The antiviral activity of the milk protein lactoferrin against the human Respiratory Syncytial Virus

Daniel Menendez1, Wesley Gladwell1 Heather Li1, Brian Elgart2, Steven Kleeberger1

1Immunity, Inflammation, and Disease Laboratory, 2Molecular Genomics Core, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709

Abstract

In absence of an effective vaccine, finding effective strategies to treat or reduce respiratory syncytial virus (RSV) infection is a global public health priority. Lactoferrin (LF) is an iron-binding glycoprotein broadly distributed within the body fluids and predominantly found in mammalian breast milk. It is a key component of the innate immune system with well-known antimicrobial effects. In this study, we aimed to evaluate the antimicrobial and immunomodulatory properties of LF within the context of its potential applications against RSV. Human and bovine LF as well as the derived acid-labile peptide lactoferrin B markedly inhibited in a dose and time-dependent manner the RSV-A2 strain entry, replication, and cytopathic effects when added before RSV infection or during the virus adsorption step in primary and cancer human and mouse cell lines. A related protein family member transferrin (TF) had no effects. In comparison to the control conditions, cells treated with the LF compounds had significantly less RSV infection based on fluorescence localization of the virus, cytopathology, and expression of RSV-F gene measured by ddPCR. Furthermore, LF treatment before the infection abolished the RSV-induced immune response at the transcriptional level. Our findings demonstrate that LF has a protective effect in response to RSV, suggesting LF as a strong candidate for an anti-RSV reagent that will be well-tolerated and effective in the prophylaxis against RSV infection.

Keywords: lactoferrin, respiratory syncytial virus, innate immune response, immune system, innate immunity, human milk

Lactoferrin, a multifunctional protein.

Lactoferrin (LF) is also known as lactoferrin, is a 80 kDa non-binding glycoprotein member of the transferrin family. It is found mainly in the saliva, tears and breast milk of mammalian species (1). LF is a key element of host defences against bacterial, viral, and parasitic infection, and it is considered an important component of the immune defense system of mucosal surfaces, including the upper and lower respiratory tract (1,2).

LF functional properties are highly conserved among mammals (4). For instance, bovine LF (bLF) is taken up by human lactoferrin receptor and exerts similar bioactivities as human lactoferrin (hLF) (4).

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is a negative-sense, enveloped, single-stranded RNA virus of the family Paramyxoviridae. Although RSV infects people of all ages, it is especially a significant cause of respiratory illness in young children and the elderly, leading to morbidity and mortality (5).

Infants who are breastfeeding are more protected against RSV severity and mortality (6). Very little is known about the effects of lactoferrin against RSV. It has been reported that LF has antiviral activity against RSV in HeLa cells (7,8). The effects of LF during RSV infection have not been studied in a lung cell model.

Objective

We suggest the protective role for lactoferrin against RVS works by: 1) inhibiting virus entry into host cells, 2) inhibiting virus replication, and 3) inhibiting virus-induced immune response, thus reducing disease severity.

We report here the effects of LF against RSV in human and mouse lung cell lines.

Summary and Conclusions

Both hLF and bLF and its derivative bLF-B, significantly limited in a dose response manner, the entry of RSV in human and mouse lung primary and cancer cell lines, including the infectivity and cytopathic effects related to the infection. Human LF has a prophylactic but not a therapeutic effect against RSV infection. LF protection occurs only when it is added before or during the infection process but not once the virus is inside the host cells.

Human LF enhanced the expression of several RSV-induced innate immune targets, including the antiviral/proinflammatory cytokines IL-1b and IL-6, and the pathogenic senescent TLR3 in a cell-dependant manner.

The antimicrobial and immunomodulatory effects against RSV specific for lactoferrin, since the related family protein transferrin had no significant effect against RSV infection.

We conclude that LF is a protective reagent for RVS works by: 1) reducing the RSV entry into the cells and 2) modulating the expression of immune response genes.

Our findings demonstrate that LF has a protective effect in response to RSV, suggesting LF as a strong candidate for an anti-RSV reagent that will be well-tolerated and effective in the prophylaxis against RSV infection.

References


Future Directions

- For a more accurate overall perspective of lactoferrin’s effect on the immune response, a transcriptional analysis of immune gene expression is currently being evaluated.
- We are establishing a mouse model to understand LF effects on RSV infection in vivo (See paper abstract # ARB0034 Gladwell et al.)
- Human clinical trials can be designed, where lactoferrin can be supplemented in concert to babies, and these studies would be observed for effects on RSV disease severity.

Acknowledgments

We would like to thank Traci Tong, Yin Li, NIEHS Core and Centers for their assistance and contributions: Molecular Genomics Core, VIP Vector Core, Food Chemistry Core, Peptide lab at Rush-Presbyterian St. Louis Medical Center for assistance. NIEHS Project 2P50 ES061002

Questions?

Please contact me at menendez@niehs.nih.gov

Figure 1. Lactoferrin reduces RSV infectivity in human lung cell lines. 

Figure 2. Specific prophylactic effects of human and bovine lactoferrin against RSV infection. 

Figure 3. Antiviral effect of lactoferrins against RSV in primary human and mouse lung cell lines. 

Figure 4. Lactoferrin reduces expression of RSV genes and proteins. 

Figure 5. Lactoferrin enhances innate immune gene expression during RSV infection in human lung cells. 

Figure 6. Specific prophylactic effects of human and bovine lactoferrin against RSV infection. 

Figure 7. Antiviral effect of lactoferrin against RSV in human lung cells.