerard, N.P., Gerard, C., 1998. Structural interactions between chemokine receptors, gp120 Env and CD4. Seminars in Immunology 10 (3), 249–257.

Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W., Houghton, M., 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244 (4902), 359–362.

Clarke, N.M., May, J.T., 2000. Effect of antimicrobial factors in human milk on rhinoviruses and milk-borne cytomegalovirus in vitro. Journal of Medical Microbiology 49 (8), 719–723.

Cohen, M.S., Mao, J., Rasmussen, G.T., Serody, J.S., Britigan, B.E., 1992. Interaction of lactoferrin and lipopolysaccharide (LPS): effects on the antioxidant property of lactoferrin and the ability of LPS to prime human neutrophils for enhanced superoxide formation. Journal of Infectious Diseases 166 (6), 1375–1378.

Compton, T., 1995. Towards a definition of the HCMV entry pathway. Scandinavian Journal of Infectious Diseases, Supplement 99, 30–32.

Crouch, S.P., Slater, K.J., Fletcher, J., 1992. Regulation of cytokine release from mononuclear cells by the iron-binding protein lactoferrin. Blood 80 (1), 235–240.

Damiens, E., El Yazidi, I., Mazurier, J., Elass-Rochard, E., Duthille, I., Spik, G., Boilly-Marer, Y., 2000. Role of heparan sulphate proteoglycans in the regulation of human lactoferrin binding and activity in the MDA-MB-231 breast cancer cell line. European Journal of Cell Biology 77 (4), 344–351.

Damiens, E., Mazurier, J., el, Y., I., Masson, M., Duthille, I., Spik, G., and Boilly-Marer, Y., 1998. Effects of human lactoferrin on NK cell cytotoxicity against haematopoietic and epithelial tumour cells, Biochimica et Biophysica Acta 1402 (3), 277–287.

De Jong, M.D., Boucher, C.A., Danner, S.A., Gazzard, B., Griffiths, P.D., Katlama, C., Lange, J.M., Richman, D.D., Vella, S., 1998. Summary of the international consensus symposium on management of HIV, CMV and hepatitis virus infections. Antiviral Research 37 (1), 1–16.

Defer, M.C., Dugas, B., Picard, O., Damais, C., 1995. Impairmant of circulating lactoferrin in HIV-1 infection. Cellular and Molecular Biology Noisy Le Grand 41 (3), 417–421.

Di Stefano, G., Busi, C., Mattioli, A., Fiume, L., 1995. Selective delivery to the liver of antiviral nucleoside analogs coupled to a high molecular mass lactosaminated poly-L-lysine and administered to mice by intramuscular route. Biochemical Pharmacology 49 (12), 1769–1775.

Di Stefano, G., Colonna, F.P., Bongini, A., Busi, C., Mattioli, A., Fiume, L., 1997. Ribavirin conjugated with lactosaminated poly-L-lysine. Selective delivery to the liver and increased antiviral activity in mice with viral hepatitis. Biochemical Pharmacology 54 (3), 357–363.

Downham, M.A., Scott, R., Sims, D.G., Webb, J.K., Gardner, P.S., 1976. Breast-feeding protects against respiratory syncytial virus infections. British Medical Journal 2 (6030), 274–276.

el Agamy, E.I., Ruppanner, R., Ismail, A., Champagne, C.P., Assaf, R., 1992. Antibacterial and antiviral activity of camel milk protective proteins. Journal of Dairy Research 59 (2), 169–175.

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

Ellison, R.T. III, 1994. The effects of lactoferrin on Gram-negative bacteria. In: Hutchens, T.W., et al. (Eds.), Lactoferrin: Structure and Function. Plenum Press, New York, pp. 71–90.

Esposito, J.J., Obljeski, J.F., 1976. Enterovirus type 70 virion and intracellular proteins. Journal of Virology 18 (3), 1160–1162.

Fiume, L., Di Stefano, G., Busi, C., Mattioli, A., Gervasi, G.B., Bertini, M., Bartoli, C., Catalani, R., Caccia, G., Farina, C., Fissi, A., Pieroni, O., Giuseppetti, R., D'Ugo, E., Bruni, R., Rapicetta, M., 1997. Hepatotropic conjugate of adenine arabinoside monophosphate with lactosaminated poly-L-lysine. Synthesis of the carrier and pharmacological properties of the conjugate. Journal of Hepatology 26, 253–259.

Fleet, J.C., 1995. A new role for lactoferrin: DNA binding and transcription activation. Nutrition Reviews 53 (8), 226–231.

Fletcher, J., Willars, J., 1986. The role of lactoferrin released by phagocytosing neutrophils in the regulation of colony-stimulating activity production by human mononuclear cells. Blood Cells 11, 447–457.

Fujihara, T., Hayashi, K., 1995. Lactoferrin inhibits herpes simplex virus type-1 (HSV-1) infection to mouse cornea. Archives of Virology 140, 1469–1472.

Genoud, S., Kajumo, F., Guo, Y., Thompson, D., Dragic, T., 1999. CCR5-mediated human immunodeficiency virus entry depends on an amino-terminal gp120-binding site and on the conformational integrity of all four extracellular domains. Journal of Virology 73 (2), 1645–1648.

Gentile, P., Broxmeyer, H.E., 1983. Suppression of mouse myelopoiesis by administration of human lactoferrin in vivo and the comparative action of human transferrin. Blood 61 (5), 982–993.

Griffiths, P.D., 1992. Studies to define viral cofactors for human immunodeficiency virus. Infectious Agents Diseases 1 (5), 237–244.

Griffiths, P.D., 1998. Studies of viral co-factors for human immunodeficiency virus in vitro and in vivo. Journal of General Virology 79 (Pt 2), 213–220.

Gross, V., Arndt, H., Andus, T., Palitzsch, K.D., Scholmerich, J., 1994. Free Radicals in Inflammatory Bowel Diseases Pathophysiology and therapeutic Implications. Hepato-Gastroenterology 41, 320–327.

Grover, M., Giouzeppos, O., Schnagl, R.D., May, J.T., 1997. Effect of human milk prostaglandins and lactoferrin on respiratory syncytial virus and rotavirus. Acta Paediatrics 86 (3), 315–316.

Hammer, J., Haaheim, H., Gutteberg, T.J., 2000. Bovine lactoferrin is more efficient than bovine lactoferricin in inhibiting HSV-I/-II replication in vitro. In: Shimazaki, K. (Ed.), Lactoferrin: Structure, Functions and Applications. Elsevier Science, Amsterdam, pp. 239–243.

Hamosh, M., 1998. Protective function of proteins and lipids in human milk. Biology of the Neonate 74 (2), 163–176.

Harmsen, M.C., Swart, P.J., de Béthune, M.P., Pauwels, R., De Clercq, E., The, T.H., Meijer, D.K.F., 1995. Antiviral effects of plasma and milk proteins: lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication in vitro. Journal of Infectious Diseases 172, 380–388.

Hasegawa, K., Motsuchi, W., Tanaka, S., Dosako, S., 1994. Inhibition with lactoferrin of in vitro infection with human herpes virus. Japanese Journal of Medical Science and Biology 47, 73–85.

Hennart, P.F., Brasseur, D.J., Delogne Desnoeck, J.B., Dramaix, M.M., Robyn, C.E., 1991. Lysozyme, lactoferrin, and secretory immunoglobulin A content in breast milk: influence of duration of lactation, nutrition status, prolactin status, and parity of mother. American Journal of Clinical Nutrition 53 (1), 32–39 Published erratum appears in Am J Clin Nutr 1991 Apr;53(4):988.

Hirai, Y., Kawakata, N., Satoh, K., Ikeda, Y., Hisayasu, S., Orimo, H., Yoshino, Y., 1990. Concentrations of lactoferrin and iron in human milk at different stages of lactation. Journal of Nutritional Science and Vitaminology, Tokyo 36 (6), 531–544.

Hoek, K.S., Milne, J.M., Grieve, P.A., Dionysius, D.A., Smith, R., 1997. Antibacterial activity of bovine lactoferrin-derived peptides. Antimicrobial Agents and Chemotherapy 41 (1), 54–59.

Ikeda, M., Sugiyama, K., Tanaka, T., Tanaka, K., Sekihara, H., Shimotohno, K., Kato, N., 1998. Lactoferrin markedly inhibits hepatitis C virus infection in cultured human hepatocytes. Biochemical and Biophysical Research Communications 245 (2), 549–553.

Ikeda, M., Nozaki, A., Sugiyama, K., Tanaka, T., Naganuma, A., Tanaka, K., Sekihara, H., Shimotohno, K., Saito, M., Kato, N., 2000. Characterization of antiviral activity of lactoferrin against hepatitis C virus infection in human cultured cells. Virus Research 66 (1), 51–63.

Iyer, S., Lonnerdal, B., 1993. Lactoferrin, lactoferrin receptors and iron metabolism. European Journal of Clinical Nutrition 47, 232–241.

Jansen, R.W., Kruijt, J.K., Van Berkel, T.J.C., Meijer, D.K.F., 1993. Coupling of the antiviral drug ara-AMP to Lactosaminated albumin leads to a specific uptake in rat and human hepatocytes. Hepatology 18, 146–152.

Johansen, B.G., 1960. Isolation of an iron containing red protein from human milk. Acta Chemica Scandinavica 14, 510–512.

Kapikian, A.Z., Kim, H.W., Wyatt, R.G., Cline, W.L., Arrobio, J.O., Brandt, C.D., Rodriguez, W.J., Sack, D.A., Chanock, R.M., Parrott, R.H., 1976. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. New England Journal of Medicine 294 (18), 965–972.

Kato, N., Ikeda, M., Mizutani, T., Sugiyama, K., Noguchi, M., Hirohashi, S., Shimotohno, K., 1996. Replication of hepatitis C virus in cultured non-neoplastic human hepatocytes. Japanese Journal of Cancer Research 87 (8), 787–792.

Kirkpatrick, C.H., Green, I., Rich, R.R., Schade, A.L., 1971. Inhibition of growth of *Candida albicans* by iron-unsaturated lactoferrin: relation to host-defense mechanisms in chronic mucocutaneous candidiasis. Journal of Infectious Diseases 124 (6), 539–544.

Kozak, S.L., Kuhmann, S.E., Platt, E.J., Kabat, D., 1999. Roles of CD4 and coreceptors in binding, endocytosis, and proteolysis of gp120 envelope glycoproteins derived from human immunodeficiency virus type 1. Journal of Biological Chemistry 274 (33), 23499–23507.

Kuipers, M.E., de Vries-Hospers, H.G., Eikelboom, M.C., Meijer, D.K.F., Swart, P.J., 1999. Synergistic fungistatic effects of lactoferrin in combination with antifungal drugs against clinical *Candida* isolates. Antimicrobial Agents and Chemotherapy 43 (11), 2635–2641.

Kuo, G., Choo, Q.L., Alter, H.J., Gitnick, G.L., Redeker, A.G., Purcell, R.H., Miyamura, T., Dienstag, J.L., Alter, M.J., Stevens, C.E., 1989. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 244 (4902), 362–364.

Laegreid, A., Kolsto Otnaess, A.B., Orstavik, I., Carlsen, K.H., 1986. Neutralizing activity in human milk fractions against respiratory syncytial virus. Acta Paediatrica Scandinavica 75 (5), 696–701.

Laquerre, S., Argnani, R., Anderson, D.B., Zucchini, S., Manservigi, R., Glorioso, J.C., 1998. Heparan sulfate proteoglycan binding by herpes simplex virus type 1 glycoproteins B and C, which differ in their contributions to virus attachment, penetration, and cell-to-cell spread. Journal of Virology 72 (7), 6119–6130.

Levay, P.F., Viljoen, M., 1995. Lactoferrin: a general review. Haematologica 80 (3), 252–267.

Levy, O., 1996. Antibiotic proteins of polymorphonuclear leukocytes. European Journal of Haematology 56 (5), 263–277.

Lonnerdal, B., Iyer, S., 1995. Lactoferrin: molecular structure and biological function. Annual Review of Nutrition 15, 93–110.

Lopez Alarcon, M., Villalpando, S., Fajardo, A., 1997. Breastfeeding lowers the frequency and duration of acute respiratory infection and diarrhea in infants under six months of age. Journal of Nutrition 127 (3), 436–443.

Lu, L., Broxmeyer, H.E., Meyers, P.A., Moore, M.A., Thaler, H.T., 1983. Association of cell cycle expression of Ia-like antigenic determinations on normal human multipotential (CFU-GEMM) and erythroid (BFU-E) progenitor cells with regulation in vitro by acidic isoferritins. Blood 61 (2), 250–256.

Lu, L., Hangoc, G., Oliff, A., Chen, L.T., Shen, R.-N., Broxmeyer, H.E., 1987. Protective influence of lactoferrin on mice infected with the polycythemia-inducing strain of Friend virus complex. Cancer Research 47, 4184–4188.

Mandel, I.D., Barr, C.E., Turgeon, L., 1992. Longitudinal study of parotid saliva in HIV-1 infection. Journal of Oral Pathology and Medicine 21 (5), 209–213.

Mann, D.M., Romm, E., Migliorini, M., 1994. Delineation of the glycosaminoglycan-binding site in the human inflam-

matory response protein lactoferrin. Journal of Biological Chemistry 269 (38), 23661–23667.

Marchetti, M., Longhi, C., Conte, M.P., Pisani, S., Valenti, P., Seganti, L., 1996. Lactoferrin inhibits herpes simplex virus type 1 adsorption to Vero cells. Antiviral Research 29, 221–231.

Marchetti, M., Pisani, S., Antonini, G., Valenti, P., Seganti, L., Orsi, N., 1998. Metal complexes of bovine lactoferrin inhibit in vitro replication of herpes simplex virus type 1 and 2. BioMetals 11 (2), 89–94.

Marchetti, M., Superti, F., Ammendolia, M.G., Rossi, P., Valenti, P., Seganti, L., 1999. Inhibition of poliovirus type 1 infection by iron-, manganese- and zinc-saturated lactoferrin. Medical Microbiology and Immunology (Berlin) 187 (4), 199–204.

Masson, P.L., Heremans, J.F., 1966. Studies on lactoferrin, the iron binding protein of secretions. Protides of the Biological Fluids 14, 115–124.

Masson, P.L., Heremans, J.F., 1971. Lactoferrin in milk from different species. Comparative Biochemistry and Physiology B 39 (1), 119–129.

Mcabee, D.D., Esbensen, K., 1991. Binding and endocytosis of apo- and holo-lactoferrin by isolated rat hepatocytes. Journal of Biological Chemistry 266, 23624–23631.

Meijer, D.K.F., Ziegler, K., 1993. Mechanisms for the hepatic clearance of oligopeptides and proteins. Implications for rate of elimination, bioavailability and cell-specific drug delivery to the liver. In: Audus, K.L., Raub, T.J. (Eds.), Biological Barriers to Protein Delivery. Plenum Press, New York, pp. 339–408.

Meijer, D.K.F., Molema, G., Moolenaar, F., De Zeeuw, D., Swart, P.J., 1996. (Glyco)-protein drug carriers with an intrinsic therapeutic activity: the concept of dual targeting. Journal of Controlled Release 39, 163–172.

Metz-Boutigue, M.H.S., 1984. Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. European Journal of Biochemistry 145, 659–676.

Mizutani, T., Kato, N., Saito, S., Ikeda, M., Sugiyama, K., Shimotohno, K., 1996. Characterization of hepatitis C virus replication in cloned cells obtained from a human T-cell leukemia virus type 1-infected cell line, MT-2. Journal of Virology 70 (10), 7219–7223.

Molema, G., Meijer, D.K.F., 1994. Targeting of drugs to various blood cell types using (neo-) glycoproteins, antibodies and other protein carriers. Advanced Drug Delivery Reviews 14, 25–50.

Molema, G., Jansen, R.W., Visser, J., Herdewijn, P., Moolenaar, F., Meijer, D.K.F., 1991. Neoglycoproteins as carriers for antiviral drugs: synthesis and analysis of protein-drug conjugates. Journal of Medicinal Chemistry 34, 1137–1141.

Montagne, P., Cuilliere, M.L., Mole, C., Bene, M.C., Faure, G., 1998. Microparticle-enhanced nephelometric immunoassay of lysozyme in milk and other human body fluids. Clinical Chemistry 44 (8 Pt 1), 1610–1615.

Muller, F., Holberg-Petersen, M., Rollag, H., Degre, M., Brandtzaeg, P., Froland, S.S., 1992. Nonspecific oral immunity in individuals with HIV infection. Journal of Acquired Immune Deficiency Syndromes 5, 46–51.

Nibbering, P.H., Ravensbergen, E., Welling, M.M., van Berkel, L.A., van Berkel, P.H., Pauwels, E.K., Nuijens, J.H., 2001. Human lactoferrin and peptides derived from its N terminus are highly effective against infections with antibiotic-resistant bacteria. Infection and Immunity 69 (3), 1469–1476.

Nikawa, H., Samaranayake, L.P., Tenovuo, J., Hamada, T., 1994. The effect of antifungal agents on the in vitro susceptibility of *Candida albicans* to apo-lactoferrin. Archives of Oral Biology 39, 921–923.

Numazaki, K., 1997. Human cytomegalovirus infection of breast milk. FEMS Immunology and Medical Microbiology 18 (2), 91–98.

Odell, E.W., Sarra, R., Foxworthy, M., Chapple, D.S., Evans, R.W., 1996. Antibacterial activity of peptides homologous to a loop region in human lactoferrin. FEBS Letters 382, 175–178.

Okutomi, T., Abe, S., Tansho, S., Wakabayashi, H., Kawase, K., Yamaguchi, H., 1997. Augmented inhibition of growth of *Candida albicans* by neutrophils in the presence of lactoferrin. FEMS Immunology and Medical Microbiology 18 (2), 105–112.

Peen, E., Eneström, S., Skogh, T., 1996. Distribution of lactoferrin and 60/65 kDa heat shock protein in normal and inflamed human intestine and liver. Gut 38, 135–140.

Peen, E., Johansson, A., Engquist, M., Skogh, T., 1998. Hepatic and extrahepatic clearance of circulating human lactoferrin: an experimental study in rat. European Journal of Haematology 61 (3), 151–159.

Plummer, G., 1973. Cytomegaloviruses of man and animals. Progress in Medical Virology 15, 92–125.

Powell, M.J., Ogden, J.E., 1990. Nucleotide sequence of human lactoferrin cDNA. Nucleic Acids Research 18, 4013–4013.

Puddu, P., Borghi, P., Gessani, S., Valenti, P., Belardelli, F., Seganti, L., 1998. Antiviral effect of bovine lactoferrin saturated with metal ions on early steps of human immunodeficiency virus type 1 infection. International Journal of Biochemistry and Cell Biology 30 (9), 1055–1062.

Pullan, C.R., Toms, G.L., Martin, A.J., Gardner, P.S., Webb, J.K., Appleton, D.R., 1980. Breast-feeding and respiratory syncytial virus infection. British Medical Journal 281 (6247), 1034–1036.

Putman, M., Koole, L.A., Van Veen, H.W., Konings, W.N., 1999. The secondary multidrug transporter LmrP contains multiple drug interaction sites. Biochemistry 38 (42), 13900–13905.

Regoeczi, E., Chindemi, P.A., Debanne, M.T., Prieels, J.P., 1985a. Lactoferrin metabolism in the rat liver. American Journal of Physiology 248, G8–G14.

Regoeczi, E., Chindemi, P.A., Debanne, M.T., Prieels, J.P., 1985b. Lactoferrin metabolism in the rat liver. American Journal of Physiology 248, G8–G14.

Retegui, L.A., Moguilevsky, N., Castracane, C.F., Masson, P.L., 1984. Uptake of lactoferrin by the liver. I. Role of the

reticuloendothelial system as indicated by blockade experiments. Laboratory Investigation 50 (3), 323–328.

Rey, M.W., Woldshuk, S.L., de Boer, H.A., Pieper, F.R., 1990. Complete nucleotide sequence of human mammary gland lactoferrin. Nucleic Acids Research 18, 5288–5288.

Sawitzky, D., 1996. Protein-glycosaminoglycan interactions: infectiological aspects. Medical Microbiology and Immunology 184 (4), 155–161.

Sawitzky, D., Voigt, A., Habermehl, K.O., 1993. A peptide-model for the heparin-binding property of pseudorabies virus glycoprotein III. Medical Microbiology and Immunology 182 (6), 285–292.

Schiff, R.D., Oliff, A., 1986. The pathophysiology of murine retrovirus-induced leukemias. Critical Review of Oncology and Hematology 5 (3), 257–323.

Semba, R.D., Miotti, P.G., Lan, Y., Chiphangwi, J.G., Hoover, D.R., Dallabetta, G.A., Yang, L.-P., Saah, A.J., 1998. Maternal serum lactoferrin and vertical transmission of HIV. AIDS 12 (3), 331–332.

Shimizu, K., Matsuzawa, H., Okada, K., Tazume, S., Dosako, S., Kawasaki, Y., Hashimoto, K., Koga, Y., 1996. Lactoferrin-mediated protection of the host from murine cytomegalovirus infection by a T-cell-dependent augmentation of natural killer cell activity. Archives of Virology 141 (10), 1875–1889.

Siciliano, R., Rega, B., Marchetti, M., Seganti, L., Antonini, G., Valenti, P., 1999a. Bovine lactoferrin peptidic fragments involved in inhibition of herpes simplex virus type 1 infection. Biochemical and Biophysical Research Communications 264 (1), 19–23.

Siciliano, S.J., Kuhmann, S.E., Weng, Y.M., Madani, N., Springer, M.S., Lineberger, J.E., Danzeisen, R., Miller, M.D., Kavanaugh, M.P., De Martino, J.A., Kabat, D., 1999b. A critical site in the core of the CCR5 chemokine receptor required for binding and infectivity of human immunodeficiency virus type 1. Journal of Biological Chemistry 274 (4), 1905–1913.

Sinicco, A., Raiteri, R., Sciandra, M., Dassio, G., Bechis, G., Maiello, A., 1997. The influence of cytomegalovirus on the natural history of HIV infection: evidence of rapid course of HIV infection in HIV- positive patients infected with cytomegalovirus. Scandinavian Journal of Infectious Diseases 29 (6), 543–549.

Sorensen, M., Sorensen, S.P.L., 1939. The protein in whey. Trav. Lab. Carlsberg 23, 55–99.

Soukka, T., Tenovuo, J., Lenander Lumikari, M., 1992. Fungicidal effect of human lactoferrin against *Candida albicans*. FEMS Microbiology Letters 69 (3), 223–228.

Spector, S.A., Wong, R., Hsia, K., Pilcher, M., Stempien, M.J., 1998. Plasma cytomegalovirus (CMV) DNA load predicts CMV disease and survival in AIDS patients. Journal of Clinical Investigation 101 (2), 497–502.

Spik, G., Legrand, D., Leveugle, B., Mazurier, J., Mikogami, T., Montreuil, J., Pierce, A., Rochard, E., 1994. Characterization of two kinds of lactotransferrin (lactoferrin) receptors on different target cells. Advances in Experimental Medicine and Biology 357, 13–19.

Superti, F., Donelli, G., 1995. Characterization of SA-11 rotavirus receptorial structures on human colon carcinoma cell line HT-29. Journal of Medical Virology 47 (4), 421–428.

Superti, F., Ammendolia, M.G., Valenti, P., Seganti, L., 1997. Antirotaviral activity of milk proteins: lactoferrin prevents rotavirus infection in the enterocyte-like cell line HT-29. Medical Microbiology and Immunology (Berlin) 186 (2-3), 83–91.

Swart, P.J., Kuipers, M.E., Smit, C., Pauwels, R., de Béthune, M.-P., De Clercq, E., Huisman, H., Meijer, D.K.F., 1996. Antiviral effects of milk proteins: acylation results in polyanionic compounds with potent activity against human immunodeficiency virus type 1 and 2 in vitro. AIDS Research and Human Retroviruses 12, 769–775.

Swart, P.J., Kuipers, M.E., Smit, C., van der Strate, B.W.A., Harmsen, M.C., Meijer, D.K.F., 1998. Lactoferrin: antiviral activity of lactoferrin. In: Spik, G., Legrand, D., Mazurier, J., Pierce, A., Perraudin, J.P. (Eds.), Advances in Lactoferrin Research. Plenum Press, New York, pp. 205–213.

Swart, P.J., Harmsen, M.C., Kuipers, M.E., Van Dijk, A.A., van der Strate, B.W.A., Van Berkel, P.H.C., Nuijens, J.H., Smit, C., Witvrouw, M., De Clercq, E., de Béthune, M.-P., Pauwels, R., Meijer, D.K.F., 1999. Charge modification of plasma and milk proteins results in antiviral active compounds. Journal of Peptide Science 5 (12), 563–576.

Tanaka, T., Kato, N., Cho, M.J., Shimotohno, K., 1995. A novel sequence found at the 3′ terminus of hepatitis C virus genome. Biochemical and Biophysical Research Communications 215 (2), 744–749.

Van Berkel, P.H.C., Geerts, M.E.J., Van Veen, H.A., Mericskay, M., de Boer, H.A., Nuijens, J.H., 1997. N-terminal stretch Arg^2, Arg^3, Arg^4 and Arg^5 of human lactoferrin is essential for binding to heparin, bacterial lipopolysaccharide, human lysozyme and DNA. Biochemical Journal 328, 145–151.

van der Strate, B.W.A., Harmsen, M.C., The, T.H., Sprenger, H.G., De Vries, H., Eikelboom, M.C., Kuipers, M.E., Meijer, D.K.F., Swart, P.J., 2000. Plasma Lactoferrin levels are decreased in end-stage AIDS-patients. Viral Immunology 12 (3), 197–203.

van der Strate, B.W.A., Harmsen, M.C., Schafer, P., Swart, P.J., The, T.H., Jahn, G., Speer, C.P., Meijer, D.K.F., Hamprecht, K., 2001. Viral load and not lactoferrin concentrations correlates with the transmission of HCMV to the preterm neonate. Clinical and Diagnostic Laboratory Immunology 8, 818–821.

Viani, R.M., Gutteberg, T.J., Lathey, J.L., Spector, S.A., 1999. Lactoferrin inhibits HIV-1 replication in vitro and exhibits synergy when combined with zidovudine. AIDS 13 (10), 1273–1274.

Voigt, A., Sawitzky, D., Zeichhardt, H., Habermehl, K.O., 1995. Cellular receptor structures for pseudorabies virus are blocked by antithrombin III. Medical Microbiology and Immunology 184 (2), 97–103.

Vorland, L.H., 1999. Lactoferrin: a multifunctional glycoprotein. Apmis 107 (11), 971–981.

Wakabayashi, H., Abe, S., Okutomi, T., Tansho, S., Kawase, K., Yamaguchi, H., 1996. Cooperative anti-*Candida* effects of lactoferrin or its peptides in combination with azole antifungal agents. Microbiology and Immunology 40 (11), 821–825.

Webster, A., 1991. Cytomegalovirus as a possible cofactor in HIV disease progression. Journal of Acquired Immune Deficiency Syndrome 1 (Suppl 4), S47–52.

Weller, T.H., 1971a. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. II. New England Journal of Medicine 285 (5), 267–274.

Weller, T.H., 1971b. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations I. New England Journal of Medicine 285 (4), 203–214.

Welsh, J.K., May, J.T., 1979. Anti-infective properties of breast milk. Journal of Pediatrics 94 (1), 1–9.

Yi, M.Y., Kaneko, S., Yu, D.Y., Murakami, S., 1997. Hepatitis C virus envelope proteins bind lactoferrin. Journal of Virology 71 (8), 5997–6002.

Ziere, G.J., Van Dijk, M.C.M., Bijsterbosch, M.K., Van Berkel, T.J.C., 1992. Lactoferrin uptake by the rat liver: characterization of the recognition site and effect of selective modification of arginine residues. Journal of Biological Chemistry 267, 11229–11235.

Zou, S., Magura, C.E., Hurley, W.L., 1992. Heparin-binding properties of lactoferrin and lysozyme. Comparative Biochemistry and Physiology B: Comparative Biochemistry 103, 889–895.