Prophylactic effect of human and bovine lactoferrin against the respiratory syncytial virus in the mouse model.

Wesley Gladwell*, Daniel Menendez†, Kevin Gerrish‡, Steven Kleeberger§

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

Abstract

Background: Lactoferrin (LF) is a multifunctional glycoprotein, belonging to the transferrin family that is found in human milk and has broad antibacterial and antiviral effects. We have shown in vitro that LF has antiviral activity against the respiratory syncytial virus (RSV) in human and mouse cells. We hypothesized that human (hLF) and bovine LF (bLF) would attenuate RSV replication and lung inflammation and function in an in vivo murine model.

Methods: The antiviral activity of hLF and bLF was evaluated in male BALB/c mice inoculated with RSV-A2 strain (10^7 PFU). LF derived compounds or mock (DPBS) were administered daily by intranasal administration at doses of 1mg/day, from 72h before until 120h post-NSV inoculation. Human transferrin (TF) treatment was included as the internal control. Mice were sacrificed 3 and 5 days post-infection and bronchoalveolar lavage fluid (BALF) and whole lung samples were collected to assess lung inflammation and RSV loads (determined by ddPCR). Weight was assessed daily in all groups.

Results: No significant difference in body weight was found between the experimental groups. hLF and bLF but not TF treatment reduced the inflammation induced by RSV in the lungs as indicated by the decrease in the number of macrophages, monocytes, eosinophils, and lymphocytes in BALF when compared to the RSV only infected group. We also found that expression of RSV F and N genes was diminished in the lactoferrin plus RSV group compared to the RSV only infected group. TF had no effect, indicating that intranasal dosing of LF specifically protects and dampens RSV infection.

Conclusions: Intranasal administration of hLF and bLF has a protective and prophylactic antiviral activity against RSV in a mouse model.

Lactoferrin

Lactoferrin (LF) is a globular glycoprotein with a mass of 80 kDa, found mainly in the saliva, tears and breast milk of mammalian species (1).

Lactoferrin is part of the immune system for the body; it has antimicrobial activity and is part of the innate defense. In particular, lactoferrin provides antibacterial activity to the respiratory tract of infants (2).

Lactoferrin antiviral activity appears correlated to the prevention of viral binding to host cells, thus inhibiting infection (3).

LF's functional properties are highly conserved among mammals (2). For instance, bovine LF (bLF) is taken up by the human lactoferrin receptor and exerts similar antibacterial activities as human lactoferrin (hLF) (4).

Respiratory syncytial virus

Respiratory syncytial virus (RSV, Paramyxoviridae pneumovirus) is aspherical or pear-shaped virus that is prevalent in young children and the elderly, leading to morbidity and mortality (4).

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 (4).

Although LF's antiviral activity against RSV in vitro (Davies et al. 1988) was noted, antiviral activity was not observed in a mouse model (Gualdi et al. 2013).

There is no vaccine licensed and very few antiviral chemotherapeutic agents approved for the prevention and treatment of RSV.

Objective

Test the hypothesis that lactoferrin has a protective role in lung cells during respiratory syncytial virus infection in vivo using a mouse model.

Nasal lactoferrin treatment reaches lung tissue in mouse model.

Lactoferrin treatment reduces RSV infectivity in mouse model.

Summary and Conclusion

- Mice treated with hLF and infected with RSV have lower weight loss compared to the mice infected just with RSV virus.
- The hLF but not TF treatment reduced the inflammation induced by RSV infection in the lungs as indicated by the decrease in the number of macrophages, monocytes, eosinophils, and lymphocytes in BALF when compared to the RSV only infected group.
- bLF but not TF treatment reduced the inflammation induced by RSV infection, though it was less efficiency when compared to hLF effects.
- hLF and bLF treatments decreased the expression of RSV F and N genes, indicating that intranasal dosing of LF specifically protects and dampens RSV infection.
- We conclude that intranasal administration of hLF and bLF has a protective and prophylactic antiviral activity against RSV infection in an in vivo mouse model.

Future Directions

To study the immunomodulatory effects of lactoferrin we will conduct a transcriptomic analysis in lungs from RSV infected mice treated with human and bovine lactoferrin.

- Assess the histopathology of the upper and lower respiratory tracts in mice infected with RSV that were pretreated with lactoferrin.
- Evaluate by plaque assay the RSV titers in lungs from mice infected with RSV and without lactoferrin dosing.
- Include a control group of mice infected with RSV and treated with human lactoferrin to compare the differences in the outcome of infection.

We established a cell model to understand LF effects on RSV infection in vitro.

Please contact me at gladwell@niehs.nih.gov.

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References