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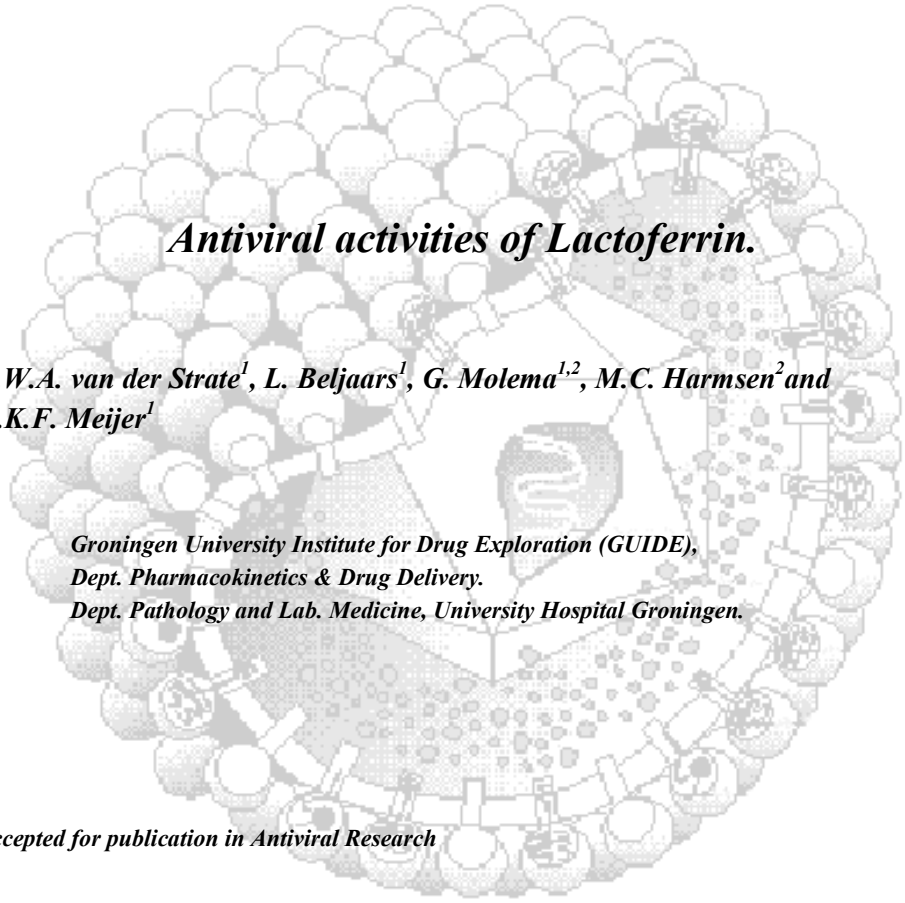

Antiviral activities of Lactoferrin.

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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 mechanisms of this protein. Lactoferrin displays antiviral activity against both DNA- and RNA-viruses, including rotavirus, respiratory syncitial 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.

1.1: 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 [76,78,93]. LF is an 80 kD glycosylated protein, consisting of 692 amino acids [93,107,114]. LF is a net positively charged protein, with a pI in the range of approximately 8.0-8.5 [76,78]. The protein consists of a single polypeptide chain, folded in 2 symmetric, globular lobes (N- and C-lobe, Fig. 1 [7]). These two lobes are connected with a "hinge region", which provides additional flexibility to the molecule [2,140]. Each separate 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 [76,78]. 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 [93].

Fig. 1: Chemical structure of LF *(adapted from Anderson et al. [2])***.**

Epithelial cells at the mucosa of many mammalian species [76,78] produce LF. As a result, LF is present in several mucosal secretions such as tears, saliva and seminal and vaginal fluids [76,78,87]. Furthermore, LF is present in the secondary vesicles of neutrophillic granulocytes [8,16,76-78]. Lactoferrin is present in low concentrations in plasma, approximately 0.2 μg/ml [9,137], and it is thought that the plasma concentrations are the net result of the spontaneous release from these granulocytes and clearance from the circulation [9,137]. In fact, a linear correlation between plasma LF concentrations and neutrophil counts has been established [137]. 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 produced post partum) and the LF concentrations in mature milk decline roughly sevenfold in time during lactation [60,61,76,78,97]. 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 [88].

1.2: Pharmacokinetic studies.

Pharmacokinetic studies in rats and mice have demonstrated a rapid clearance of LF from the bloodstream by the liver [92,105,112,113,147]. Both hepatocytes [89,147] as well as Kupffer cells [104,105] are responsible for uptake of LF. However, higher dosages of LF resulted in prolonged plasma levels [10]. Plasma elimination curves were best described by a two compartiment model. The initial plasma half life (t1/2) was found to approximately 8 min, while the second component mounted to 220 min. The volume of distribution (V) was found to 25.1 ml and the the initial clearance (Cli) was 0.57 ml/min. and an increase in the dosage resulted in an increased plasma t1/2 of several hours [10]. 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 [10]. LF was also detectable in plasma after i.p. administration. The bioavalailability was 0.6%, but could be increased to 3.6% after repeated administration [10].

Two classes of binding sites for LF on cell membranes have been described. LF can bind with high affinity to a 105 kD 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 [65,78,127]. In addition, the LDL remnant receptor [112,147] and the 45kD subunit of the asialoglycoprotein receptor [13] have been demonstrated to act as receptors for LF.

1.3: Biological functions of Lactoferrin.

Since the discovery of LF in bovine [124] and human milk 1960 [67], 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 [18,76,78,111]. 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 [76,78,140]. 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 [3,11,44,62,77,99]. Binding of LF to lipopolysaccharides (LPS) of gram-negative bacteria may be one of the antibacterial mechanisms of LF[30,42,43,102]. In addition, this binding of LF to LPS prevents priming of neutrophils, leading to an inhibition of superoxide anion production [6,30].

Furthermore, fungicidal activity, in particular against *Candida* species, has been described [12,70,72,76,78,100,125]. 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 [12,76,78,141]. 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 [137].

1.4: 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 diarrhoea, that were mediated by bacterial infections. In addition, fewer infections with rotavirus, Respiratory Syncitial virus (RSV) or Vesicular Stomatitis Virus (VSV) were observed [25,79,145].

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 [41,57,74]. 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.

1.4.1: Antiviral activities of LF: Hepatitis C virus.

Hepatitis C virus (HCV) is a member of the *flaviviridae* family [69,133]. 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 [28,73]. At least 6 major HCV genotypes have been identified, which are subdivided into more than 50 subtypes [24,110]. This genetic diversity of HCV plays a role in the immune evasion of HCV and has held back the development of an adequate vaccine. Treatment of HCV infections has not been very successful. Interferons have been used, however only with a low success rate of up to maximally 30% [64,120]. More success was obtained when a combination therapy of interferons and ribavirin was applied [90].

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 [94] and Ikeda et al [64] 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 [63,64]. 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 [63]. 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 [63,64].

LF can prevent adsorption to target cells by the fact that it binds to the envelope proteins of HCV E1 and E2 [146]. 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 [63], LFcin did not bind to these envelope proteins E1 or E2 [146].

1.4.2: Antiviral activities of LF: Rotavirus.

Rotavirus is a member of the *reoviridae*-family [14,15,68]. The genome of rotavirus consists of 10 different segments of double stranded RNA, packaged within a three-shelled capsid [14]. Rotavirus infections are the most frequent cause of non-bacterial gastro-enteritis in neonates and children in the world, causing approx. 1 million death cases world-wide every year [15,68].

LF displays a potent inhibition of a simian rotavirus SA11 by LF *in vitro* [128]. 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. 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 [129], it is thought that LF cannot compete with rotavirus for binding to its cellular receptors [128]. 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 [55] failed to show any antiviral effect of LF.

1.4.3: Antiviral activities of LF: Friend virus.

Friend virus complex (FVC), a murine retrovirus, causes an erythroleukemia in mice within 3 months after infection [80]. In the early eighties, Lu et al. [80] already published an effect of human LF and transferrin on disease progression in mice infected with FVC. Later studies, [26,81,140] confirmed the antiviral effect of human LF against FVC in their mouse leukaemia 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 hrs 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 [81]. LF was shown to decrease myelopoiesis in bone marrow and the spleen [22,23,51]. Infectivity of FVC is associated with the DNA-synthesis phase of the cycle of the target cell [117]. It is postulated that LF is able to accomplish a decrease in cycling status of hematopoietic progenitor cells *in vivo*. This is confirmed by the regulatory effects of LF in myelopoiesis [5,19-21,48] and the ability of LF to act as a transcription factor [47].

1.4.4: Antiviral activities of LF: Poliovirus.

Poliovirus is an enterovirus from the *picornaviridae* family [14]. 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 [14]. Infections with poliovirus lead to poliomyelitis, which can cause paralysis of limbs. As a result of vaccination programs, poliovirus has been eradicated in the

industrialised West. However, poliovirus infections are still a problem in developing countries.

Marchetti et al. [86] 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 [45].

1.4.5: Antiviral activities of LF: Respiratory Syncitial 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 [14]. Breast milk has a protective effect against illness from RSV infections [40,109]. 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 [74]. Moreover, human LF displayed antiviral effect against RSV in concentration ranges well below normal LF levels in breast milk [55]. The antiviral mechanism of LF against RSV has not been elucidated yet.

1.4.6: Antiviral activities of LF: HIV.

Human Immunodeficiency Virus (HIV), is a member of the lentiviridae. 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 [8,82] but also decreases in

LF levels were observed [37,98]. 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.[118] 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 defence against HIV *in vivo*.

Bovine as well as human LF are potent inhibitors of HIV-infection *in vitro*. [58,108,130,131]. The combination of LF with zidovudine could have synergistic inhibitory effects [138].

The antiviral mechanism of LF against HIV takes place in an early phase of infection, probably during adsorption of the virus to target cells [58,108]. 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 to negatively charged albumins [131]. The negatively charged hinge region of LF was responsible for the binding to 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 [27,50,71,122].

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.

1.4.7: Antiviral activities of LF: Herpesviridae.

Herpes simplex virus types 1 and 2 (HSV-1 and –2) are members of the α-herpes virus family [14]. 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 [56]. Antiviral treatment with acyclovir or its derivatives is successful in limiting the infection. However, this treatment is increasingly complicated by antiviral resistance [31]. 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 [59]. Later, Fujihara et al. [49] 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 &-2 [56,84,85,121]. 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 [54,59]. The metal saturation of LF did not play a significant role in the inhibition of HSV [85]. 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 [84,85].

Not only intact LF was capable of inhibiting HSV; a tryptic digest of LF was also antivirally active [56,121]. Further purification of the tryptic digest resulted in 4 different fractions, both from the C- and N-lobe, that displayed antiviral activity [121]. Hammer et al.[56] 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 [56,121].

1.4.8: Antiviral activities of LF: Cytomegalovirus.

Cytomegalovirus (CMV) is a member of the β-herpes virus family. Like other herpesviruses, CMV causes a latent and persistent infection [1]. 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% [1,101]. CMV is able to reactivate under circumstances of immunosuppression. Reactivations in these immunocompromised hosts such as AIDS patients, transplant recipients or preterm neonates, cause severe morbidity and mortality [106,143,144]. HIV-infected subjects, who are also seropositive for CMV, progress more rapidly to AIDS [52,53,123,142]. In

fact, symptomatic AIDS patients who suffer frequently from CMV-reactivations with high viral loads are associated with a decreased survival time [17,36,126].

The antiviral effect of LF against CMV *in vitro* was established in 1994 [59], later studies confirmed this effect [29,58,130,132]. LF probably interferes with the entry of virus into the target cell, since preincubation of target cells with LF is essential for its antiviral effect. Low affinity binding of LF to heparan sulphate proteoglycans (HSPGs) [34,83,134,148] prevents the virus from docking to the target cell [32]. 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 [130,132]. The potency 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 [130].

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 [119]. 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 [33,35,76,78].

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 [4,101]. 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 [136].

Summary.

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 it inhibits 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 [32,75,115,116,139]. 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 apo-LF 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 [45]. 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 to the partially or fully metal saturated isoforms of LF.

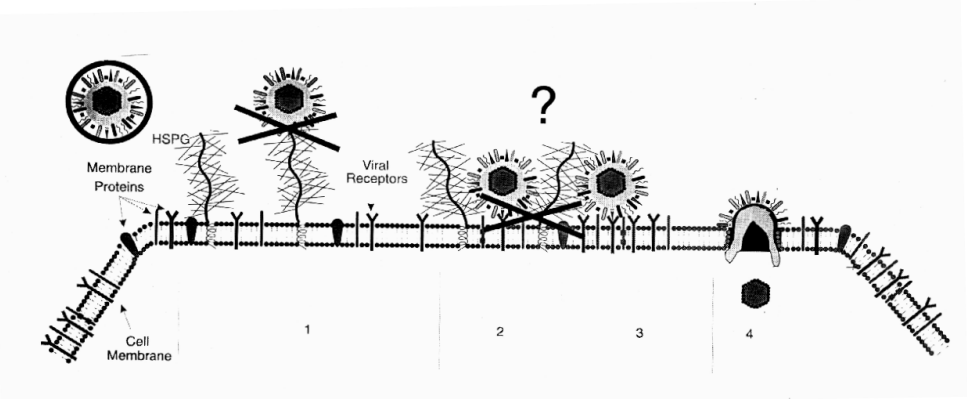

Fig. 2: Schematic representation of the antiviral mechanisms of LF*. LF prevents infection of the host cell by virus particles by either direct binding to virus particles. In addition, docking of the virus is prevented by binding to HSPGs or by direct binding to viral receptors of the host cell. Finally, an intracellular activity of LF has been postulated.*

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 mechanism 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.

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 to 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 [91,95,96]. Specific delivery of this drug-carrier-conjugate may prevent the backdraws 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 [66]and others for Hepatitis B-virus targeting using lactosaminated proteins and polymer carriers [38,39,46,66].

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 [72,103]. Combination of LF with the anti-CMV drug cidofovir resulted in enhanced inhibition of CMV-infection[135]. In concordance, synergistic activity against HIV was suggested on the combination of LF with the nucleoside analogue AZT [138].

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.

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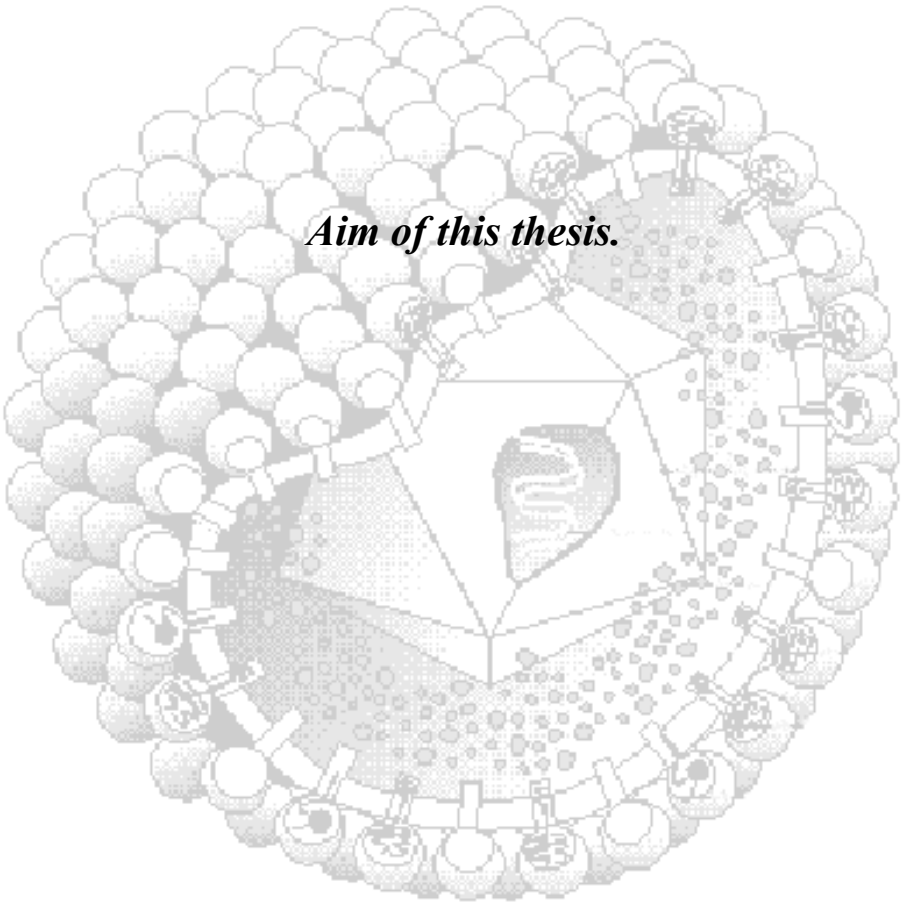

Aim of this thesis.

Cytomegalovirus (CMV) is a member of the ß-herpes virus family. Like other herpes viruses, the virus establishes a latent or persistent infection after primary infection. Primary infections with CMV are generally unnoticed due to the lack of symptoms. Approximately 60% of the western population have experienced an infection with CMV, but dependent on socio-economic status or population density this number may reach values up to 100% [1,11].

In the immunocompromised patients such as transplant recipients, premature neonates and AIDS patients, primary infections or reactivations of CMV can cause severe morbidity or even mortality [1,12,19,20]. In fact, CMV can enhance the progress of AIDS in HIV-infected subjects [6,7,16,18] and frequent reactivations of CMV with high viral loads are associated with a decreased survival time of AIDS patients [4,5,17]. In transplant recipients, primary infections or reactivations of CMV have been associated with the development of both acute and chronic transplant dysfunctions [2,13,14].

Currently, treatment of CMV infections is complicated by the development of severe side effects and drug resistance [3,21 10,15]. Especially in the immunocompromised patients, who need continuous treatment due to the lack of a proper immune response, side effects and drug resistance are problematic. These side effects would be less pronounced if the antiviral drugs were specifically delivered to infected cells only. In addition, a lesser amount of drugs would be needed to gain the same effect. As a result of the selective delivery of antiviral drugs, more potent, and thus more toxic, drug could be used.

In this drug targeting concept, (chemically modified) glycoproteins, which are specific for a certain tissue or cell type, can be used as a carrier molecule for the specific delivery of conventional antiviral drugs. The use of intrinsically antivirally active carrier proteins in combination with conventional antiviral drugs with different mechanisms of action may result in a simultaneous inhibition in different steps of viral entry and replication of the virus. As a result, the development of drug resistance may be prevented or at least delayed.

Since LF had displayed a potent antiviral activity against CMV *in vitro* [8,9], we were interested in the application of lactoferrin (LF) as an intrinsically active drug carrier. *Aim of this thesis* was to investigate whether LF represents a potential carrier for the selective delivery of antiviral drugs. Therefore, we decided to study the mechanism of antiviral activity of LF *in vitro*. In addition, combinations of

conventional antiviral drugs with LF were tested against CMV *in vitro*. Finally, two rat models for CMV infection were employed to evaluate the antiviral effect of LF *in vivo.*

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