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Lactoferrin inhibits human papillomavirus binding and uptake in vitro

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Abstract

Lactoferrin (LF), a member of the transferrin family, is a bi-globular protein secreted in milk, saliva, tears, seminal fluid, endocervix and vaginal secretions. LF is an important player in the defence against pathogenic microorganisms and has also been shown to have activity against several viruses including herpesvirus, adenovirus, rotavirus and poliovirus. The antiviral activity of LF is directed against the early steps of viral infection and the LF antiviral effect against herpesvirus is mediated through LF binding to the herpesvirus receptor heparan sulfate. Human papillomavirus (HPV) causes genital warts and is a prerequisite for cervical cancer. HPV can also use heparan sulfate on the cell surface as a receptor. We studied the inhibition by LF on HPV entry by incubating HaCaT cells and HPV-16 virus-like particles (VLPs) with either human (HLF) or bovine lactoferrin (BLF). LF inhibited internalization of HPV-16 particles using CFDA-SE-labelled VLPs that only fluoresce after internalisation. By using a western blot assay we also found dose-dependent LF inhibition of HPV-16 VLP binding to the HaCaT cell surface. BLF was a more potent inhibitor of HPV entry than human LF. It was also clear that LF acted early in the HPV uptake process.

Keywords: Lactoferrin; Human papillomavirus; Heparan sulfate; Antiviral activity

1. Introduction

Lactoferrin (LF) is a bi-globular (N- and C-lobe) protein consisting of a single polypeptide chain divided by a hinge region. The two globes can both bind one metal ion, such as Fe^{2+}, Fe^{3+}, Cu^{2+} or Zn^{2+}. The molecular weight of LF is 80 kDa and the gene shows a high degree of homology between different species. LF is a member of the transferrin family and can be found in mucus secretions, milk, saliva, tears, seminal fluids and vaginal secretions. The highest concentrations of LF is found in colostrum with concentrations up to 7 g/l (Cohen et al., 1987). LF is expressed in human endocervix and by most of the endocervical glands (Farley et al., 1997). In human vaginal mucus the concentration varies from 62.9 to 218 μg/mg of protein after menses and 3.8 to 11.4 μg/mg of protein before menses. Women taking oral contraceptives show no variation in LF level during the menses cycle. This suggests hormonal control of LF levels (Cohen et al., 1987). Furthermore, LF can be found in the secondary vesicles of neutrophilic granulocytes (Hansen et al., 1975).

LF plays an important role in the primary defence against pathogenic microorganisms. It has antimicrobial activities against both Gram-negative and Gram-positive bacteria and fungicidal activities against *Candida* (Bellamy et al., 1993). It also has antiviral activities against rotavirus, herpesviruses, poliovirus, respiratory syncytial virus, hepatitis C virus, human immunodeficiency virus (van der Strate et al., 2001), adenovirus (Di Biase et al., 2003), enterovirus 71 (Lin et al., 2002)), hepatitis B virus (Hara et al., 2002) and hantavirus (Murphy et al., 2001). Furthermore, lactoferricin, the tryptically cleaved N-terminal part of LF, has been shown to have bactericidal (Vorland et al., 1999), fungicidal (Bellamy et al., 1993) as well as antiviral activity. For virus inhibition lactoferricin is active against herpes simplex virus (Andersen et al., 2003), human cytomegalovirus (Andersen et al., 2001), adenovirus (Di Biase et al., 2003) and feline calicivirus (McCann et al., 2003). The interactions and capacities of LF to reduce infections are exerted in different ways between virus

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species. It has been suggested that LF bind directly to rotavirus (Superti et al., 1997), poliovirus (Marchetti et al., 1999), HIV-1 (Swart et al., 1996) and hepatitis C virus (Yi et al., 1997), thereby preventing adsorption of the virus to target cells in vitro. In a different manner, the antiviral activity of LF against herpes simplex virus (Marchetti et al., 1996) and human cytomegalovirus (Andersen et al., 2001) is probably due to the capability of LF to bind to heparan sulfate proteoglycans on the cell surface and in that way interfere with the binding of the virus to the target cell. There are several receptors described for LF, one is a 105 kDa receptor (Spik et al., 1994) and another is glucoseaminoglycans (Di Biase et al., 2003; Marchetti et al., 1996). The positively charged N-terminal part of LF is responsible for binding of LF to glycosaminoglycans (Di Biase et al., 2003; Wu et al., 1995).

Human papillomavirus (HPV) belongs to the papillomaviridae family and is a 8 kb non-enveloped double stranded DNA virus. Infection of certain HPV types is a prerequisite for cervical carcinoma (zur Hausen, 2002). HPV is also the causative agent of condyloma acuminata, larynx papilloma and skin warts. Because, HPVs are highly tissue specific and are strongly linked to the differentiation stage of the epithelial cells, HPV have been difficult to propagate in vitro. Recently infectious virions have been produced and used in cell culture infection assays (McLaughlin-Drubin et al., 2003; Meyers et al., 2002; Ozbun, 2002a; Ozbun, 2002b). For studying the early stages of HPV infection, virus-like particles (VLPs) from different types of HPVs have been produced consisting of either the capsid protein L1 alone or both capsid proteins L1 and L2 (Hagensee et al., 1993; Kirnbauer et al., 1992; Park et al., 1993; Rose et al., 1993). When these proteins are expressed in eukaryotic cells they self-assemble into empty particles with similar characteristics with virions, which can be used to study viral uptake and binding. HPV have been reported to bind to several different cell lines (Muller et al., 1995; Qi et al., 1996; Roden et al., 1994; Volpers et al., 1995). α-6 Integrin has been suggested as a candidate receptor for HPV (Evander et al., 1997; Yoon et al., 2001) and the cell surface glycosaminoglycan heparan sulfate have been shown to be required for infection (Combita et al., 2001; Drobni et al., 2003; Giroglou et al., 2001; Joyce et al., 1999; Shafti-Keramat et al., 2003). It is not known how HPV is affected by components of the innate immune defence, such as LF and since, LF has the capability to interact with heparan sulfate and thereby, inhibit virus-receptor interactions for several studied viruses, we wanted to investigate the anti-viral activity of LF against HPV.

2. Materials and methods

2.1. VLPs and CFDA-SE labelling

HPV 16-L1 VLPs were produced using previously described recombinant baculovirus in Sf-21 insect cell lines (Qi et al., 1996). The recombinant baculovirus was a kind gift from Ian Frazer, CICR, Brisbane, Australia. Fluorescent VLPs were constructed by adding carboxy-fluorescein diacetate, succinimidyl ester (CFDA SE) (Molecular Probes Inc., Eugene, OR) to VLPs. CFDA SE stock was prepared by dissolving 500 μg CFDA SE in 90 μl DMSO supplied by the manufacturer. Then, 10 μg VLPs were mixed with 100 μM CFDA-SE in a final volume of 1 ml in PBS (pH 7.4) and incubated over night in room temperature in the dark. The particles were then dialysed against 3 × 2 l of PBS.

2.2. Proteins and antibodies

Bovine lactoferrin (BLF) from milk and human lactoferrin (HLF) from milk was purchased from Sigma chemical Co. (St. Louis, MO). Anti HPV 16-L1 antibody (MAB 885) was purchased from Chemicon International Inc. and horseradish peroxidase (HRP) conjugated antibodies were from Dakopatts AB (Älvsjö, Sweden)

2.3. Internalization assay

HaCaT (human epithelial) cells were grown to approximately 80% confluence in a 75 cm^2 bottle in DMEM plus 10% FCS (Life Technologies, Inc., Gaithersburg, MD). Cells were trypsinated, dislodged, diluted to 10 ml in DMEM and grown in suspension for 2 h at 37 °C with occasional swirling. The CFDA-SE-labelled VLPs (300 ng) were mixed with appropriate concentration of LF for 60 min at 37 °C in 200 μl serum-free medium and then added to 2×10^5 cells. Control cells were incubated with CFDA-SE-labelled VLPs without LF. After incubation for 2 h the cells were washed twice in PBS/2% BSA and then analysed by flow cytometry as described previously (Drobni et al., 2003)

2.4. VLP Binding assay

The HaCaT cells were grown, trypsinated, dislodged and grown in suspension as above. HPV-16 VLPs (300 ng) were incubated with the appropriate concentration of LF in 200 μl serum-free medium for 30 min at 0 °C. The mixture was then added to cells (2×10^5) and incubated for 30 min at 0 °C. For analysis of binding, cells were washed twice in ice cold PBS before resuspending in 15 μl SDS-PAGE loading buffer. Western blot using a antibody directed against HPV L1 was used for detection. The bands were quantified using the GelPro analyser software (Media Cybernetics, Silver Spring, MD)

2.5. Addition time point assay

The HaCaT cells were grown, trypsinated, dislodged and grown in suspension as above.

CFDA-SE-labelled HPV 16 VLPs (500 ng) were added to the cells and incubated at 37 °C. Then 100 μg/ml HLF or 10 μg/ml BLF was added together with 200 μl of serum-free medium after different time points ranging from 0 to 150 min.

The samples were then incubated at 37 °C for 1 h. Positive controls were treated as 150 min samples but without addition of LF. Negative controls were incubated with labelled VLPs for 30 min on ice. The cells were washed twice with PBS and were then analysed by flow cytometry as described previously (Drobni et al., 2003).

3. Results

3.1. LF inhibits HPV internalization

The inhibitory effect of both HLF and BLF on HPV-16 VLP internalisation in HaCaT cells after pre-treatment with LF was studied. To detect internalization of HPV we directly labelled HPV-16 L1 VLPs with the carboxy-fluorescein diacetate, succinimidyl ester (CFDA SE) (Drobni et al., 2003). Inside cells, acetate groups on the CFDA SE are cleaved off by intracellular esterases and yields highly fluorescent particles. The uptake was then studied by measuring fluorescent cells using flow cytometry. A dose-dependent inhibition of HPV VLP internalization was detected when using BLF, but not with HLF (Fig. 1). The most effective inhibition was by BLF with an IC_{50} of 35 μg/ml. HLF was not as effective and only reduced uptake by 20% even at concentrations of 250 μg/ml (data not shown).

3.2. HPV binding was inhibited by bovine and human LF

Although, BLF was a potent inhibitor of HPV uptake it did not completely inhibit HPV VLP CFDA internalization. Therefore, we decided to study the effect of LF on HPV binding. The unlabelled HPV-16 VLPs were mixed with LF and added to HaCaT cells at 0 °C and the amount of surface bound virus was studied by western blot, using antibodies directed against the HPV L1 capsid protein. A dose-dependent inhibition of binding by both BLF and HLF was observed (Fig. 2). BLF inhibited binding at lower concentrations than HLF, with an IC_{50} of 30 μg/ml and a maximum of inhibition of 90% at 100 μg/ml in comparison to HLF with an IC_{50} of 90 μg/ml and a maximum of inhibition of 70% at 200 μg/ml (Fig. 2).

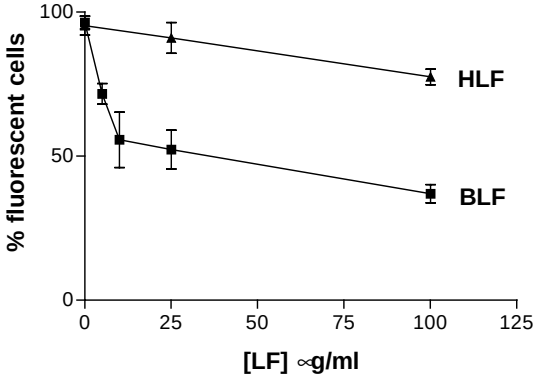

Fig. 1. Lactoferrin inhibited HPV uptake. Dose response curve of inhibition of HPV-16 VLP uptake by bovine lactoferrin (BLF) and human lactoferrin (HLF). CFDA SE-labelled HPV-16 VLPs and HaCaT cells were incubated for 2 h at 37 °C with different concentrations of either BLF or HLF. Uptake was analysed by measuring the fluorescence by flow cytometry. Data are mean ± S.E. of three separate experiments in duplicates performed with each LF.

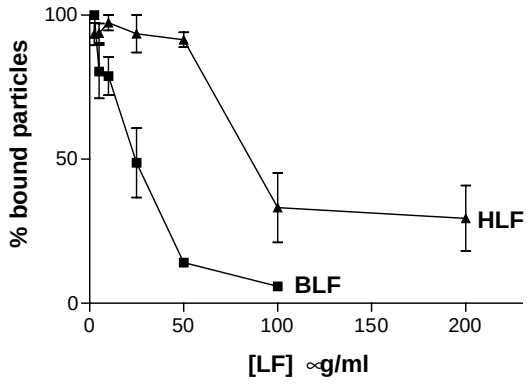

Fig. 2. Lactoferrin inhibited HPV binding. Dose–response curve of inhibition of HPV-16 VLP binding at 0 °C by bovine lactoferrin (BLF) and human lactoferrin (HLF). Binding was detected with an antibody against the L1 capsid protein. The amount of bound HPV VLPs to untreated cells was set to 100% and the relative values were calculated using the GelPro analyser software. Data are mean ± S.E. of three separate experiments performed with each LF.

3.3. Addition time point assay

To investigate if LF could inhibit papillomavirus uptake after virus adsorption to the cell surface, HaCaT cells were incubated with CFDA-SE-labelled HPV-16 VLPs and HLF or BLF was added at different time points. The amount of LF used in the assay was determined on the basis of the lowest LF concentrations where the internalization inhibition reached a plateau (Fig. 1). When LF was added only a few minutes after the virus there was an immediate decrease of inhibition (Fig. 3). Thus, LF seemed to exert the antiviral activity early in the HPV entry process and did not have high inhibitory effect when cells were incubated with virus for more than 25 min. When LF was added two hours after VLPs neither BLF nor HLF could reduce uptake with more than 10% (Fig. 3).

4. Discussion

This is the first study showing that the entry of papillomavirus could be inhibited by LF. We showed here that the inhibition of uptake and binding by BLF was very specific with an IC_{50} of 35 μg/ml and 30 μg/ml, respectively, for HPV-16 VLPs. This is similar to other studies where BLF show an IC_{50} for HSV-1 of 10 μg/ml (Marchetti et al., 1996) and for enterovirus 71 of 10–25 μg/ml (Lin et al., 2002). Several other viruses are also inhibited by LF and for viruses belonging to the herpesvirus family it has been shown that the LF antiviral effect is exerted by inhibiting viral entry (Andersen et al., 2001; Marchetti et al., 1996). In fact, glycosaminoglycan

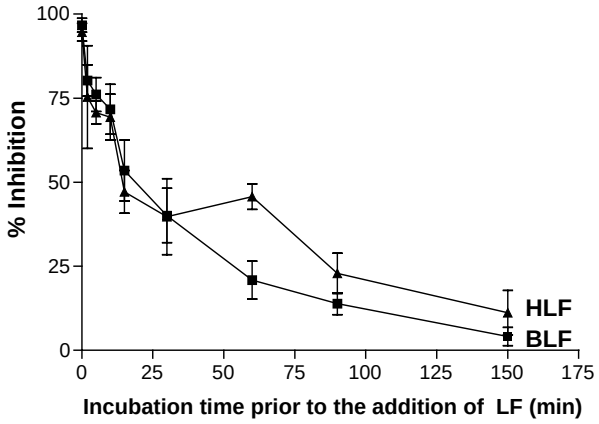

Fig. 3. Addition of lactoferrin after virus adsorption. The timeframe in where lactoferrin (LF) exerts its inhibitory effect was studied by incubating HaCaT cells with CFDA SE-labelled HPV-16 VLPs for certain time points before adding either 10 (g BLF or 100 (g human LF (HLF). The LF concentrations were chosen where inhibition reached a plateau (Fig. 1 and the absolute inhibition at zero time point was 45% for BLF and 21% for HLF. The sample where LF was added simultaneously with VLPs was set to 100% inhibition and relative values from other time points were calculated. Uptake was analysed by measuring the fluorescence by flow cytometry. Data for BLF are mean ± S.E. of three separate experiments in duplicates. Data for HLF are mean ± S.E. of one experiment in duplicate.

chains on the cell surface have been shown to interact with LF (Pierce et al., 1991) suggesting that LF binding to heparan sulfate blocks the herpesvirus receptor. For papillomavirus, a similar interaction is likely since, papillomavirus have been shown to interact with heparan sulfate on the cell surface for initial binding (Combita et al., 2001; Drobni et al., 2003; Giroglou et al., 2001; Joyce et al., 1999; Shafti-Keramat et al., 2003). However, we could not completely inhibit HPV uptake and we cannot rule out that HPV binds to another receptor than heparan sulfate, e.g. α-6 integrin. BLF was more efficient than HLF in inhibiting HPV uptake into HaCaT cells. Bovine and human LF differs in their structure and within the molecules the glycan chains and the number of disulphide bridges vary (Metz-Boutigue et al., 1984). Furthermore, commercial preparations of human LF might have lost N-terminal arginine residues which might effect the antiviral activities of the LF protein (van der Strate et al., 2001). A quenching effect of LF has also been described (Xiao and Kisaalita, 1997), but we did not detect any differences in quenching of CFDA SE fluorescence using BLF and HLF (data not shown).

Uptake of HPV-16 VLPs was inhibited by adding LF to the target cells together with the virus, but when preincubating cells with HPV for increasing periods before LF was added the antiviral effect of LF gradually disappeared. This indicated that the inhibition was directed at the receptor binding step and once the virus had bound to the receptor, LF had very small effect. The uptake process of HPV in vitro is slow. For uptake of CFDA SE-labelled HPV-16 VLPs into HaCaT cells we have previously shown that the uptake starts almost immediately but do not reach a plateau until after 2 h (Drobni et al., 2003). Using infectious virion uptake assays, it has been reported that HPV-33 VLPs have a halftime of uptake of 3 h (Selinka et al., 2003) while 50% of bovine papillomavirus-1 VLPs are taken up by 4 h (Day et al., 2003). Since, our CFDA SE VLP assay measure uptake into the cytosol and not transport to the nucleus it has a shorter halftime of uptake. Interestingly heparin, VLP antiserum and certain monoclonal antibodies against HPV capsid proteins are able to neutralize HPV after attachment to the cell surface (Christensen et al., 1995; Giroglou et al., 2001; Selinka et al., 2003). Heparin was able to completely neutralize HPV-33 pseudoinfection up to 2 h after attachment (Giroglou et al., 2001). The mechanisms for the heparin antiviral effect against HPV is by binding to the virus particle, while LF most probably inhibited HPV interaction with heparan sulfate on the cell surface. For rotavirus, poliovirus, HIV-1 and hepatitis C virus it has been reported that LF interacts directly with the virus thereby, preventing adsorption of the virus to target cells in vitro (Marchetti et al., 1999; Pietrantoni et al., 2003; Superti et al., 1997; Swart et al., 1996; Yi et al., 1997). Although, our results suggest that LF inhibit HPV entry by binding to heparan sulfate we cannot rule out that LF also can bind directly to HPV VLPs. HPV VLPs composed of both the L1 and L2 capsid proteins will most likely be inhibited by LF, since, CFDA SE-labelled HPV L1 VLPs have the same internalization efficiency as HPV L1+L2 VLPs (Drobni et al., 2003). The minor capsid protein L2 probably has a role in transport to the nucleus, but not in initial attachment to the cell surface.

There was a difference in efficacy of LF inhibition between HPV binding and HPV uptake, which may indicate that the LF-cell interaction is less stable at 37 °C and a higher LF concentration is needed for inhibition than at 0 °C. It is tempting to speculate that HPV may directly use secondary uptake receptors to enter cells at 37 °C, while at 0 °C only heparan sulfate may be available. This would suggest a heparan sulfate independent pathway of uptake for HPV-16 VLPs in HaCaT cells. Interestingly, it was recently shown that the affinity of lactoferricin analogues for heparan sulfate was shown to only be partly responsible for the anti-HSV activity (Andersen et al., 2003).

LF is present in mucus secretions, milk, saliva, tears, seminal fluids, vaginal secretions and endocervix. It is not known whether the frequency of infection of mucosal HPVs is correlated to the level of LF in the oral- and genital-tract. It is tempting to speculate that LF, as well as other antimicrobial peptides within the innate immune system, play a role in the defense against HPV. For home-therapy against anogenital warts caused by HPV, two treatments are recommended. Podophyllotoxin or podofilox, a purified extract of the podophyllum plant inhibits mitotic division of the HPV host cell and imiquimod, a nucleoside-like compound acts as an immune response inducer leading to wart regression (von Krogh et al., 2000). The clearence rates are 60–80% for podophyllotoxin (Claesson et al., 1996; Petersen et al., 1995; Strand et al., 1995) and 56% for imiquimod (Edwards et al., 1998). These therapies do not resolve all HPV caused warts

but interestingly, for herpes simplex virus 1 and 2 infection in vitro, a synergy effect of infection inhibition was seen using LF or lactoferricin combined with acyclovir (Andersen et al., 2003). Possibly LF could act alone or in synergy with other substances to inhibit HPV infection.

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References

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 Res. 51, 141–149.

Andersen, J.H., Jenssen, H., Gutteberg, T.J., 2003. Lactoferrin and lactoferricin inhibit Herpes simplex 1 and 2 infection and exhibit synergy when combined with acyclovir. Antiviral Res. 58, 209–215.

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. Med. Microbiol. Immunol. (Berl) 182, 97–105.

Christensen, N.D., Cladel, N.M., Reed, C.A., 1995. Postattachment neutralization of papillomaviruses by monoclonal and polyclonal antibodies. Virology 207, 136–142.

Claesson, U., Lassus, A., Happonen, H., Hogstrom, L., Siboulet, A., 1996. Topical treatment of venereal warts: a comparative open study of podophyllotoxin cream versus solution. Int. J. STD AIDS 7, 429–434.

Cohen, M.S., Britigan, B.E., French, M., Bean, K., 1987. Preliminary observations on lactoferrin secretion in human vaginal mucus: variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with Neisseria gonorrhoeae. Am. J. Obstet. Gynecol. 157, 1122–1125.

Combita, A.L., Touze, A., Bousarghin, L., Sizaret, P.Y., Munoz, N., Coursaget, P., 2001. Gene transfer using human papillomavirus pseudovirions varies according to virus genotype and requires cell surface heparan sulfate. FEMS Microbiol. Lett. 204, 183–188.

Day, P.M., Lowy, D.R., Schiller, J.T., 2003. Papillomaviruses infect cells via a clathrin-dependent pathway. Virology 307, 1–11.

Di Biase, A.M., Pietrantoni, A., Tinari, A., Siciliano, R., Valenti, P., Antonini, G., Seganti, L., Superti, F., 2003. Heparin-interacting sites of bovine lactoferrin are involved in anti-adenovirus activity. J. Med. Virol. 69, 495–502.

Drobni, P., Mistry, N., McMillan, N., Evander, M., 2003. Carboxyfluorescein diacetate, succinimidyl ester labeled papillomavirus virus-like particles fluoresce after internalization and interact with heparan sulfate for binding and entry. Virology 310, 163–172.

Edwards, L., Ferenczy, A., Eron, L., Baker, D., Owens, M.L., Fox, T.L., Hougham, A.J., Schmitt, K.A., HPV Study Group. Human PapillomaVirus, 1998. Self-administered topical 5% imiquimod cream for external anogenital warts. Arch. Dermatol. 134, 25–30.

Evander, M., Frazer, I.H., Payne, E., Qi, Y.M., Hengst, K., McMillan, N.A., 1997. Identification of the alpha6 integrin as a candidate receptor for papillomaviruses. J. Virol. 71, 2449–2456.

Farley, J., Loup, D., Nelson, M., Mitchell, A., Esplund, G., Macri, C., Harrison, C., Gray, K., 1997. Neoplastic transformation of the endocervix associated with downregulation of lactoferrin expression. Mol. Carcinog. 20, 240–250.

Giroglou, T., Florin, L., Schafer, F., Streeck, R.E., Sapp, M., 2001. Human papillomavirus infection requires cell surface heparan sulfate. J. Virol. 75, 1565–1570.

Hagensee, M.E., Yaegashi, N., Galloway, D.A., 1993. Self-assembly of human papillomavirus type 1 capsids by expression of the L1 protein alone or by coexpression of the L1 and L2 capsid proteins. J. Virol. 67, 315–322.

Hansen, N.E., Malmquist, J., Thorell, J., 1975. Plasma myeloperoxidase and lactoferrin measured by radioimmunoassay: relations to neutrophil kinetics. Acta Med. Scand. 198, 437–443.

Hara, K., Ikeda, M., Saito, S., Matsumoto, S., Numata, K., Kato, N., Tanaka, K., Sekihara, H., 2002. Lactoferrin inhibits hepatitis B virus infection in cultured human hepatocytes. Hepatol. Res. 24, 228.

Joyce, J.G., Tung, J.S., Przysiecki, C.T., Cook, J.C., Lehman, E.D., Sands, J.A., Jansen, K.U., Keller, P.M., 1999. The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. J. Biol. Chem. 274, 5810–5822.

Kirnbauer, R., Booy, F., Cheng, N., Lowy, D.R., Schiller, J.T., 1992. Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc. Natl. Acad. Sci. U.S.A. 89, 12180–12184.

Lin, T.Y., Chu, C., Chiu, C.H., 2002. Lactoferrin inhibits enterovirus 71 infection of human embryonal rhabdomyosarcoma cells in vitro. J. Infect. Dis. 186, 1161–1164.

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 Res. 29, 221–231.

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. Med. Microbiol. Immunol. (Berl) 187, 199–204.

McCann, K.B., Lee, A., Wan, J., Roginski, H., Coventry, M.J., 2003. The effect of bovine lactoferrin and lactoferricin B on the ability of feline calicivirus (a norovirus surrogate) and poliovirus to infect cell cultures. J. Appl. Microbiol. 95, 1026–1033.

McLaughlin-Drubin, M.E., Wilson, S., Mullikin, B., Suzich, J., Meyers, C., 2003. Human papillomavirus type 45 propagation, infection, and neutralization. Virology 312, 1–7.

Metz-Boutigue, M.H., Jolles, J., Mazurier, J., Schoentgen, F., Legrand, D., Spik, G., Montreuil, J., Jolles, P., 1984. Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. Eur. J. Biochem. 145, 659–676.

Meyers, C., Bromberg-White, J.L., Zhang, J., Kaupas, M.E., Bryan, J.T., Lowe, R.S., Jansen, K.U., 2002. Infectious virions produced from a human papillomavirus type 18/16 genomic DNA chimera. J. Virol. 76, 4723–4733.

Muller, M., Gissmann, L., Cristiano, R.J., Sun, X.Y., Frazer, I.H., Jenson, A.B., Alonso, A., Zentgraf, H., Zhou, J., 1995. Papillomavirus capsid binding and uptake by cells from different tissues and species. J. Virol. 69, 948–954.

Murphy, M.E., Kariwa, H., Mizutani, T., Tanabe, H., Yoshimatsu, K., Arikawa, J., Takashima, I., 2001. Characterization of in vitro and in vivo antiviral activity of lactoferrin and ribavirin upon hantavirus. J. Vet. Med. Sci. 63, 637–645.

Ozbun, M.A., 2002a. Human papillomavirus type 31b infection of human keratinocytes and the onset of early transcription. J. Virol. 76, 11291–11300.

Ozbun, M.A., 2002b. Infectious human papillomavirus type 31b: purification and infection of an immortalized human keratinocyte cell line. J. Gen. Virol. 83, 2753–2763.

Park, D.S., Selvey, L.A., Kelsall, S.R., Frazer, I.H., 1993. Human papillomavirus type 16 E6, E7 and L1 and type 18 E7 proteins produced by recombinant baculoviruses. J. Virol. Methods 45, 303–318.

Petersen, C.S., Agner, T., Ottevanger, V., Larsen, J., Ravnborg, L., 1995. A single-blind study of podophyllotoxin cream 0.5% and

podophyllotoxin solution 0.5% in male patients with genital warts. Genitourin Med. 71, 391–392.

Pierce, A., Colavizza, D., Benaissa, M., Maes, P., Tartar, A., Montreuil, J., Spik, G., 1991. Molecular cloning and sequence analysis of bovine lactotransferrin. Eur. J. Biochem. 196, 177–184.

Pietrantoni, A., Di Biase, A.M., Tinari, A., Marchetti, M., Valenti, P., Seganti, L., Superti, F., 2003. Bovine lactoferrin inhibits adenovirus infection by interacting with viral structural polypeptides. Antimicrob. Agents Chemother. 47, 2688–2691.

Qi, Y.M., Peng, S.W., Hengst, K., Evander, M., Park, D.S., Zhou, J., Frazer, I.H., 1996. Epithelial cells display separate receptors for papillomavirus VLPs and for soluble L1 capsid protein. Virology 216, 35–45.

Roden, R.B., Kirnbauer, R., Jenson, A.B., Lowy, D.R., Schiller, J.T., 1994. Interaction of papillomaviruses with the cell surface. J. Virol. 68, 7260–7266.

Rose, R.C., Bonnez, W., Reichman, R.C., Garcea, R.L., 1993. Expression of human papillomavirus type 11 L1 protein in insect cells: in vivo and in vitro assembly of viruslike particles. J. Virol. 67, 1936–1944.

Selinka, H.C., Giroglou, T., Nowak, T., Christensen, N.D., Sapp, M., 2003. Further evidence that papillomavirus capsids exist in two distinct conformations. J. Virol. 77, 12961–12967.

Shafti-Keramat, S., Handisurya, A., Kriehuber, E., Meneguzzi, G., Slupetzky, K., Kirnbauer, R., 2003. Different heparan sulfate proteoglycans serve as cellular receptors for human papillomaviruses. J. Virol. 77, 13125–13135.

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. Adv. Exp. Med. Biol. 357, 13–19.

Strand, A., Brinkeborn, R.M., Siboulet, A., 1995. Topical treatment of genital warts in men, an open study of podophyllotoxin cream compared with solution. Genitourin Med. 71, 387–390.

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. Med. Microbiol. Immunol. (Berl) 186, 83–91.

Swart, P.J., Kuipers, M.E., Smit, C., Pauwels F R, deBethune, M.P, de Clercq, E<J>, Meijer, D.K, Huisman, J.G, 1996. Antiviral effects of milk proteins: acylation results in polyanionic compounds with potent activity against human immunodeficiency virus types 1 and 2 in vitro. AIDS Res. Hum. Retroviruses 12, 769–775.

van der Strate, B.W., Beljaars, L., Molema, G., Harmsen, M.C., Meijer, D.K., 2001. Antiviral activities of lactoferrin. Antiviral. Res. 52, 225–239.

Volpers, C., Unckell, F., Schirmacher, P., Streeck, R.E., Sapp, M., 1995. Binding and internalization of human papillomavirus type 33 virus-like particles by eukaryotic cells. J. Virol. 69, 3258–3264.

Vorland, L.H., Ulvatne, H., Andersen, J., Haukland, H.H., Rekdal, O., Svendsen, J.S., Gutteberg, T.J., 1999. Antibacterial effects of lactoferricin B. Scand. J. Infect. Dis. 31, 179–184.

von Krogh, G., Lacey, C.J., Gross, G., Barrasso, R., Schneider, A., 2000. European course on HPV associated pathology: guidelines for primary care physicians for the diagnosis and management of anogenital warts. Sex Transm. Infect. 76, 162–168.

Wu, H.F., Monroe, D.M., Church, F.C., 1995. Characterization of the glycosaminoglycan-binding region of lactoferrin. Arch. Biochem. Biophys. 317, 85–92.

Xiao, R., Kisaalita, W.S., 1997. Iron acquisition from transferrin and lactoferrin by Pseudomonas aeruginosa pyoverdin. Microbiology 143 (Pt 7), 2509–2515.

Yi, M., Kaneko, S., Yu, D.Y., Murakami, S., 1997. Hepatitis C virus envelope proteins bind lactoferrin. J. Virol. 71, 5997–6002.

Yoon, C.S., Kim, K.D., Park, S.N., Cheong, S.W., 2001. alpha(6) Integrin is the main receptor of human papillomavirus type 16 VLP. Biochem. Biophys. Res. Commun. 283, 668–673.

zur Hausen, H., 2002. Papillomaviruses and cancer: from basic studies to clinical application. Nat. Rev. Cancer 2, 342–350.