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Antiviral activity of human lactoferrin: Inhibition of alphavirus interaction with heparan sulfate

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Abstract

Human lactoferrin is a component of the non-specific immune system with distinct antiviral properties. We used alphaviruses, adapted to interaction with heparan sulfate (HS), as a tool to investigate the mechanism of lactoferrin's antiviral activity. Lactoferrin inhibited infection of BHK-21 cells by HS-adapted, but not by non-adapted, Sindbis virus (SIN) or Semliki Forest virus (SFV). Lactoferrin also inhibited binding of radiolabeled HS-adapted viruses to BHK-21 cells or liposomes containing lipid-conjugated heparin as a receptor analog. On the other hand, low-pH-induced fusion of the viruses with liposomes, which occurs independently of virus-receptor interaction, was unaffected. Studies involving preincubation of virus or cells with lactoferrin suggested that the protein does not bind to the virus, but rather blocks HS-moieties on the cell surface. Charge-modified human serum albumin, with a net positive charge, had a similar antiviral effect against HS-adapted SIN and SFV, suggesting that the antiviral activity of lactoferrin is related to its positive charge. It is concluded that human lactoferrin inhibits viral infection by interfering with virus-receptor interaction rather than by affecting subsequent steps in the viral cell entry or replication processes.

Keywords: Lactoferrin; Antiviral; Heparan sulfate; Receptor; Liposome; Sindbis virus; Semliki Forest virus

Introduction

Human lactoferrin (hLF) is an 80-kDa cationic glycoprotein belonging to the transferrin family of Fe^{3+}-transporting proteins (see Kanyshkova et al., 2001 for review). hLF is produced by epithelial cells and, as a result, is present in mucosal secretions such as tears, saliva, nasal exudate, gastrointestinal fluids, and seminal and vaginal fluids. Furthermore, hLF is present in high concentrations in human breast milk. Breast-feeding is known to protect newborns against a variety of infections including viral infections (Hanson and Korotkova, 2002). Viruses against which lactoferrin possesses antiviral activity include human herpes simplex virus types 1 and 2 (Hasegawa et al., 1994), adenovirus (Arnold et al., 2002), human immunodeficiency virus (Harmsen et al., 1995; Puddu et al., 1998; Swart et al., 1999), hepatitis C virus (Ikeda et al., 2000), human cytomegalovirus (Harmsen et al., 1995; Hasegawa et al., 1994; Swart et al., 1999), poliovirus (Marchetti et al., 1999), hantavirus (Murphy et al., 2000), and enterovirus 71 (Lin et al., 2002) (for a review, see van der Strate et al., 2001).

Most of the above studies on the antiviral activity of hLF suggest that the protein inhibits virus entry into cells rather than later phases of viral replication. Lactoferrin, in principle, can prevent virus cell entry by binding to the virus particle or by binding to cell-surface molecules that

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viruses use either as receptors or co-receptors. In either case, lactoferrin would prevent viral attachment to the cell surface (Meijer et al., 2001; van der Strate et al., 2001). It has been suggested that binding of hLF to cell-surface heparan sulfate glycosaminoglycans (HSPGs) is involved in inhibition of viral infection (van der Strate et al., 2001). Numerous studies have shown that viruses from different families interact with HSPGs. Examples include Sindbis virus (Byrnes and Griffin, 1998; Klimstra et al., 1998), Venezuelan equine encephalitis virus (Bernard et al., 2000), Ross River virus (Heil et al., 2001), adeno-associated virus type 2 (Summerford and Samulski, 1998), foot-and-mouth disease virus (Jackson et al., 1996; Sa-Carvalho et al., 1997), herpesviruses (Birkmann et al., 2001; Compton et al., 1993; Shukla and Spear, 2001; Trybala et al., 2002), human immunodeficiency virus (Patel et al., 1993), echovirus (Goodfellow et al., 2001), dengue virus (Chen et al., 1997; Germi et al., 2002; Hilgard and Stockert, 2000), and yellow fever virus (Germi et al., 2002). It is important to note that the ability of viruses to interact with HSPGs is often acquired by cell culture adaptation. However, affinity for HSPGs has also been demonstrated in clinical isolates, e.g., for Herpes Simplex virus type 1 (Trybala et al., 2002) and echovirus (Goodfellow et al., 2001).

In order to determine whether hLF indeed exerts antiviral activity through interference with virus binding to HSPG receptors, we studied the effects of hLF on the cell entry and receptor binding of alphaviruses, which are adapted to interaction with HSPGs. Alphaviruses are enveloped positive-strand RNA viruses belonging to the family *Togaviridae* (reviewed by Strauss and Strauss, 1994). Cell entry of alphaviruses is mediated by the heterodimeric E1/E2 envelope glycoprotein (Kielian, 1995). Infection of a host cell is initiated by the interaction of the E2 glycoprotein with an attachment receptor on the cell surface (Davis et al., 1986; McKnight et al., 1996; Russel et al., 1989; Tucker et al., 1997), after which the receptor-bound virion is internalized through endocytosis. This is followed by E1-mediated fusion of the viral envelope with the endosomal membrane, which delivers the viral RNA to the cytosol (Helenius et al., 1980; Marsh et al., 1982, 1983). For Sindbis virus (SIN), Klimstra et al. (1998) identified positively charged amino acid substitutions in the viral spike protein E2 after passaging of the virus on BHK-21 cells. These mutations are responsible for interaction of the virions with heparan sulfate (HS) moieties of HSPGs.

In the present study, the antiviral activity of hLF on an HS-adapted SIN mutant (TRSB), a non-adapted SIN strain (TR339), and tissue-culture-adapted Semliki Forest virus (SFV) was determined. Using a model system involving liposomes containing lipid-conjugated heparin (HepPE) as an attachment receptor analog for the virus (Smit et al., 2002), we demonstrate that hLF prevents infection of cells by alphaviruses through blocking of the viral attachment receptors on the cell surface.

Results

Inhibition of HS-adapted SIN and SFV infection by hLF

To determine whether hLF has antiviral activity against HS-adapted alphaviruses, we performed a plaque titration in the presence of hLF. CHO cells were pre-incubated with 200 μg/ml hLF. The same concentration of hLF was maintained throughout the infection and in the overlay. The cells were infected with SIN strains TR339 and TRSB, and a laboratory-adapted strain of SFV. TR339 is a non-HS-adapted SIN strain, whereas TRSB and the laboratory strain of SFV are known to use HS as a cell-attachment receptor (Klimstra et al., 1998; Smit et al., 2002). Infection of CHO cells by the SIN strain TR339 was not inhibited by hLF (Fig. 1, black bar). By contrast, in the presence of hLF the plaque titer of the HS-adapted SIN strain TRSB was strongly reduced (Fig. 1, gray bar). The laboratory strain of SFV was also strongly inhibited by hLF (Fig. 1, white bar). No cytotoxic effect of hLF on the cells was observed during the experiments.

The above results were confirmed by the use of recombinant SFV carrying the LacZ gene. The virus was derived from an SFV clone generated from a laboratory strain passaged on BHK cells (Liljeström and Garoff, 1991; Liljeström et al., 1991). In the presence of 200 μg/ml hLF, infection of BHK-21 cells by SFV-LacZ was completely abolished (Fig. 2). A standard cytotoxicity assay excluded the possibility that hLF was cytotoxic to the cells. In addition, hLF did not change the pH of the medium significantly up to a concentration of 2.5 mg/ml (not shown).

Taken together, the results shown in Figs. 1 and 2 clearly demonstrate that hLF inhibits the infection of cells by HS-

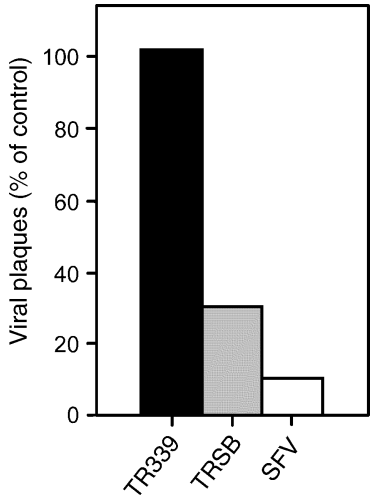

Fig. 1. Effect of hLF on infectivity of SIN TR339, SIN TRSB, and SFV. CHO-K1 cells were pre-incubated with medium containing 200 μg/ml hLF or with medium alone. Each virus was titrated on the cells in the presence or absence of 200 μg/ml hLF. The average titers in the presence of hLF, determined from two dilutions plated in duplicate, are presented as a percentage of the average titer in the absence of hLF.