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MINI-REVIEW

Antiviral activities of whey proteins

Tzi Bun Ng[1] · Randy Chi Fai Cheung[1] · Jack Ho Wong[1] · Yan Wang[1] ·
Denis Tsz Ming Ip[1] · David Chi Cheong Wan[1] · Jiang Xia[2]

Abstract Milk contains an array of proteins with useful bioactivities. Many milk proteins encompassing native or chemically modified casein, lactoferrin, alpha-lactalbumin, and beta-lactoglobulin demonstrated antiviral activities. Casein and alpha-lactalbumin gained anti-HIV activity after modification with 3-hydroxyphthalic anhydride. Many milk proteins inhibited HIV reverse transcriptase. Bovine glycolactin, angiogenin-1, lactogenin, casein, alpha-lactalbumin, beta-lactoglobulin, bovine lactoferrampin, and human lactoferrampin inhibited HIV-1 protease and integrase. Several mammalian lactoferrins prevented hepatitis C infection. Lactoferrin, methylated alpha-lactalbumin and methylated beta-lactoglobulin inhibited human cytomegalovirus. Chemically modified alpha-lactalbumin, beta-lactoglobulin and lysozyme, lactoferrin and lactoferricin, methylated alpha-lactalbumin, methylated and ethylated beta-lactoglobulins inhibited HSV. Chemically modified bovine beta-lactoglobulin had antihuman papillomavirus activity. Beta-lactoglobulin, lactoferrin, esterified beta-lactoglobulin, and esterified lactoferrin displayed anti-avian influenza A (H5N1) activity. Lactoferrin inhibited respiratory syncytial virus, hepatitis B virus, adenovirus, poliovirus, hantavirus, sindbis virus, semliki forest virus, echovirus, and enterovirus. Milk mucin, apolactoferrin, Fe^{3+}-lactoferrin, beta-lactoglobulin, human lactadherin, bovine IgG, and bovine kappa-casein demonstrated antihuman rotavirus activity.

✉ Tzi Bun Ng
b021770@mailserv.cuhk.edu.hk

✉ Randy Chi Fai Cheung
chifaicheung@cuhk.edu.hk

[1] School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

[2] Department of Chemistry, Faculty of Science, The Chinese University of Hong Kong, Hong Kong, China

Introduction

Milk and colostrum are an abundant source of proteins/peptides with pivotal roles in both neonates and adults. Milk proteins and peptides are potential health-enhancing nutraceuticals. They may be utilized as nutraceuticals in the treatment of asthma, cancer, dental diseases, diarrhea, hypertension, immunodeficiency, mineral malabsorption, and thrombosis. Proteins and peptides in milk are characterized by bioavailability and safety. Lactoferrin exhibits antiviral, antifungal, antibacterial, antitumor, antiparasite, and immunoenhancing activities. Lactoperoxidase has antibacterial activities. Lysozyme prevents dental decay. Casein and its hydrolyzate protect against bacteriemia and display antitumor activity. Glycomacropeptide derived from kappa-casein exerts antithrombotic and antibacterial actions. Alpha-lactalbumin possesses antiviral, antitumor, anxiolytic, antistress, antidiarrhea, and antihypertensive activities. Proline-rich polypeptide enhances T cell maturation and protects against autoimmune diseases (Zimecki and Kruzel 2007; Kanwar et al. 2009; Almehdar et al. 2015). The intent of the present article is to review the antiviral effects of various whey proteins, and their different antiviral actions are shown in Fig. 1.
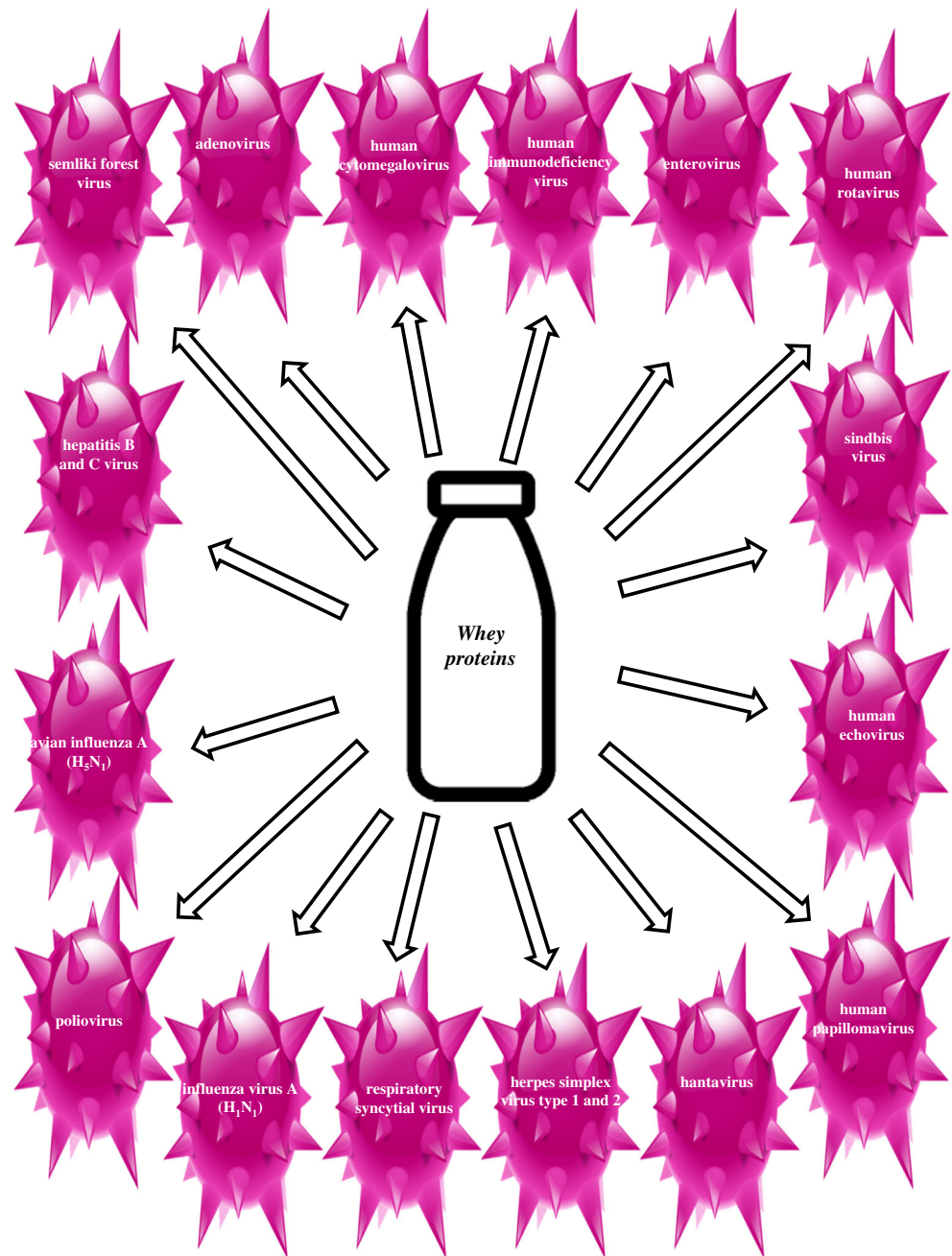
Fig. 1 Different antiviral actions
of milk proteins

Antihuman immunodeficiency virus (anti-HIV) activity

Secretory leukocyte protease inhibitor, a protein found in milk and other body secretions like saliva, inhibited HIV type 1 in vitro. The level of secretory leukocyte protease inhibitor in infant saliva did not correlate with HIV-1 transmission overall. However, among breast-fed babies who were HIV-1 uninfected at 1 month, elevated salivary levels of secretory leukocyte protease inhibitor correlate with a reduced risk of HIV-1 transmission through breast milk (Farquhar et al. 2002).

Purified milk mucins but not crude breast milk inhibited HIV-1 in vitro (Habte et al. 2008; Mthembu et al. 2014). This is because MUC1 in crude milk is surrounded by fat globules, and to act against the virus, it has to be liberated from the fat globules (Habte et al. 2008). Angiogenin is a milk protein with both ribonuclease and anti-HIV activities (Bedoya et al. 2006; Cocchi et al. 2012).

Tenascin-C in breast milk is an extracellular matrix protein with a pivotal role in fetal development and wound healing. It neutralized HIV-1 by binding HIV-1 envelope protein at the chemokine coreceptor site CD4. This process was inhibited by

monoclonal antibodies directed against V3 loop-(19B and F39F) and chemokine coreceptor binding site. This accounts for protection of most of the HIV-1-exposed breast-fed babies against mucosal HIV-1 transmission (Fouda et al. 2013).

Chemical modification of lysine residues in casein by aromatic acid anhydrides, such as 3-hydroxyphthalic and trimellitic anhydrides and trimellitic anhydride chloride, ensued in potent inhibition by casein of (i) binding between the HIV-1 gp 120 envelope glycoprotein and the CD4 cell receptor, due to binding to CD4, and (ii) HIV-1 infection. Modified bovine milk proteins demonstrated potent inhibition of HIV-1 and may be potentially useful for anti-HIV-1 prophylaxis (Neurath et al. 1995).

Bovine lactoferrin in apo-form or forms saturated with ferric, manganese, or zinc ions potently suppressed HIV-1 replication and syncytium formation and curtailed HIV-1 DNA in the C8166 T cell line when added prior to HIV infection or during the viral adsorption step. The mechanism of action may involve inhibition of HIV binding to or entry into C8166 cells although a postadsorption effect cannot be eliminated (Puddu et al. 1998).

The polyanionic reagents dextran sulfates, heparin sulfates, and anionic proteins display anti-HIV activity in vitro. The chemical modification product of beta-lactoglobulin formed by reaction with 3-hydroxyphthalic anhydride possessed a considerably elevated HIV inhibitory activity. Addition of anionic carboxyl groups along the polypeptide backbone engendered repulsion within the protein molecule and consequent modifications in secondary and tertiary structures, producing an unstructured protein. Similarly, alpha(S2)-casein and alpha-lactalbumin gained anti-HIV activity after modification by 3-hydroxyphthalic anhydride (Berkhout et al. 1997).

Unlike some chemically modified milk proteins, unmodified alpha(s2)-, beta-, and kappa-casein, as well as several negatively and positively charged fragments, were devoid of a repressive effect on HIV replication. Bovine lactoferrin suppressed HIV-1 with an IC_{50} of 0.4 μM. Lactoferricin, a positively charged domain of bovine lactoferrin, manifested moderate inhibitory activity, signifying that domains of the lactoferrin molecule in addition to lactoferricin contribute to the HIV inhibitory activity of bovine lactoferrin. Bovine lactoferrin suppressed HIV-1 variants that employ either the CXCR4 or the CCR5 coreceptor. In order to elucidate the mechanism of action of bovine lactoferrin, an HIV-1 variant demonstrating resistance to the milk protein was utilized. The viral envelope protein, which harbors two mutations associated with changed virus-host interaction and receptor-coreceptor interaction, plays a role in the resistant phenotype. The data show that bovine lactoferrin targets HIV-1 entry (Berkhout et al. 2002).

Two peptides composed of nine and 18 amino acids of human lysozyme (HL9 and HL 18), corresponding to residues 98-115 and 107-115, inhibited HIV-1 infection and replication with EC_{50} of 50 to 55 nM, similar to intact lysozyme. Scrambling the sequence or replacement of tryptophan or arginine residues abrogated the antiviral activity. HL9, with the sequence RAWVAWRNR, which forms a pocket with superficial basic residues, displays an alpha-helical structure in native human lysozyme, in a region separate from its muramidase catalytic site. However, the tendency to form helix showed no correlation with antiviral activity as revealed by Monte Carlo peptide folding energy minimizing simulation modeling and circular dichroism studies. The HL9 peptide modified gene expression of HIV-infected cells, impacting pathways implicated in stress, survival, transforming growth factor-beta, NF kappaB, p53, protein kinase C, and hedgehog signaling (Lee-Huang et al. 2005).

Wang et al. (2000) assayed a number of milk proteins for human immunodeficiency virus-1 reverse transcriptase, alpha-glucosidase, beta-glucosidase, and beta-glucuronidase inhibitory activities. Alpha-lactalbumin, beta-lactoglobulin, and casein displayed hardly any inhibitory effect. Lactoferrin demonstrated the highest inhibitory potency. Lactoperoxidase, lactogenin, angiogenin-1, and glycolactin inhibited HIV-1 RT activity with reduced potencies. Alpha-lactalbumin, beta-lactoglobulin, casein, glycolactin, and human lactoferrin exhibited augmented inhibitory effect following succinylation. The various milk proteins had little inhibitory effect on the activities of alpha-glucosidase, beta-glucosidase, and beta-glucuronidase. Succinylation enhanced the suppressive activity of milk proteins on alpha-glucosidase, but there was no similar effect on their inhibitory activity toward beta-glucosidase and beta-glucuronidase (Wang et al. 2000). A protein with an N-terminal sequence resembling polymeric immunoglobulin receptor and HIV-1 reverse transcriptase inhibitory activity has been isolated from bovine milk (Ng and Ye 2004). Although alpha-lactalbumin, beta-lactoglobulin, and casein had some HIV-1 protease inhibitory and integrase inhibitory activities, HIV-1 reverse transcriptase was not inhibited. Lactoferrin was a potent inhibitor of HIV-1 reverse transcriptase, but its HIV-1 protease inhibitory and integrase inhibitory activities were low. Glycolactin and angiogenin-1 mildly inhibited HIV-1 reverse transcriptase but more powerfully suppressed HIV-1 protease and integrase. In comparison with the other milk proteins, glycolactin strongly inhibited HIV-1 protease and integrase and mildly inhibited HIV-1 reverse transcriptase. Lactogenin exerted a marked suppressive action on HIV-1 integrase. Its HIV-1 reverse transcriptase inhibitory activity and HIV-1 protease inhibitory activity were, respectively, moderate and weak (Ng et al. 2001). Human lactoferricin potently inhibited HIV-1 reverse transcriptase with an IC_{50} of 2 μM. Bovine lactoferricin and bovine lactoferrampin derived from bovine lactoferrin were less potent, with an IC_{50} of 10 and 150 μM, respectively. Human lactoferrampin and human and bovine lactoferrin (1-11) at 1 mM concentration were devoid of any inhibitory effect on

HIV-1 reverse transcriptase. All peptides manifested a weak inhibitory action (from slightly below 2 to 6 % inhibition) on HIV-1 protease. Human and bovine lactoferrampins inhibited HIV-1 integrase significantly at 37 and 18.5 μM, respectively. Human and bovine lactoferrampins dose-dependently inhibited HIV-1 integrase. The other peptides were devoid of HIV-1 integrase inhibitory activity. Thus, some of the lactoferrin fragments inhibited HIV-1 reverse transcriptase and HIV-1 integrase (Wong et al. 2014).

Antihuman cytomegalovirus (anti-HCMV) and anti-herpes simplex virus type 1 and 2 (anti-HSV-1 and 2) activities

Introduction of positive charges to lactoferrin by amination led to an enhancement of its anti-HCMV activity and a fall in its anti-HIV activity. The N-terminal part of lactoferrin contributes to this anti-HCMV activity (Swart et al. 1998).

Unmodified milk proteins with the exception of lactoferrin are devoid of inhibitory activity toward HIV and HCMV. Chemical modification which endows several other milk proteins with a polyanionic or polycationic characteristic enables them to acquire anti-HIV activity and anti-HCMV activity, respectively (Florisa et al. 2003).

Methylated alpha-lactalbumin and methylated beta-lactoglobulin, their peptic hydrolysates, and L-polylysines attenuated the infectivity of cytomegalovirus in MRC-5 fibroblasts (Chobert et al. 2007).

Reaction of alpha-lactalbumin, beta-lactoglobulin, and lysozyme with the chemical modification agent 3-hydroxyphthalic anhydride produced compounds with antiviral activity toward HSV-1 before, during, or after infection, but devoid of activity against porcine respiratory corona virus and bovine parainfluenza virus type 3. Peptic, tryptic, chymotryptic digestion of the proteins yielded anti-HSV fragments (Oevermann et al. 2003).

Lactoferrin or lactoferricin exhibited a synergistic action with acyclovir against HSV-1 and HSV-2. The 50 % effective concentration for the antiviral agent and the milk protein/peptide, when used in conjunction with acyclovir, could be reduced by 2-fold to 7-fold than when they were applied singly (Andersen et al. 2003).

Lactoferrin and lactoferricin thwarted the entry of HSV into host cells (Jenssen 2005). Methylated alpha-lactalbumin, methylated and ethylated beta-lactoglobulins, and their peptic hydrolyzates manifested a protective action against HSV-1, albeit less potent than those of acyclovir and L-polylysines, when tested against acyclovir-sensitive and acyclovir-resistant strains of HSV-1 using fixed or suspended Vero cell lines. Methylated alpha-lactalbumin inhibited HSV-1 replication as shown by real-time PCR (Sitohy et al. 2007).

Cells exposed to bovine lactoferrin and also cells exposed to bovine lactoferricin exhibited a marked reduction in HSV-1 cellular uptake compared to control cells which had been infected but not treated with milk proteins. The few internalized virus particles exhibited delayed intracellular trafficking (Marr et al. 2009).

Lactoferrin was localized at the cell surface in cells expressing heparin sulfate while lactoferricin exhibited a random intracellular distribution. Heparan sulfate at the cell surface is crucial to the antiviral activity of both lactoferrin and lactoferricin. In order to manifest antiviral action, lactoferrin has to be located at the cell surface while lactoferricin can do so when present inside the cells (Andersen et al. 2004).

Lactoferrin potent inhibitor human herpes simplex type 1 (HSV-1) and 2 (HSV-2). Bovine lactoferrin inhibited viral infection by binding to heparin sulfate glycosaminoglycans which are receptors for human herpetic viruses. The antiviral action of bovine lactoferrin did not necessitate gC-heparan sulfate interaction as the milk protein was active on both wild-type virus and mutant virus. The mutant virus HSV-2 gC-neg1 demonstrated higher sensitivity than the wild-type virus to human lactoferrin, suggesting interaction of human lactoferrin with viral structures covered by gC in wild-type viruses. Bovine lactoferrin displayed higher efficacy than human lactoferrin against HSV-2 infection. Distinct from the case of HSV-1, bovine lactoferrin suppressed plaque-forming activity of HSV-2 also in cells not expressing glycosaminoglycan. The findings suggest that the bovine milk protein may block a virus receptor of non-glycosaminoglycan nature (Marchetti et al. 2009).

Both lactoferrin and lactoferricin suppressed infection of GMK cells by HSV-2. Lactoferricin but not lactoferrin suppressed HSV-2 infection in vivo. When lactoferricin was mixed with HSV-2 before inoculation, disease development was inhibited, and the viral load in a mouse model of genital HSV-2 infection was reduced. The production of chemokines CCL3, CCL5, CXCL1, or CXCL2 by mouse splenocytes in vitro was not affected by the milk proteins. However, when tested in vivo, both lactoferrin and lactoferricin elicited vaginal CCL5 production and lactoferrin induced CXCL2 production. Neither lactoferrin nor lactoferricin prevented HSV-2 infection when administered on the day before HSV-2 infection. Lactoferricin delayed the onset of the disease by 3 days and lowered the viral load by nearly 15-fold when administered 1 day after infection (Shestakov et al. 2012).

Antihepatitis B virus (anti-HBV) activity

Lactoferrin exerted its antiviral action at an early phase of HBV infection probably by interacting with host cell surface molecules. Human lactoferrin and seven synthetic peptides derived from human lactoferrin1-47 were tested for ability to

inhibit HBV infection and replication using the HepaRG and HepG2.2.2.15 cell lines. Four of the peptides elicited 40–75 % inhibition of HBV infection in HepaRG cells, with HLP1-23 containing the GRRRR cationic cluster exhibiting the highest potency. This GRRRR cationic cluster is one of the two glycosaminoglycan binding sites of native human lactoferrin associated with antiviral activity. The mechanisms of action of HLP1-23 and the full-length protein are distinct. Preincubation of the peptide with the virus but not with the target cells inhibited HBV infection. The positively charged cluster on the peptide enables it to interact with residues on the virion envelope carrying the opposite charge. The lack of the second glycosaminoglycan binding site accounts for its inability to efficiently adhere to the cells. HLP1-23 may hold promise for interfering with HBV entry by neutralizing the viral particles (Florian et al. 2013).

Lactoferrin interacted with HCV and inhibited HCV infection in cultured PH5CH8 human hepatocytes. Preincubation of PH5CH8 cells but not HBV with bovine or human lactoferrin had an inhibitory effect on HBV infection. Bovine transferrin, lactoalbumin and casein were devoid of anti-HBV activity (Hara et al. 2002).

Antihepatitis C virus (anti-HCV) activity

Bovine lactoferrin but not lactoferricin, beta-lactoglobulinor casein, inhibited HCV infection in PH5CH8 human liver cells which are susceptible to HCV infection and in human MT-2C T cells, by direct interaction with HCV. Inhibition of HCV infection by lactoferrin forestalled infection with hepatitis G virus, a distant relative of HCV (Ikeda et al. 2000).

Camel lactoferrin exerted a suppressive effect on HCV entry into Huh7.5 cells by directly interacting with the virus and HCV amplification in Huh7.5 cells. The N and C lobes of camel lactoferrin manifested anti-HCV activity in Huh7.5 cells (Liao et al. 2012).

Human lactoferrin, and also counterparts from the cow, sheep, and camel interfered with the entry of HCV into HepG2 cells by interacting directly with the virus rather than bringing about changes in the target cells, as well as reduced viral amplification in HepG2 cells infected by HCV. Camel lactoferrin was the most potent (El-Fakharany et al. 2013). The N-lobe fragment of camel lactoferrin possessed higher anti-HCV activity than camel lactoferrin which was more active than the C-lobe fragment of camel lactoferrin (Redwan et al. 2014).

Anti-adenovirus activity

Lactoferrin suppressed adenovirus infection by binding to adenovirus particles and competing for common glycosaminoglycan receptors. The targets are viral III and IIIa structural polypeptides (Pietrantoni et al. 2003).

Lactoferrin, but not alpha-lactalbumin, beta-lactoglobulin, or mucin, repressed adenovirus replication. Lactoferrin was capable of inhibiting viral replication when prior to or during the viral adsorption step, or throughout the adenovirus replicative cycle. Hence, lactoferrin exerts its action on an early phase of viral replication (Arnold et al. 2002).

Anti-hantavirus activity

The antiviral efficacies of bovine lactoferrin and ribavirin were determined against Seoul type hantavirus (SR-11 strain) in vitro. Hantaviral foci number in Vero E6 cells infected with SR-11 underwent a decline after exposure to lactoferrin for 5 days following infection to obtain a 50 % effective dose (ED50) of 2500 μg/ml, while pre-exposure to lactoferrin was highly effective yielding an ED50 of 39 μg/ml. However, pre-exposure to ribavirin for 1 h did not produce any inhibition of viral focus formation but decreased the number of viral foci (ED_{50}=10 μg/ml) when used from the time of viral infection. Pre-exposure of the cell monolayer to lactoferrin for 1 h followed by addition of ribavirin disclosed a synergism between the actions of lactoferrin and ribavirin against SR-11. Exposure of SR-11 to lactoferrin for 1 h prior to cell inoculation yielded an ED50 of 312.5 μg/ml. Rinsing the lactoferrin-pretreated cell monolayer with phosphate-buffered saline demonstrated minimal decrease of foci, indicating light attachment of lactoferrin to cells (Murphy et al. 2000). Subsequently, the anti-hantaviral mechanisms of action of bovine lactoferrin and ribavirin were elucidated. Hantavirus focus formation at 48 h was 15 % of the control value in cells exposed to 400 μg/ml lactoferrin for 1 h at 37 °C before viral infection and 2.5 % of the control value in cells exposed to 100 μg/ml ribavirin after viral infection. Hantavirus focus formation was totally abolished by preinfection exposure to lactoferrin and postinfection exposure to ribavirin. Synthesis of viral glycoprotein (G2) and nucleocapsid protein was postponed in lactoferrin-pretreated cells up to 24-h postinfection but became similar to the control value by 48-h postinfection. Lactoferrin suppressed viral shedding at 24 h but not at 48-h postinfection. Lactoferrin hinders viral adsorption to cells. On the other hand, ribavirin interferes with viral RNA synthesis. The effects of preinfection exposure to lactoferrin and postinfection exposure to ribavirin in hantavirus-infected suckling mice were investigated. Preinfection exposure to lactoferrin at 40 and 160 mg/kg 1 day before challenge with the virus increased the survival rates to 15 and 70 %, and to 85 and 94 %, respectively, 2 and 1 day before challenge with the virus. Ribavirin at the concentrations of 25 and 50 mg/kg yielded survival rates of 68 and 81 %, respectively. Hence, both

lactoferrin and ribavirin are effective in preventing hantavirus infection in vivo (Murphy et al. 2001).

Anti-alphavirus (Sindbis virus and Semliki Forest virus) activity

Lactoferrin inhibited infection of baby hamster kidney BHK-21 cells by heparin sulfate-adapted, but not by nonadapted Sindbis virus or Semliki Forest virus. Lactoferrin also inhibited binding of radiolabeled heparin sulfate-adapted viruses to BHK-21 cells or liposomes containing lipid-conjugated heparin as a receptor analog. However, viral fusion with liposomes induced by a lowpH, which takes place independently of virus-receptor interaction, was unaltered. Preincubation of virus or cells with lactoferrin disclosed that lactoferrin blocks heparin sulfate moieties on the cell surface but there is no binding to the virus. The antiviral activity of lactoferrin is associated with its cationic characteristic since human serum albumin, which acquired a cationic characteristic by charge modification, displayed analogous antiviral activity against heparin sulfate-adapted Sindbis virus and Semliki Forest virus. Thus, human lactoferrin interferes with virus-receptor interaction and thereby inhibits viral infection (Waarts et al. 2005).

Anti-avian influenza A (H5N1) activity

β-Lactoglobulin, lactoferrin, esterified β-lactoglobulin, and esterified lactoferrin manifested antiviral activity against A/chicken/Egypt/086Q-NLQP/2008 HPAI (H5N1) strain of clade 2.2.1, with the latter two proteins being more potent. α-Lactalbumin and esterified α-lactalbumin exhibited only weak antiviral activity (Taha et al. 2010).

Anti-influenza virus A subtype H1N1 activity

Lactoferrin or lactoperoxidase administered by gavage mitigated pneumonia in mice that had been infected intranasally with influenza virus A/PR/8/34 (H1N1). The serum concentration of pro-inflammatory interleukin-6 and the number of infiltrated leukocytes in the bronchoalveolar lavage fluid fell although there was no effect on the virus yield in the lavage fluid (Shin et al. 2005). MDCK cells infected with influenza virus subtype H1N1, upon exposure to methylated β-lactoglobulin, demonstrated a fall in hemagglutinating activity. The inhibition of the virus was escorted by a protection of MDCK cells. When the virus was treated with methylated β-lactoglobulin in the absence of MDCK cells, there were diminutions of intact virus particles and viral viability as well

as deleterious changes in the ultrastrctural appearance of the virus particles (Sitohy et al. 2010).

All main subtypes of influenza virus, including H1N1 and H3N2, are inhibited by bovine lactoferrin and its C-lobe but not its N-lobe. With the help of molecular docking, it was found that three fragments of the C-lobe bound to the HA(2) region of viral hemagglutinin and suppressed viral hemagglutination and infection at fentomolar concentrations (Ammendolia et al. 2012). Bovine lactoferrin suppressed cytopathic effects brought about by influenza virus when incubated with the cells after the adsorption step, and this activity was unrelated to ion saturation or carbohydrate content of the milk protein (Pietrantoni et al. 2012).

Antirespiratory syncytial virus activity

Lactoferrin demonstrated antiviral activity against respiratory syncytial virus in vitro but not in an in vivo mouse model. Whether the same is true in infants at risk for infection by respiratory syncytial virus awaits elucidation (Gualdi et al. 2013).

Antihuman papillomavirus activity

Pronounced inhibitory activity of 3-hydroxyphthalic anhydride-modified bovine beta-lactoglobulin, against human papillomaviruses, including HPV6, HPV16, and HPV18, has been reported. The anti-HPV activity showed correlation with the percentage of modified lysine and arginine residues in JB01. This modified milk protein showed no cytotoxicity at 1 mg/ml, and stability at room temperature and 37 °C for at least 3 months was noted. Hence, JB01 is a promising candidate for development into an anti-HPV agent (Lu et al. 2013).

Antihuman echovirus, antienterovirus, and antipoliovirus activities

Native bovine lactoferrin, and especially lactoferrin completely digested in the gastric phase with pH reduced to 2.5, suppressed echovirus 5 in vitro, when added prior to or concomitantly with the virus onto the cells. Bovine lactoferrin may prevent infection by gastrointestinal virus (Furlund et al. 2012).

Bovine lactoferrin thwarted echovirus 6-elicited cytopathic effect and antigen synthesis and suppressed viral replication prior to, during, and subsequent to the viral adsorption step. Lactoferricin also impeded viral binding (Pietrantoni et al. 2006).

Enterovirus type 71 is an etiologic agent of hand-foot-and-mouth disease. Recombinant porcine lactoferrin expressed in

the milk of transgenic mice protected the pups from EV71 infection (Chen et al. 2008).

Bovine lactoferrin suppresses poliovirus infection of monkey embryo kidney cells by attaching to the surfaces of susceptible cells and inhibiting viral adhesion. Hence, lactoferrin can be used to combat food-borne infections caused by enteric viruses like poliovirus (McCann et al. 2003).

Bovine and human lactoferrin but not alpha-lactalbumin, beta-lactoglobulin, or mucin prevented poliovirus infection in Vero cells. Apolactoferrin, native lactoferrin, or lactoferrin fully saturated with ferric, manganese, or zinc ions inhibited viral replication throughout the poliovirus infection cycle or during viral adsorption. Only zinc lactoferrin potently suppressed viral infection when incubated with the cells following viral attachment, with the inhibition showing a positive correlation with the extent of zinc saturation. All aforementioned lactoferrins acted on an early phase of poliovirus infection. Zinc lactoferrin was the only form of lactoferrin that could suppress an infection stage after viral internalization into the host cells (Marchetti et al. 1999).

Antihuman rotavirus activity

An elevated titer of antirotavirus antibodies was detected in camel milk. The lactoperoxidase system had no inhibitory effect on rotavirus (El-Agamy et al. 1992).

A complex of human milk glycoprotein mucin has elevated levels of antirotavirus activity which declines after deglycosylation. Human milk mucin bound to rotavirus and suppressed viral replication in vitro and in vivo (Yolken et al. 1992).

Apolactoferrin, Fe^{3+}-lactoferrin, and beta-lactoglobulin suppressed rotavirus replication, with apolactoferrin exhibiting the highest activity. Apolactoferrin bound to the viral particles, impeded viral attachment to cell receptors, suppressed both rotavirus hemagglutination and binding to human enterocyte-like HT-29 cells. Apolactoferrin exerted its inhibitory effects in the early phases of rotavirus infection. It undermined rotaviral antigen synthesis and yield in HT-29 cells during viral adsorption or the early hours of infection (Superti et al. 1997).

Rotavirus causes gastroenteritis in infants and may result in severe and even fatal dehydration. Lactadherin, a milk fat globule protein, may contribute to the higher protection of breast-fed children against infection. Human lactadherin attenuated the infectivity of rotavirus. Bovine MUC1 and a bovine macromolecular whey protein fraction CM3Q3 with bovine IgG as the main constituent were also active in reducing the infectivity of the mouse EMcN rotavirus strain as indicated by viral shedding in stools and inhibition of appearance of viral antibodies in serum in adult BALB/c mice (Bojsen et al. 2007).

The mechanism of suppressive action of bovine κ-CN on HRV entails binding to virus particles through glycan residues. The thermolabile structures in κ-CN may contribute to the maintenance of κ-CN binding to HRV (Inagaki et al. 2014).

Conclusions

From the foregoing account, it can be seen that a diversity of whey proteins exhibit antiviral activity (Table 1), sometimes only after chemical modification. The antiviral action is expressed on a large number of viruses pathogenic to humans including human immunodeficiency virus, human cytomegalovirus, hepatitis C virus, avian influenza A, influenza virus A subtype H1N1, respiratory syncytial virus, herpes simplex virus type 1 and type 2, human papillomavirus, human echovirus, enterovirus human rotavirus. It is possible that the milk proteins are also active against other viruses which have not been explored to date. As more investigations are conducted, it is likely that the list of milk proteins that are active against a particular virus can be expanded. This is because not all milk proteins have been tested for antiviral activity toward different viruses, for instance, whether apolipoprotein H-like milk protein, lactoribonuclease, lactogenin, and glycolactin (Ye et al. 1999; Ye and Ng 2000a, b, c) have antiviral activity awaits elucidation. The presence of a multitude of antimicrobial proteins in milk endows milk with health-promoting and antipathogenic properties. Regular consumption of milk is recommended by nutritionists and physicians. In fact, some milk formulas have been fortified with lactoferrin with the intent to promote health. Many of the milk proteins other than lactoferrin should be valuable nutraceticals. The advantages of milk proteins are that they have good bioavailability and safety and that some of them retain activity in the presence of proteolytic enzymes. It is not known whether the various milk proteins manifest synergistic actions with each other. Potentiation of the action of antiviral therapeutics has been demonstrated for some milk proteins especially in the case of bovine lactoferrin (Viani et al. 1999). This would facilitate the synergistic effect with antiviral drugs and the lowering of the drug dosage. The use of combination therapy reduces the likelihood of developing drug-resistant mutants and cytotoxicity due to lower dosage requirements for antiviral drugs (Andersen et al. 2003). Usually, the antiviral mechanisms of the milk proteins and antiviral agents are distinctly different, and they inhibited different steps in the viral replication cycle (van der Strate et al. 2003). Lactoferrin has antimicrobial properties against bacteria, fungi, and several viruses (Jenssen and Hancock 2009) which were included in this review like human immunodeficiency virus, human cytomegalovirus, hepatitis B and C viruses, adenovirus, poliovirus, hantavirus, sindbis virus, semliki forest virus, avian influenza A (H_5N_1), influenza virus A H_1N_1, respiratory syncytial virus,

Table 1 Inhibition of viral infections by milk proteins

Target	Milk protein types	Mode of action	Reference
Human immunodeficiency virus	Chemically modified casein, β-lactoglobulin and α-lactalbumin	binds to HIV-1 gp 120 envelope glycoprotein at CD4 cell receptor inhibits HIV-1 replication	(Neurath et al. 1995; Berkhout et al. 1997)
	Bovine lactoferrin and lactoferricin	blocks viral entry into host cells and CXCR4 or CCR5 attachment	(Berkhout et al. 2002)
	Bovine lactoferrin in apo-form or forms saturated with ferric, manganese or zinc ions	suppresses HIV-1 replication and syncytium formation, inhibits viral binding and entry into host cells	(Puddu et al. 1998)
	Secretory leukocyte protease inhibitor	inhibits HIV-1 replication	(Farquhar et al. 2002)
	Peptides composed of residues 98-115 and107-115 of human lysozyme	disrupts viral particle, prevents its binding and entry into target cells	(Lee-Huang et al. 2005)
	Angiogenin	inhibits HIV-1 replication	(Bedoya et al. 2006; Cocchi et al. 2012)
	Milk mucin	inhibits HIV-1 infectionby physically aggregation with the virus through a charge interaction with its negatively charged carbohydrate side chains containing highsialic acid and sulphate content	(Habte et al. 2008; Mthembu et al. 2014)
	tenascin-C	neutralizes HIV-1 by binding the viral envelope protein at the chemokine coreceptor site CD4	(Fouda et al. 2013)
Human cytomegalovirus	Lactoferrin	blocks viral replication	(Swart et al. 1998)
	Methylated α-lactalbumin and methylated β-lactoglobulin	interacts with the viral genomic DNA, perturbs replication or transcription activities of the virus.	(Chobert et al. 2007)
Herpes simplex virus type 1and 2	Chemically modified α-lactalbumin, β-lactoglobulin and lysozyme	inhibits HSV-1 multiplication	(Oevermann et al. 2003)
	Lactoferrin	inhibit the first steps in HSV infection, exhibits synergistic effect in combined therapy with acyclovir	(Andersen et al. 2003)
	Lactoferrin and lactoferricin	inhibits HSV replication, entry into host cells	(Jenssen 2005; Andersen et al. 2004; Marr et al. 2009)
Hepatitis B virus	Methylated α-lactalbumin, methylated and ethylated β-lactoglobulins	inhibits interactions between viral and cellular proteins, limits virus entry and protects the cells.	(Sitohy et al. 2007)
	Human lactoferrin and its synthetic derivativespeptides	inhibits viral entry by neutralizing the viral particles	(Florian et al. 2013)
Hepatitis C virus	Human and bovine lactoferrin	interferes with viral attachment and entry	(Hara et al. 2002)
	Bovine lactoferrin	neutralizes the virus and blocks its invasion into host cells	(Ikeda et al. 2000)
	Human, cow, sheep and camel lactoferrins	interacts with the virus and suppresses viral entry and amplification in the host cells	(Liao et al. 2012; Redwan et al. 2014; El-Fakharany et al. 2013)
Adenovirus	Bovine lactoferrin	prevents viral replication during the entire replicative cycle, neutralizes infection by binding to virus particles and that its targets are viral III and IIIa structural polypeptides	(Pietrantoni et al. 2003; Arnold et al. 2002)
Hantavirus	Bovine lactoferrin	inhibits virus adsorption to host cells and viral shedding	(Murphy et al. 2000, 2001)
Sindbis virus and semliki forest virus	Human lactoferrin	inhibits viral infection by interfering with virus-receptor interaction	(Waarts et al. 2005)

Table 1 (continued)

Target	Milk protein types	Mode of action	Reference
Avian influenza A (H_5N_1)	β-lactoglobulin lactoferrin, esterified β-lactoglobulin, and esterified lactoferrin	inhibits interaction with viral nuclear proteins (PB1, PB2, PA and NP), which catalyze the transcription of viral RNA and disturbs the overall replication pathways	(Taha et al. 2010)
Influenza virus A (H_1N_1)	Lactoferrin	suppresses infiltration of inflammatory cells in the lungs	(Shin et al. 2005)
	Methylated β-lactoglobulin	reduces viral RNA replication	(Sitohy et al. 2010)
	Bovine lactoferrin	suppresses cytopathic effects, binds to the HA(2) region of viral hemagglutinin and suppresses virus-induced hemagglutination and infection	(Ammendolia et al. 2012; Pietrantoni et al. 2012)
Respiratory syncytial virus	Lactoferrin	binds to the F1 protein subunit of the virus	(Gualdi et al. 2013)
Human papillomavirus	Chemically modified bovine β-lactoglobulin	inhibits the early stage of viral replication, particularly the viral entry process	(Lu et al. 2013)
Human echovirus	Bovine lactoferrin and lactoferrin	inhibits viral binding, exhibits cytopathic effect and antigen synthesis, inhibits viral replication prior to, during and subsequent to the viral adsorption step	(Pietrantoni et al. 2006; Furlund et al. 2012)
Enterovirus	Porcine lactoferrin	blocks the adsorption or receptor-mediated binding of the virus to the target cell membrane	(Chen et al. 2008)
Poliovirus	Bovine lactoferrin	targets viral adsorption, binds to the surfaces of host cells and prevents attachment of virus particles, competes for viral receptor interaction	(McCann et al. 2003; Marchetti et al. 1999)
Human rotavirus	Milk mucin	binds to rotavirus and suppressed viral replication	(Yolken et al. 1992)
	Apolactoferrin, Fe^{3+}-lactoferrin and β-lactoglobulin	binds to viral particles, impedes viral attachment to cell receptors, suppresses both rotavirus hemagglutination and binding to cellular receptor	(Superti et al. 1997)
	Human lactadherin and bovine IgG	inhibits rotavirus infectivity	(Bojsen et al. 2007)
	Bovine κ-casein	binds to virus particles through glycan residues	(Inagaki et al. 2014)

herpes simplex virus type 1 and 2, echovirus, enterovirus, and human rotavirus. Readers interested in antiviral properties of lactoferrin may consult a good review by van der Strate et al. (2001). The mechanism of antiviral action of milk proteins has not been uncovered for all viruses. It also remains to be elucidated whether the aforementioned different antiviral effects are demonstrable in animal models infected with the viruses. Since the fragments of lactoferrin like lactoferricin have high potency in some biological activities, fragments of the other milk proteins may likewise have considerable antiviral activity and can be chemically synthesized and used instead of the parent milk proteins.

A great deal of effort has been directed to the search of natural products with suppressive activity on various viruses (Harnett et al. 2005; Tshikalange et al. 2008; Klos et al. 2009; Ghosh et al. 2010; Chen et al. 2013; Mulder et al. 2013; Shin et al. 2013; Alfajaro et al. 2014; Wang et al. 2014; Xu et al. 2014; Dang et al. 2015). We have in milk a combination of proteins with activity against a diversity of viruses. Thus, milk has potential therapeutic application as well as good nutritional value.

Conflict of interest The authors have declared that no competing interests exist.

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